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CATTLE OF SOUTHERN INDIA

BY

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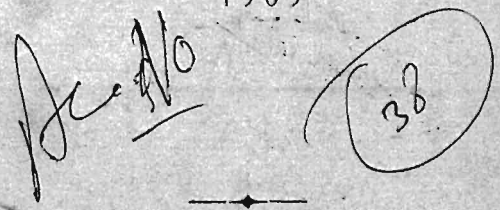

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CATTLE OF SOUTHERN INDIA.

THE cattle of the Madras Presidency have long been famous, and of the several breeds which are to be found in this part of the country those designated the "Mysore," and the "Ongole"—sometimes also known as the "Nellore," are undoubtedly pre-eminent. On account of its prepotency the former is most assuredly entitled to first honours as a visit to all the various large cattle fairs held in the southern districts of Trichinopoly, Madura, and Tinnevelly, also in the more northerly districts of Anantapur and Bellary will show how predominating is this type.

The "Ongoles" are very beautiful in appearance, and for their special purpose are unsurpassable, but they differ in almost every respect from the Mysore. They are huge in size, extremely docile, and suitable for steady heavy draught, whereas the Mysore cattle are specially adapted to road work, as they are quick, very high spirited, and have extremely hard sound feet.

Indian cattle, like those of Europe, vary in most districts either as to size, form, and symmetry, or as to the growth and length of their horns, according to the varying local peculiarities of the climate, soil, and lastly, but not least, fodder. It may be stated that both natural and artificial fodder tends to influence the form, size, and character of the animal. Ordinarily the native who lives on a meal of rice, and perhaps a few herbs to season the

same with, expects that his cattle will, in like manner, pick up what they can in the way of pasture about the village or its adjacent lands, so that he never troubles himself to grow green food, or prepare dry fodder for them; the same plant which supplies him with grain feeds his cattle also with its straw. In most towns and villages cattle are driven out at all seasons to graze abroad, and in the dry season they more frequently lick the dust only, and return home with their stomachs as empty as when they started, to receive perhaps a few handsful of straw or rubbish just sufficient to sustain life.

Madras is essentially a cattle-raising province, and consequently the animal wealth is enormous, but, as in other presidencies in India, large herds of village or mongrel cattle are to be met with everywhere. Many of them are worthless, being too weedy even to put into the lightest plough, and they are allowed to exist and eat the ration of the more profitable ones. Undoubtedly religious sentiment among the vast majority of the people is averse to destroying cattle, as among the Hindus the bull has always been considered to be sacred, and indeed is worshipped under the name of *Nundi*, it having formed the vehicle of their deity Shiva during his peregrinations. A Hindu would consider it a most grievous sin to kill them, and utter pollution to partake of their flesh, yet he freely partakes of their milk.

The three great centres of cattle raising are shown in the accompanying map, and from these they are taken by dealers, who form a very large community, to the numerous annual and weekly markets held in this presidency. For guidance a list of the fairs and the markets held weekly, also the approximate number of animals which are brought for sale, has been added at the end of the paper. Frequently these drovers have their regular

customers, and they receive payments by instalments, but this custom is principally limited to the north and western districts. Mysore has enjoyed from a very early period a just renown for a superior breed of cattle. The generally mild and salubrious climate of the plateau, with an extensive pasture on which cultivation has not made much inroad favoured cattle breeding, and attracted Gollas and other nomadic tribes from the north, who brought with them their excellent breeds which, being established for generations in the country, and mixing with the indigenous population, could not fail to improve them. In a country in which 90 per cent. of the population subsist by agriculture, and in which cattle play a most important part, a demand for them is never wanting. Cattle manure is used as fuel or serves to enrich the soil exhausted by cultivation. The operations of ploughing, harrowing, sowing, and thinning the crop, of lifting water from wells for irrigation purposes are carried on almost entirely by bullock power. The crop when cut is removed to the threshing floor, and there trodden out by the cattle, and transported by them to the market; and in fact it would be difficult for the Indian cultivator to get on without his cattle which indeed constitute the life and soul of agriculture. The substance of the ryot is usually estimated by the number of cattle he owns, and the number of ploughs he works. Moreover cattle are intimately associated with the domestic incidents of the people. The present of a cow with a few acres of land to the bridegroom is a noticeable part of the marriage ceremony. The present of a cow and land is also part of the Brahminical obsequies. As a propitiatory offering, when a relation has died, a young bull is presented to the deity to be eventually turned loose into the herd to eventually become a sire.

Mysore Breeds.

All over the presidency so far as cattle breeding is concerned two descriptions of cattle exist side by side, and this is particularly noticeable in Mysore, and also on the East Coast where the Ongole breed are to be found. The first is known as *Nadudana* or *Natudana*, really village cattle, which are by far the most numerous, of small size, compact frame, and various colours; every village in the province teems with them. They constitute the bulk of the agricultural stock, and are the main source of the dairy supply, such as it is. The second is termed the Doddadana meaning large cattle, and consists of the less numerous but more efficient and valuable kinds, of more uniform size and colour; they are more often used in conveying the traffic of the country than in agriculture, and are largely sold in cattle markets. Doddadana and Nadudana are particularly Mysore terms. The Doddadana embrace the Amrat Mahal, Hallikar, Chitraldroog, Alumbadi, or Mahadeswarabetta, and kindred breeds. Cattle of this description are only owned by well-to-do ryots and breeders. There are professional breeders, but every ryot who has a little capital adds to his agricultural occupation that of rearing a few head of cattle. There are parties who keep their herds of cows and bulls for breeding purposes mostly in the vicinity of grazing hills and lowland forests. Calves of a year old or so are bought from them by the ryots, who attend them with much care for two or three years, and exhibit them for sale to the best advantage at the cattle fairs.

The whole breeding operations of this country are carried on by means of three descriptions of bulls:—

(*a*) Choice specimens of the *Doddadana* breeds, either kept in villages and homefed, which are licensed to

graze on village crops or may be kept in the herds, and freely moving with them in their jungle pastures ; these may be styled *special superior breeding bulls.* The large majority of these have been dedicated to temples, and are thus held to be sacred.

(*b*) The calves of *Doddadana* bought when young and reared in villages, destined for agriculture or sale after castration, but employed as sires meanwhile. These may be styled *casual good breeding sires.* They are moderately good though inferior to the first named for breeding, and being permitted to cover before castration they make, it is said, less efficient agricultural and draught cattle.

(*c*) The numerous small sized and more or less ill-shaped young males of the "Nadudana" class herding with the village cattle, and these undoubtedly lead to degenerate breeding ; these may be called *Nadu bulls.* Nadudana or village cattle are left entirely to the course of nature without any control, and without any of those artificial restrictions by which alone a breed can be saved from degeneration. Seldom is any selection made of breeding cows and bulls with reference to their fitness for producing a healthy progeny. Nor are inferior and defective bulls generally castrated ; and the common practice of driving all the village cattle, male and female, together in one herd leads to indiscriminate breeding.

In some parts however it is the custom for one or two villages to club together and subscribe for a superior bull which is carefully selected and purchased when young. It is the common property of the villagers, and being allowed every license, even to the extent of grazing on private fields, keeps in excellent condition. Such bulls follow the herd during the day, and being accustomed to graze on rich crops seldom pay heed to the

poor grazing on the village common. They run to the field crops and graze their fill either after the herd returns home for the night, or before it is let out in the morning. With the better class animals great care and attention is paid to the selection of both cows and bulls, and the conditions under which they are reared afford facilities for the regulation of breeding. Cows of the *Doddadana* are kept in the villages, they are homefed and under shelter, in which case only the very best bulls are secured for serving them. Each herd has its own special superior bull sometimes selected from the same herd, but more often from some other herd to prevent in-and-in breeding. As the bull grows old and deficient in vigour, a young bull is similarly selected and kept in the herd to take its place. The young one in many cases only acts the part of a teaser. No sooner does it apperceive symptoms of a cow being in heat than it approaches, and then keeps constantly attending on it. The skittish habit of the cow, being tired of the importunity seeks the protection of the elder bull which the young one dare not approach and which then serves the cow. It may be of interest to know what the breeding experts consider to be the best points of a Mysore bull of the best quality, and these, though they cannot be met with in a single specimen, are more or less searched for in all careful selections :—

(1) A long and stretching frame.

(2) A good height—say 52 inches measured behind the hump.

(3) A long and tapering head, with a narrow and prominent forehead.

(4) Small but prominent and bright eyes.

(5) Small and erect ears.

(6) Thin, fairly long and graceful set of horns, the difference between their thickness at the base and at the end being small.

(7) Strong fairly long neck with a small well-shaped hump.

(8) Thin and short dewlap.

(9) Broad and full chest.

(10) Well formed and strong shoulders and hind quarters.

(11) Strong and well rounded ribs.

(12) Level back and broad loins.

(13) Narrow flanks.

(14) A level croup. An abruptly falling croup being condemned ("goose rumped" in horsey phraseology).

(15) A thin short whip like tail reaching to or very little below the point of the hock.

(16) A well projecting anus ring, so that the ejected dung may fall clear of the body. It should not be situated in a niche-like hollow, as in cows and old animals.

(17) A sheath having a little or no pendulous growth.

(18) Legs of medium length and well proportioned, having strong and fairly thick bones and moving with a swing in perfect rhythm, and straight not turned sideways or brushing against each other.

(19) Short fetlocks, and hard and small hoofs with equal halves having a very narrow cleft between them. A long shank bone is considered a weakness.

(20) The colour of the horns, hoofs, muzzle and skin should be black.

(21) The skin should be thin and satiny, having short and soft hair. Bluish and iron grey colours are preferred.

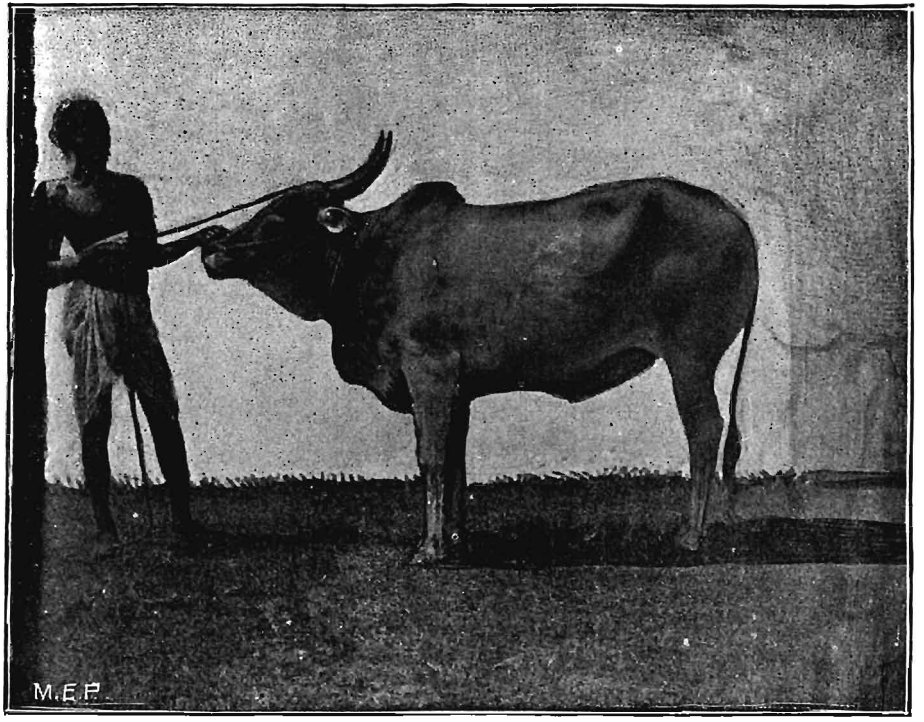
(22) A compact body free from all pendulous growths.

(23) The animal should be sound in every way, of symmetrical features, of good temper and pure breed, and free from hereditary diseases. In the selection of the cow no such special attention is bestowed as in the case of the bull, which, considering the number of animals it is likely to influence, is most carefully selected. The main points looked for in cows are good size, length, shapely head, and horns, broad hips and loins, and nice colour.

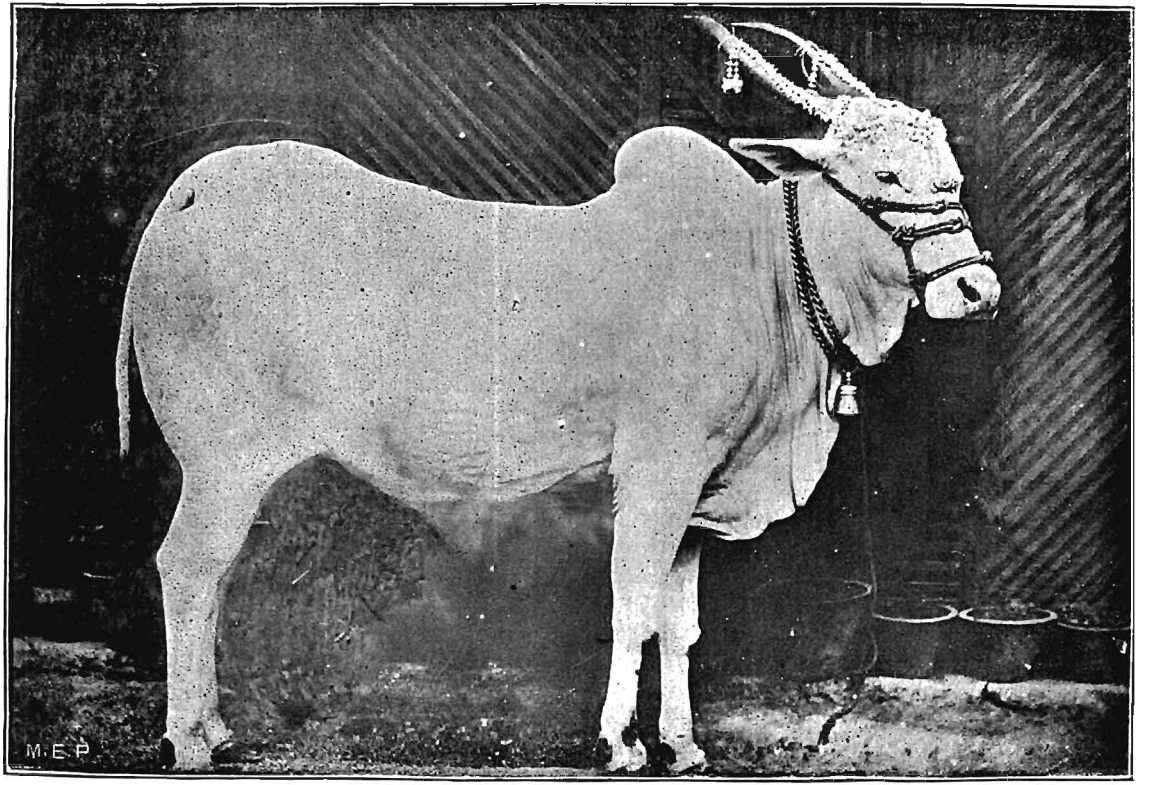
Amrat Mahal Breed.

Among the breeds found in Mysore the first place is undoubtedly due to the Amrat Mahal. The Amrat Mahal, literally Milk Department, is an establishment for the breeding of a race of cattle peculiar to the country of Mysore, and the present cattle comprise three principal varieties called Hallikar, Hagalvadi and Chitraldroog from the districts which originally produced them, and so distinctive is this breed that they may readily be distinguished from every other breed in India. The different breeds composing the present Amrat Mahal cattle owe their origin to the cattle of the tribe of Gollas and their sub-tribe of Hallikars who, with their superior cattle, are believed to have migrated in ancient times in several successive waves from the north, and settled in different parts now comprised in the Chitraldroog and Tumkur districts.

The *Karuhatti* establishment of the Vijayanagar Viceroy (some time between 1572 and 1600) at Seringapatam consisted of Hallikar cows imported from Vizianagar. This may be said to be the nucleus of the Amrat Mahal cattle. The Seringapatam cattle passed into the hands of the Wodayars of Mysore, some of whom,



Mysore Heifer.

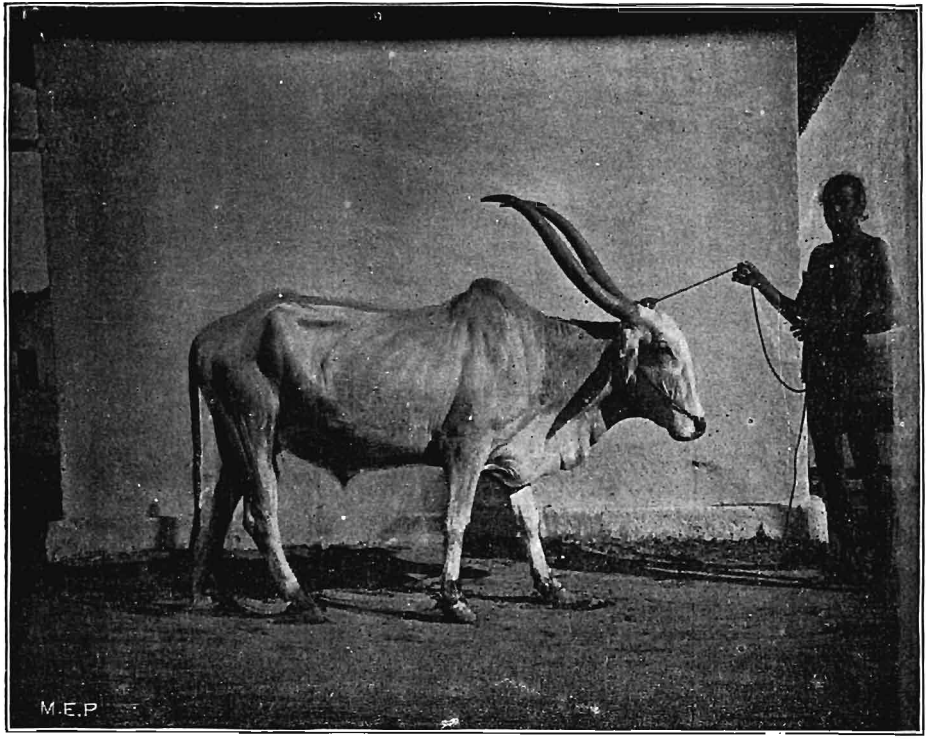


This was the favorite Cow at the Mysore Palace.

notably Shamaraj Wodayar (1617—1636), Kantirava Narasa Raj Wodayar (1638—1658), and the celebrated Chikka Devaraj Wodayar (1672—1704) made their own additions to them from time to time, assigning "Kavals" in different parts of the kingdom. It was in Chikka Devaraj Wodayar's time that the cattle establishment obtained recognition as one of the departments of the administration. It was called "*Benne Chavadi*" or establishment of cows both as a breeding stud and to furnish milk and butter for the palace. He introduced for the first time the system of branding them with his initial ದೆ (Dé). The accumulated herds of the Rajas of Mysore passed on to Hyder Ali when he usurped the throne. In extending his conquest, and in reducing the numerous rulers who had held sway over more or less extensive tracts in Mysore, he acquired also the herds of the superior cattle belonging to them. Among these may be mentioned the Polłegars of Chitraldroog, Tarikere, and the Raja of Nagar. Hyder seems to have made extensive use of the cattle which he had appropriated in the movements of his army equipage, and is popularly credited with having kept at least 60,000 bullocks in different parts of the province, though they were not organised as carefully or in as minute details as was afterwards done by Tippu, on a system which has, in essential points, been adhered to ever since. Upon succeeding to the throne of his father Tippu added to these herds those of the Pollegar of Hagalvadi, Chikka Devaraj Wodayar's and the suggestive name of "*Benne Chavadi*" was changed in his time into the more pompous one of Amrat Mahal from *Amruta* :—nectar. Tippu took great interest in these cattle and issued a "*Hukumnama*" or regulations for the department, the greater part of which continued to be observed after

the taking of Seringapatam, and the same system was afterwards followed by the British officers. The Dairy department seems to have been on a large scale. The *Amildars* were expected to train the young bulls. These were given absolute freedom and were allowed to graze in the ryots' fields. They were afterwards classified when they were required as gun bullocks, pack bullocks and plough bullocks, etc. There was an annual muster of the herds, and Tippu frequently attended it in person and distributed rewards. Such was the composition of the Amrat Mahal cattle inaugurated by Chikka Devaraj Wodayar, reconstituted by Hyder Ali, and thoroughly organised by Tippu Sultan.

The attention of the British was first called to the excellence of the breed when it enabled Hyder Ali to march 100 miles in two days and a half to the relief of Chellumbrum, and after every defeat to draw off his guns in the face of the enemy; and when Tippu Sultan was enabled to cross the Peninsula of Southern India in one month for the recovery of Bednore, and to march 63 miles in two days before General Meadows. It also enabled the Duke of Wellington to execute those marches of unexampled rapidity which are the admiration of military men, and the Duke brought it prominently to the notice of the then Commander-in-Chief Lieutenant-General Stuart. Captain Davidson, in a report on the Amrat Mahal cattle attached to the Bombay column of the English Army in Afghanistan in 1842, says:—"No draught cattle in either army were so efficient as the 230 Mysore bullocks which accompanied the Bombay troops to Afghanistan. It was entirely due to this very superior description of cattle that no part of the Bombay Park was required to be abandoned when the troops were returning to India over the almost



Amrat Mahal Bullock.

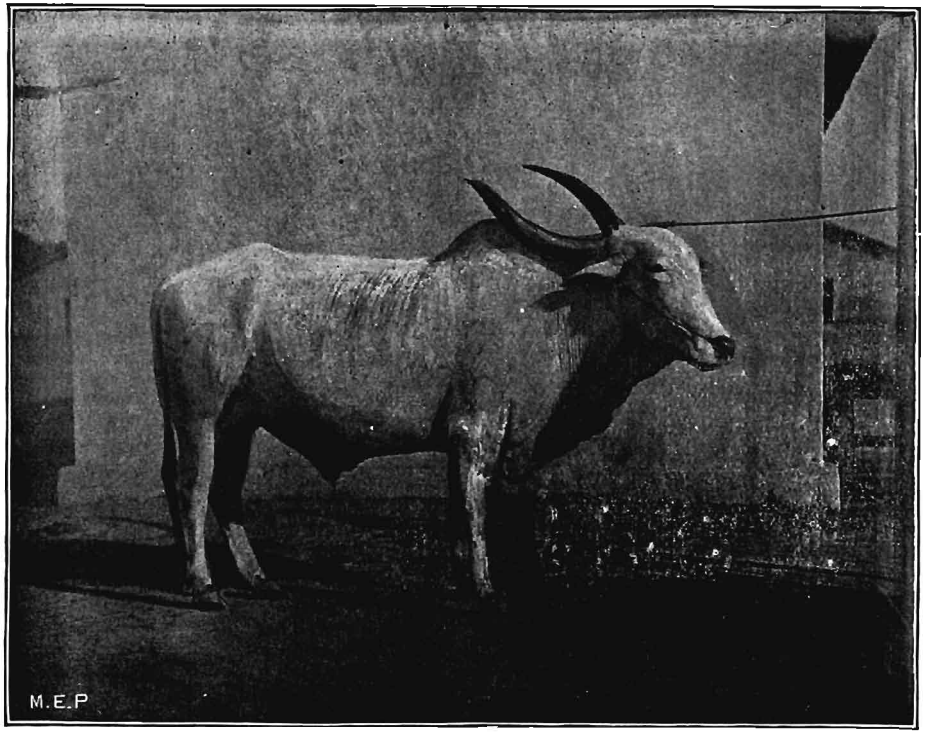
impracticable roads through the Tirah Mountains. These cattle were frequently upwards of sixteen hours in yoke. The draught bullocks of the Bengal army were the property of Government, and were not in my opinion as fine animals as the Mysore bullocks." Other memorable military events might also be cited to the credit of the Amrat Mahal cattle. It is said that during the Peninsular War the Duke of Wellington often regretted that he had not the services of the cattle of this breed. In 1808 the Commissioner of Mysore said of them :—They are active, and fiery, and walk faster than troops ; in a word they constitute a distinct species, and are said to possess the same superiority over other bullocks in every valuable quality that Arabs do over other horses. Professor Wallace remarked in 1899, that the breed as a whole occupies among cattle a position for form, temper and endurance strongly analogous to that of the thorough bred among horses.

On the fall of Seringapatam the whole of the cattle became the property of the British Government, the management of the herds being allowed to remain with the Maharaja of Mysore on the condition of his supplying a certain number of bullocks. It was probably imagined that the same attention would be given to the establishment as has been extended to it by the former Government, but Tippu Sultan had depended upon it for the efficiency of his army, and the new Government could be actuated by no such motive. The consequence was that the establishment was left to the servants who had charge of it, and by them neglected and abused ; the British Government were disappointed in their expected supplies, and the cattle were allowed to degenerate to such a degree that after a period of thirteen years it became necessary to resume charge of it in order to

preserve the breed from extinction. In 1813 the Amrat Mahal cattle, together with the pasture lands were handed over to Captain Harvey of the Madras Commissariat. The herds then rapidly improved and doubled in numbers in the course of but ten years. In 1840 the Maharaja's Amrat Mahal herds and grazings were amalgamated with those of the British Government, and the whole placed under the orders of the Mysore Commission. In 1860 from motives of economy Sir Charles Trevelyan ordered the establishment to be broken up, and the herds to be sold ; this appears to have been a fatal error, alike in policy and economy, and the results were fatal to the public service. The price of cattle soon became prohibitive (Rs. 150 each) and it was, with the cordial approval and assistance of the late Maharaja, re-established in 1866 by the purchase of such cows and bulls of the old breeds as were procurable in the Mysore country ; very few were obtained owing to the Pasha of Egypt having secured most of the best blood. Fortunately however the late Maharaja was a large purchaser when the old establishment was broken up, and the Madras Government was able to obtain sufficient stock to fairly start again in 1870, the complement being 4,000 cows and 100 bulls.

In 1883 the British Government handed over this valuable property to the Government of His Highness the Maharaja for two and a quarter lacs. It is now entirely under its control, and every effort is made by careful elimination of doubtful stock to restore the old breed to its former excellence.

Stud books have been opened, and the cattle are mustered by name and brand. Births and deaths are registered and reported in monthly returns, and frauds on the part of subordinates have been to a great extent



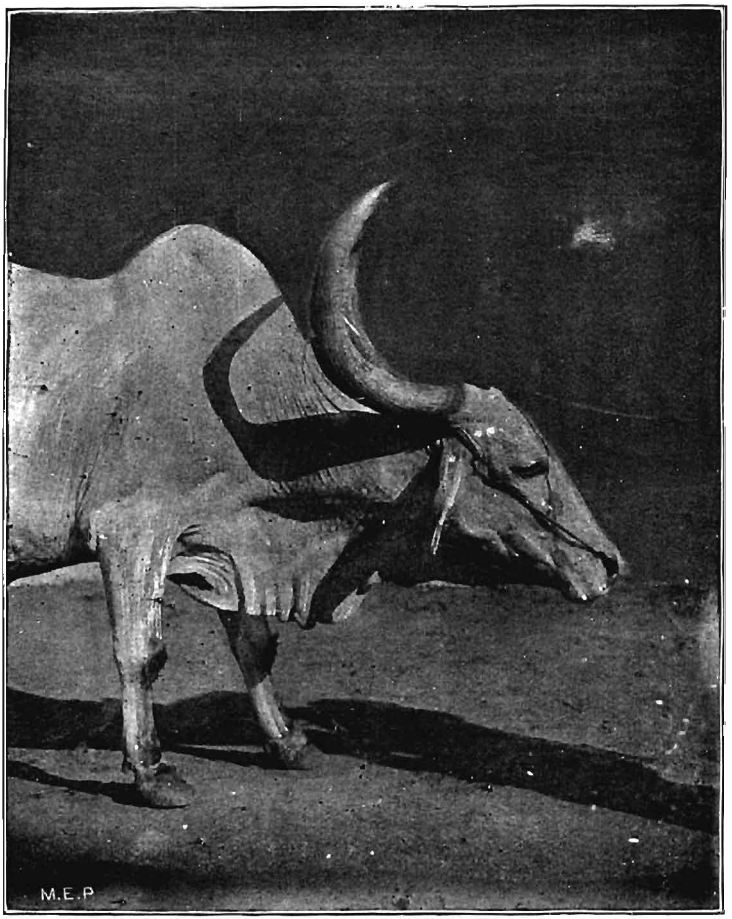
Amrat Maha Bullock.

prevented. The Madras Government receive from the establishment 200 bullocks annually. Private individuals may obtain bulls by writing to the military adviser to the Government of Mysore, Bangalore, the prices of which are about a hundred rupees each. There is frequently some delay in procuring them, as the herds have to be rounded up, the young bull selected and secured, after which they undergo a process of training. This is very necessary as they have been living in practically a wild state.

As has already been stated the cattle of this breed originally comprised three varieties : 1 (*a*) Hallikar, (*b*) Hagalvadi and (*c*) Chitraldroog. Prior to the abolition of the department in 1860, the several herds seized by Hyder and Tippu Sultan seem to have been maintained for the most part unmixed as separate "Serwés," the distinguishing peculiarity of each breed being thus kept unadulterated. In 1866 when the department had to be reorganised by repurchasing the stock it was found impracticable to get back in their original purity all the cattle sold six years before. At this juncture the men, to whom the work of collecting the cows was entrusted on promise of appointing them "Serwegars," freely mixed the three main varieties of the old Amrat Mahal. A large number of inferior cows of every other breed, including their own bred cattle known as "Swanta Gosu" (mixed breed), and a large number of the Mahadeswarbetta cows are also said to have been passed off for the reconstruction of the Department. During recent reductions and reconstitutions of "Serwés," since 1877, many herds have been broken up and distributed among others, new herds have been formed out of the excess stock of the old ones, and exchanges of stock are often being made between different "Serwés" all tending to promote

mixture. At present the Amrat Mahal breed cannot be said to be as pure as it was prior to 1860, although careful selection and uniformity of treatment in recent years seem to have erased a good many points of difference, which must have necessarily existed at the time of reconstitution of the herds in 1866. The different breeds of Hallikar, Hagalvadi and Chitraldroog vary but slightly, their general characteristic being the same. Some special characteristics developed by local peculiarities in the different herds may however be noticed. The cattle reared in the "Kavals" or reserved pastures are of much larger size than those found in the north. They are more bony, carry thicker and rather less gracefully set horns, having comparatively thicker tails; their hoofs are said to be not so tough as those of cattle in Chitraldroog from which they differ in having a somewhat pendulous sheath and dewlap. The cattle in the Tumkur, Hassan, and Kadur districts, though slightly smaller in size are very much like those of the Amrat Mahal. The herds of the eastern parts of the Chitraldroog consists of cattle of smaller size but more compact and hardly frame with a finer tail, thinner and more gracefully set horns, and stronger hoofs. The cattle of the western Chitraldroog and Channagiri taluks resemble the last named variety, and differing from them only in being slightly bigger in size.

The Amrat Mahal cattle are kept in their grazing grounds which are called "Kaval" about 210 in number, and these are distributed over the greater portion of the western and central parts of Mysore, and cover an immense tract of country. They comprise varieties of soils, often undulating and covered with scrub jungle growth. The cattle feed on various grasses, though "vunaga" (*Heteropogon contortus*) is by far the most



Amrat Mahal Bullock.

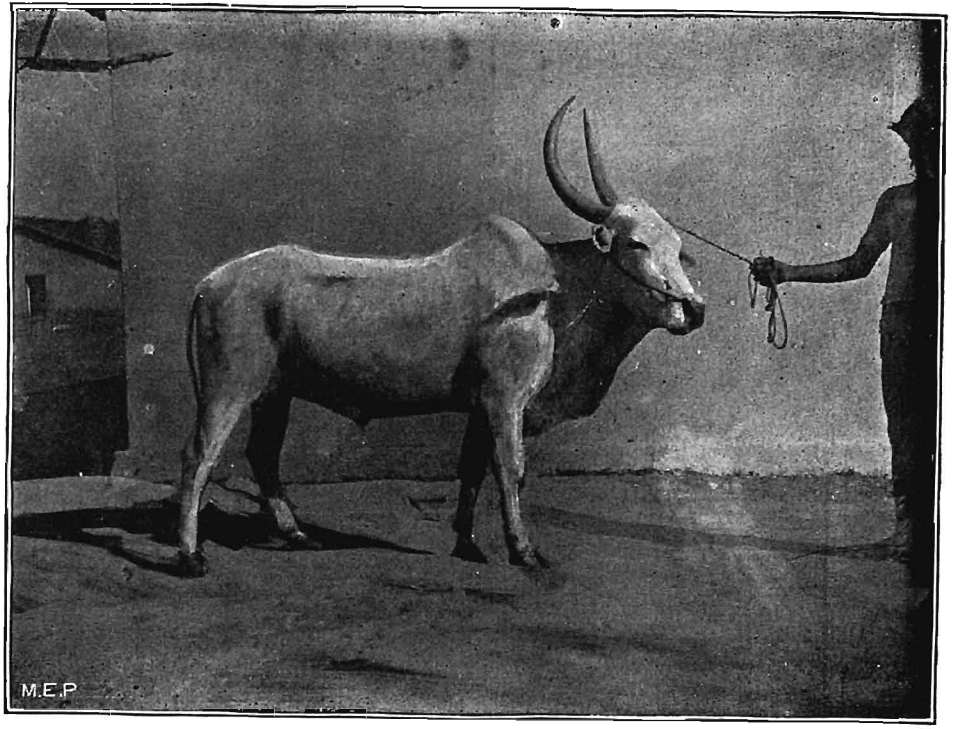
predominant. The grazing in the Kavals situated in the valleys is most nutritious. As the country becomes more elevated the grass becomes more scanty, and inferior in quality. The "Kavals" are divided into (*a*) hot weather, (*b*) wet weather and (*c*) cold weather Kavals" according to the season of the year at which they are most suitable for grazing. The hot weather "Kavals" are generally in the beds of tanks in which grass springs up during the hot months, and where generally there are trees capable of affording shade to the cattle during the heat of the day. The cold and wet "Kavals" are those where grass dries up during the hot weather. The cattle are driven for about four months in each year from May to September to these "*malnad* kavals". The herds in the west are taken to the south-western jungles on the Coorg frontier, and those in the north to the Lukkavalli and Channagiri Forest "Kavals" in the Kadur and Shamoga districts; where the first showers of the advancing monsoon ensures an early and abundant growth of grass. They then return to their maidan, or plains "Kavals" about the beginning of September in each year when the supply of grass is plentiful all over Mysore.

The whole of the cattle are divided into "Serwés" or herds, each of which, with attendants attached to it, is kept separate and distinct. The establishment of each herd is fixed at two hundred breeding cows, one hundred heifers, twelve bulls, and twenty "Peshros" or leaders, with the calves of both sexes and all ages, the actual produce of the herd the number of which varies according to circumstances; but which, generally speaking, raises the total number of animals in each herd to five of six hundred head. Each "Serwé" orherd is placed in charge of a "Serwegar" assisted by two "Mundals" each of

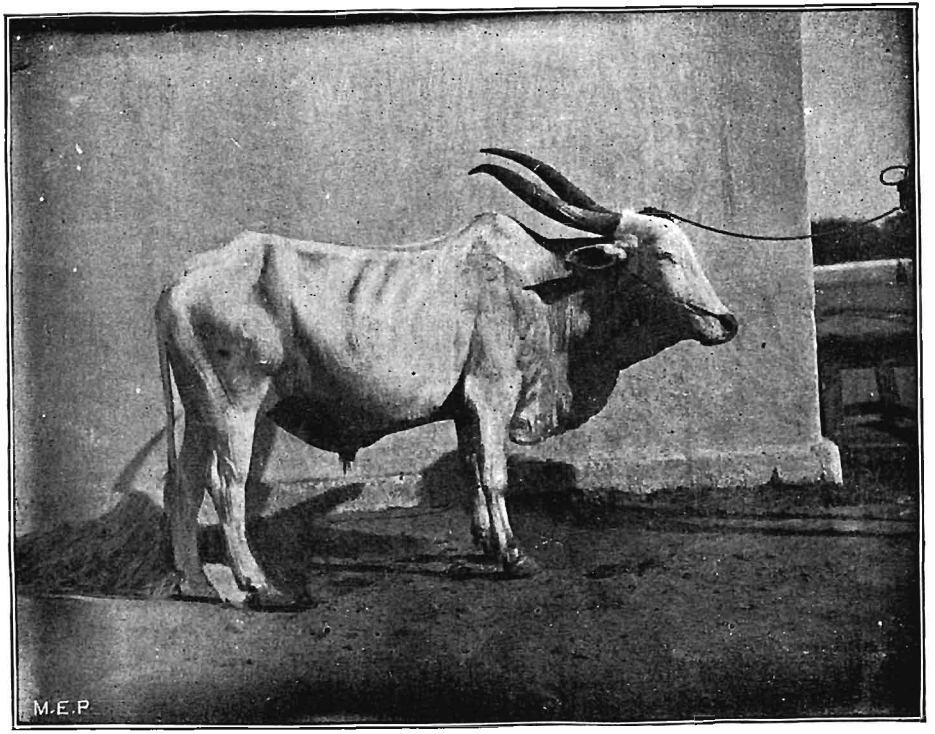
whom is responsible for the proper management of the cattle under his charge. An establishment of graziers and other attendants is likewise attached to the herd. The number of "Kavals" allotted to each herd varies from three to nine according to the size of the "Kavals," and the quantity of pasturage they afford, and although the herds are not supposed to be permanently attached to particular "Kavals" still they are not removed from those which have been allotted to them without special reasons. The whole of the herds are divided into fourteen "Tukries" or divisions, some composed of two and others of three herds, the "Kavals" belonging to each herd being, of course, conveniently situated. Each Tukri is placed under the superintendence of a "Daroga" whose duty it is to frequently inspect the herds, to muster the cattle, to check, and report all irregularities on the part of the attendants in charge of them, and also to arrange as far as may lay in his power any difference which may rise with the inhabitants or local authorities. At the annual inspection of the cattle which takes place in the vicinity of the grazing farm in the months of July and August each herd is separated, carefully examined, all inferior cattle removed, and unmarked cattle branded.

Early castration is the rule in the Department, and the calves are castrated when they are eighteen months old in the cold season.

The bullocks are separated from the herds after four years of age, and those sold to the Madras Government are transferred to the Public Cattle Department at Hunsur and when turned five years old they are thoroughly trained to work. They are in their full vigour at seven years, and past it at twelve. They work until they are fourteen or fifteen, after which they rapidly decline and die at about eighteen years of age.



Amrat Mahal Bullock.



Mysore Bullock.

to Rs. 90, average cows Rs. 40 to Rs. 60. A pair of first-class bulls are said to fetch in Hyderabad as much as Rs. 500 and even Rs. 800. People in the Dharwar Collectorate eagerly seek these cattle and pay good prices for young bulls from two to four years old even as much as Rs. 100 to Rs. 150. It was said that a pair of bullocks was sold there for Rs. 800 having won a race in dragging a heavily laden cart through sandy soil.

The "Serwegars" of the Amrat Mahal Department have been allowed the privilege of keeping their own cattle with the Government herds with the consequence that the Amrat Mahal bulls have crossed the Nadudana cows and the result has been mongrel, and these, from long association, have taken on all the characteristics for the Amrat Mahal. They are now known by the name of Egosu or Swantagosu cattle.

Hallikar Breed.

The history of this breed has already been given under the head of the Amrat Mahal cattle of which the Hallikar breed is the most important and valuable member. An absurd legend is current among the herdsmen of the Department regarding the origin of the Hallikar. They state that Hyder Ali, after one of his trips to the south, brought back to the Mysore country a number of the cows of the small Brahmini caste. These cows were turned loose in a "Kaval" in the Tumkur district in which there were great numbers of antelope, and a cross between the big black buck and the small Brahmini cows gave rise to the present Hallikar breed. In support of this statement they point to the small spot below the inner canthus of the eye which is common to the antelope and the Hallikar cattle. It is curious that while the name of Gollas has disappeared among cattle

that of Hallikar, their sub-tribe, has survived in the cattle which they introduced into Mysore. Hallikar cattle, besides chiefly comprising the Government Amrat Mahal herds, are to be found in Tumkur, Hassan and Mysore districts, the cheif centres being parts of the Nagamangala, Kunigal and Gubbi taluks. The area over which the breed prevails is not by any means extensive, and it is thinly scattered even within those limits. The reason is that there are no extensive pasture lands in the habitat of these cattle, and the tracts being populous they are mostly homefed and are not maintained in large numbers except by a few breeders in the taluks just named. They are frequently bred by small ryots who have only a few cows, and special attention is paid to the mating of the cows and the rearing of the young stock.

Mr. Wallace in his "India in 1887" gives an excellent description of the breed. He says:—The head is well shaped, long and tapering towards the muzzle which is generally black, the forehead bulges out slightly, and is narrow and furrowed in the middle. The horns are unique in shape, and differ considerably from most other breeds. They are usually large, set well back on the crest of the frontal bone; springing close together, they diverge, inclining backwards each in a straight line for nearly half their length, and then with a gentle and graceful sweep bend forwards usually lightly inclining inwards towards their points, which are black tipped and exceedingly sharp. At times when the head is down, as when feeding, the horns can almost touch the neck in front of the hump. They thicken gradually as the head is approached, and are very strong near the base which seems to extend, apparently to give strength, down the forehead between the eyes as a distinct ridge on each

side, thus forming a perpendicular groove or depression in the centre of the forehead.

The colour is of a more or less uniform grey, varying from light to a deep iron grey with a darker shade over the shoulders and hind quarters. Broken colours are being carefully weeded and sold. The neck is thin, for the size of the cattle, but is long and sinewy. The dewlap is thin, and does not extend very far back. The ears are small and taper to a point, being carried in a horizontal position.

The hump is well developed in the bull. The tail is thin, and tapers like a whip. The legs are clean, strong and sinewy, standing well apart. The hoofs are small, well formed, black and hard, with a very close cleft between. This breed seldom attain a very large size. In shape they are remarkably neat, with muscles like whipcord.

The cows have a very masculine appearance and vary only with having a thinner hump and horns. In colour they are invariably of a light grey. They have a small compact udder with small and hard teats. They are poor milkers, though the milk is rich and sweet with a high percentage of butter fats. They are of high mettle, and, though mostly homebred, are not gentle or tractable.

There is always a great demand for these cattle, and as the number annually produced is not sufficient to meet it, high prices prevail. The average market value is: Very good breeding bulls Rs. 80 to Rs. 120, average bulls Rs. 50 to Rs. 75. Very good cows Rs. 60 to Rs. 100, average cows Rs. 40 to Rs. 60. Bull calves of one year Rs. 20 to Rs. 40.

Gujmavu is the most valuable variety of the Hallikar breed, and it is to be found at Karadahalli in the

Nagamangala taluk. Good Gujmavu cows of this locality are little different from the Amrat Mahal cows. The shape of the head, face, muzzle, eyes, ears, horns, neck, legs and barrel is exactly of the same type. The similarity extends even to the masculine look of the cow. One peculiar point which is very highly prized in this variety is the very long back which is supposed to give them a greater mechanical strength and advantage. The cattle owners of Karadahalli treat their animals after the fashion of the Amrat Mahal Department, they send their herds to distant jungles in the Heggadevankote taluk for the benefit of the early season pasture.

Superior bulls are kept at Karadahalli for breeding purposes. Cows, even from distant places, are taken to these bulls for service upon payment of a fee from Re. 1 to Rs. 4 for each service. It is said that so highly are the Gujmavu prized that ryots of the neighbouring taluks of Mandaya, Seringapatam and Closepet advance to the breeders of Karadahalli Rs. 50 to Rs. 100, for calves still in the mother's womb. If a cow calf is dropped the advance is returned, as it is not customary for the breeders of Karadahalli to sell the cows of their breed. If a bull calf is born it is sold according to the original agreement. In some cases such sales are subject to the provision that the calf should be reared for two years, and resold to the original owner for its full value at the time of its resale which is usually from Rs. 100 to Rs. 200. This system of selling and reselling is a common custom and it affords a convenient division of labour. The Karadahalli bulls do not attain a large size, the average height being 49 inches measured behind the hump. They are very difficult to tame at first, but when once tamed they are far more amenable to being handled and worked by men than the Amrat Mahal cattle which,

to the last, retain more or less their impatience of strangers.

Chitraldroog Breed.

The breed bears a close resemblance to the Hallikar cattle differing from them only in some minor points. The head is smaller and shorter than the Hallikar, but not stumpy like the Mahadeswarabetta cattle. The forehead resembles that of the Hallikar though owing to the shortness of the head it does not appear to be so narrow, and the furrow is absent. The horns are thinner, longer, and taper more gradually; but as they grow upwards they get farther apart from each other, and bend forward with a deeper curve. The neck, tail, and dewlap are thinner. White is the predominant colour, and they are smaller in size than the Hallikar.

These cattle are kept in "Roppas" as well as in villages, and are bred and treated in the same manner as the Mahadeswarabetta cattle. Cows of this breed are said to come to early maturity and take the bull when they are two years old, but this is really regulated by the quality and quantity of the food given to them. When the early rains commence all the large breeds are driven to salt lands, and are freely allowed to lick earth salt tending thenceforth to improve their condition by grazing in fresh pastures.

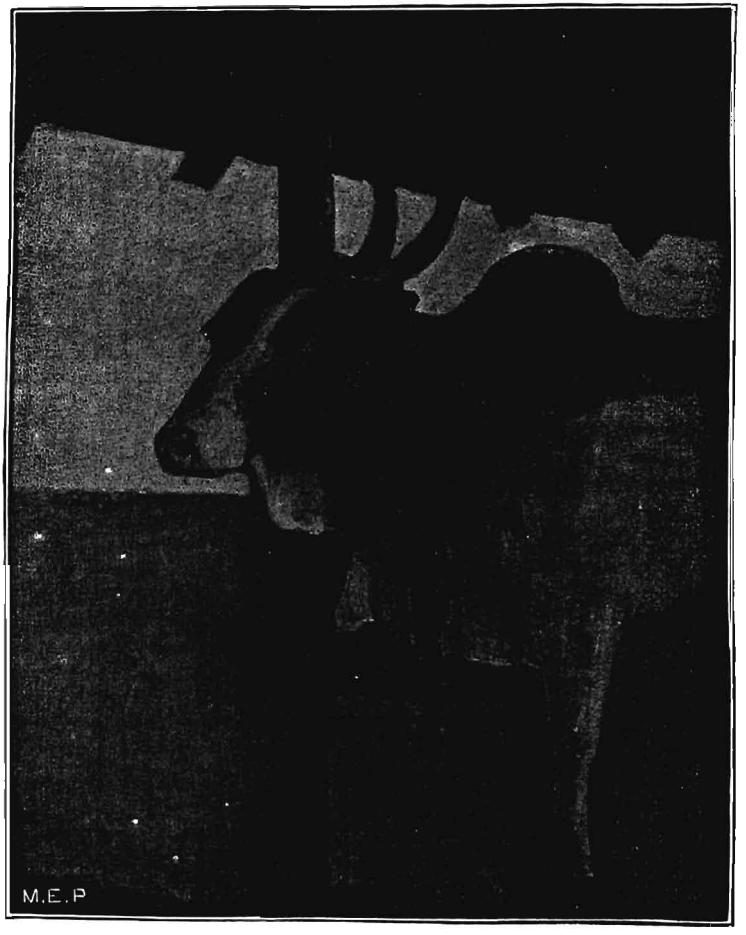
These cattle are, though small, exceedingly active, and are largely used for single bullock traffic.

Mahadeswarabetta or Alumbadi Breed.

This breed derives its name from its chief and fountain market Mahadeswarabetta in the Kollegal taluk of the Coimbatore district, where two large cattle fairs are held in February and in October at which cattle exhibited are mostly of this description. It is also called

the Betsal or Cauvery breed from its hilly home on both sides of the Cauvery, but probably a more common name is that of Alumbadi called after a village of that name on the bank of the Cauvery. The chief habitat of this race of cattle is in the Kankanahalli taluk of the Bangalore district, and the northern taluks of the Coimbatore and Salem districts which are divided from Mysore by the river Cauvery. The reason for these regions teeming with great herds of cattle is the wide expanse of forest land which is scarcely fit or has not yet been taken up for cultivation, and with only patches of tillage in favoured spots, and affording herds of cattle abundant pasture and wide and unrestrained roaming ground. The tracts are stony in elevations and humous in the valleys. The forest growth being all deciduous, the pasture lands are thoroughly baked in the hot weather by the heat of the sun so peculiarly intense in the valleys in the low hilly regions. Another reason, though of secondary importance, is the noble stream of the Cauvery whose waters are utilized for irrigation higher up in Mysore, and fertilizes some of the richest tracts in Southern India lower down, and which runs in these regions through scenery of wild grandeur on a bed too deep for irrigation purposes, and affords cattle a perennial supply of water in seasons when the country becomes parched up and thirsty.

Beyond these jungle centres, but bordering on them, large herds of this breed are also kept in villages commanding extensive pasture. Cows and bulls of this breed, in small numbers, purchased from these large herds are taken away and reared in "Maidan" villages of the Kolar, Bangalore and Mysore districts. It is from these breeding-tracts that all the cattle of this kind are exported to other districts, and to foreign ports such as



Alumbadi Bullock.

Penang, Singapore, Java and Colombo. An average of nine thousand large powerful animals have been exported from Negapatam to Penang alone during the past few years. Nearly all the large cart bullocks used in Southern India are obtained from this source. The whole habitat of the breed is favourable to the development of bone. These cattle are more massive and are of larger build than those of the Mysore type found elsewhere, though they are often found wanting in their symmetry of form. A good specimen should have the following points :—

Head.—Short and stout, with thick muzzle and broad forehead.

Horns.—These are not so uniform as those which are to be found among the Amrat Mahal and allied breeds, but are more like those of the village cattle. They are much shorter and stouter than the horns of the Amrat Mahal cattle, and have, in most cases, a rather sharp turn backwards towards the upper half of their length and are very close together.

Colour.—Frequently black, and many also may be light reddish brown but the majority I have seen are white.

Eyes.—More or less prominent, black and gentle to dullness with the surrounding thick skin overhanging.

Neck.—Short and thick.

Dewlap.—Thick, broad and in folds, sometimes continuing backwards to the sheath.

Ears.—Long and generally erect.

Hump.—Large and well developed.

Legs.—Short and with plenty of bone.

Feet.—Large, with the coronary band large and the halves usually unequal and cleft wide. Consequently

the feet are rather tender, and require shoeing when the cattle are used for cart work on hard roads.

Back.—Is very seldom straight, and usually inclines from the croup to the "Cowlick" and from there gently rising to the hump.

Ribs.—Well arched and strong.

Sheath.—Deeply pendulous.

Rump.—Droops from the croup to the tail. It is usually narrower than is consistent with symmetry.

Tail.—Long and thick at the root tapering abruptly.

Skin.—Thick and loose—generally of a brown colour.

Nellore Breed.

The Nellore breed of cattle has a wide reputation throughout India, and even beyond its limits. Formerly the principal breeding localities were situated within the northern taluks of the Nellore district of the Madras Presidency, but recently these taluks have been included in the new district of Guntúr, so the cattle should not rightly be given the name of Nellore but rather that of Ongole, from which tract the best specimens of the breed are to be obtained.

It was formerly noticeable that cattle breeding received most attention in those parts of the country where circumstances of one kind or another were adverse to the extensive prosecution of agriculture. The cultivators were repeatedly deprived of the results of their labour, and were consequently considerably harassed: they, therefore, as a substitute devoted their time to raising large herds of cattle of a superior kind which were then much in demand, and which they saved from the grasp of the officials by moving them from place to place. Under more secure Government, these cattle breeders

settled down, and being a fairly wealthy class retained their pride in the fine cattle in their possession, with the result that many beautiful specimens may now be seen in this part of the country. The very best examples are to be found in the villages of Karumanchi, Nidamanur, Pondur, Jayavaram, Tungutur and Karavadi in the Ongole taluk, and in Elapalapadan, Nennurpad and the hamlets along the banks of the Musi in the Kandukur taluk. Fine cattle of this breed may also be found in the taluks of Vinukonda and Narasaraopet in the Kistna district. In the southern part of the Nellore district, where wet crops are grown, the cattle are much inferior not being so well cared for or fed as in the places above named.

The system of feeding observed by the ryots of the different parts of this country naturally depends upon the extent of the pasturage. In the lowlying parts where paddy is principally grown, a certain portion of dry land is often kept as a pasturage for cattle. Most of the cattle, however, leave the villages during September and October—the southern rainy season—and are sent to the western taluks where there are extensive waste and jungle tracts. Part of the working cattle will occasionally follow the other cattle during November and December, should the pasture land of the village not be sufficient. The ryots often club together and send their cattle away in large herds. For this purpose, prior to the departure of the cattle from the village, arrangements are made for renting pasture blocks at a fixed sum for the season (October to February), or engagements are entered into with the holders of the pasture farms for the pasturing of the whole herd for the season at a small fee per head for each full grown animal. Should the north-east monsoon be favourable and extend

till late in the season, the cattle are only pastured until January when the paddy is harvested, after which there is very fair grazing. The whole of the jungle grazing is not open to the cattle at all times, for after a heavy burst of rain, invariably experienced about October, the best portion of the pasturage is preserved and kept clear of cattle for one or two months until the grass has grown up well, when the working cattle alone are turned in and kept thereon so long as the pasturage suffices, another portion being similarly kept for the other cattle. Towards the end of January when the sorghum crop on the higher land has come to ear, the young shoots termed Zadu, which are not likely to mature, are removed and given to the bullocks, which are often picketed close to the fields. Occasionally fields in the midst of cultivation are set apart for pasture, and are planted with the black and white varieties of Acacia. After being under grass for ten or twelve years, the ground is cleared of trees and broken up for cultivation. The trees shade the ground and favour the growth of grass, whilst the pods form good fodder. The pasture land held in this way is invariably distinct for each ryot, and is generally kept exclusively for working cattle, young stock, and milch cows.

The country where the best cattle are raised is undulating highland interspersed with small hills, and is mostly composed of light red or dark alluvial soil, where good sorghum, other millets and legumes are raised. The well-to-do ryots in these parts find their pleasure and pride in raising fine cattle.

There is no greater truism than that which refers to the necessity of feeding young stock, and the very great care which the best breeders bestow on the young animals, both male and female, most certainly accounts for the well-deserved reputation of the Ongole cattle. The young

calves are allowed to suckle the whole of the cow's milk, and when they are three months old are given grass and a small amount of mixed grains. In every way they are treated and tended as the family pets, and it is only by going into the houses of the villages that the best calves can be seen.

That there is considerable profit in the undertaking is evidenced by the high prices which the animals realize. Young bulls sell from Rs. 80 to Rs. 250. Several fine animals have been exported to South America. A good Nellore cow is worth from Rs. 80 to Rs. 150 according to the amount of milk she yields.

Ghee (clarified butter) is made in fairly large quantities, and sold to merchants for export, being seldom used by the ryots, who prefer gingelly oil for cooking, and reserve the ghee for sale. A good Nellore cow gives from 11 to 14 lb. of milk daily.

The breed is probably not so hardy as the Mysore or Alumbadi, but for slow heavy work they are unsurpassable, and they are universally employed for drawing very heavy loads in the city of Madras, frequently amounting to five tons.

The characteristics of the breed are—

Head.—Face moderately long; muzzle fine; forehead broad; eyes elliptical in shape, large and mild; skin round the eyes is black for about half an inch; ears long and drooping; horns short and inclined to be stumpy; in cows the horns are longer than in bulls they are directed outwards and slightly backwards.

Neck.—Short and thick.

Hump.—Well developed.

Body.—Massive, long and deep, but some are inclined to be flat-sided. In fine specimens, the girth

behind the hump is about 84 inches, and height behind the hump 63 inches.

Back.—Of moderate length and invariably higher at the croup.

Quarters.—Strong, with a considerable droop.

Sheath.—Pendulous: cows have also a fold of skin in the position of the sheath.

Tail.—Long, fine and tapering.

Legs.—Strong, and somewhat coarse.

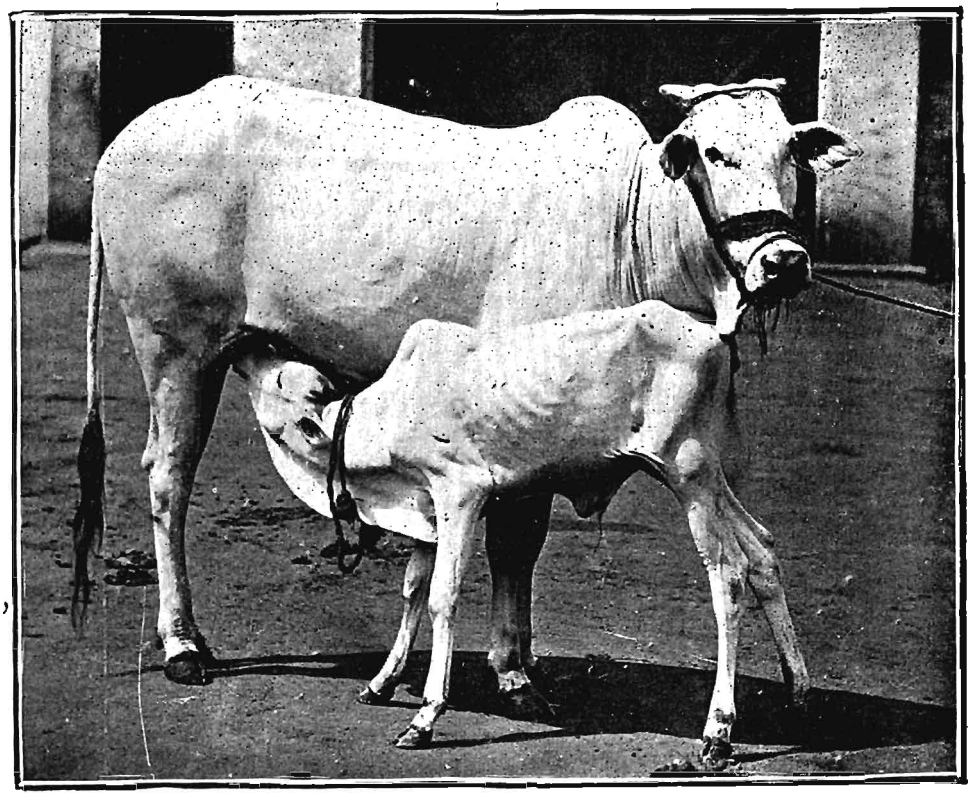
Feet.—Large and somewhat soft looking.

Colour.—Black and white and pure white, the latter is now most esteemed, but formerly black and white was the predominating colour.

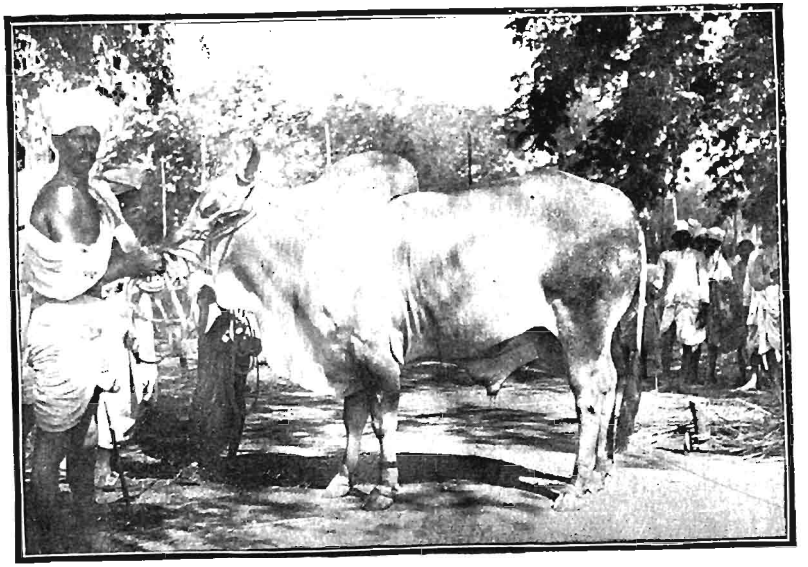
Temper.—Very docile.

The extensive pasturage obtainable in this part of the country is no doubt responsible in a great measure for the large cattle breeding industry, which Government has always been careful to foster. Previous to 1867 a poll tax used to be levied, but in that year this was abolished in Nellore, and a principle was laid down for the future that, out of the waste of each village, an area equal to 30 per cent. of the area occupied by cultivation should in future be reserved for common grazing to be equally enjoyed by all villagers free of charge; the surplus waste, if sufficient in extent to make it worth while to adopt the system, may be leased out for one or two years at a time to the highest bidder.

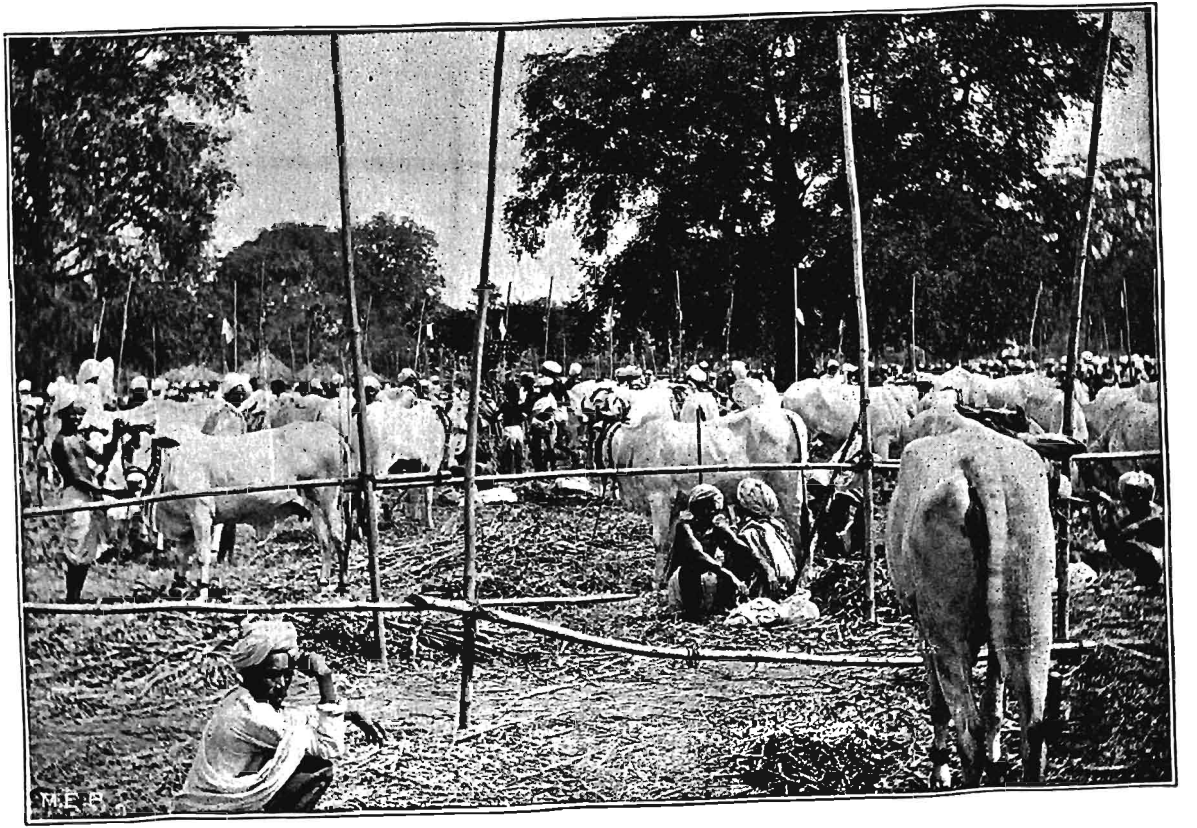
In order further to develop and encourage the breeding of good stock, an annual cattle show was established so far back as 1858, and continued uninterruptedly until 1871. During these twelve years a total of over Rs. 18,000 was distributed in prizes. The cattle show was resuscitated in 1904 with most successful results, and it is doubtful whether such a large collection of bulls and cows



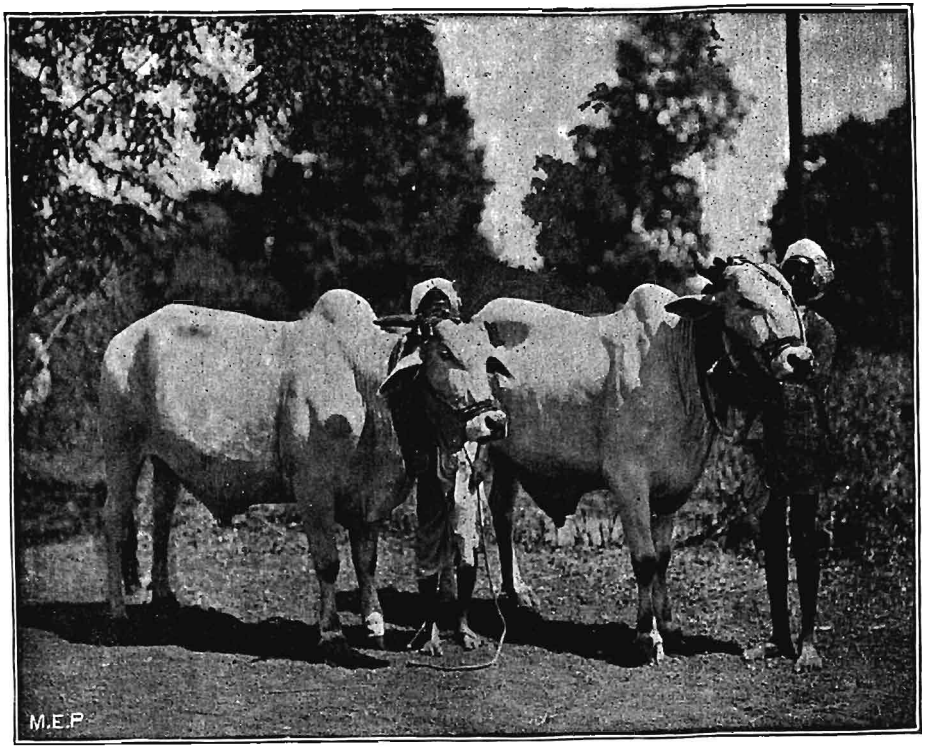
Ongole Cow and Calf.



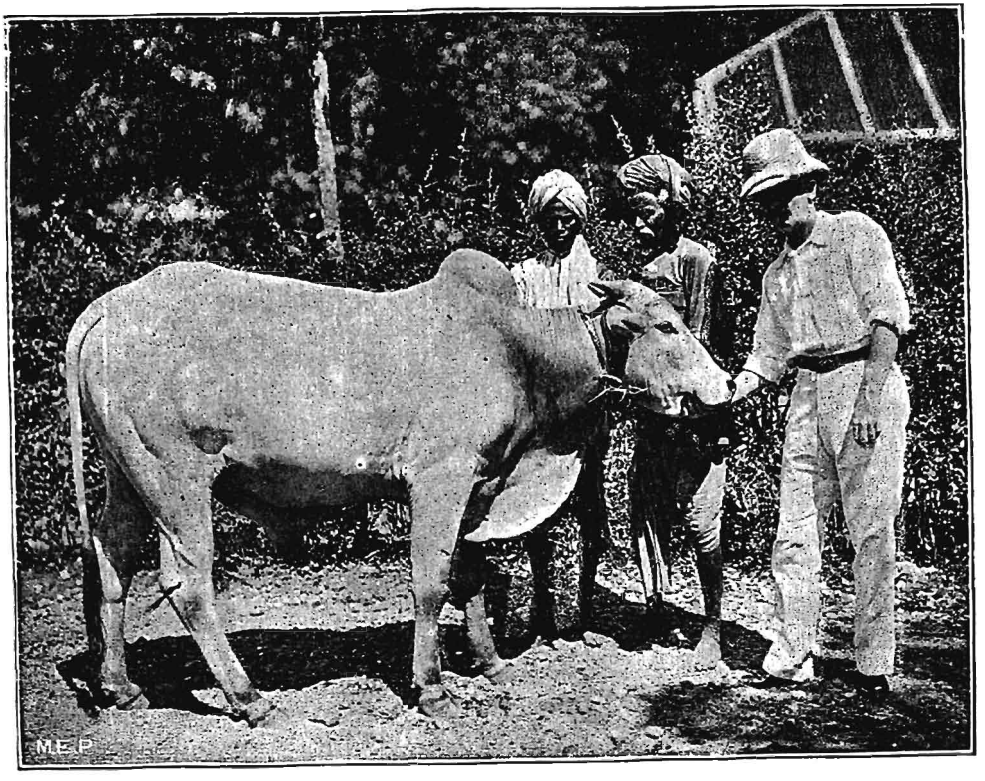
Ongole Bull.



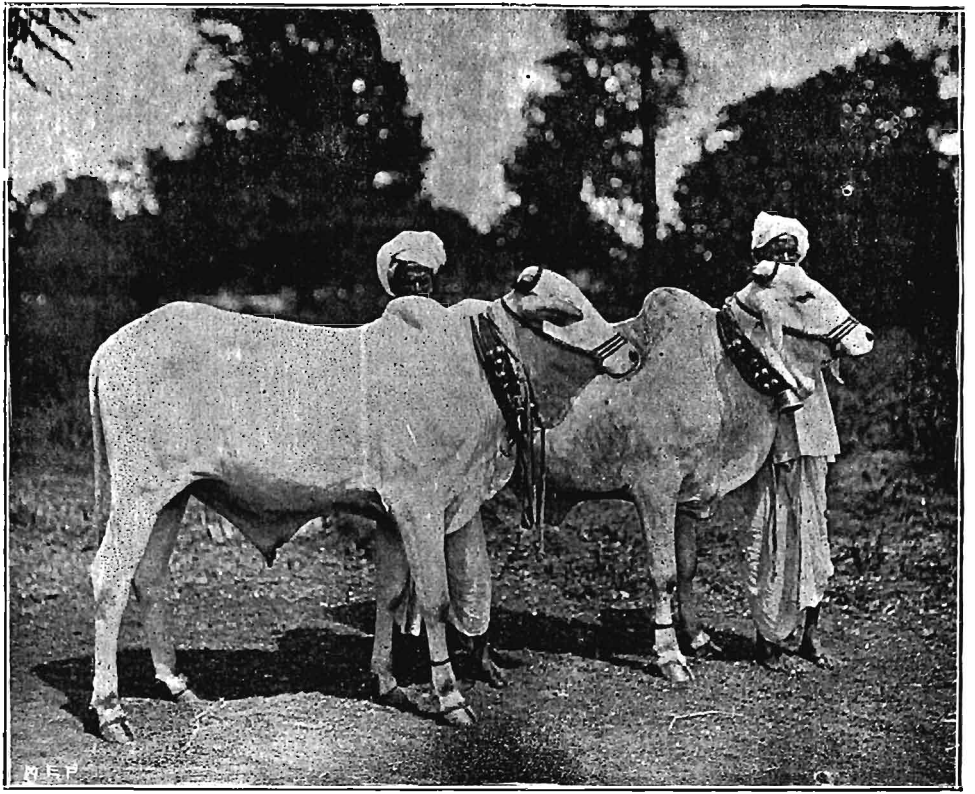
Ongole Cattle Show.



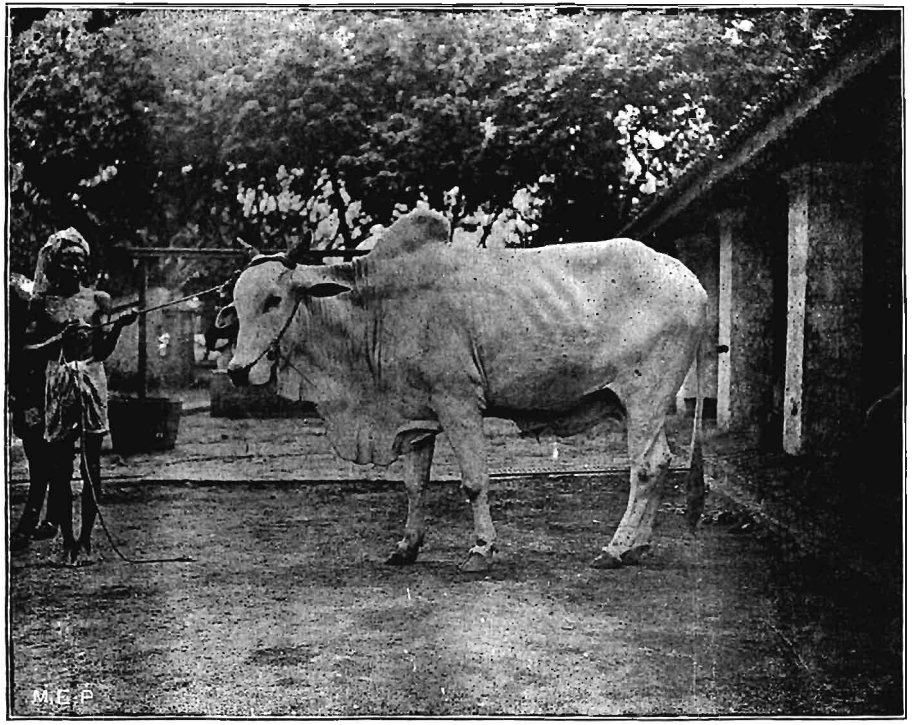
Pair of Kistna Cattle—Ongole Breed.



Vizagapatam Breed Bull Calf.



Vizagapatam bred pair of young Bulls.



Ongole Bullock.

of one breed have ever been brought together before in India. There were exhibited—

Brahmini bulls	45
Bulls	120
Bull calves	83
Bullocks, single	22
,, pairs	31
Cows	166
Heifers	132
Buffaloes, bulls	1
,, bullocks	9
,, cows	6
Rams	38
Ewes	3
Goats, he	11
,, she	3
Total ..	670

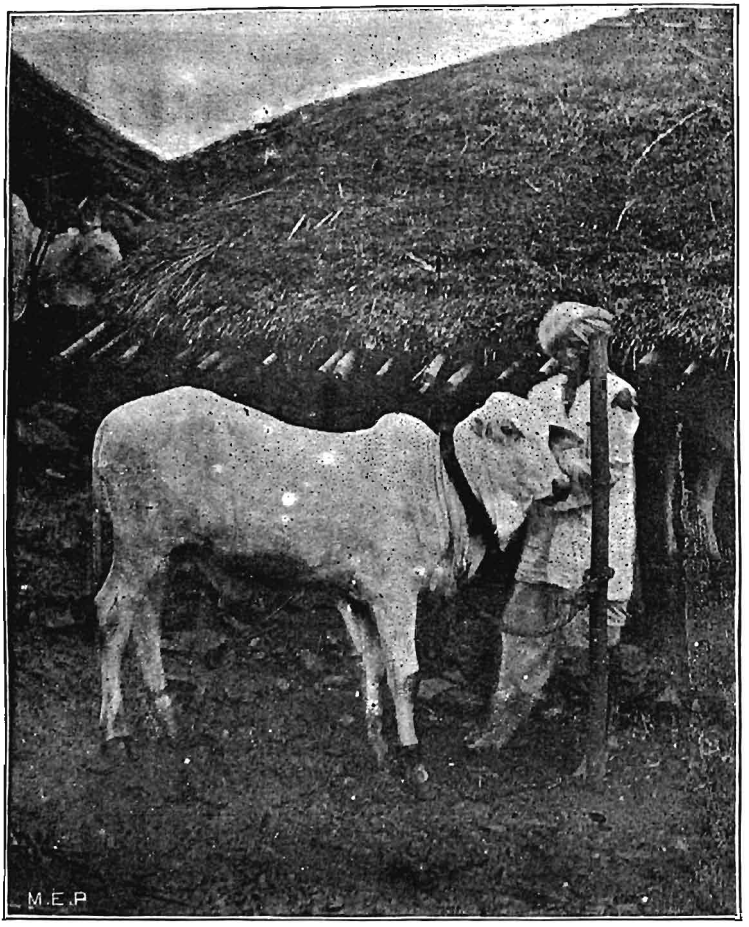
The heifers and young bulls were an exceedingly good lot, and it is most sincerely to be hoped that, with the encouragement held out by the Madras Government, the show will be held annually and equally well represented. The Cattle Show at Ongole has now become a regular institution, and has continued to grow in popularity. It would therefore be a very great pity if it were allowed to drop for want of funds and Government support, as this breed has become world renowned. During 1906 buyers came from Brazil and about 200 young stock were taken away to this distant country where the breed has become very popular.

Great care is taken in the selection of the village bull, and the collection of *Brahmini* bulls brought into the show at Ongole consisted of forty-five handsome upstanding animals in splendid condition. Almost every village has one or two so-called Brahmini bulls, which

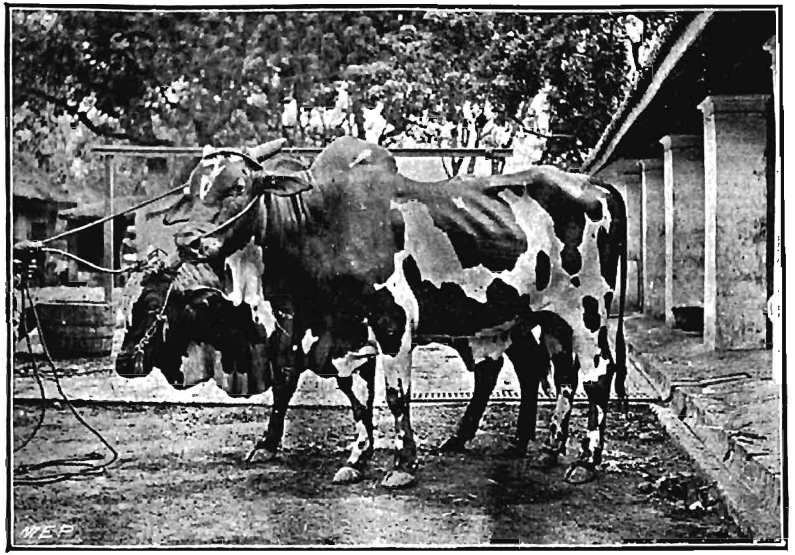
are common property, having been presented by the relations of a deceased villager as a memorial, or by some wealthy ryot, or having been purchased by public subscription. Such animals are always branded with a sacred mark.

Like all agricultural classes the ryots of the east coast are very superstitious. They are usually very unwilling to exhibit a favourite cow owing to the influence of the Evil Eye (*Drishti*). A bullock whose tail has the root of the tuft of the hair situated above the hock is said to have *Eru-val* and to bring ill luck. This is not objectionable in the cow. A bullock having white hair, skin, horn and hoofs is considered of weak constitution and should not be purchased. A black bullock is generally considered to be a rogue; if not a rogue, he is considered a great value. The saying is:—"A black bullock is but the fourth of a bull, but if he is guileless he is a bullock and a quarter." A bullock with numerous small spots over the body "like a deer" is considered very lucky.

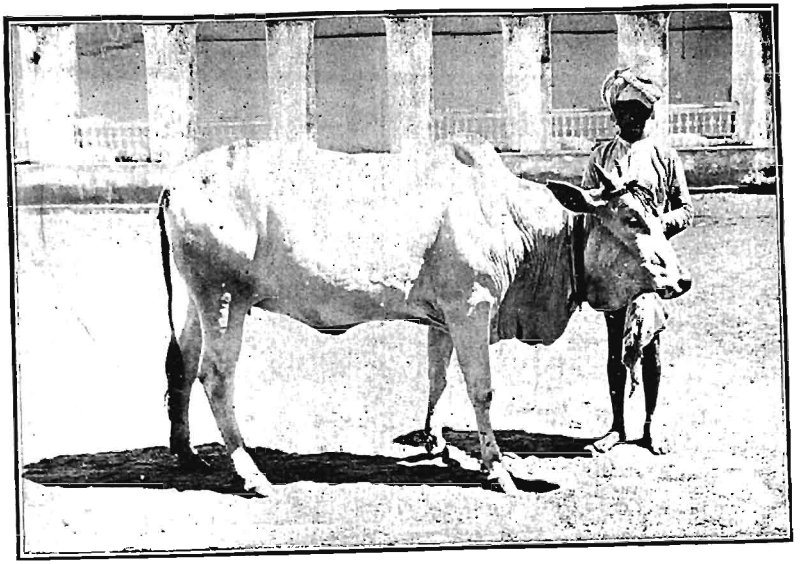
The form of the horns is supposed to indicate many things and receives as many names. For instance, "*Madakombu*" means horns bent backwards, and is considered an excellent sign in a cow. There is an old saying:—"Let any man, who does not know how to select a cow, purchase one with horns bent backwards." Straight horns are liked. Horns pointed forwards "*Kopadi*" indicate spirit. Irregular, twisted horns *churuttai* are not objected to. Those which appear hollow and have light coloured patches "*Kollikombu*" are considered to be very disastrous. Horns with white tops "*Punkombu*" are also bad. If a cow at the time of purchase voids urine, it is considered a very good omen,



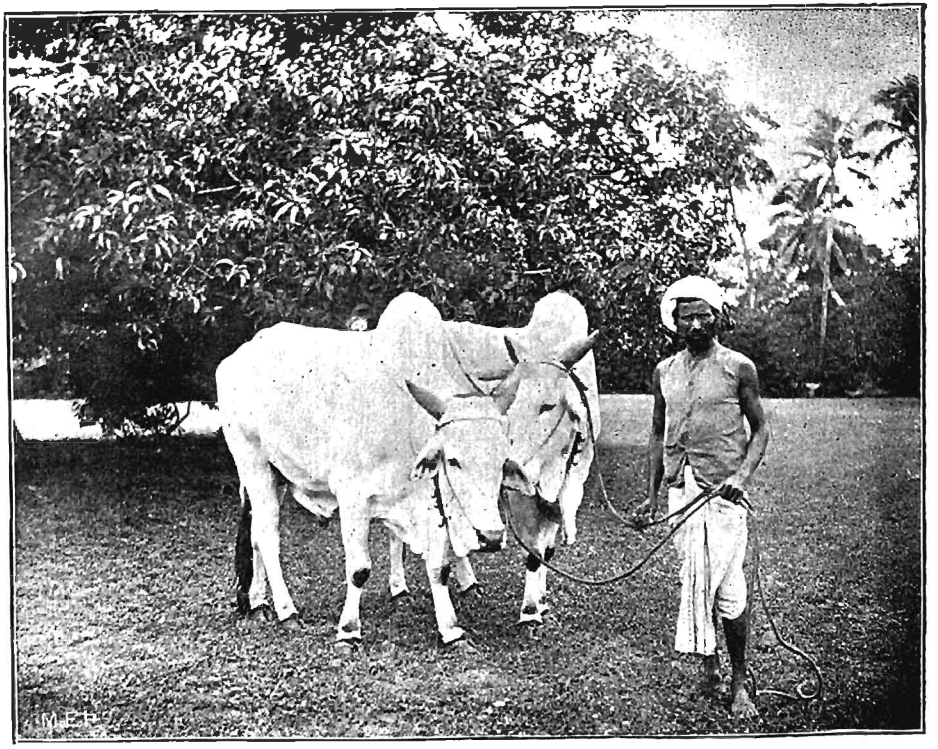
Ongole Bull Calf.



Pair of Ongole Bullocks.



Ongole Cow.



Pair of Ongole Bullocks.

but if she passes dung it is a bad sign. The reverse is the case with the bullock.

A bullock, which fails to cut the fourth pair or corner incisors, is called "*Arukatti-Madu*" and is considered lucky. The saying is :—"He who purchases a bullock with only six permanent teeth (incisors) will become rich enough to purchase an elephant." A bullock which cuts only seven permanent front teeth is unlucky to its owner, and is responsible for the saying that "He who purchases such a bullock should have the preparations for his funeral made ready."

Certain observances are most scrupulously carried out by both purchaser and seller at cattle sales, and, in fact, have become unwritten law resting for authority on long consent. Disregard of these details in the procedure is seriously believed to imperil the prosperity of the owner, the seller, and the innocent animal. The following are the principal ones :—

(1) After the price has been fixed the buyer hands the seller a silver coin, either a two-anna bit or a rupee as earnest money.

(2) The balance of the money may be paid at once or at any stated time afterwards.

(3) The seller has to pay the purchaser a four-anna or eight-anna bit for what is called *Maralu labham* of Pathi Vithamalu (cotton seed). It is intended that this money should be used by the buyer for fodder for the animal for that day. The purchaser is always careful to go with four-anna pieces in the event of the seller not having change for a rupee.

(4) The buyer must never tie up the animal with his own rope, and, therefore, a purchaser never carries one.

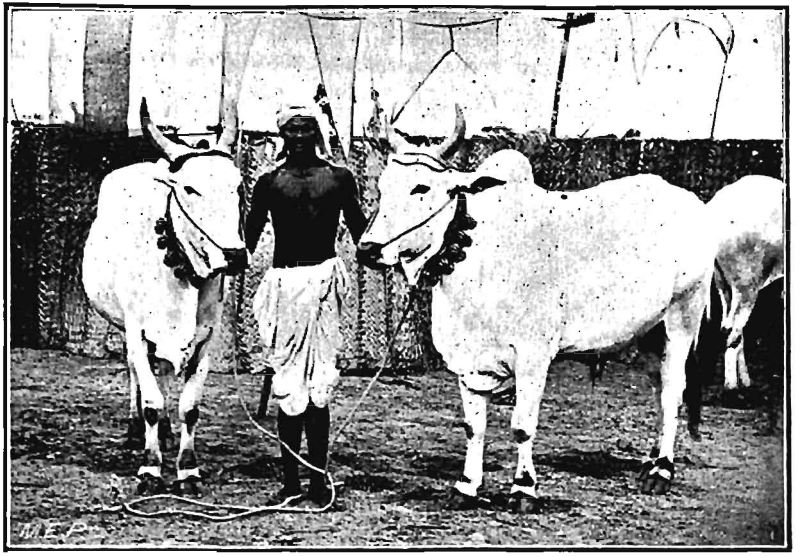
(5) The seller must always supply the purchaser with a new rope, and if it is not available, he gives the purchaser raw material which must be braided or twisted into a rope. The seller must never give the rope already used by the animal.

(6) The seller in company with the purchaser should for a short distance lead the animal himself with the fresh rope and then transfer the rope to the hands of the purchaser who then takes the animal home. This settles the sale contract and is never disputed. The conditions of sale are never reduced to writing.

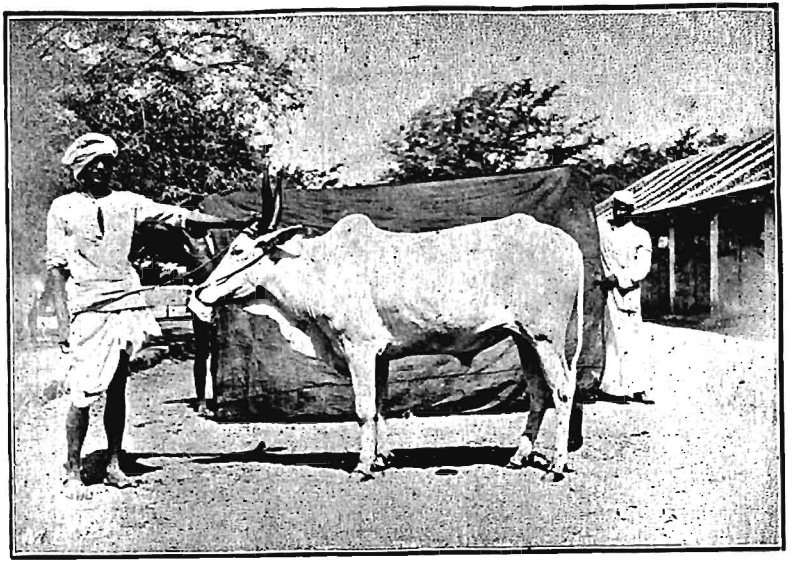
Kangayam Breed.

This breed is also known by the names *Kanganad*, *Kongu* and *Kangaen* and derives its name from the taluk of the Kangayam.

These cattle are bred in the southern and south-eastern taluks of Coimbatore. There are said to be two varieties, a large and a small. The smaller are found to be more numerous in the Kangayam, Dhárápuram, Udamalpet, Pollachi, Palladam and Erode taluks, while the larger variety are more prevalent in Karúr, Aravakurichi, and Dindigul taluks. It is understood that the celebrated Kangayam breed are not the common cattle of Kangayam, but are the property and produce of large breeders such as the Pattagar of Palaiyakottai and his family, the Kadiyar Munsiff Monigar, etc., who maintain herds of from 500 to 1,000 head, and keep large numbers of cows and bulls for breeding only. Many ryots, however, own from 10 to 20 head of cattle reared for sale. These cattle are sold, not at ordinary markets, but to dealers who come to the district for the purpose, or at large cattle fairs such as Avanashi, Mahadeshwara Hill near Kollegal, and Madura.



Kangyam Bullocks half bed. The cross is with the Ongole.



Kangyam Bullock.

The breed in its purest form may be seen in the herd of the Pattagar of Palaiyakottai who has been an extensive breeder for many years.

It is curious to note that the animals seen at the several cattle fairs held in what is practically the home of this breed are not nearly so good as animals of the same breed which are sold at fairs in the southern districts. There are probably two explanations for this, firstly, that it is not profitable to export any but selected animals, and secondly, purchasers select the very young male stock and these are taken away to good grazing grounds where they are specially fed up, but the fact remains that better pairs of bullocks of this breed are to be met with in the Madura and Tinnevelly districts than in the neighbourhood of Kangayam.

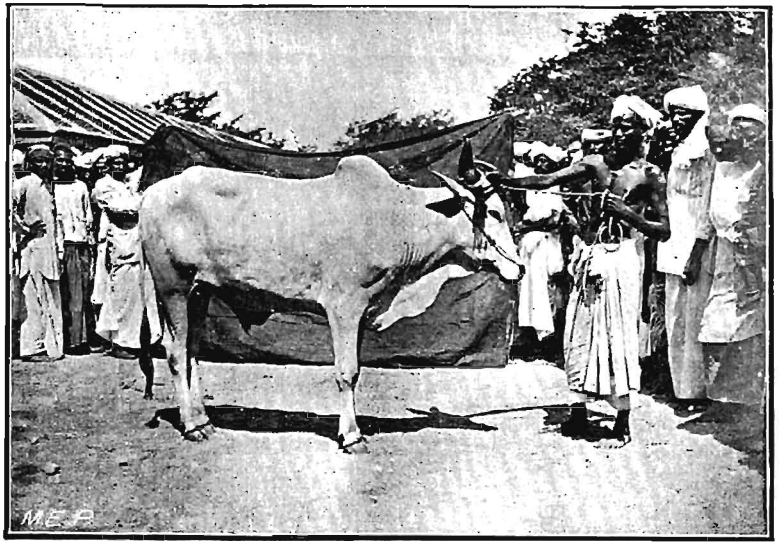
In the breeding of the ordinary cattle of the district there does not appear to be much, if any, care taken in setting apart bulls. Every ryot keeps his cows and other cattle in his own fields, which are all fenced, a practice which scarcely is to be seen beyond the limits of Coimbatore. As the young stock are often parted with at a very early age, and there is an exchange from hand to hand always going on, it is impossible to trace out the origin of the majority of the stock.

Regarding the Pattagar's cattle however this is different. His herds show a very different system of management, there is a careful selection of sires and dams, the young stock are properly reared and consequently attain a development rarely seen among cattle of the same breed met with in other parts of the district. In the first place he provides a considerable area of permanent pasture land. His land being fenced it is easy to separate the herds of different ages and sexes, the heifers run in one herd, and the young bulls in

another, the cows in a third and so on. He states that he keeps the breed pure by using sires only from his own herd, though the appearance of some of the cows and heifers was such as to give the impression that they had a distinct strain of the Ongole blood in them, and as a matter of fact he possessed several pure bred Ongole cows. One herd belonging to the Pattagar consisted of about thirty-five young heifers about three years old with a bull running with them. This herd was a remarkably fine one, the heifers being in fine condition, and were a very level lot, showing great quality. The prevailing colour was white with grey markings about the hump and quarters, though there were many fawn, fawn white and even light reds. The latter are not thought so highly of as the whites or greys, although they are just as well made. The bull was a dark grey, verging to black on head, hump, and quarters with the characteristic broad face, short, thick, but pointed horns of the breed. The pasture on which the herd was grazing, although rather bare owing to the absence of rain, showed a strong sward, and was evidently well able to carry the stock on it, and which received no additional food.

Another herd consisted entirely of young bulls, all of them between one and two years old. These were a very even lot, showing however a considerable variation in colour. Another herd consisted of young bullocks which had been castrated at three years old, and were chiefly intended for sale. The Pattagar informed me that he castrated his bulls at three years old, and sold nothing but bullocks.

It is doubtful whether there is another landowner in India who pays so much attention, or carries out the systemic cattle breeding on such good lines as this



Kangyam Bullock.

Pattagar, and this only shows what can be done if due attention is paid to the essential requirements.

Both varieties of this breed are strong, active animals with compact bodies, and short stout legs. In the larger variety the horns are much longer, and of different shape to those of the small. They curve outwards and almost complete a circle at the point where they approach the tips, and for a distance of three or four inches have a sharp backward curve. The prevailing colour is pure white. The Pattagar of Palaiyakottai is the breeder of the small variety, a description of which is as follows :—

Head.—Short with broad level forehead, eyes dark and prominent, ears short and erect, horns spreading apart, straight, short and thick with sharp points.

Neck.—Short and thick.

Hump.—Moderately developed.

Dewlap.—Thin and reaching to the sternum only.

Body.—Compact and well ribbed up.

Back.—Short, broad and level.

Quarters.—Strong, slightly drooping.

Sheath.—Not pendulous.

Tail.—Short and thin.

Legs.—Short and of good bone.

Feet.—Small and hard.

Colour.—The prevailing colour is white, grey and white with grey shoulder and quarters, also red, black and broken colours are to be found. Among the ryots there is no selection of bulls, the Swami or Brahmini bull is usually the village herd sire, but there can be little doubt that cows are also served by the young uncastrated stock.

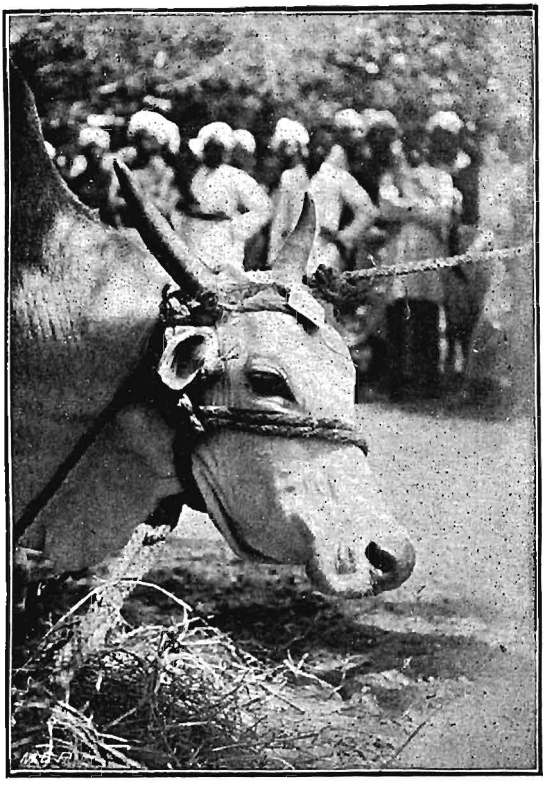
The origin of the Brahmini bull is somewhat interesting. The tradition is that one of the early Hindu

kings in the course of his travels saw what poor specimens the herd bulls were, and having given this matter much thought he decreed in honour of *Siva* and of *Nandi* the bull, who was the vehicle of the God in his peregrinations—that all well-to-do persons should, on the death of a relative, select the best young bull calf they could find, and present it as an offering to the God. These animals thus became the property of the community, and were allowed to roam and feed where they liked, and thus became the sires of the village herd.

So long as this was carried out in the spirit of the decree fine animals were produced, but gradually, although the custom is still kept up, persons bought the cheapest young bull they could find, as a salve to their conscience, and turned this loose, to eventually become the Brahmini bull. And this will probably account for some of the wretched bulls which are to be seen everywhere among the village herds.

The cows of this breed are said to be fairly good milkers, and are preferred by some people on account of their small well-set frame. The price of small bullocks varies from Rs. 40 to Rs. 70 per pair, but the larger variety command much higher prices. Cows when in full milk may be obtained for about Rs. 40.

The custom in South Coimbatore is that if the ryot possesses only a garden, he keeps his cattle within his garden with the exception of the cows which are kept at home. If the cultivator possesses dry lands he grazes his cattle during the day, and keeps them in the house compound during the night. Some keep their cattle night and day on these dry lands. There seems to be a strong objection to keep cattle on red soil if it can be helped, as it is considered to be heating. On the contrary black cotton soil is said to be cooling.



Kángyam Cow.

It is not customary to afford shelter against the sun or rain, but the cattle are protected against the furious winds which prevail in Southern Coimbatore by screens of bamboo mats and branches which are formed into a pen. These pens are moved at intervals of one or two days, and in this way the whole field becomes manured. In this way also ticks, which are a great pest to cattle, and increase where cattle are confined, are prevented from fixing on them.

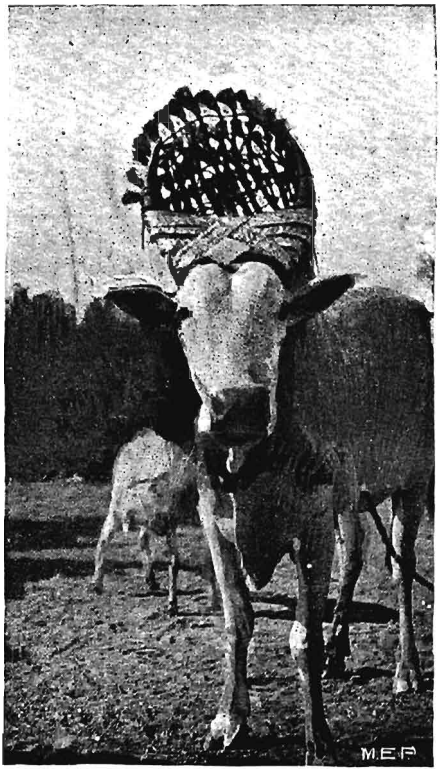
The following is the daily routine of cattle worked in gardens. At 6 A.M., the ryot gives his cattle water either with or without bran, after which they work at the plough or wells till 12 noon; they are then tied up under the shade of a tree, watered and fed. Special food consisting of bran, cotton seed, and gram which is soaked and ground is given in addition to a full ration of straw or as much as it can eat. At 2 P.M., work is resumed and continued until 6 P.M. The animals are then tied to pegs in the garden or taken to the house, and straw is given in small quantities at intervals during the night. The straw ration consists of stalks of cholum, cumbu, ragi, tenai, samai, varagu, paddy, chola chukka (top of the cholum containing ears after the removal of the grain) and collu thiri (the pods of horsegram after removal of the seeds). As a rule no special feeding is given to cows or bullocks not in work. The cattle graze on the harvested fields, and the few fields which lie fallow or on any waste land. Some few ryots in Kangayam reserve some lands for grazing on which Kolakuttai grass is grown.

Bulls are put ploughing at two and a half years of age, at three and a half they are yoked to carts, and at four and a half they are trained to work at water-lifts.

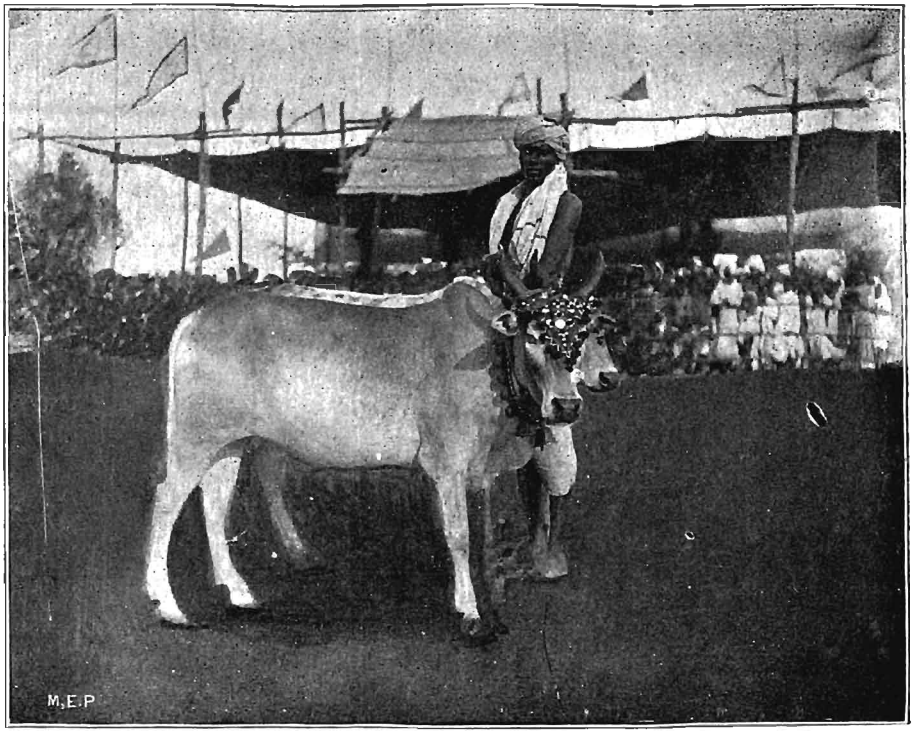
Fodder crops, grown as such, are rare, but the practice is known throughout the district and is occasionally

followed. In the Kangayam division of Dhárápuram where the best cattle are still reared, there is a regular practice in February and March of growing either cholum (*Sorghum vulgare*) or kumbu (*Penicillaria spicata*), chiefly the former under well irrigation, this is called *adar* (meaning close or crowded) *cholum* from its being sown closely so as to yield heavily, and of thinner stalk, and is grown at such time that fodder may be most needed. It is cut down before earing, and affords considerable provision during the hot weather. Fodder crops are not grown on dry lands; there is a sufficiency of pasture except in the hot weather, and as it is unusual to get rain sufficient even for ploughing during the period reckoning from the end of December to the 15th April, no such crops are possible except on garden lands. Cholum straw is a favourite fodder, and is carefully stacked for future use; the numerous stacks that dot the black cotton soil of Udamalpet and all gardens, are an agreeable feature in the landscape. Paddy (*Oryza sativa*), samai (*Panicum miliare*) and ragi (*Eleusine coracana*), straw are equally approved of, cumbu not so much. The ryot excuses himself from growing fodder by alleging, and with some reason, that as his cholum fodder is little injured by growing to maturity, he grows cholum as a grain crop rather than as a fodder crop for the double yield. The expenses of well irrigation in the hot weather are considerable, and few can afford to lose the grain of the crop. Nevertheless it is probable that a gram crop could more profitably be raised during the April-May rains rather than let it be fed off by stock or ploughed in as green manure.

Young bulls are emasculated at the age of about two and a half years by the process of crushing which is universal in India. They are at that age allowed to



Jellicut Bull.



Pulikolum Breed—Jellicut Bull.

cover cows, and heifers are put to the bull when their first pair of permanent incisors have appeared.

Pulikolum or Jellicut Breed.

Other names have been given to this breed and they are sometimes known as Kilakad or Kilkattu. This is a very numerous breed, and although a fair number are bred in the various villages in the Madura district by far the largest breeding operations are conducted in the south and south-west of Madura; large numbers are also raised in the vicinity of the Cumbum valley and the Perraya river where there are grazing grounds of vast extent. There is a big breeder living near Madura at Cholavandanniya, by the name Iyengottai Mamzak-karar, who owns about 1,000 head of cattle, and who exhibited some at the Madura Cattle Show held in 1907. The young bulls which he stated to be typical of the breed were rather small, but otherwise there was little fault to be found with them. They were very compact, with stout legs and hard feet. The loins, shoulders, and neck very powerful, and both were capable of doing very good work. He wanted Rs. 100 each for these bulls, but this class of cattle may usually be purchased for about Rs. 35 to Rs. 50 each. These cattle, as a rule, are comparatively small in size, but are very active and capable of much endurance. In many of the villages in South Madura certain of the small bulls are kept for the purpose of bull fighting or rather bull baiting, and are known as *Jellicut* which means an ornament of leaves, from the fact that the horns of the bull are usually decorated with a vividly coloured cloth. The method of baiting is as follows. A coloured cloth is firmly tied round the horns of a bull, and he is then set free. A number of men then attack it and endeavour to untie the

cloth which the bull, having now become extremely excited, resents. It naturally shows fight, and has generally to be thrown down, and both hind legs held before the cloth can be untied. It is no uncommon occurrence for men to be injured, and even killed, but nevertheless the sport is most popular. Bulls selected for this purpose are fed and well cared for. They are kept apart and fed by the owner only, so as to make them savage towards strangers. To show how popular is this sport I may say that hundreds of these bulls are kept for this sole purpose.

In many points they resemble the smaller sized variety of the Konga or Kangayams, but they are finer bred, and give the idea that they have in them a strain of the Mysore blood, and this is highly probable. The larger ones are extensively used for coach work, and they are capable of trotting continuously 5 or 6 miles an hour. The cows are poor milkers.

Description.

Head.—Face of moderate length, muzzle fine, forehead fairly broad, horns wide-spread and curved inwards having sharp points, ears short and erect, and eyes quick and prominent.

Neck.—Short and stout.

Hump.—Well developed.

Dewlap.—Narrow, thin, and extending to the chest only.

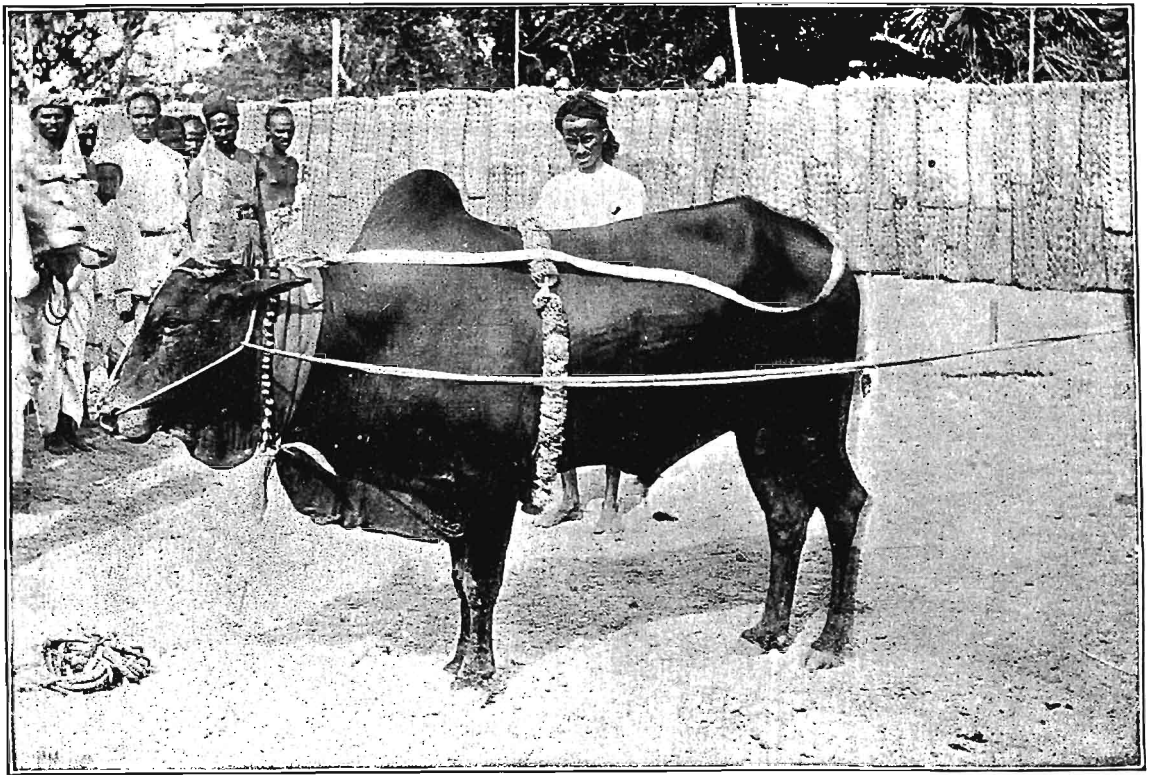
Body.—Compact, and well ribbed up.

Back.—Broad, short, and slightly higher at croup.

Quarters.—Strong and level.

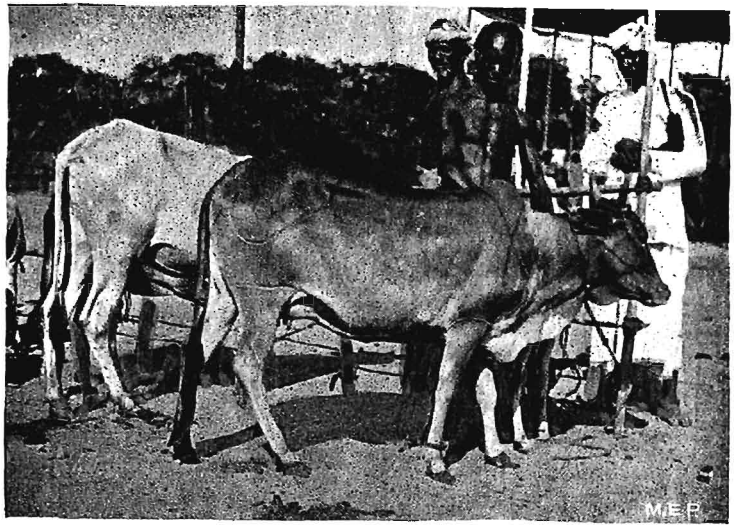
Sheath.—Adherent to the body.

Tail.—Long, with large tuft of hairs.

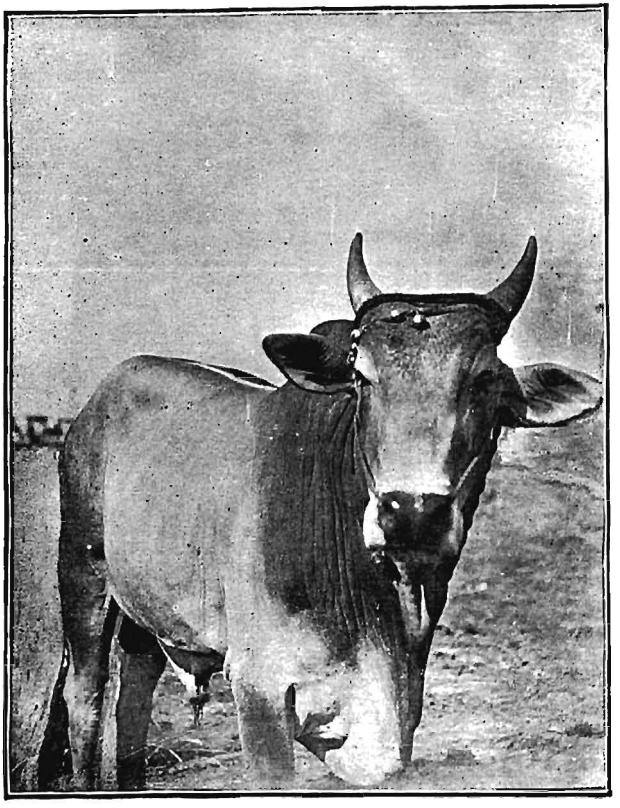


Jellicut Bull--Madura.

Pulikolum Bull.



Jellicut Cows.



Pulikolum Bull.

Legs.—Strong, short, and well set apart, usually grey and white, but other colours prevail.

Kappiliyan Breed.

A tribe of people called the Kappiliyans of Cumbum in the Madura district have a herd, not very numerous of a somewhat distinctive breed. This tribe are of Canarese origin, and still speak that language. Mr. Francis in the Gazetteer of Madura gives a most interesting account. In describing them he says they are small, active, round barrelled animals well known for their trotting powers, which the people themselves declare to be descendants of some cattle they brought with them when they first came to these parts. They are called the *Devaru Avu* in Canarese or in Tamil the *Tambiran Madu*, both of which phrases mean "the sacred herd."

The cows are never milked, and are only used for breeding. Members of the herd which die are buried, and are not, as elsewhere, allowed to be desecrated by the chuckler's skinning knife.

The leader of the herd is called "the King Bull" *Palladu Avu*, and when he dies a successor is selected in a quaint manner, with elaborate and expensive ceremonial. On the auspicious day fixed for the election, the whole herd is assembled, and camphor, plantain, betel and nut, and so forth are solemnly offered to it. A bundle of sugarcane is then placed before it, and the attendant Kappiliyans watch eagerly to see which of the bulls of the herd will approach and eat this. The animal which first does so is acclaimed as the New "King Bull" and is formally installed in his office by being daubed with saffron and kunkuman and garlanded with flowers. Thereafter he is treated by the whole caste as a God, is given the holy name of *Nandagopalaswami*, and is allotted to

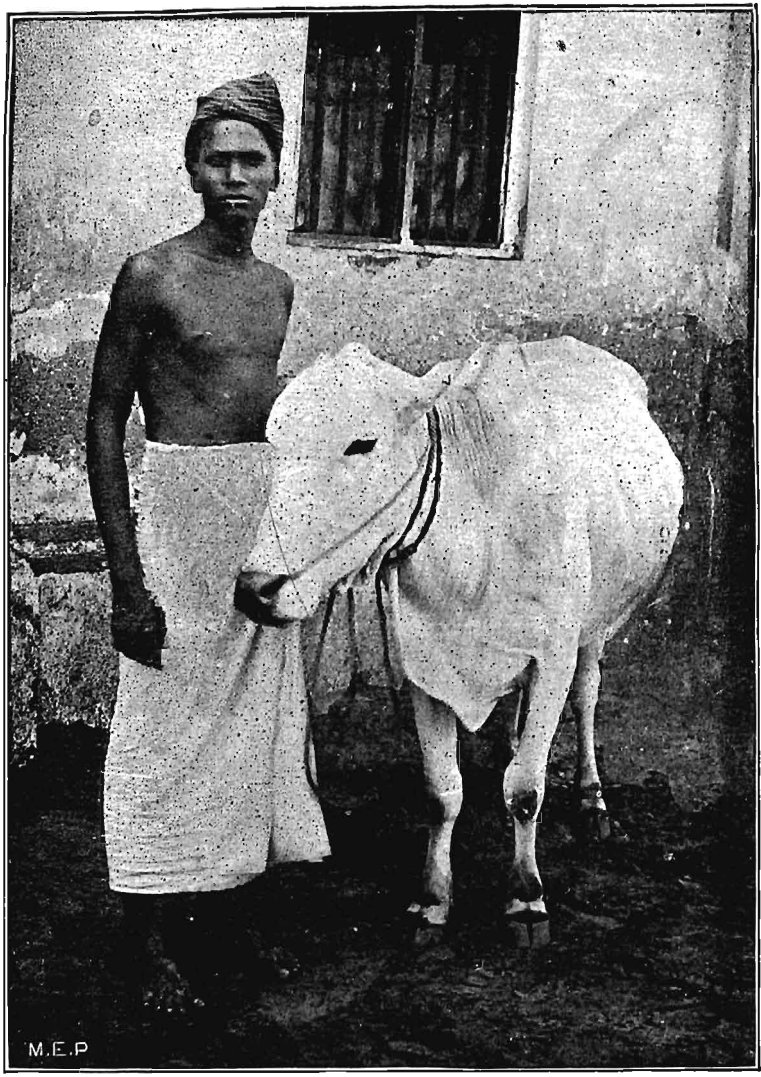
watch over and worship him a special attendant, who enjoys the inams which stand in his name, and is the custodian of the jewels and the copper grants which were presented in days gone by to his predecessors. There are now nine of these grants, but they do not state the Sakka year in which they were drawn out, and the names of the Rulers are not identifiable.

The "King Bulls" are credited with having performed many miracles, many of which stories are still eagerly related, and their opinion is still solicited on matters of importance. The herd is not taken to the hills for the hot weather until its King has signified his approval by accepting some sugar and milk placed near him. His attendant always belongs to a particular subdivision of the caste, and when he dies his successor is selected in as haphazard a fashion as the King Bull himself. Before the assembled Kappiliyans puja is offered to the sacred bull, and then a young boy is seized by divine inspiration and points out the man who is to be the new holder of the office.

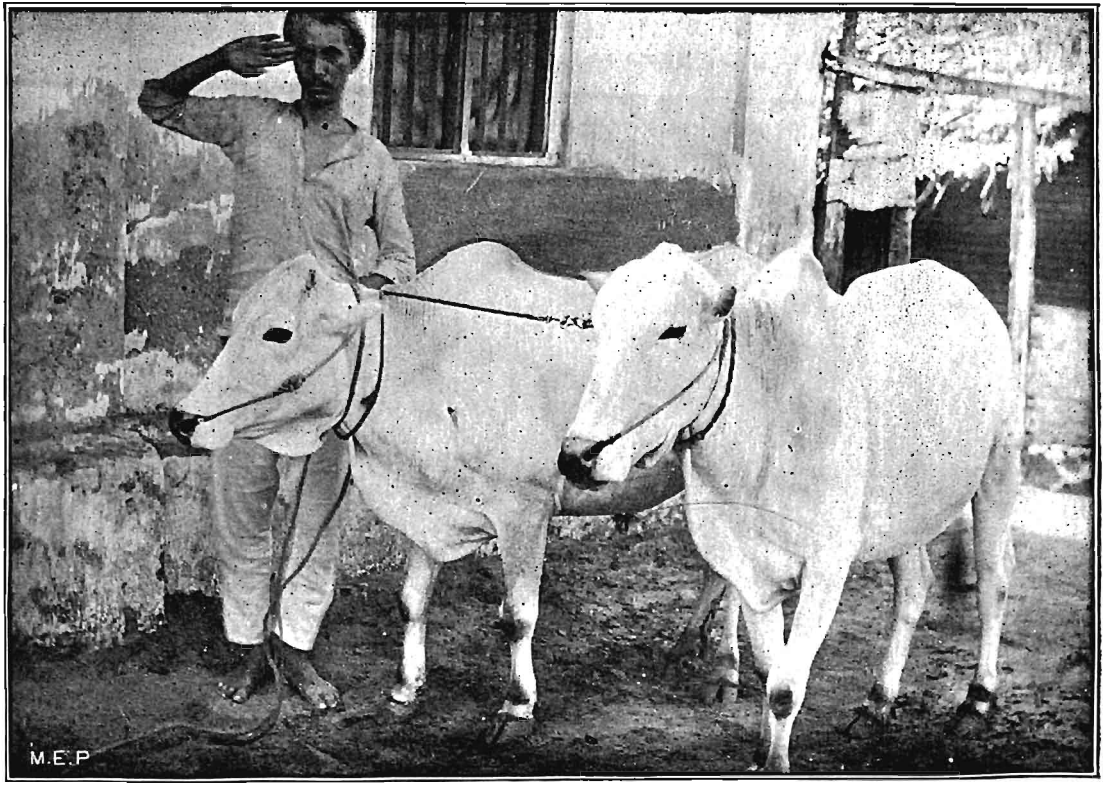
The herd receives recruits from outside owing to the Hindus round about dedicating to it all calves which are born on the first day of Thai, but these are not treated as being quite of the elect. The Kappiliyans have recently raised Rs. 11,000 by taxing all members of their caste in the Periyakulam taluk for three years, and have spent this sum in building roomy masonry quarters at Cumbum for the sacred breed. Their chief grievance is that the same grazing fees are levied on their animals as on mere ordinary cattle which, they urge, is equivalent to treating Gods as equals of men.

Tanjore Polled Cattle.

At first sight these animals would appear to be of a distinct breed, and quite different from any animals found



Tanjore Bullock.



A pair of Tanjore horned cattle.

in the Presidency, but this is due to the early destruction of their horns, giving the head an appearance of that of the British polled cattle. In this district it is the custom to purchase cattle from the cattle dealers who bring them chiefly from Salem and Coimbatore, but many are brought from the breeding grounds in the Cumbum valley in the Madura district. The offspring which are calved in the Tanjore homesteads are, at about the age of six months, dehorned by their owners. The procedure is to take some hairs from the tail, and this is mixed with jaggery or coarse sugar, and the mixture is applied over the young horns; then a heated iron is drawn backwards and forwards until the part is sufficiently burnt. The burnt part takes two or three weeks to heal, and prevents the growth of the horns. It is also customary to cut two or three inches off the ears, as this is supposed to improve the appearance of the animal. The destruction of the horns is supposed to increase its strength and render it more docile, and, to a certain extent, it has this effect. This process alters the appearance of the cattle, and most certainly give the impression that it is a special breed. It is only in certain parts of Tanjore that it would be possible to breed cattle, as nearly the whole of it consists of delta land. Except in the appearance of the head these animals present all the chief characteristics of the Kangayam breed.

Description.

Head.—Short, forehead broad, eyes, bright and prominent, horns absent, and ears shortened.

Neck.—Short and thick.

Hump.—Moderately developed.

Dewlap.—Thin, and reaching to the sternum only.

Body.—Compact.

Back.—Slightly rising towards the croup.

Quarters.—Strong and drooping.

Sheath.—Adherent to the body.

Tail.—Rather long, and with a large tuft, sometimes nearly reaching to the ground.

Legs.—Short and strong.

Feet.—Good, being small and hard.

Colour.—White or light grey prevails.

Height.—From 44 to 48 inches behind the hump.

Goomsur Breed.

This is a very small variety of animal found to the north of the Ganjám district. They are very symmetrical in shape and active, and are largely used for cart work in the district. All over the taluk of Goomsur there is extensive grazing ground which is very suitable for rearing of cattle but they never attain any size. In 1908 a cattle show was inaugurated at Berhampur, the chief town of the district, and handsome prizes were offered for the best cattle, but it being the first show ever held in the district it was unfamiliar to the people. It is, however, anticipated that in time it will become a valuable institution, as on the first occasion it was very well attended. The following is a description of a typical Goomsur bullock :—

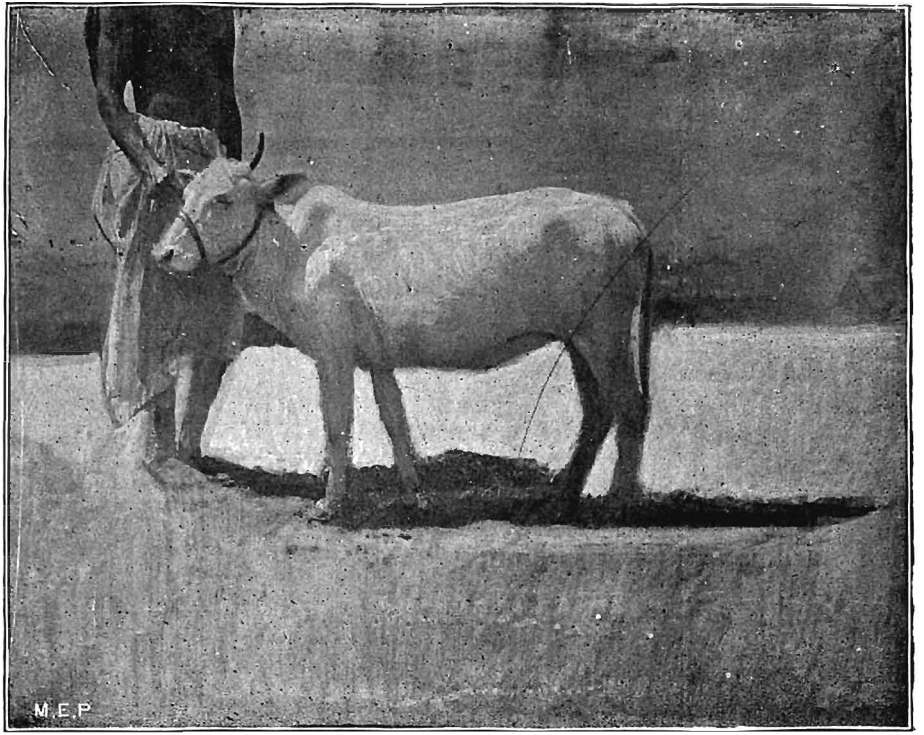
Head.—Small, with a flat face, broad forehead, and mild but intelligent eyes. The horns are medium sized curving outwards and inwards.

Body.—Square, compact, and well ribbed up, with good strong loins. The hump is fairly well developed.

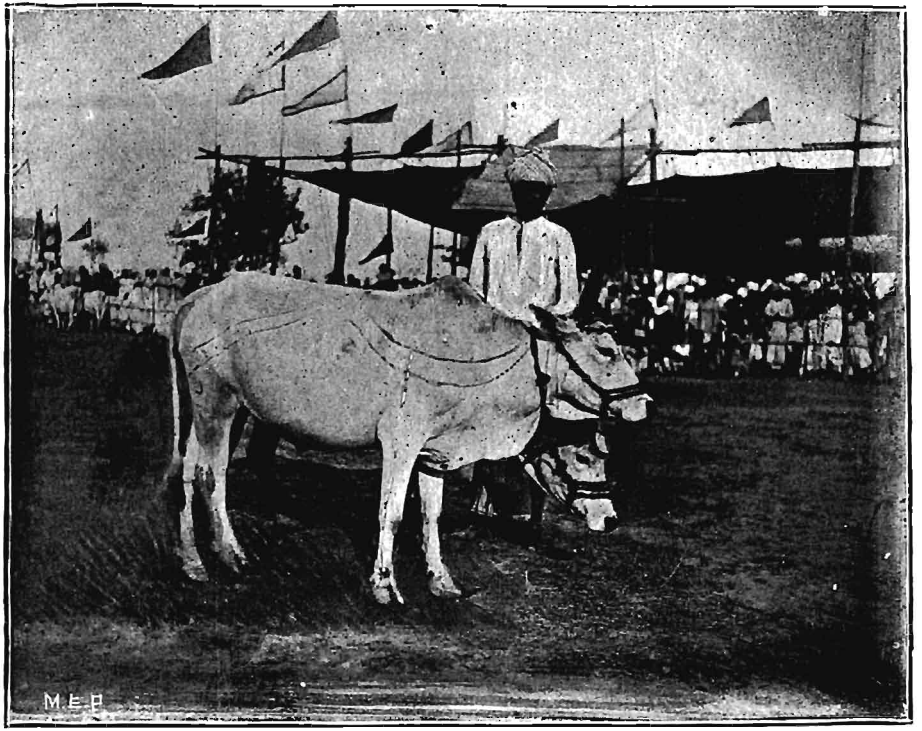
Dewlap.—Thin, and reaching to the sternum only.

Sheath.—Adherent to the body.

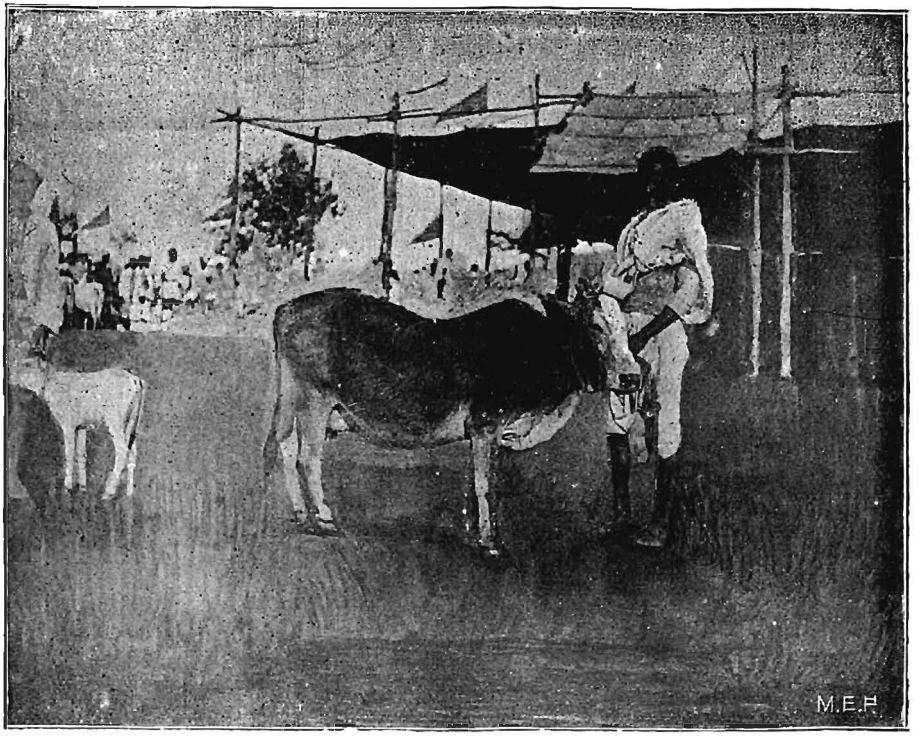
Tail.—Reaching to the hocks, fine and "whippy" with a large tuft of hair at the end.



Gumsoor Cow.

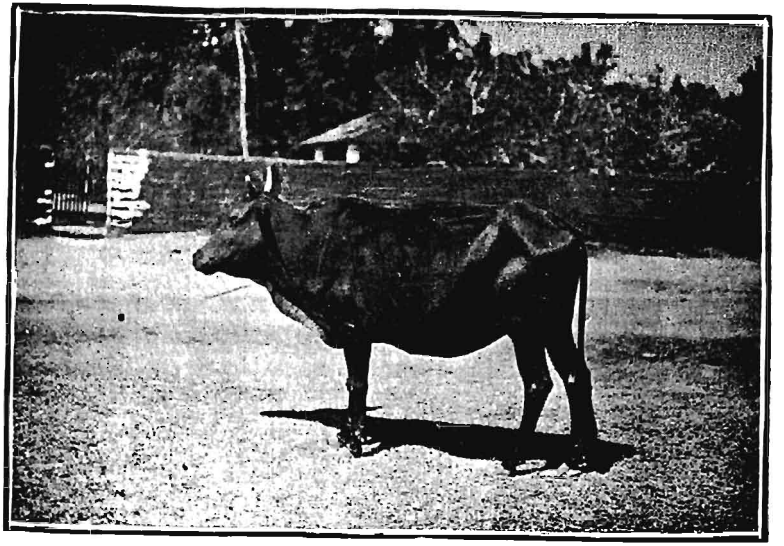


Pair of Gumsoor Cattle.

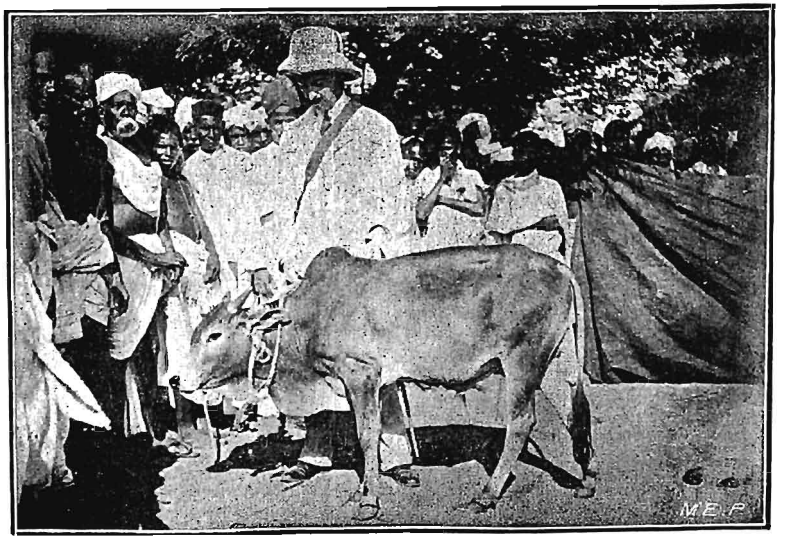


Gumsoor Cow.

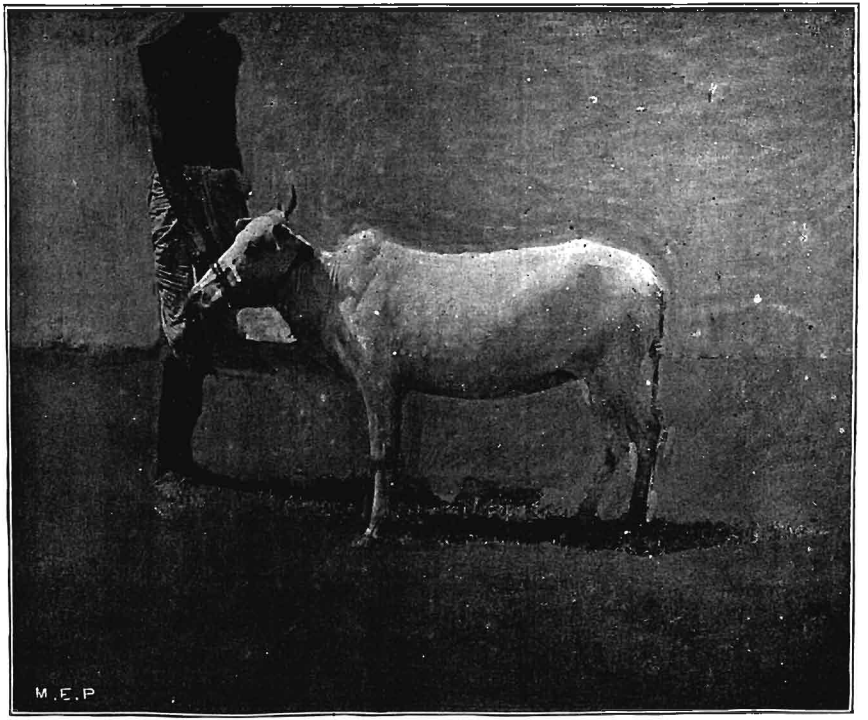
Malabar Cow.



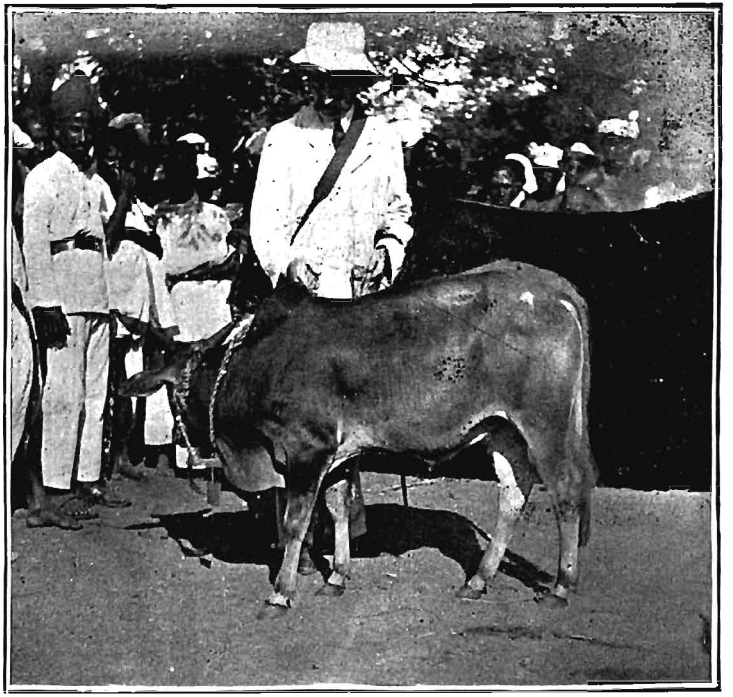
West Coast (Malabar) Cow.



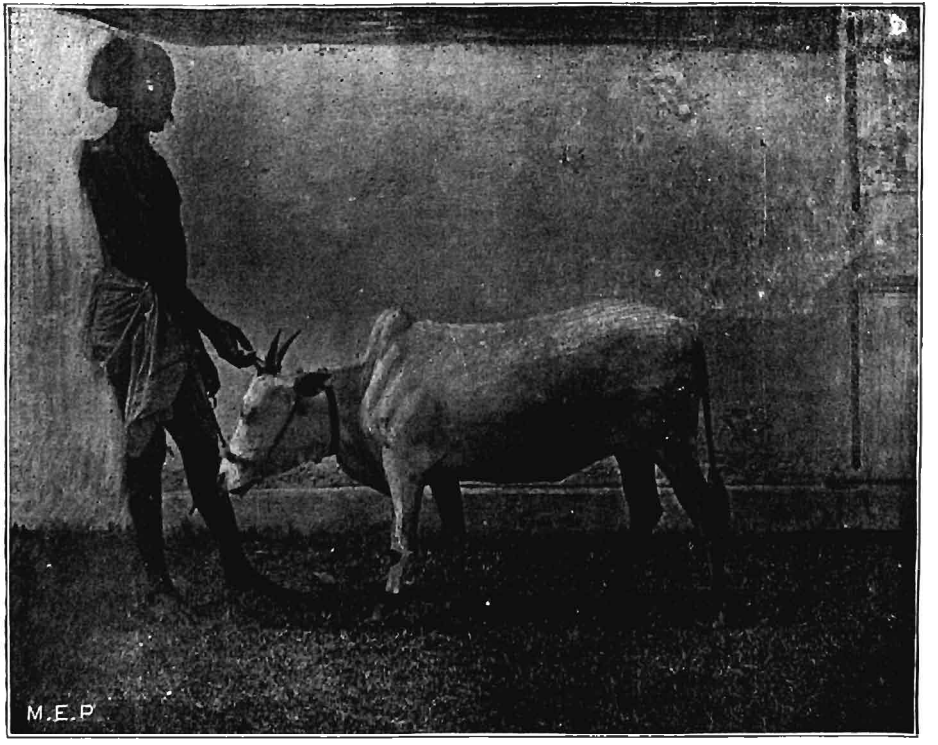
Diminutive Bull—4 years old.



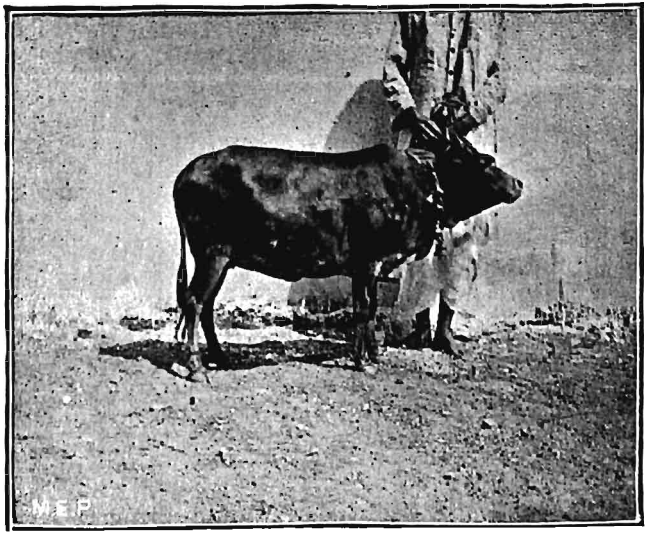
Diminutive Breed—Cow.



Diminitive bull.



Diminutive Cow.



Diminutive Cow.

Feet.—Good, hard, and sound.

Colour.—Usually light grey, but other colours also prevail.

Height.—From 42 to 46 inches measured behind the hump.

Lucky Marks.

(1) *Támani Suli.*—A ridge of hair along the middle line of the animals' back, about the centre. "Támani" means a herd, and this mark indicates that the purchaser will acquire a large number of cattle.

(2) *Irattai-kavam* consists of two ridges of hair, one on each side of the brisket. A single hair mark on one side of the brisket (*Ottai-kavam*) is most unlucky, and forebodes the loss of all other cattle in the house, and also the death of the purchaser.

(3) *Bháshikam Suli* is a crown on the forehead above the line of the eyes. "*Bháshikam*" is the name of the wreath worn by bride and bridegroom during the marriage ceremony. If the purchaser be a bachelor or widower, this mark indicates that he will marry soon. If the purchaser be a married man, he will either have the misfortune to lose his wife and marry again, or the good fortune to obtain two wives.

(4) *Gopura Suli.*—A crown upon, in front of, or immediately behind, the hump. This is considered to be a very lucky mark.

(5) *Nir Suli* is a crown situated on the middle line of the belly just opposite to the opening of the urethra. Regarding this the saying is that "the family will either be reduced to ashes, or swell like a river." The hair-mark is thus of doubtful signification. An intending purchaser, rather than incur the risk of evil consequences, will avoid the purchase. The ryots say that if a little

earth be taken and rubbed on this hair-mark the bullock will void urine.

(6) *Eurupuran* (ascending centipede).—A ridge of hair on the hind quarters curving up to the back is a sign of coming prosperity. If the ridge does not curve upwards to the back it is called *Irangupúrán*, (descending centipede) and indicates adversity to the purchaser.

(7) *Lakshimi Suli* is a hair-mark situated on one side of the neck at some distance from the dewlap. "*Lakshumi*" is the goddess of fortune. This is considered to be the most lucky hair-mark, but is rarely met with. A bullock with such a mark is highly esteemed, and fetches a long price.

Unlucky Marks.

(1) *Mukkanti Suli* or *Agni Suli.*—Three crowns on the forehead arranged in form of a triangle said to represent the three eyes of Siva of which the one in the forehead will, if opened, burn up all things within the range of vision.

This mark forebodes ruin to the purchaser.

(2) *Kudai-mel-kudai.*—Two crowns one over the other on the forehead predict disaster after disaster.

(3) *Ottai-kavam.*—A single hair-mark on one side o the brisket close to the middle line forebodes loss of all other cattle in the house, and the death of the purchaser.

(4) *Vilangu Suli* (fetters).—Hair-marks on the fetlocks of either pair of legs indicate that the purchaser will soon be in gaol.

(5) *Padai Suli.*—Two ridges of hair on the back on either side of the middle line indicate that the purchaser will soon need a coffin.

(6) *Irangupúrán* (descending centipede).—A ridge of hair on the hind quarters not curving upwards to the back indicates adversity.

(7) *Nagappadam.*—A ridge of hair on the haunch spreading out at one end like the hood of a cobra. If the direction of the hood is upwards it is termed *Munnágam*, and if downwards, *Pinnágam.*

(8) *Tattu Suli* (obstacle).—A crown situated on the back between the points of the hips indicates that any business undertaken by the purchaser will fail.

(9) *Tudaippa Suli.*—A hair-mark on the side of the tail near the root, sometimes extending as ridge over the back.

BUFFALOES.

The buffalo or *Bos babulus* stands next to the ox in its utility to man, and still exists in a wild state in large numbers. There is scarcely an animal upon which domesticity has made so little impression as the buffalo, and yet it is universal, and found all over the Presidency, distinguished by its large flat horns, some curved and some long, measuring sometimes as much as five and six feet in length. The colour varies from a black to a light slate colour, with very scanty hair, but with tufts of hair in various parts of the body.

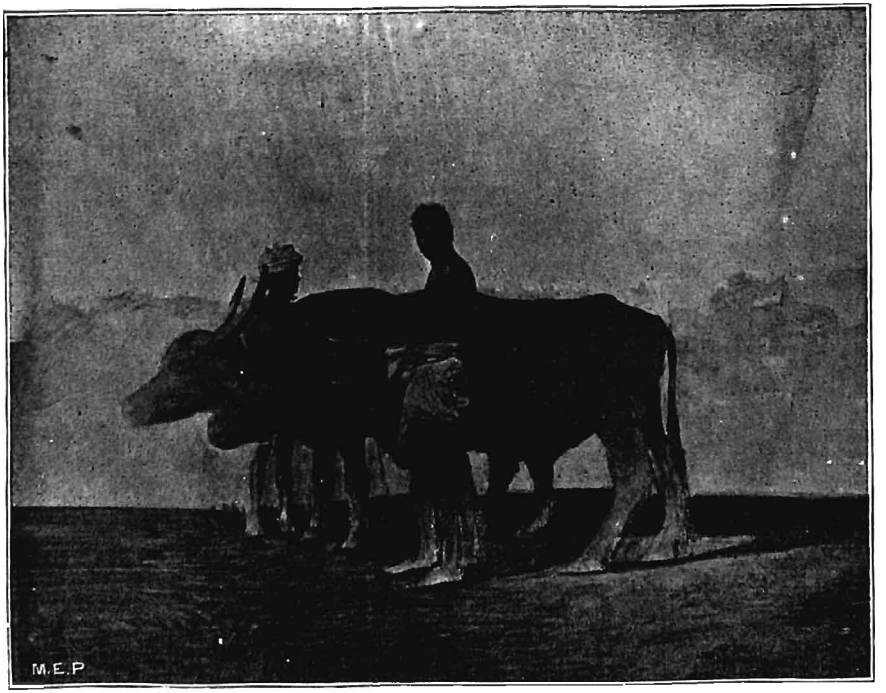
These animals are very largely used where wet cultivation is in vogue. Although slow they are excellent workers, particularly in the ploughing of paddy fields and sugar mills.

In Southern India there are probably four distinct varieties, *viz.* :—The Toda which are found on the plateau of the Nilgiri hills. The Parlákimedi on the Ganjám hills on the East Coast. Malabar, the best of which are to be found in the south-western portion of that district and South Canara, and the small non-descript variety which may be seen everywhere.

Toda Buffalo.

The Toda buffalo is pretty well known to the frequenters of the Nilgiri hills where each Toda Mund possesses a herd. They differ from the kind generally met with on the plains, and appear to be indigenous to those hills alone. They are of exceedingly powerful build and long in carcase, they have scarcely any hump; the chest is broad and deep; the legs short and sturdy; the head large and heavy, and surmounted by horns set wide apart and curved differently to those of the animals seen

Toda Buffaloes.



Toda Buffaloes.

on the plains, the points being recurved inwards, outwards, and forwards. They carry their heads low, and from this peculiar curvature of the horns it gives them at first sight a bull dog appearance. Along the crest of the neck, hump and back, there is a thick growth of hair like a mane which imparts a bison-like appearance to them. They are known to be fierce and rather dangerous to approach incautiously. At the sight of a stranger they throw up their heads, run back for some distance, when they abruptly halt, and turn towards the object of these fears, at whom they fiercely stare with heads erect, cautiously advance, and retire, then gather together in a compact mass prepared for attack.

At other times the whole herd starts suddenly into an impetuous rush with their heads down, and overrun, gore or trample to death the object that has excited their anger. In this manner tigers and other beasts of prey are often kept at bay, or killed by the simultaneous rush of the herd.

They are good milkers, yielding daily from five to nine quarts of very rich milk. The milk has an unusually well flavoured taste. Beyond this they are put to no other use whatever.

The cows are milked both at night and in the morning, but the principal dairying operation is conducted before sunrise. The "tuel" or pen is a circular enclosure varying in size according to the numbers comprising the herd, built of loose stone with a single entrance guarded by powerful wooden stakes in which the herds are shut in for the night. It is generally located in some sheltered spot, and embanked to the height of three or four feet. During the heavy rains the windward side of the pen is bushed with brushwood to protect the herd from the cold

and piercing winds. These pens have no covering whatsoever, the cattle are exposed at all seasons to the rains and sun, while the floor is covered with an accumulation of their own droppings. The young calves however, prior to their being weaned, are very carefully looked after, and kept under shelter at all times. During the day the calves either accompany their mothers or are grazed separately in charge of an attendant. The Toda buffalo enters very largely into the ceremonials of this peculiar people who interest all visitors to the Nilgiris. The Todas have no history, no written character, and only a very elementary language. They are supposed to have lived on this plateau for upwards of 800 years, and their only means of livelihood is the keeping of herds of buffaloes. Before the advent of the British Government they were considered to be "lords of the soil," but when the encroachment of the town of Ootacamund began to envelope the grazing lands of these people, the question of the title was fully gone into, and now the Toda have become tenants of the over-lord. Under the recent survey it was arranged that about fifty acres of land, ordinarily to a considerable extent woodland, should be demarcated as the site or reserve of each Mund or homestead, the total being about seventy. On this the Todas pay an assessment of two annas per acre, the lowest under the present assessment, and this is the only tax they pay.

Soon after the birth of a child a young buffalo calf is brought into the presence of the family. The father takes three measures and pours water from the third measure into the other two holding them close to the hind quarters of the calf on the right side. The meaning of this singular rite is not clear, but it probably has reference to the future supply of milk for the infants' sustenance.

Early betrothals are common among Todas, and an interchange of buffaloes ratifies the agreement; later on when the marriage is consummated, another exchange of buffaloes takes place. At the funeral a small herd of buffoloes is driven along with the *Cortége*, and all the friends of the deceased and neighbouring villagers to do honour to the dead. Arrived at the place of burning each buffalo has a little bell hung round its neck, and they are then driven close to the pile with the words "*Aran od atu*," "Go with him." Then all the mourners take handfuls of earth and throw them at the buffaloes, and afterwards ask the corpse whether they may kill buffaloes for it.

There is an annual ceremonial in commemoration of all those who have died during the year. During the first day the animals intended for sacrifice are driven in where they are safely enclosed in a kraal, two or three for each of the dead to be commemorated. The young men now throw off their blankets and rush among them, hanging on to the animals by their horns and neck, whilst a bell is hung on to the neck of each. On the second day the bier is brought out, a hole is dug at the entrance of the kraal, and the priest "does puja." After this the "pujari" approaches with garlands of creepers which he throws at the buffaloes. This is the signal for the "*Coup de grace.*" The poor terrified creatures who have been half maddened by the treatment which they have already undergone from the young men who have spent the preceding horns in exciting them in every way, rush madly about, and sometimes leap the kraal wall, and make their escape to some distance before they can be caught and despatched. The bodies are dragged back and placed in a line with the bier beside them, and mourned.

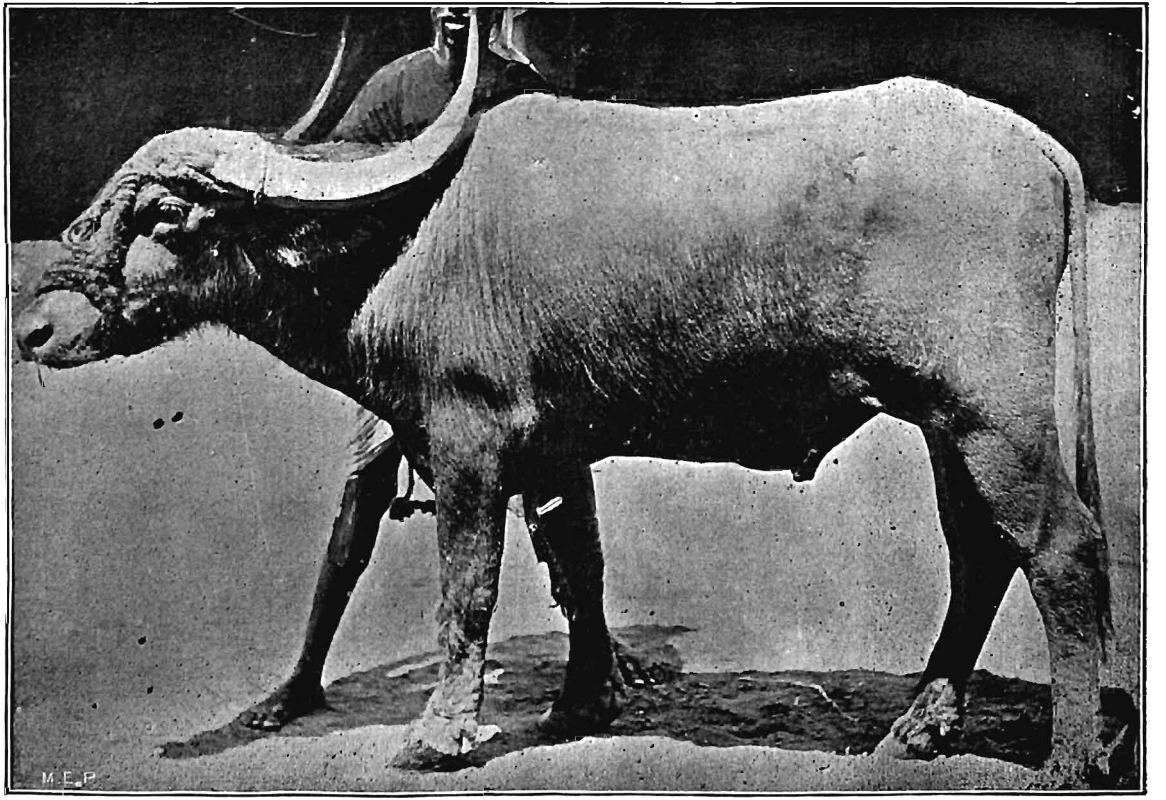
What follows next is weird and cruel, and the Todas evidently fear that Government may prohibit it on the

score of cruelty, for they "make a secret" of this part of the proceedings. A buffalo, cow and calf are produced, the latter is held by three men while the former receives a blow between the horns which stuns without killing her. A gash is then made under the foreleg of the poor animal, and the *Varzhal* or inferior priest, dipping some pieces of bark into the wound, gives some of the blood to the kinsmen, who smear it upon the "bier" saying at the time, "May the sin run away."

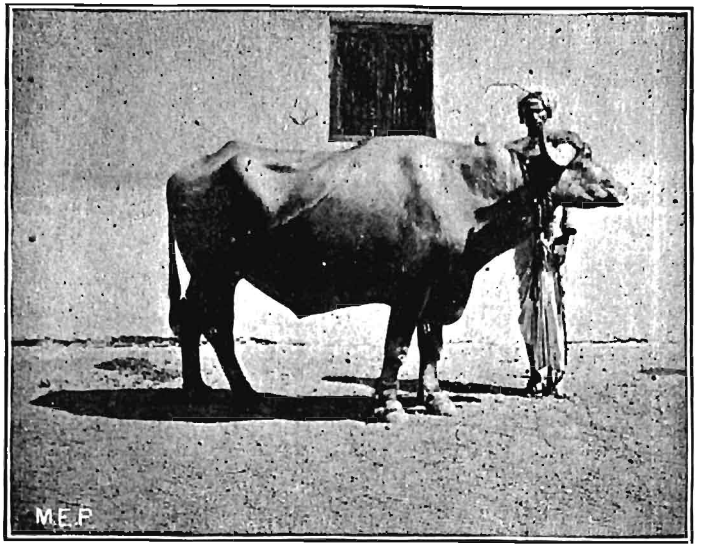
Formerly there used to be reckless slaughter, but Government has stepped in and stopped it on the ground of the cruelties practised. None were gainers by the deaths of these poor animals except the *Kotas* who attend on these occasions as musicians, and they claim the carcasses of all the buffaloes. When the Todas are asked why they give the Kotas all the carcasses, they exclaim "when the buffaloes are alive they are ours, when they are dead they are the Kotas."

Buffaloes—South Canara.

There is a sect of Hindus known as the Jain Bants who own a fine hardy breed of buffaloes, and some of the best may be seen in the neighbourhood of Mangalore on the West Coast. The nature of the cultivation carried on in this part of the country necessitates the use of these animals as the indigenous cattle are small and insignificant. Buffalo racing forms one of the great amusements of these people, and every rich Bant keeps his "Kambla" field sacred to buffalo racing, for the details of which I am greatly beholden to Mr. E. Thurston. The best pairs of buffaloes used for this purpose are valued up to Rs. 500, and are splendid animals, and, except for occasional plough drawing, are kept for racing all the year round



West Coast Buffalo Bull.



Parlákimidi Buffalo.

Each pair of buffaloes runs the course alone and is judged by the assembled crowd for pace, style, and most important of all, the height and breadth of the splash which they make. Sometimes a kind of gallows, perhaps twenty feet high, is erected on the course and there is a round of applause if the splash reaches up to or above it. The course is generally a wet paddy field about 150 yards long, full of mud and water. The driver stripping himself to the necessary minimum of garments mounts a little flat board about 12 by 18 inches, on which is a small elevation or stool. His friends hold on to the buffaloes for all they are worth, and he places one foot on the stool and one on the pole attaching the so-called car to the yoke, his whip is held aloft in one hand, and one of the buffaloes' tails in the other. He has no reins. In this way he comes down the course shouting with all his might with the plank on which he stands throwing up a sort of Prince of Wales' feather of mud and water round him. They have frequent spills, but the falling is quite soft and it is seldom that any one comes to grief. Marks are given for the pace, style, sticking to the plank and throwing up the biggest and widest splash. At a big meeting perhaps a hundred pairs will be entered brought from all the surrounding country and the big men always send their buffaloes to the races headed by a local band.

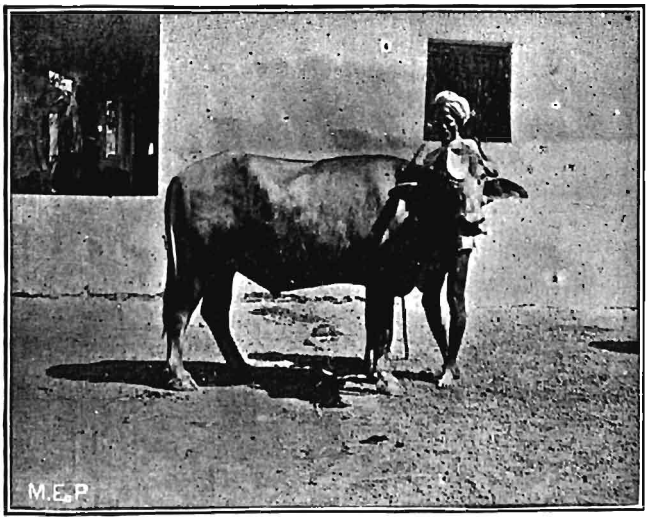
Parlakimedi Buffaloes.

Three distinct breeds of buffaloes are found in this part of the Ganjám district, namely, the "Desi" or "Manda," the "Jerangi" and the "Pedda Kimedi" breed. The buffaloes commonly met with are of the "Desi" or "Manda" breed. They are larger than the animals found elsewhere in the district, and some of them

are imported from the Kalahandi State. In colour they are dark grey. The buffaloes of the "Jerangi" breed are smaller, and have short horns and short tails. They are noted for their hardiness and smartness; and they are somewhat darker than the "Desi" breed. Jerangi is in the Parlákimedi Maliahs, and it is here where most of these animals are bred.

The "Pedda Kimedi" buffaloes are much larger and stronger than those of the Desi and Jerangi breeds. They cannot however stand the sun so well, but are exceedingly useful for slow heavy work. It appears from such information as is available that they are brought by the Pedda Kimedi people from Kalahandi and other parts of the Samalpur district of Bengal, and are not bred in Pedda Kimedi. Cow-buffaloes of "Jerangi" and "Pedda Kimedi" breeds are rarely met with outside their native borders, as they do not fare so well, nor do they yield as much milk as they generally do when found in their native tract, on account of change in food. These two breeds are not therefore very frequently met with outside their own country, and apparently no attempt is made to produce a mixed breed with "Desi" cows and "Jerangi" or "Pedda Kimedi" bulls.

Buffaloes are largely used for agricultural purposes, and for heavy draft work which the bullocks of these parts are incapable of performing. On the Parlákimedi Estate the ryots keep a far larger number of buffaloes than bullocks, as the former are the more useful for preparing land during the rains for transplantation of paddy. No special care is taken, nor is it necessary to provide pasture for the animals, as, in addition to the grazing lands and the cultivated lands after the crops are cut, plenty of fodder (paddy straw) is available in every village throughout the year, and this with rice bran,



Gódávari Buffalo.

horse gram, and gingelly oil-cake, which is generally given, is sufficient to sustain the animals in good condition. What is absolutely necessary in regard to these animals is that they should be washed twice daily. Young bulls sell from Rs. 30 to Rs. 100. A good cow is worth from Rs. 25 to Rs. 60 according to the milk she yields. A cow-buffalo is generally considered to be one of the requisites of a family, as she helps to support the family by yielding plenty of milk and ghee. A good cow-buffalo gives from 12 to 18 lb. of milk daily.

Several poor families earn their livelihood solely by maintaining one or two cow-buffaloes and selling milk-curd, buttermilk and ghee. The regular dealers in milk and ghee who are called "Gowdus" maintain herds of cow-buffaloes. The herds do not contain very many fine specimens. These are always kept outside villages and towns, and live on the grazing they can get in fields and jungles. They are not given any rice bran, horse-gram, oil-cake, etc., and are rather wild. There are weekly fairs held at Uppalada and Balada where some very fine animals are brought for sale. Many animals are purchased for export to other taluks in the district, and also to the adjoining districts. The following are some of the signs which are believed to be good in a cow-buffalo :—

(1) The head should be small.

(2) The back should be low.

(3) Fore legs should be shorter than the hind legs.

(4) Horns should be formed in such a curve so as not to allow water which may be poured over them to fall on the hoofs of the fore legs when the animal is standing.

The chief feature that is looked for, and to which much importance is attached in the bull-buffaloes is the

formation of the horns. If the two horns are of equal length bending backwards with tips facing each other, this is considered to be a good specimen. An animal that stretches out its tongue and turns it from side to side is said to have "*Pamu Naluka*" (snake tongue) and is considered to be destructive to the owner. So also is the animal which strikes, with its horns, the post to which it is tied, the action being called "*Kommu Kotta-dam*" (horn-beating).

Particulars (place, time, etc.) of Cattle Fairs held during 1906–1907 in the Madras Presidency.

District and taluk.	Name of place or village.	Month.	Approximate number of cattle.	Different breeds brought for sale.	Remarks.
Ganjám ...	Berhampur	February	1,000	Goomsur and mixed breeds.	
Gódávari ...	Draksharama cattle fair	Every Monday	500	.....	
	Cocanada do.	Every Sunday	100	.....	
	Pithápur do.	Every Saturday	2,500	.....	Annual cattle show, March.
	Yelésvaram do.	Every Thursday	200	.....	
	Tuni do.	Every Sunday	320	.....	
Kistna ..	Ellore do.	March	...	Principally Ongole and Kistna.	Annual cattle show, February.
	Palacole do.	Every Saturday	1,400	.....	
	Undi do.	Every Tuesday	200	Principally Ongole and Kistna.	
	Pentapad do.	Every Sunday	800	.....	
Guntúr ...	Ongole cattle show	March	1,200	Pure Ongole	A splendid selection of bulls and cows.
Bellary ..	Mylar cattle fair	30th January 1907 ...	1,000	.....	
	Kuruvalli do.	12th February 1907 ...	10,000	.....	
	Chitwadigi do.	Every Saturday	25 to 30	Ongole and Mysore ...	

Particulars (place, time, etc.) of Cattle Fairs held during 1906-1907 in the Madras Presidency—cont.

District and taluk.	Name of place or village.	Month.	Approximate number of cattle.	Different breeds brought for sale.	Remarks.
Bellary—*cont.*	Adóni cattle fair ...	Every Friday	180	.....	
	Kosigi do. ...	Every Tuesday	230	.....	
South Arcot ...	Tiruvannámalai do. ...	Prohibited	..	.....	
	Tennanai do. ...	Every Tuesday	...	.....	
	Kilpennathur do. ...	Every Thursday	200 to 500	.....	
	Vattavalam do. ...	Every Wednesday ...	100	.....	
	Mailam do. ...	Every Sunday	400 to 500	.....	
	Do. do. ...	Every month	500	.....	
	Ginjee do. ..	Every Friday	300	.....	
	Ongur do. ...	Every Tuesday	500	Mysore and Kangayam.	
	Brahmadesam do. ...	Every Thursday	...	.....	
	Valatti do. ...	Every Sunday	40	.....	
	Tívenúr do. ...	Every Monday	...	.....	
	Kottattai do.	1st May 1907	1,000	.....	
	Kattuparur do. ...	Every Sunday	100 to 300	.....	
	Kuvagam do. ...	Prohibited	...	.....	
	Parikal do. ..	Do.	..	.....	
	Vandipálaiyam do. ...	Every Tuesday	10 to 15.	.....	
Vizagapatam ...	District Agricultural Association show changed annually.	February	1,000	East Coast breeds and buffaloes	

Salem ...	Méchéri car festival fair	27th February o 2nd March 1907.	...	.....	
	Nangavalli car festival fair	27th February to 2nd March 1907.	500	.....	
	Berikai cattle fair	Not held	....	.....	
	Adamankotah Jatra fai	1st to 10th April 1907 ...	1,000	Kangayam....	
...	Nathapatti cattle fair	29th April 1907 .	500	.....	
	Kunthi Amman Jatra fair ...	16th to 23rd January 1907.	100	.....	Annual cattle and pony show.
Coimbatore ...	Tiruppúr	une	1,500	Kangayam and Mysore.	Ponies are brought to the fair.
Trichinopoly ...	Samayavaram Ekadasi festival cattle fair.	January 1907	2,500 pairs or 50,000.	.....	
Madura ...	Madura cattle fair	End of April 1907 ...	15,000	..... Kangayam and Mysore breeds.	
	Attur do.	19th to 23rd March 1907.	9,000	Pallikulam breeds.	
	Palni do.	1st to 3rd February and 29th March to 5th April 1907.	1,500	.....	
Tinnevelly ...	Muthulapuram cattle fair ...	6th to 31st July 1906 .	15,000	.....	
	Adi Tapasu fair at Sankaranayinárkóvil.	3rd August to 3rd September 1906.	5,000	.....	
	Tiruchendur cattle fair	17th to 31st August 1906.	2,000	.....	
	Timmarajapuram cattle fair ...	22nd September to 14th October 1906.	2,500	.....	

Particulars (place, time, etc.) of Cattle Fairs held during 1906-1907 in the Madras Presidency—cont.

District and taluk.	Name of place or village.	Month.	Approximate number of cattle.	Different breeds brought for sale.	Remarks.
Tinnevelly—*cont.*	Taipusam fair at Kazhugumalai	26th January to 9th February 1907.	12,000	.....	
	Masi fair at Tiruchendur	25th February to 17th March 1907.	8,000	Mysore and Palkikulam breeds.	
	Panguni Uttiram fair at Kashugumalai.	26th March to 8th April 1907.	2,000	.....	
	Panguni Uttiram fair at Virudupatti.	15th March to 25th April 1907.	2,000	.....	
	Chittrai fair at Sivalaperi ..	11th to 30th April 1907.	15,000	Alambadi, Kangayam and local breeds.	
	Vaikasi fair at Azwartirunagiri ...	23rd May to 8th June 1907.	3,000	.. ...	
Malabar ...	Kizhur fair	9th to 19th December 1907.	10,000	.....	
	Kodaramba fair	In abeyance	...	.....	
South Canara.	Kulgunda fair	Do.	...	.....	
	Subramania fair	November	Very large collection of cattle.	.....	

Particulars (place, time, etc.) of Cattle Fairs held in the Province of Mysore.

District and taluk.	Name of place or village.	Month.	Approximate number of cattle.	Different breeds brought for sale.	Remarks.
Kolar district.					
Kolar ...	Vakkaleri	February	16,000	Nearly all of Mahadeswarabetta breed; Nandi and Mastidana and "Nadudana."	
	Vanarasi	April	15,000		
Sidlaghatta ...	Mailur	Do.	10,000		
	Talakayalabetta	February	4,000		
Bowringpet ...	Bowringpet	May	5,000		
	Tornahalli	February	5,000		
Malur ...	Chiktirupati	April	7,000		
	Chalaganahalli	Do.	4,000		
	Bhupatemmanabetta	Do.	10,000		
Mulleagal ...	Avani	March	15,000		
	Yaldur	May	10,000		
Chikballapur.	Nandi	March	18,000		
Tumkur district.					
Koratageri ...	Srikyamanahalli	January	3,000	Mahadeswarabetta a d Hallikar breeds and a few of Pavgada breed.	

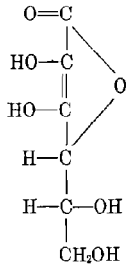
Particulars (place, time, etc.) of Cattle Fairs held in the Province of Mysore—cont.

District and taluk.	Name of place or village.	Month.	Approximate number of cattle.	Different breeds brought for sale.	Remarks.
Tumkur district—cont.					
Pavgada ...	Nagalmadiké	January	3,000	Pavgada breed, a few of Mahadeswarabetta breed and "Nadudana."	
Shimoga district.					
Honnali ...	Rampur	February	6,000	Mahadeswarabetta, Chitraldroog, Amrat Mahal and "Nadu."	
Mysore district.					
Yadatore ...	Chunchanakatté	January	7,000	Hallikar, Bettadapur, Metikuppé and Mahadeswarabetta breeds and Nadudana.	

pared a cortical extract by means of organic solvents. This method of preparation has been improved still further so that adrenalectomized animals may be maintained in good health for years.

Recently Wintersteiner and Pfiffner (1935) and Kendall et al. (1934) have isolated a crystalline compound which maintains life in adrenalectomized animals. The substance contains only carbon, hydrogen and oxygen. Whether there is more than one substance has not been determined, nor has any substantial opinion been advanced in regard to the structural formula of the crystals.

In addition to the cortical hormone, vitamin C was isolated in crystalline form, in large amounts, from the adrenal cortex by Szent-Gyorgyi (1929). This substance was originally called ascorbic acid but the name has recently been changed to cevitamic acid. Its chemical formula



Cevitamic acid

FIG. 94

is $C_6H_8O_6$, and its generally accepted structural formula is shown in figure 94.

Cevitamic acid is a white or slightly yellowish-white, odorless, crystalline powder which melts between 189 and 192°C., and is freely soluble in water, soluble in alcohol, but practically insoluble in chloroform and ether. It oxidizes on exposure to air and light. Whether vitamin C is stored or synthesized in the cortex, is still uncertain. Its significance in the adrenal cortex is unknown.

Method of Assay. The only test for cortical hormone is biological. In common with all complicated biological tests, it is subject to wide individual variations, depending on the animals. Such factors as diet, salt and water intake, must be taken into consideration. Pfiffner, Swingle and Vors (1934) suggest the "dog unit" or that amount of hormone which will prevent an increase of 100 per cent in the blood urea of

an adrenalectomized dog in the course of from seven to ten days. This is not the most satisfactory test, the best method (and the most difficult one) is the one by means of which the lives of adrenalectomized animals are prolonged beyond the maximum survival time of control animals.

Relation of the Adrenal Glands to Other Glands of Internal Secretion

1. To the Pituitary. The adrenotropic (interrenotropic) principle.

The anterior pituitary gland stimulates the adrenal cortex. Philip E. Smith (1930) was the first to observe that hypophysectomy in rats was followed by atrophy of the adrenal cortex, and that the normal condition was restored by intramuscular implantations of fresh rat hypophyses. Evans, Meyer and Simpson (1932) confirmed Smith's observations, and in addition produced cortical repair by means of pituitary extracts. Collip, Anderson and Thompson (1933) found the adrenotropic substances to be closely associated with the thyrotropic, and eventually succeeded in separating the extracts. Houssay et al. (1933) were able to stimulate the adrenal cortex of hypophysectomized dogs with pituitary extracts.

Clinically it has been well known that destruction of the hypophysis is followed by marked atrophy of the adrenal cortex (Fahr, 1918; Jaffee and Tannenberg, 1928). Pituitary tumors and acromegaly may cause hypertrophy of the adrenal cortex (Wieth-Pedersen, 1931; Salmon, 1933; Bauer, 1933; Cushing, 1933; Berblinger, 1932). Furthermore, in anencephalic monsters having maldevelopment of the hypophysis there is usually found an atrophy and hypoplasia of the adrenal cortex (R. Meyer, 1912; Kohn, 1924)

The adrenotropic principle is best tested by means of the hypophysectomized rat. Anselmino, Hoffmann and Herold (1934) suggest the use of the infantile mouse, in which adrenotropic hormone produces a hypertrophy of the fascicular and glomerular zones. They ascribe to the adrenotropic hormone the following properties:

It is soluble in water and 50 per cent alcohol, but insoluble in 70 per cent alcohol, ether, acetone and chloroform.

It passes through a collodion membrane, and from this they deduce that it is not attached to a protein, and that it is rather a small molecule.

It is not destroyed by dilute acid or alkali, but is destroyed by concentrated acids and alkalies, especially upon heating.

It can be heated in neutral solution up to 60°C. without loss of potency.

It is not identical with the growth, gonadotropic, thyrotropic, parathyrotropic, pancreatropic, or lactation hormones.

It is conceivable that Addison's disease may be due in some cases to a failure of the adrenotropic hormone, and in other cases to (local) disease in both adrenal glands.

There is no evidence of any atrophy of the medulla following hypophysectomy. Houssay et al. (1933) report a decrease in the epinephrine content in the medulla of dogs after the administration of pituitary extract. Anselmino, Herold and Hoffman (1934) report the finding of a separate hormone in the anterior hypophysis that acts upon the medulla. They describe histological changes in the fat staining properties of the medulla and chromaffine system which are already in evidence two hours after injection, reach a maximum in nine hours and rapidly diminish after twenty-four hours.[1]

To the Gonads. There is a definite interrelationship between the gonads and the adrenals, but the mechanism and significance are not entirely clear. These interrelationships have been studied clinically and experimentally.

To the Ovary. Precocious puberty has been noted in the presence of adrenal tumors or hyperplasia (Thomas, 1926). Other tumors may give rise to masculinization (virilism). Addison's disease is frequently accompanied by hypoplasia or atrophy of the ovaries (Karakoscheff, 1906; Matthies, 1926; Halban, 1925). This is quite understandable if we assume the Addison's disease to be due here to a failure of the adrenotropic hormone. A definite hypertrophy in adrenal cortex occurs during pregnancy (Aschoff, 1910; Aschner, 1912). This pertains particularly to the fascicular zone, wherein there is also a definite increase in the lipoids. Menstruation is also associated with a hypertrophy of the adrenal cortex, though to a lesser extent (Stoerk and Haberer, 1908; Wehefritz, 1923). Castration is followed by hypertrophy of the cortex, especially the fascicular zone (Kohn, 1924; Anderson and Kennedy, 1932).

Pregnancy prolongs the survival of an untreated adrenalectomized dog (Rogoff and Stewart, 1927). A non-pregnant dog will survive four to nine days, and a pregnant dog thirteen to thirty-nine days. Whether this is due to foetal adrenals, or the corpus luteum of pregnancy, or the placenta is at present unknown.

The adrenal medulla does not undergo noticeable change during pregnancy or following castration. Nor has there been any agreement as to the epinephrine content of the adrenals during these conditions.

Blotevogel (1927) showed an increase in the chromatofillic cells in the sympathetic ganglion of the plexus cervicalis of the mouse, during pregnancy and following the injection of follicular hormone. The significance of this is unknown.

To the Testes. Clinically, a hypernephroma may produce precocious sexual maturity. No satisfactory explanation has been offered for this phenomenon.

Castration leads to a hypertrophy of the adrenal cortex (Schenk, 1910; Masui and Tamura, 1926). An increase in lipoids occurs. The significance of these changes is not understood. The medulla does not seem to be involved.

Adrenalectomy frequently leads to an inhibition of spermatogenesis and development of the secondary sex characteristics (Novak, 1914; Jaffe and Marine, 1923). The simultaneous removal of the testes and adrenals produces less marked castration atrophy in the rat than the removal of the testes alone (Kishikawa, 1925). The conclusion is then drawn that the adrenal cortex sensitizes the secondary sex characteristics for the male sex hormone. Poll (1933) and Nürnberger (1932) made some interesting observations on the effect of injections of follicular and testicular hormones upon the adrenal cortex. The studies are based on certain histological pictures present in the adrenal cortex of mice. During the *infantile* state both males and females possess a reticular zone. In the young male the reticular zone disappears with the first appearance of sperm in the testes, so that in the adult male the reticular zone is absent, and in its place a connective tissue zone develops. The adult female mouse possesses a reticular zone only during the first half of its active sex life. The zone then gradually disappears.

If male sex hormone (about one half of a capon unit) is injected into infantile males and females, then the reticular zone rapidly disappears in a few days. If an infantile male is castrated, then the reticular zone persists and continues to develop. This process can be hastened and accentuated by the injection of estrin (16 M.U. of Progynon). The process can then be reversed by the injection of one Capon Unit of male sex hormone, i.e. the reticularis degenerates. If an adult male is castrated there is a slight tendency for the reticularis to grow back again, but nowhere as complete as in the previous case. Castration of an infantile female leads to persistence of the reticularis, but it can be made to disappear by the injection of male sex hormone. The injection of estrin into an old mouse (200 days) causes a considerable reforma-

tion of the reticular zone. The injection of male sex hormone into an old intact male, or into a castrated young (not infantile) male leads to a reformation of the reticular zone.

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CHAPTER X

THE THYROID GLAND AND THE THYROTROPIC HORMONE

An interrelationship exists between the thyroid gland and the adenohypophysis that is similar to that between the latter gland and the gonads. The parallelism is striking.

Rogowitsch (1888) noted an enlargement of the pituitary of dogs and rabbits following thyroidectomy. A similar enlargement was observed clinically in cases of myxoedema (MacCallum and Fabyan, 1907), cretinism (Boyce and Beadles, 1892), and cachexia thyreopriva (Wegelin, 1925). Adler (1914) destroyed the pituitary anlage in larval amphibia and prolonged the larval state, while on the opposite extreme Gudernatsch (1912–1914) produced precocious metamorphosis in tadpoles by thyroid feeding. In a classical experiment Philip E. Smith (1922) demonstrated that hypophysectomy in tadpoles produced atrophy of the thyroid, and that repair could be brought about by homologous transplants of the anterior pituitary or by intraperitoneal injections of bovine extracts of the hypophysis. This work was extended by him to the rat. The atrophy of the thyroid resulting from the hypophysectomy caused a lowering of the metabolic rate by as much as 35 per cent (Foster and Smith, 1926). Meanwhile Spaul (1923, 1930) produced hypertrophy of the thyroid and acceleration of metamorphosis of tadpoles by injection of anterior pituitary extracts. Clinically, patients suffering from hypopituitarism have a subnormal metabolism (Cushing, 1927), and in pituitary cachexia (Simmonds disease) atrophy of the thyroid occurs (Graubner, 1925).

Hyperplasia of the thyroid of the guinea pig was produced by Loeb (1929) and Aron (1929) by means of anterior pituitary extracts. This was also demonstrated for other species of animals, and the same phenomenon could be demonstrated *in vitro* (Eitel and Loeser, 1933). Homotransplants and autotransplants of thyroid tissue in animals responded by hyperplasia when the animals were injected with anterior lobe extracts (Houssay, Biasotti and Magdalena, 1932).

Aron and Klein (1930) report the finding of thyrotropic hormone in the urine. Smith and Moore (1933) were unable to confirm this. Gumbrecht (1935) reports the finding of thyrotropic hormone in the urine of patients with menopause symptoms, as well as during menstrua-

tion. He believes that the symptoms such as sweats, palpitation and hot flushes are due to an excess of this hormone. Hence, in order to inhibit this pituitary overactivity (in the production of thyrotropic hormone) he recommends the administration of diiodotyrosine. (One frequently sees these symptoms in patients with a low metabolic rate!)

Chemistry of Thyrotropic Hormone

The hormone has not been isolated in pure form. It is in all probability a protein molecule. It is not identical with the growth, gonadotropic, adrenotropic or lactogenic hormones. It is rapidly destroyed by heat. Peptic digestion or autolysis of the anterior lobe destroys the gonad stimulating before the thyrotropic factor (Qugenot, Pouse, Vallette and Dottrens, 1934).

The Physiology of the Thyrotropic Hormone

The *unit* of thyrotropic hormone is as follows: Collip (1935)—the minimum amount which "when administered daily in two injections, will cause a rise of 20 per cent in the metabolism of the hypophysectomized rat by the fourth day."

Action of Thyrotropic Hormone (figs. 95A and 95B). Injections of thyrotropic hormone into normal animals produce:

a. Enlargement and hyperplasia of the thyroid (Loeb, 1929; Aron, 1929).

b. An increase in the metabolic rate (Siebert and Smith, 1930).

c. An increase in the heart rate (Schittenhelm and Eisler, 1932).

d. A reduction of the iodine content of the thyroid gland (Schockaert and Foster, 1932).

e. An increase in the alcohol-insoluble iodine of the blood (Closs, Loeb and MacKay, 1932).

f. A marked decrease in liver glycogen (Eitel and Loeser, 1932).

g. An increase in the excretion of creatine in both the normal and the hypophysectomized rat (Pugsley, Anderson and Collip, 1934).

h. An increase in the excretion of calcium by the intestine (Pugsley and Anderson, 1934).

The symptoms of hyperthyroidism are absent when thyroidectomized animals are injected with thyreotropic hormone, except exophthalmus, which, according to Marine and Rosen (1934), is due to direct stimulation of the centers in the midbrain controlling the sympathetic innervation of the eye. Smelser (1936) showed that exophthalmos is due to an increase in orbital structures. It is readily obtained by the

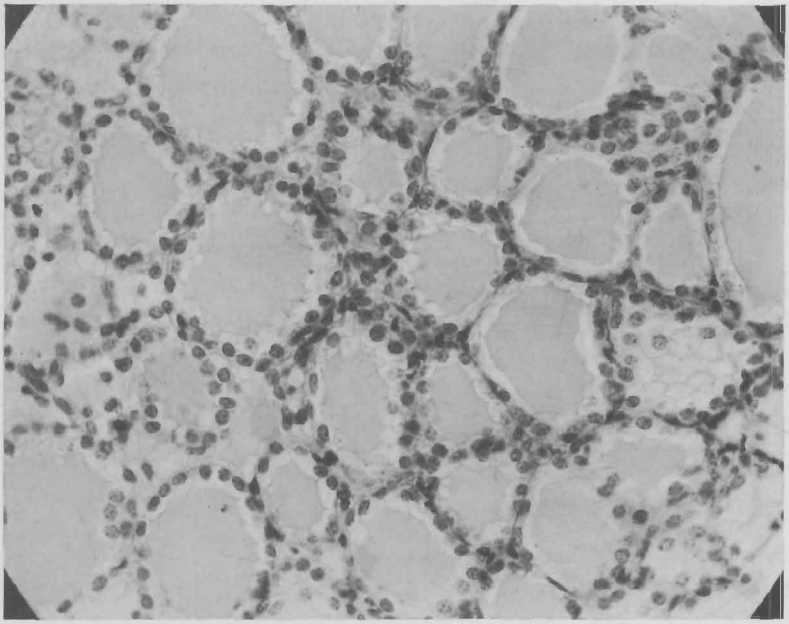


FIG. 95A. Guinea pig thyroid. Normal. Epithelium low, acini filled with colloid. ×520. (Courtesy of Dr. S. C. Werner.)

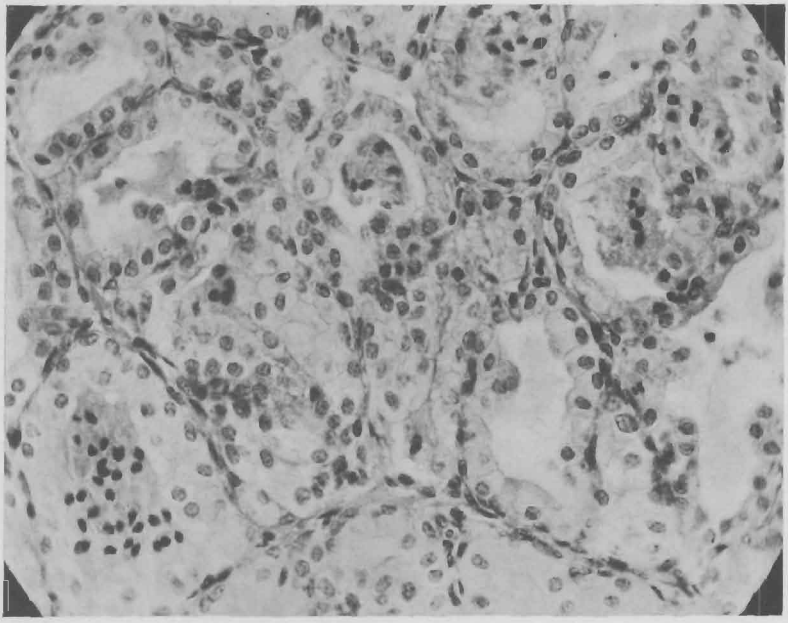


FIG. 95B. Guinea pig thyroid. Treated with thyrotropic hormone. Epithelium is high and very active. Almost complete absence of colloid. (Courtesy of Dr. S. C. Werner.)

injection of beef anterior lobe extracts in thyroidectomized, but not normal, guinea pigs of both sexes.

The increase in metabolic rate which results from the injection of thyreotropic hormone into *normal* rats is plus 26 per cent (Collip, 1935). Larger doses do not result in higher values. On the contrary, goiterous animals show a far greater increase, from plus 95 to plus 162 per cent. Hypophysectomized rats show a decline in the metabolic rate to minus 29 per cent. Such animals are more sensitive to thyrotropic hormone than normal ones. If the injections of thyrotropic hormone into normal rats are continued for several weeks the metabolism drops to minus 29 per cent or to the level of metabolism of the hypophysectomized rat. (The explanation will be considered below.)

The iodine metabolism of the thyroid is controlled by the thyreotropic hormone. According to Oswald (1901) the iodine content of the thyroid varies in general with the amount of visible colloid. Marine et al. (1908, 1909) showed that the iodine store in general varied inversely with the degree of hyperplasia, and in extreme degrees of thyroid hyperplasia the iodine store was exhausted. The thyrotropic hormone by inducing hyperplasia thus lowers the iodine content of the gland. The normal blood iodine ranges from 10 to 12 gamma per 100 cc. The iodine occurs in two fractions, the inorganic which is alcoholic soluble, and the organic which is alcohol insoluble. Their ratio is as 1:2. In Graves' disease the blood iodine is usually increased to from 30 to 60 gamma per 100 cc., the increase being due to a rise in the organic or alcohol insoluble fraction, which, as we have seen, results from an increase in thyrotropic hormone.

The Antithyrotropic Hormone

It might be supposed that by constantly injecting thyrotropic hormone it would be possible to stimulate the thyroid indefinitely. *But the contrary fact is true.* Korenchevsky (1930) injected pituitary extracts into rats for six to eleven weeks and noticed a decrease in weight of the thyroid, which reached 17 per cent below the control weight. Similarly, the metabolic rate at first showed a rise which reached a peak by the tenth day and then a return to the normal level (Siebert and Smith, 1930) (Verzar and Wahl, 1931). If the injections of thyrotropic hormone were carried out still longer, then the metabolic rate dropped below normal (Lee and Gagnon, 1930; Evans and Sarka, 1933). This phenomenon was extensively studied by Collip and his associates (1934, 1935) and by Werner (1936).

Collip showed that continued injections of purified thyrotropic hormone produced the following effects:

a. A rise in the metabolic level to plus 28 per cent during the first week.

b. A return to the preinjection level during the second or third week.

c. A continued fall in the metabolic rate to minus 29 per cent by the fifth week. This corresponds to the low metabolic level of the hypophysectomized rat. Histologically, the thyroid at this stage resembles the thyroid following hypophysectomy, i.e., atrophic.

d. The pituitaries of animals thus treated for a long time give a negative response for thyrotropic hormone and a positive response for growth hormone.

What is the nature of this inhibition? Collip found it to be a substance present in blood serum, and therefore named it the antithyrotropic hormone. When blood serum from an animal treated for many weeks with thyrotropic hormone is injected into a hypophysectomized animal (0.5 to 1.0 cc. per day for 3 days), it prevents a rise in the metabolic rate even when 200 times the required minimal effective dose of thyrotropic hormone is injected.

The antithyrotropic substance is unstable. Boiling at pH 5 for 3 minutes destroys it. Standing at room temperature destroys its potency, while standing in the refrigerator inhibits its potency. Collip (1935) reports finding this antihormone in certain patients with low metabolic rates. Its presence also explains the numerous clinical failures resulting from injections of thyrotropic hormone.

How does the antithyrotropic substance work? It does not inhibit the action of thyroxin; Collip (1935) states that when animals are treated for several weeks with thyrotropic hormone and then injected with thyroxin, a rise in metabolism occurs. The combined administration of antithyrotropic substance and thyroxin does not prevent a rise in metabolism. Abelin (1931, 1932) has shown that diiodotyrosine inhibits the action of thyroxin, hence this effect is not similar to the action of the antithyrotropic substance.

Collip's concept of an antithyrotropic hormone has recently been challenged by Werner (1936). The development of refractoriness has been found to depend on the type of extract in which the hormone is administered. By using a flavianate preparation of thyrotropic hormone (prepared by Dr. Karl Meyer) Werner demonstrated a continued thyroid stimulation, treatment being continued as late as the 80th day.

A control series subjected to thyrotropic hormone made by the Dyke-Wallen Lawrence method, showed the refractoriness as described by Collip.

When certain mixtures of thyrotropic and antithyrotropic hormone are injected into a hypophysectomized rat it is possible to produce hyperplasia of the thyroid gland *without* raising the metabolic rate, which is about 30 per cent below normal (Collip, 1935). The nature of the hyperplasia of the thyroid is not clearly understood. Marine (1927) believes that it is due to a functional insufficiency of the gland. He gains support for this contention from the following fact. When thyrotropic hormone is injected, the first effect noted is an outpouring of organic iodine from the thyroid gland, and is coincident with the appearance of vacuoles in the colloid of the gland and a rise in the metabolic rate. A later secondary effect is the hyperplasia. Collip stresses the fact that the metabolic changes are not directly related to the histological picture. Effects may be produced histologically with physiological change and vice versa.

The introduction of the concept of antihormone introduces new vistas into endocrine dysfunctions. An equilibrium may be assumed to exist between the hormone and its antihormone. (It has not been shown that all hormones have antihormones—estrin has no antihormone?) Thus, hyperfunction could be due to an excess of the hormone, or lack of the antihormone. Hypofunction could be due to a lack of hormone or excess of antihormone. Returning to the thyroid, hyperthyroidism as seen in acromegaly can be due to an excess of thyrotropic hormone. In Graves' disease no histological changes in the pituitary are to be found and the disease could be due to an insufficiency of the antithyrotropic substance.

The Physiology and Chemistry of the Thyroid Gland

The thyroid gland occupies a central position in the endocrine system in that its single hormone—*thyroxin*—is linked with the oxidative processes of all the body cells.

Nerve Supply. The thyroid gland derives its nerve supply from both sympathetic and parasympathetic fibers. The former arise from the middle cervical ganglion, while the latter pass in with the superior and inferior laryngeal nerves. Kuntz (1934), in reviewing the evidence for the nervous control of the thyroid glands, points to the mass of conflicting evidence regarding this point. Variations in the blood supply to the gland bring out variations in function.

Chemistry. The thyroid elaborates a specific hormone containing *iodine.* Bromine is also found in the gland, but its biological significance is unknown. The iodine in the thyroid is in organic combination with a protein, free iodine is present only in traces. This iodine compound is stored mainly in the colloid, and the iodine content of the gland corresponds to its richness in colloid.

The Iodine Compounds of the Thyroid Gland. Baumann (1896) was the first to discover the presence of iodine in the thyroid.

Three compounds containing iodine have been isolated from the thyroid gland, namely:

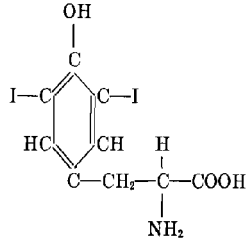
1. Iodothyroglobulin (Oswald, 1899).
2. Diiodotyrosine (Drechsel, 1896).
3. Thyroxine (Kendall, 1914).

Iodothyroglobulin. This substance is a globulin which contains iodine, and exists as such in the gland. Iodothyroglobulin is as active as the powder obtained by drying the gland. By digesting the thyroglobulin with trypsin and then pepsin Harington and Salter (1930) obtained crystalline thyroxin. Foster (1929) obtained from iodothyroglobulin 33 per cent of the iodine as diiodotyrosine and 16 per cent as thyroxin. All iodine in thyroglobulin is calorigenically active (Lerman and Salter, 1934). The iodine content of thyroglobulin from persons with hyperthyroidism decreases as the basal metabolism increases (Cavett, Rice and McClendon, 1934). The activity of the thyroglobulin is probably that of the thyroid itself.

Diiodotyrosine. Figure 96 shows the constitution of diiodotyrosine according to Wheeler (1930). It is a crystalline compound containing 58.7 per cent iodine. It is dextrorotary and is easily soluble in dilute ammonia, alkalies and acids. Both Harington (1929) and Foster (1929) obtained it from thyroglobulin by hydrolysis. Thompson et al. (1935) state that its calorigenic activity per millimol is only about one ten-thousandth that of thyroxine. As stated above, Abelin believes that diiodotyrosine antagonizes thyroxine, and uses their quantitative imbalance as an explanation for various thyroid dysfunctions. It is capable of lowering the high basal metabolic rate of Graves' disease, but so is inorganic iodine. In all probability diiodotyrosine represents a step in the synthesis of thyroxin by the body.

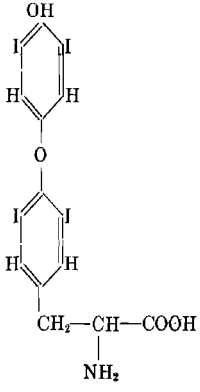
Thyroxine. This substance was first isolated by Kendall and Osterberg (1919). Harington (1926) established the structural formula for thyroxin, and in 1927 together with Barger synthesized this compound. Thyroxin has the structural formula given in figure 97.

Thyroxin contains 65 per cent of iodine. It is very sparingly soluble in water or in organic solvents, but readily soluble in alcohol after addition of alkali or an acid salt. When heated to 231–233°C. it begins to decompose, liberating free iodine. As with all biological compounds the stereochemistry is very interesting. Due to racemization during



Diiodotyrosine

FIG. 96



Thyroxine

FIG. 97

the extraction of the compound from the gland thyroxin is optically inactive. Harington (1928) resolved dl-thyroxine into its optically active isomers. The laevo compound is three times as active as the dextro, and the former has been isolated as such from the thyroid by the action of proteolytic enzymes (Harington, 1930). The high potency of l-thyroxin is to be expected, for the laevo compounds are the

ones usually found in the body. According to Plummer (1921) the human thyroid secretes about 1 mgm. of thyroxine daily.

Physiology of the Thyroid Gland. *1. Effect on Metabolism.* The most important physiological action of the thyroid gland is to increase the oxidative processes of the body. This is also true of thyroxin (Boothby, 1924), 2.5 mgm. of thyroxin being equivalent to 1 gram dried thyroid gland. This increased oxygen utilization manifests itself also in the various *isolated* organs (Asher and Rohrer, 1924). The duration of motility of sperm is increased by thyroxin (Carter, 1932).

Effect on Carbohydrate Metabolism. Both thyroid extract and thyroxin cause a loss of liver glycogen. This was first observed by Cramer and Krause (1912–1913). It is also true of heart muscle but not of skeletal muscle. The loss of glycogen is accompanied by a loss of ability to store glycogen in response to carbohydrate feeding. This is not accompanied by a lowering of the tolerance for sugar, nor is glycosuria observed. The cause for this loss of glycogen is probably the oxidation of the mobilized sugar at a high rate. The reason for this action is that thyroid hormone sensitizes the liver to stimuli which promote the discharge of glycogen. Thus thyroid feeding increases adrenalin hyperglycaemia and thyroidectomy diminishes it. When such a loss of liver glycogen occurs as a result of thyroid feeding a small amount of insulin will produce a fatal hypoglycaemia (Marks, 1925). This is due to the failure of the compensatory discharge of sugar.

Effect on Protein Metabolism. In myxoedema the excretion of urea is diminished, and the administration of thyroid substance increases it. This effect is less noticeable in normal animals. Creatinuria occurs in Graves' disease and is diminished when the condition is alleviated by treatment with iodine (Palmer, Carson and Sloane, 1928). In general, it may be stated that thyroid extract increases protein catabolism, affecting all the protein whether endogenous or exogenous.

Effect on Fat Metabolism. Myxoedema is accompanied by an increased fat deposit due to lessened activity. Adequate treatment with thyroid extract causes a loss of fat due to increased muscular movement. There may also be a direct effect on fat metabolism, for Abelin and Kürsteiner (1928) have observed that thyroid feeding caused a loss of body fats within 16 hours, i.e., before the general metabolic effect of the hormone was detectable.

Effect on Water Balance. Eppinger (1917) in his monograph on oedema cites many instances of increased water and salt excretion after the administration of thyroid extract. Mahnert (1925) has noted the

same effect in the oedema of pregnancy. This diuretic effect persists after section of the cervical cord, hence the action of the hormone must be peripheral (Fujimaki and Hildebrandt, 1924). Changes in the viscosity and surface tension of the blood, as well as changes in tissue permeability have been offered as explanations for this diuresis.

Effect on Calcium Metabolism. Hyperthyroidism shows an increase in calcium excretion (Aub, Bauer, Heath and Ropes, 1929). The extent of excretion was not in relation with the extent of increase in the metabolic rate. The increased excretion in the urine may reach more than 200 per cent above the normal. Cases of myxoedema showed an abnormally low calcium excretion, which was increased upon the administration of thyroid. The high calcium excretion of Graves' disease is decreased upon the treatment with iodine. Together with the loss of calcium there is a loss of phosphorus suggesting its derivation from the calcium phosphate of the bones, hence the osteoporosis of Graves' disease (Hunter, 1930). There is no increase in the blood calcium in Graves' disease, in contradistinction to the rise in blood calcium as the result of parathyroid hormone where there is also an increase in calcium excretion through the urine. The reason for this heightened excretion in Graves' disease is unexplained.

Effect on Growth and Differentiation. Gudernatsch (1913–1914) found that the rate of metamorphosis of various species of tadpoles was markedly accelerated by thyroid feeding. When fed on ordinary meat tadpoles showed distinct hind legs 53 days, and forelegs 73 days, after the beginning of the feeding, metamorphosis being complete after 104 days. When thyroid was substituted for ordinary meat, hind legs appeared in 9 days, forelegs in 11 days, and metamorphosis was completed in 18 to 20 days. The same effect has been accomplished with 1:1,000,000 synthetic thyroxin. General body growth was not produced in these animals, for they remained pygmies and died within a few days. In fact, growth of white rats is inhibited by thyroid or thyroxin feeding (Cameron and Carmichael, 1920, 1921). At the same time, the heart, liver, kidneys and suprarenals showed some evidence of growth, probably as the result of extensive catabolic activity on the part of these organs. A number of workers have utilized the acceleration of the metamorphosis of tadpoles for purposes of biological assay. If the tadpoles are fed thymus instead of thyroid they continue to grow but do not undergo metamorphosis (Gudernatsch, 1916; Romeis, 1923). Thus thyroid produces dwarf frogs and thymus, giant tadpoles. Metamorphosis of tadpoles may also be induced by diiodotyrosine (Romeis, 1923) and by parenteral feeding of inorganic iodine (Abelin, 1928).

It has been demonstrated that the ionic concentration of the milieu in which the tadpoles are kept has a marked influence on the metamorphosis induced by thyroid feeding (H. Zondek and Ucko, 1924, 1925, 1926). Calcium ions in definite, small concentration inhibit, and under given conditions, even reverse the effect of thyroid hormone, while potassium ions stimulate it. Similarly, it appears that the metamorphosis is a direct function of the hydrogen ion concentration. Thus, at a pH of 7.08 there is no effect while at 6.88 there is a marked acceleration in the metamorphosis.

For a further consideration of the other physiological properties attributed to the thyroid gland the reader is referred to the excellent monographs of Harington (1933) and Laqueur (1934).

Interrelationship between the Thyroid and the Sex Glands

It has been known clinically that the thyroid gland enlarges during puberty, menstruation, lactation and pregnancy. The nature of the interrelationship is still uncertain, but it is more than likely that it is through the mediation of the anterior pituitary gland. Thyroid feeding produces an inhibitory effect upon estrus (Abelin and Wiedner, 1932). DaCosta and Carlson (1933) showed that large doses of desiccated thyroid retarded sexual maturity of white rats of both sexes while small doses tended to accelerate it.

Tagliaferro (1933) found that thyroid enlargement occurred in rats and guinea pigs following injections of estrogenic substance, when the injections were limited to five or ten days. But, when the injections were continued for more than twenty days involution of the thyroid occurred. Both hypothyroidism and hyperthyroidism lead to sterility (Döderlein, 1929). As stated previously, the effects produced are best understood when the rôle of the anterior hypophysis in its relation to the gonads and thyroid, is considered. The thyrotropic and gonadotropic hormones are produced by the cells of the adenohypophysis. It might well be expected that a stimulus acting upon the thyrotropic hormone would extent to the gonadotropic hormone. Thus, small doses of follicular hormone stimulate the production of gonadotropic hormone while large doses inhibit it. The same holds true for thyroid extract. Tagliaferro's results are then readily understandable. Small doses of estrin stimulate the production of gonadotropic hormone. The same group of adenohypophyseal cells being affected, there occurs an increased production of thyrotropic hormone, hence, the enlargement of the thyroid. Large doses of oestrogenic substance have the opposite effect upon the pituitary, hence the opposite effect upon the thyroid gland.

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CHAPTER XI

THE PANCREAS

One of the most brilliant discoveries in medicine is the fact that removal of the pancreas produces a fatal diabetes. This honor belongs to Oskar Minkowski (1858–1931). In a joint publication with O. V. Mering (1889–1890) he states: "After complete removal of the organ, the dogs became diabetic. It has not to do simply with a transient glycosuria, but a genuine lasting diabetes mellitus, which in every respect corresponds to the most severe form of this disease in man" (Major, 1932). The honor of discovering insulin belongs to Frederick G. Banting who was then (1922) assistant in Physiology in Macleod's laboratory in Toronto. He was assisted by C. H. Best, then a medical student. They showed that extracts of the pancreas influenced carbohydrate metabolism in a characteristic manner. It is interesting to note that J. de Meyer in 1909–1910 employed the name "insulin" for the active substance in the islet system.

Both the islets and the acinae arise from an entodermal pouch in the upper intestine. The relationship between the islet cells and the acinar cells is not entirely clear. The direct transformation of the latter into the former has frequently been suggested but not proved.

The pancreas derives its nerve supply from the coeliac plexus by way of the hepatic, superior mesenteric and splenic plexus (Kuntz, 1934). Both sympathetic and parasympathetic nerves traverse the coeliac plexus, and thus pass into the pancreas. Their anatomical separation is impossible. There is some experimental evidence to show that the right vagus is the secretory nerve to the pancreatic islets (De Corral, 1918). MacLeod (1930) advanced the opinion that the vagus center sends secretion impulses to the islet cells in response to the stimulating effect of increased concentration of glucose in the blood. The rôle of the sympathetic is as yet unknown.

The Chemistry of Insulin

Banting and Best (1922) realized the deleterious effect of trypsin upon insulin. Their first method consisted of tying off the pancreatic ducts and thus allowing the acinal to atrophy. Later they used calf embryos in whom the islet system was fully developed, and in which

trypsin was not as yet produced. Together with Collip (1922) they utilized acid alcohol extraction of fresh glands as routinely obtained from the abattoir. Insulin may be obtained directly from the islets of teleostean fishes in which the islets consist of a separate organ.

Insulin has been obtained from other organs. In all probability it is not produced there, but merely stored.

Insulin was first crystallized by Abel (1926, 1927). It has the following empirical formula $C_{45}H_{69}O_{14}N_{11}S + 3H_2O$. Its biological activity is given as 1 mgm. = 40 International Units. It gives positive Biuret, Pauly, Millon and ninhydrin reactions. It is an albumose, and is composed of well known amino acids, namely: tyrosine, 12 per cent, cystine, 12 per cent, glutamic acid, 21 per cent, leucine, 30 per cent, arginine, 3 per cent, histidine, 8 per cent, lysine, 2 per cent. Tryptophane, aspartic acid, hydroxyglutamic acid and glycine appear to be absent. According to Wintersteiner (1928) the largest amount of the sulphur in insulin is in the disulphide form (—S—S—), about two-thirds of which is accounted for by the cystine.

Crystalline insulin is irreversibly inactivated by pepsin and trypsin, for these enzymes break up the protein molecule (Charles and Scott, 1930). High temperatures destroy insulin, especially in the presence of strong acids. Krogh and Hemmingsen determined that at 20° in a slightly acid medium insulin will lose only one-half of its activity in 10 years. Heating with alkalies rapidly destroys it. The carboxyl groups (—COOH) in insulin may be esterified with acid alcohol. This inactivates the hormone, but treatment with dilute alkali almost completely restores its activity (Carr et al., 1929). Acetylation of insulin reduces its activity by four-fifths. Careful esterification almost completely reactivates the acetyl compound (Jensen and Geiling, 1928). Reduction of crystalline insulin with cystein and glutathione results in inactivation, which is not reversed by either oxygen or alkali (Du Vigneaud et al., 1931). It is believed that the disulphide groups have been reduced to sulphhydryl groups (—SH). Iodine inactivates insulin immediately, probably for the same reason.

Crystalline insulin is optically active and laevorotatory, the degree of rotation depending upon the pH of the solvent. Its molecular weight determined by means of the ultra-centrifuge is about 35,000.

Physiology of Insulin

1. Effect on Carbohydrate Metabolism. It might be stated at the outset that we do not know the exact mechanism of insulin action. Many of the effects obtained by the earlier investigators are now known

to be due to pharmacologically active impurities. Effects obtained by crystalline insulin alone are of significance.

The most striking effect noted after the subcutaneous injection of insulin is the *hypoglycemia*. Glucose disappears from the blood. What happens to this sugar? It is either broken down or it is utilized for the building up of high molecular aggregates, such as glycogen. Both phenomena occur in muscle and in the liver, in the former the breaking-down processes predominate, while in the latter the synthetic predominate. Many investigations show that the glucose enters the musculature. There sugar catabolism proceeds in two distinct phases (Embden, 1925 and Meyerhof, 1930); first, a phase of glycolysis which is anaerobic in character, and which results in the formation of two molecules of lactic acid from one molecule of glucose; second, an oxidative phase involving the oxidation of the lactic acid. The energy yielded by this oxidation converts part of the lactic acid back into glycogen. The glycogen thus formed is in the liver and in the muscle. Bornstein, Guesbach and Holm (1924) noted, on the basis of studies of the respiratory exchange in animals, and the perfusion of isolated muscle, that insulin stimulates the burning up of sugar and the synthesis of glycogen. These observations were confirmed by Bissinger, Lesser and Zipf (1926) and Lesser and Ammon (1928). They formulated the concept that the Meyerhof process is stimulated, namely, the combination of the oxidation of glucose plus the glycogen synthesis. These phenomena take place in the muscle (Best, Holt and Marks, 1931).

2. Effect on Fat and Protein Metabolism. Our exact knowledge here is even less than that which dealt with carbohydrate metabolism. Insulin increases the alkali reserve (Klein and Holzer, 1928) and diminishes the amount of acetone bodies in the blood (Pucsko, 1928). Diabetic lipemia disappears after insulin administration (Donomae, 1928). Liver lipoids are decreased by insulin (Theis, 1928). Wiechmann (1924) and others describe a diminution of the amino acids of the blood. Milhorat and Chambers (1928) report an increased nitrogen excretion. Goldblatt and Ellis (1931) are of the opinion that insulin does not influence protein or nitrogenous metabolism, and considering the large number of contradictory reports, their opinion is probably correct.

3. Effect on Water Metabolism. It was observed very early in the use of insulin, that there was a tendency for the occurrence of water retention and edema. Meyer-Bisch (1927), who reviewed this question, believes it to be due to three factors, namely:

a. Through the carbohydrate metabolism.

b. Through the water interchange between the blood and the tissues.

c. Through the nervous centers in the midbrain.

Klein (1926) believes that there is an accumulation of salt in the tissues. When insulin is taken for the relief of undernutrition and then discontinued, there is no increased excretion of water (Fonseca, 1926).

Insulin raises the renal threshold for sugar (Van Creveld and Van Dam, 1925; Sarge, 1931). This holds true only in some species of animals, and not always in man.

With regard to the ionic concentration in the blood, the following has been reported: an increase in potassium (Harrop and Benedict, 1923), a decrease in calcium (Rothschild and Jacobsohn, 1926), a decrease in phosphorus (Kerr and Blish, 1932).

Gibson (1926) lowered the hydrogen ion concentration of diabetic patients by the administration of glucose plus insulin. Kylin (1925) found that potassium ions increase, and calcium ions decrease the intensity of insulin action.

The Insulin Unit. The insulin unit is measured by means of the drop in the blood sugar of rabbits. A rabbit unit is that amount of insulin which will lower the blood sugar of a 2 kgm. fasting rabbit to 45 mgm. per 100 cc. of blood. Frequently hypoglycemic twitchings ensue at this point. A *clinical unit* is one-third of a rabbit unit. Well fed animals may not show this drop in blood sugar (Hoskins and Snyder, 1927). In view of the fact that reactivity of different animals varies, it is advisable to use a large number of animals when standardizing an unknown insulin solution.

Factors Controlling the Production, Excretion and Destruction of Insulin

La Barre and his co-workers (1931–1932) believe that the nervous system controls the production of insulin. The control reaches from a center in the thalamus through the vagus. This appears to be supported by an observation of Dworkin (1931), who noted an intensification of the insulin action after sympathectomy in the cat. On the other hand, many workers believe in the humoral regulation of insulin production and action, that is, control by the level of the blood sugar (Mason and Matthew, 1925; Gayet and Guillaumie, 1933). Staub (1925) noted that a second intravenous injection of sugar does not produce a further rise in the blood sugar, and offers as an explanation for this that the further addition of sugar caused the production of a new supply of insulin and this prevented the rise in the blood sugar. The

"Staub effect" cannot be produced with lactose, galactose and maltose (Sawada, 1933). The effect of levulose varies according to different observers.

Insulin disappears rapidly from the blood stream (Heymans, 1927). Red blood corpuscles inactivate it (Walter, 1932). The addition of glucose to insulin in vitro does not inactivate it (Du Vigneaud, 1927). Some insulin is probably excreted through the kidneys, because following double nephrectomy a prolonged and intensified insulin effect can be obtained (Loeper, 1928). Whether insulin is present in urine has been a disputed question.

The question whether insulin can pass through the placenta from mother to foetus has not been definitely settled. Pack and Barber (1929) and Corey (1932) believe it can pass the placental barrier, while Olow (1930) believes that it cannot. Aron (1929) reports that insulin may pass over only in the latter part of pregnancy.

Relation to Other Glands of Internal Secretion

A. To the Pituitary. The work of Anselmino and Hoffman (1933) shows that the anterior hypophysis stimulates the production of insulin. They administered anterior pituitary extracts and obtained an increase in the size and number of islands of Langerhans in the pancreas of the rat. These led to a very slight decrease in the blood sugar of dogs and rabbits and to a disappearance of the liver glycogen in the rat. Koster (1930) observed some atrophy of the pancreas after hypophysectomy, but was unable to observe any specific changes in the islands of Langerhans. Aron (1933) reported that the intrauterine injection of thyrotropic hormone in guinea pigs produced an increase in the number of the islands of Langerhans in the foetal pancreas. It is quite possible that the effect may be due to the pancreatropic substance being carried along as an impurity.

It is advisable to discuss at this point two substances liberated by the anterior hypophysis, that are closely associated with carbohydrate metabolism. They are (1) the blood sugar raising principle and (2) the ketogenic principle.

I. The Blood Sugar Raising Principle. The effect of the anterior pituitary gland on the blood sugar and other carbohydrate metabolism effects is best studied on the hypophysectomized and the hypophysectomized plus pancreatectomized animal. Houssay and his coworkers have done masterful work in this field. Their work has been confirmed by Lucke, Barnes and Regan, and by Collip and his coworkers.

The cardinal fact about which this work revolves is that the symptoms following complete pancreatectomy—glycosuria, hyperglycemia and acidosis—are markedly relieved when hypophysectomy follows the first operation.

The influence of the anterior pituitary gland on carbohydrate metabolism can best be studied if we note the effects that follow (1) hypophysectomy and (2) pancreatectomy plus hypophysectomy.

a. Effect of Complete Hypophysectomy. 1. Blood sugar—a well fed animal shows no change, while fasting often leads to a fatal hypoglycemia unless relieved by injections of glucose (Houssay et al., 1931, 1932). This has been confirmed by Collip (1935) on the monkey.

2. Sugar tolerance curve—Canus and Roussay (1914) and Houssay et al. (1922) believe there is no change in the dog, while Lucke and his co-workers (1933) note a decreased tolerance. These variations may be due to the nutritional condition of the animal.

3. Respiratory quotient—no change has been noted in the dog (Gaebler, 1929).

4. Insulin—the dogs are extremely sensitive to this hormone. Doses that are well tolerated by a normal animal will here lead to a fatal hypoglycemia (Houssay and Magenta, 1929).

5. Epinephrine—produces a hyperglycemia in the fed and a lesser effect in the fasting dog (Braier, 1931).

6. Glycogen content of liver—in the dog there is little change (Houssay et al., 1922).

b. Effect of Pancreatectomy Followed by Hypophysectomy (Houssay Dog). Animals having undergone these operations survive from six to nine months, but their undernourishment increases and they finally die of cachexia (Houssay, 1931).

1. Blood sugar—shows marked variations ranging from 100 mgm. in the fasting to 300 mgm. per hundred cubic centimeters in the fed dog. Glycosuria may follow feeding.

2. Insulin—produces a marked hypoglycemia (Houssay, 1933). This is why we speak of the contra-insulin hormone of the anterior hypophysis. In the intact or pancreatectomized animal the hypoglycemic effect of insulin is counterbalanced by the blood sugar raising principle of the anterior pituitary gland. This principle is lost following hypophysectomy, hence the increased sensitivity of the Houssay dog to insulin.

3. Respiratory quotient—rises after the administration of glucose (Houssay and Biasotti, 1931). In pancreatic diabetes, there is an inhibition of oxidation when the blood sugar principle of the anterior

pituitary gland is present. In the absence of this principle oxidation of glucose does take place.

4. Liver glycogen—can be stored (Houssay, 1933).

c. Effect of Injections of the Blood Sugar Raising Principle. 1. Into normal animals—there is a rise in the blood sugar (Lucke, 1933). If insulin is injected the resultant hypoglycemia is decreased by simultaneous injection of the blood sugar raising principle.

2. Into pancreatectomized animals—the hypoglycemic effect of insulin is here less markedly exhibited than in the normal animal.

3. Into pancreatectomized and hypophysectomized animals,—the resistance to insulin is increased (de Benedetto, 1932) and continued injections may lead to diabetic coma (Lucke, 1933).

The Site of Action of the Blood Sugar Raising Hormone. Lucke et al. (1933) believe that the blood sugar raising principle acts upon a brain center, which in turn, acting by way of the sympathetic, stimulates the adrenals. Adrenalectomy or adrenal degeneration prevents the action of this diabetogenic hormone. On the contrary, Houssay and Biasotti (1933) find that the only essential organ is the liver for, according to these writers, complete evisceration, or adrenalectomy, or thyroidectomy, or castration fails to inhibit the action of the hormone. When the blood sugar raising hormone is injected, a considerable latent period elapses before the blood sugar rises (Houssay, 1933), but if the injection is given directly into the spinal fluid, the effect is instantaneous (Lucke, 1933). This seems to support Lucke's contention of action by way of a brain center.

The hormone is excreted into the urine (Houssay and Biasotti, 1931) and small amounts are found in the placenta.

Chemistry. The blood sugar raising hormone is not identical with any of the previously described anterior pituitary hormones. It is soluble in water and in 60 per cent alcohol, but insoluble in absolute alcohol, benzene, ether, acetone and chloroform. It is destroyed by boiling, does not pass through ultrafilters and is easily absorbable (Houssay and Biasotti, 1931). It is found in the pituitary of man.

II. The Ketogenic Principle. This substance is also known as the fat metabolism hormone. The first observation was made by Burn and Ling (1928), who observed that rats kept on a high fat diet showed an increased excretion of acetone bodies after the injection of a dilute alkaline pituitary preparation. This observation was confirmed and extended by Hoffmann and Anselmino (1931) and Magistris (1933).

Physiological Properties. 1. The ketone content of the blood, par-

ticularly beta-oxybutyric acid, is greatly increased (Hoffmann and Anselmino, 1931).

2. There is increased excretion of acetone bodies (Burn and Ling, 1928).

3. It decreases the lipoid content of the blood (Magistris, 1932).

4. Increases the glycogen content of the liver, especially so, when given with thyroid hormone (Magistris, 1932).

5. Increases the specific dynamic action of proteins in the rat (Hoffmann and Anselminó, 1931).

6. Depresses basal metabolism of the rat (Hoffmann and Anselmino, 1931).

7. Chronic administration decreases the blood sugar in the rat (Magistris, 1932).

Chemistry. Magistris (1932) uses aqueous extraction of acetone-stored pituitaries. His final product is ultrafiltrable and dialyzable. It is soluble in water and dilute alcohol and insoluble in absolute alcohol, ether and chloroform. The ketogenic substance is readily destroyed by heat.

Collip (1935) has definitely shown that the blood sugar raising hormone and the ketogenic hormone are two distinct substances, not identical with any known hypophyseal hormones.

Standardization. Magistris (1932) uses male rabbits weighing 2 kgm. that have been kept on an oat diet. They are fasted two to five hours before the test. One unit of ketogenic hormone is that amount of this principle which doubles the total acetone bodies in the blood two hours after injection.

Just what relationships the diabetogenic hormones have to the so-called pituitary adiposities is at present unknown. There is an interesting observation by Evans and his co-workers (1933) that in one of their dogs, chronic administration of an anterior lobe extract led to marked adiposity.

C. To the Gonads. There are several observations noting the effect of the sex hormones on blood sugar. The results of injections of pituitary-like substances from pregnancy urine are not in agreement. Eidelsberg (1932) reports an increase in the blood sugar. Snoeck (1932) finds a decrease in sugar tolerance only, without any effect on the blood sugar. Dingemanse and Kober (1933) believe that the effects are due to impurities. Barnes, Regan and Nelson (1933) reported that estrogenic hormone decreased blood sugar in pancreatectomized animals. They ascribed this effect to an inhibition of pituitary func-

tion by the follicular hormone. We have made the same observation in a case of diabetes mellitus that was resistant to insulin. Fifty thousand rat units of Progynon B definitely lowered the blood sugar. The effect lasted over several weeks.

Investigators are not unanimous as to the effect of insulin on the estrous cycle. Thus Vogt (1927) reports an inhibition of estrus in rodents by means of insulin. Del Castillo and Calatroni (1929) did not confirm this. During pregnancy or lactation there are no his tological changes in islets of the dog (Allen, 1930). An existing pregnancy does not influence experimental diabetes (Markowitz and Soskin, 1927).

Houssay and Magenta (1929) report that in their effect upon blood sugar oxytocin is a greater antagonist of insulin than vasopressin.

Clinically it has been observed that amenorrhea is frequently associated with diabetes mellitus. This is especially true of the juvenile diabetics.

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CHAPTER XII

THE PARATHYROID GLANDS

There are usually four parathyroids in man. They are in intimate contact with the lateral lobes of the thyroid. They were first recognized as independent organs by Sandstroem (1880). Gley (1891) was the first to associate their experimental removal with tetany. MacCallum and Voegtlin (1909) showed that tetany resulting from the experimental removal of the parathyroids was associated with a fall in the calcium content of the blood to about half its normal value (from 10–11 mgm. per 100 cc. to 4–7 mgm.). Collip (1925) prepared the first concentrated active extract in 1924.

The nerve supply is derived from the sympathetic. Section of the nerve supply does not interrupt the function of the gland. The metabolic disturbances following parathyroidectomy suggest that the parathyroid hormone exerts an inhibitory influence on the sympathetic nervous system.

Blood Calcium

In view of the fact that the chief function of the parathyroids is to regulate calcium metabolism it is advisable to study the state of the blood calcium. Probably all of the blood calcium occurs in the plasma. Calcium determination is done on the blood serum but whether there is any change in the calcium distribution during clotting is unknown. All the calcium in the serum is precipitated by ammonium oxalate if sufficient time elapses and an excess of oxalate is present. The distribution in the blood is as follows:

$$\text{Blood Calcium} \begin{cases} \text{Organic (non-diffusible) Ca (50\%)} \\ \text{Inorganic (diffusible) Ca (50\%)} \begin{cases} \text{ionized Ca} \\ \text{non-ionized Ca} \end{cases} \end{cases}$$

The organic calcium is probably in loose combination with protein. It is at present impossible to state in what form the non-ionized calcium is held. Which fraction (or possibly both) falls after parathyroidectomy is unknown.

Physiology

Upon the removal of the parathyroids tetany sets in. The following changes occur.

1. A drop in blood calcium.
2. A reduction of phosphorus excretion.
3. Slight increase in blood phosphate.

The underlying disturbance in tetany is a shift in the ionic ratio of the blood and tissues. Nervous irritability depends on the concentration of sodium, potassium and hydroxyl ions on one hand, and calcium and hydrogen ions on the other. Any increase in any one of the first three, or any decrease in either of the last two, leads to tetany. A reversal of this shift removes an established tetany. The hydrogen ion concentration of the blood must be of great importance, for changes in its concentration could change the concentration of the different ions.

The injection of the active extract (parathormone) results in the following effects:

1. A rise in the blood calcium. This may reach 18 mgm. per 100 cc. serum, the peak being reached in from twelve to eighteen hours. This is followed by a slow fall. The process is hastened by intravenous injection. Which calcium fraction is raised has not been decided.

2. Repeated injections of hormone produce a marked rise in blood calcium (20 mgm. per 100 cc.). The animals have attacks of vomiting and diarrhea. Marked depression sets in. The blood calcium drops. The animals pass into a state of collapse and die. There is marked kidney damage as seen from the severe rise in blood urea and non-protein nitrogen. The volume of urine is decreased (Collip, 1926). There is a decrease in the blood volume and a thickening of the blood. There is first a compensated alkalosis, then a condition of compensated acidosis, and just prior to death an uncompensated acidosis.

Chemistry

Parathormone has not been obtained in crystalline form. It is a protein, giving the protein color reactions. It is precipitated by picric and picrolonic acids. Parathyroid hormone is soluble in water and in 80 per cent alcohol, but insoluble in ether, acetone and pyridine. It is completely destroyed by boiling with dilute acids or alkalies, and is digested by pepsin or trypsin, hence it is ineffective when given orally. It does not dialyze through a collodion membrane.

Method of Assay

A biological unit is equal to $\frac{1}{100}$ of the dose necessary to produce a rise of 5 mgm. in the blood calcium of a 20 kgm. dog. The rise must be effective in five hours (Collip). Animals show a considerable variation in the response, hence the average of a fairly large number is necessary.

Relation to Other Glands of Internal Secretion

1. To the Anterior Pituitary Gland. Hertz and Kranes (1934) and Hoffmann and Anselmino (1934) have reported that extracts of the pituitary lead to proliferation of parathyroid cells. Hertz and Albright (1934) reported the finding of a parathyrotropic substance in the urine of a patient suffering from hyperparathyroidism due to hyperplasia of the parathyroids. P. E. Smith (1927) and Koster (1930) reported a parathyroid atrophy after hypophysectomy in the rat. Simultaneous hypophysectomy and pancreatectomy lead to marked degenerative changes in the parathyroids of the dog (Collip, 1935; Houssay and Biasotti, 1930). Hypophysectomy in young animals, in which the calcium metabolism is more easily disturbed, causes a lowering of the blood calcium (Geesink and Koster, 1928). Gerschman (1931) did not confirm this in the adult animal. Hoffmann and Anselmino (1934) reported the preparation of a hypophyseal extract that increased the blood calcium. The extract was ineffective after parathyroidectomy. These observations support the idea of a parathyrotropic principle in the anterior hypophysis.

2. To the Gonads. Symptoms of tetany are occasionally seen during pregnancy (Seitz, 1909). Histological changes were not found in the parathyroids.

Experimentally it has been demonstrated that a latent tetany due to parathyroid insufficiency will become activated during pregnancy, estrus, or lactation (Chandler, 1932). Pregnant dogs die more rapidly after parathyroidectomy than normal ones (Carlson, 1913).

The injection of ovarian and gonadotropic hormones has no influence on the blood calcium (Dixon, 1933). The appearance of tetany in animals with parathyroid hypofunction, during estrus, pregnancy and lactation can be prevented by the administration of irradiated ergosterol. More ergosterol is needed if it is administered during pregnancy than before or after (Kozelka, 1933).

These observations seem to show that the foetal parathyroids are not able to compensate for the maternal hypoparathyroidism. The

injection of 100 biological units of parathyroid hormone into the foetus of a dog does not produce a rise in the blood calcium of the mother, because the hormone cannot pass through the placenta (Hoskins and Snyder, 1933).

The removal of the ovaries in dogs does not lead to any change in the blood calcium (Cheymol and Guinquand, 1932).

Relation of Parathyroid Hormone to Vitamin D

The relation to calciferol or vitamin D is still an open question. A deficiency of the vitamin may be associated with a low blood calcium, and the administration of the vitamin brings the blood calcium back to normal. On the other hand, overdosage of the vitamin leads to hypercalcaemia and to metastatic deposition of calcium salts.

Hess and his co-workers (1929) believed that the effect of vitamin D is to stimulate the parathyroids, and that in their absence the rise in blood calcium failed to occur. Other workers (Holtz et al., 1934) could inhibit the appearance of tetany in parathyroidectomized dogs by large doses of irradiated ergosterol. The same has been accomplished clinically in post operative tetany. The present evidence seems to indicate that vitamin D controls the absorption of calcium from the intestine or depresses its excretion into the intestine. The parathyroid hormone probably controls the osteoblasts and osteoclasts and thereby calcium deposition and denudation. The hormone may be controlled by the level of the blood calcium.

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CHAPTER XIII

THE THYMUS

Investigators have not agreed upon the fact whether the thymus is a gland of internal secretion or a vestigial structure. It arises together with the parathyroids from the fourth branchial clefts. Microscopically it consists of a cortex and a medulla, and within the latter are the Hassall corpuscles. The nature of these bodies is in doubt.

The results of thymectomy have been variously reported by different investigators. Thus, Riddle (1931) reported negative results in pigeons; Morgan and Grierson (1930) in hens; Allen (1926) in rabbits; De Jongh (1931) in guinea pigs; and Anderson (1932) in rats. On the contrary, Paton (1926) noted definite developmental defects in the guinea pig, and Pighini (1922) observed the same in hens and rats. Soli (1924) observed a diminution in the weight of eggs, and a softening of the shells. Messini and Cappo (1932) found retarded healing of fractures. Implantation of thymus fragments stimulates growth and activity (Demel, 1922).

The consensus of opinion is that the thymus attains its greatest growth at puberty, and thereafter diminishes in size. Thus some interrelationship between the thymus and the gonads seems to be established. The mechanism is not as yet clear. Castration inhibits the regression of the thymus, while pregnancy accelerates it (Jolly and Lieure, 1929). Castration leads to a hyperplasia of the thymus (Kiyouari, 1929; Madruzza, 1929). Thymus extracts diminish the size of the testes (Balawenetz, 1930); Da Re (1929) reports a decrease in the thymus after ovarian transplants. Loewe and Voss (1931) observed some inhibition of the estrus cycle in rats after the administration of thymus extract.

The experiments with thymus extract have been very inconclusive, primarily because of the absence of a definite test object, as well as the absence of a potent extract. The effect observed could be due to impurities. Katsura (1922) observed an increased rate of growth of tissue cultures. Mino and Cerutti (1929) noted increased contractibility of the non-pregnant rabbit uterus. Daneff (1931) suggested that the foetal thymus is a factor in the initiation of labor. (The use of thymophysin during the first stage of labor should be remembered at

this point!) Asher, in a series of papers, reported the results obtained with a watery extract of the thymus, called "thymocrescin." Asher and Novinsky (1930) noted a growth regulatory effect with this substance. An alcoholic extract relieved muscle fatigue (Asher, Held and Schenfinkel, 1928). The latter effect had previously been observed by Müller (1917).

Rowntree, Clark and Hanson (1934) injected rats intraperitoneally with a thymus extract prepared by Hanson. Several succeeding generations were thus injected. Each succeeding generation from the third on showed accruing precocity in growth and development. The animals matured much earlier than the controls, although the adult animals were no larger than the controls. The injected animals show small adrenal glands and lymphatic hyperplasia, but show no susceptibility to sudden death from shock. The continued injection of the extract depresses blood pressure and causes death through auriculoventricular heart block. Whether this action is due to the vagus hormone or to some impurity cannot be determined at the present state of knowledge. It does offer, though, an explanation for the sudden death in status thymolymphaticus. This condition is associated with an enlarged thymus, hyperplasia of the bone marrow, a small heart and aorta.

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CHAPTER XIV

MENSTRUATION

Definition

By *menstruation* we imply regularly recurring bleeding from a *premenstrual* endometrium. This is to differentiate it from *cyclical bleeding* which implies regularly recurring bleeding from a *postmenstrual* endometrium.

It is our belief that the same patient may on occasions vary the type of bleeding. When the bleeding occurs from a premenstrual endometrium it implies that a corpus luteum has been formed, hence, nidation of a fertilized ovum could have occurred. Therefore, menstruation terminates a potentially *fertile cycle.* On the contrary, cyclical bleeding occurs from a postmenstrual endometrium, hence in the absence of ovulation, and of a corpus luteum. Nidation is not possible, therefore, cyclical bleeding terminates a *sterile cycle.*

The hormones directly involved in menstruation originate in the anterior pituitary gland and the ovary. The thyroid, the adrenals and the pancreas are also closely related to the menstrual cycle, for dysfunction of any of these glands may lead to abnormalities of the cycle. Indeed, it may be stated that all glands of internal secretion may give rise, sooner or later, to abnormalities of the menstrual cycle, providing their dysfunction is extensive enough. The problem of the menstrual cycle has recently been still further complicated by the assumption of a sexual center in the mid-brain. Again, not only are we aware of the *number* of the different hormones involved in the cycle, but in two cases (follicular and corpus luteum hormones) we know the *amount* of hormone essential for menstruation (fig. 98).

It is customary to date the *new* menstrual cycle from the first day of bleeding. This is, in a way, a misnomer, for the onset of bleeding marks the terminal phase of the *previous* menstrual cycle. It would be more appropriate to date the new menstrual cycle from the cessation of bleeding, but that is not a clear end point, for a slight show may be present for several days. Even the onset of menstrual bleeding as noted by the patient follows the actual onset by twelve to twenty-four hours.

Duration of the Menstrual Cycle

Many patients will maintain that their periods are absolutely regular, and are greatly surprised to find that when their periods are dated on

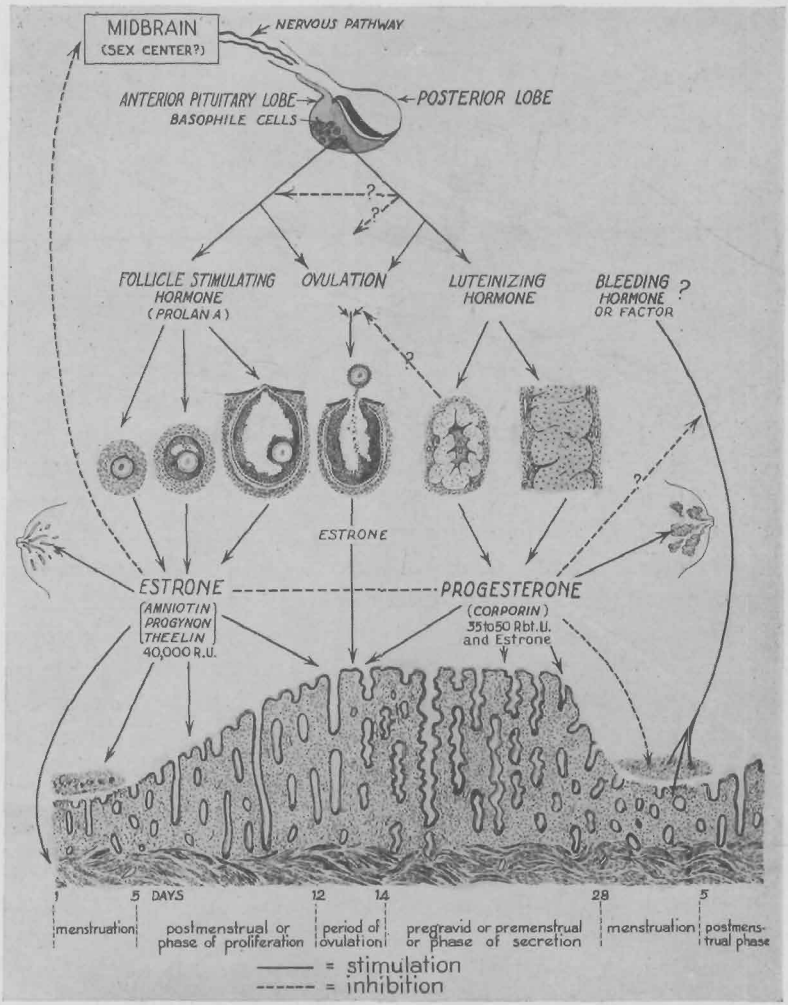

FIG. 98. Human menstrual cycle. Hormonal interrelationship

the calendar, they are not regular at all, but show irregular variations. In fact, the only regularity about the menstrual cycle is its irregularity. Fluhmann (1934) has reviewed 747 accurately recorded menstrual cycles of 76 healthy young women, and observed a marked variability in their

length. The cycles varied from 18 to 100 days, although the vast majority were between 18 and 42 days. The mean was 30.4 days, and the standard deviation 11.53. The average duration of the hemorrhage in 823 menstrual periods was 4.6 days, and no relation between the length of the menstrual cycle and the duration of the flow could be demonstrated. E. Allen (1933) noted a similar irregularity in a group of 131 women. King (1933) studied two series of cases, and concluded that 56.7 per cent of 354 periods are included in the twenty-sixth to the thirtieth days, while in another series, 523 periods, the twenty-fifth to the twenty-ninth days included the greatest number, 66.7 per cent. Hajek (1933) reported a series of 1,480 women and found that 82 per cent menstruated regularly, 12.9 per cent always menstruated irregularly, and 4.4 per cent bled irregularly or only occasionally. Only 56.6 per cent of the entire series had a regular 28-day cycle. These recent reports agree in the opinion that the menstrual cycle in normal women is subject to considerable variation.

The Question of Nervous Influence on the Ovary and Menstruation

The menstrual cycle is under hormonal control. Section of the spinal cord (van Wegenen, 1932) in an adult primate precipitates menstruation, but does not terminate the process, for regular cycles are resumed again. Clinically, a complete transverse myelitis of the cord does not affect menstruation. The ovary is abundantly supplied by nerve fibers arising from the ovarian plexus which in turn arises from the aortic and renal plexuses. The fibers accompany the ovarian artery. Afferent fibers are supplied by the tenth thoracic nerve. According to Kuntz (1919) the nerve supply to the ovary terminates in the blood vessels and fibromuscular tissue of the stroma, but no nerve fibers penetrate the ovarian follicles. Nerve fibers were observed in the theca folliculi and within well developed corpora lutea, but the fibers seemed merely to accompany the blood vessels. Ganglion cells were not observed within the ovary. Hence the finding of Hinsey and Markee (1934) that ovulation was induced by the injection of pregnancy urine into rabbits in the complete absence of functional nerve fibers to the ovary, was not at all unexpected. But at the same time it must not be supposed that the autonomic nervous system is without influence upon ovarian activity. Adrenalin and insulin are subject to control by the autonomic nervous system. Kraul (1927) treated both mice and rats with adrenalin and pilocarpin and obtained a loss of

estrus. Pilocarpin, which stimulates the vagus, caused the production of many small follicles, leading to small cystic degeneration of the ovary and an absence of luteinization. Adrenalin, which stimulates the sympathetic nervous system, inhibited follicle formation and retarded corpus luteum degeneration. Hirsch-Hoffman (1934) reached similar conclusions.

The Hormonal Control of Menstruation

In considering the hormonal control of menstruation it is advisable to consider it from the viewpoint of the various tissues and hormones involved, namely:

1. The sexual center in the hypothalamus.
2. The anterior pituitary gland. a. The follicle stimulating hormone. b. The luteinizing hormone.
3. The ovary. a. Follicular hormone or estrone. b. Corpus luteum hormone or progesterone.
4. The uterus. a. The endometrium. b. The myometrium.

The Sexual Center in the Hypothalamus. Evidence for the presence of such a center is as yet completely lacking. The work of Hohlweg and Junkmann suggests such a possibility. The location of this hypothetical center could be in the floor of the third ventricle.

The Anterior Pituitary Gland. If we consider for the moment that the presence of a sexual center has not been fully proved, then the source of the rhythmical stimuli necessary for the menstrual cycle must originate from the anterior pituitary gland. Histological variations in the anterior pituitary gland during the cycle have not been demonstrated, although Smith (1929) presented evidence of a correlation between the amount of gonad-stimulating hormone present in the pituitary of the guinea pig and the stage of the reproductive cycle. Both the follicle stimulating and the luteinizing hormones are essential for the stimulation of the entire cycle in the ovary. The hormones act in rotation, first the follicle stimulator and then the luteinizer, although it is also true that the action of one overlaps the other at about the time of ovulation (Hisaw, Greep and Fevold, 1935). Not only are both hormones essential for the production of the menstrual cycle, but there must be a quantitative relationship between them. For if the follicle stimulating hormone is present in an inadequate amount, or if its period of activity is shortened, the follicle will not develop completely, and an immature follicle is incapable of normal luteinization.

But luteinization of the follicle is a function of the luteinizing hormone, hence, this hormone does not exhibit activity until the follicle stimulating hormone has displayed its complete effect (the mature follicle). The further consequences of this lack of luteinization will be considered in the discussion of cystic and glandular hyperplasia of the endometrium. Furthermore, a failure of the luteinizing hormone to act would *only* give rise to a proliferative endometrial phase, and the bleeding that would result from such an endometrium would be cyclical bleeding and not menstruation. A sterile cycle would be the result. An excess of the luteinizing hormone would probably give rise to a persistent corpus luteum. In the presence of such a corpus luteum menstruation would not occur and a new follicle could not form. The follicle stimulating hormone cannot exhibit its function in the presence of an excess of the other. The balance is delicately maintained.

The Ovary. The ovary, having received the hormonal impetus from the anterior pituitary gland, liberates its own hormones. The follicle stimulating hormone induces the formation of a follicle and this in turn secretes follicular hormone or estrone. The luteinizing hormone converts the ruptured follicle into a corpus luteum and the latter secretes progesterone. The ovary undergoes histological as well as hormonal changes during the cycle. The ovarian hormones are delicately balanced and disturbance of one prevents the proper exhibition of the other. The administration of too large quantities of estrin prevents the proper action of progestin, as evidenced by their action on the endometrium. The action of estrone is maintained throughout the cycle, for it is produced by the follicle in the first half of the cycle, and by the corpus luteum during the second half (Allen, Pratt, Newell and Bland, 1930). Progesterone is the predominating hormone during the second half of the cycle. Since progestin has to date not been demonstrated in either blood or urine (nonpregnant), it is impossible to state whether small traces originating in the corpus luteum of the previous cycle might not be active during the first half of the new cycle. Zondek (1930, p. 57) maintains that even in the presence of histological change in the corpus luteum (at the time of menstruation) it is not safe to draw conclusions as to its function. Such traces of corpus luteum hormone might be necessary to prevent excessive proliferation of the endometrium due to estrin.

The amounts of estrone and progesterone required for the production of a complete cycle in a castrate, or in primary amenorrhea, have been demonstrated by Kaufmann (1932) to be 40,000 R.U. of estrone

and 35–50 rabbit units of progesterone. This is a distinct step forward, for we now know not only what ovarian hormones are responsible for each phase of the menstrual cycle, but know also the quantities required. It goes without saying that this figure is not absolute, for the structure of the molecules (compare estrone with estradiol!), the nature of the solvent, the frequency of injections, the rate of excretion and utilization of the hormones, and their storage, play important rôles in determining the exact dosage required. It might be asked whether 40,000 R.U. of estrin and 35–50 rabbit units of progestin represent the *entire quantity* of these hormones required by the organism, for their function extends definitely beyond the local genital sphere. This question is all the more pertinent in view of the observation of Marrian and Parkes (1930) that while one mouse unit of estrin was sufficient to produce a cornified vaginal epithelium, 200 M.U. were required to duplicate *all* the phenomena of the estrous cycle. On that basis Parkes (1932) calculated that for the human a complete replacement therapy would involve the subcutaneous administration of about 500,000 M.U. (100,000 R.U.) of estrin. Considering the fact that the benzoic acid ester of estradiol (progynon-B) is about three times as active as estrone, Parkes' theoretical figure was therefore established as correct by the clinical tests of Kaufmann. Small amounts of estrin (2800 R.U.) are sufficient to produce growth of the endometrium and breasts in patients whose sexual organs have undergone castration atrophy (Werner and Collier, 1933). On the other hand, it is our impression that in cases of primary amenorrhea and long standing secondary amenorrhea the very large doses suggested by Kaufmann were essential to produce the full psychic and physical changes involved in the menstrual cycle.

Observations on Ovarian Ocular Implants. The possibility of actually observing an ovary during its various functional phases by transplanting it into the anterior chamber of the eye was first suggested by Schocket (1920). He noted follicle growth, rupture, and was able to demonstrate histologically an ovum in the anterior chamber. The problem was revived recently by Schocket and Markee (1928), Allen and Priest (1932) and by Podleschka and Dworzak (1933). The latter workers experimented with rabbits and reached the following conclusions. When both ovaries are transplanted follicular growth and degeneration occur. Ovulation was observed only once. After copulation corpora lutea were formed, and these functioned, for a pregravid endometrium was formed and uterine mobility was inhibited. The same phenomena were observed after injection with prolan or anterior

pituitary gland extract. The transplantation of one ovary into the eye, and the retention of the other in its normal position, changes the character of the response. Only when the ovary in situ is removed does the transplant show the usual follicle growth. The reason for the inhibition of the transplant by the ovary in situ is not known. It may be due to severance of the nerve supply when the transplantation is done. Intact nervous pathways may be essential to normal ovarian function.

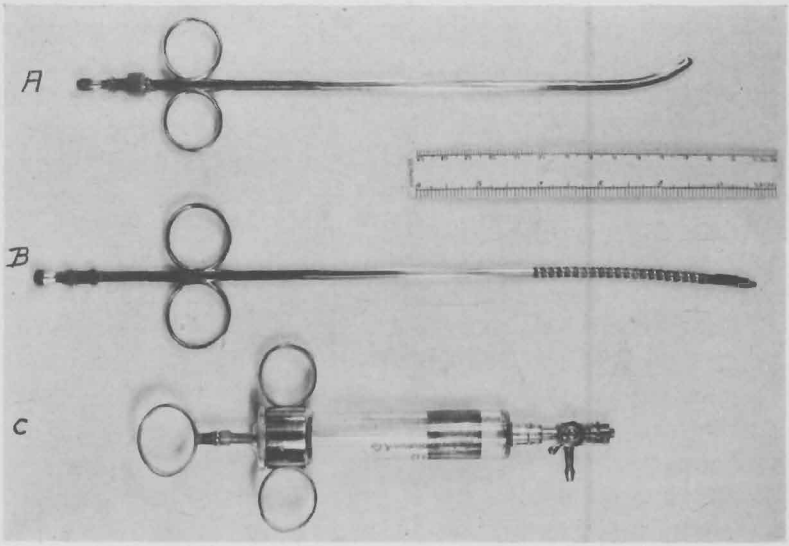

FIG. 99. Modified suction curettes for the removal of endometrial biopsies. (*A*) As modified by Kurzrok. The end of the cannula has a moderately sharp edge. It is moved along the surface of the endometrium and suction is exerted by means of the syringe. A specimen is removed from each of the uterine walls. (*B*) As modified by Cassidy. The end of the cannula is flexible, and it is inserted through the cervix by rotating the cannula counterclockwise until the fundus is reached. It is then removed slowly from the uterine cavity by rotating clockwise and, at the same time, suction is exerted. (*C*) Syringe with automatic lock and two-way valve.

The Endometrium

The methods of demonstrating endometrial change were previously carried out by an examination of curettings or by the examination of entire uteri removed at operation. Further progress was made with the suction curette (fig. 99) for with this instrument, or any of its modifications, it is possible to remove endometrial fragments repeatedly at various phases of the cycle without inconveniencing the patient to any extent. More recently Markee (1933) has studied menstruation

in ocular endometrial implants. This method promises to add a great deal to our knowledge of this subject.

Markee used monkeys in his experiments. He used homologous endometrial implants and noted the following: The first evidence of approaching menstruation was vasoconstriction lasting six to twelve hours, and the bleeding in the eye preceded that from the cervix by several hours. Nor was the onset of bleeding in all transplants simultaneous—a fact noted in the menstruating human uterus by Bartelmez. Subepithelial hematomas formed and small papillae appeared on the epithelial surface. The papillae ruptured and bleeding continued for from twenty-five to seventy minutes. No desquamation of epithelium occurred during the first few hours and only small bits during the first day. Regeneration occurred from outgrowths of the free extremities of the glands. What effect the intraocular pressure and the peculiarities of vascularization in the eye have on this form of endometrial bleeding is still unsettled. Bleeding from a free surface (in the uterus) must vary from that against a resistance (in the eye), in view of the fact that the pressure behind the endometrial bleeding cannot be very great.

Endometrial Bleeding

Endometrial bleeding may be of two types:

1. Menstrual.
2. Non-menstrual.

Menstrual bleeding here applies only to bleeding from a premenstrual endometrium conditioned by the presence of a corpus luteum. Non-menstrual bleeding may occur every month, hence be cyclical in character. The endometrium is always postmenstrual in character. Cyclical bleeding implies the absence of ovulation.

In order to understand the problem of menstrual bleeding it is advisable to consider the factors that produce non-menstrual bleeding.

1. Oöphorectomy—in the *first half* of the menstrual cycle (bilateral). This is common knowledge to all gynecologists. The bleeding sets in within a few days of operation. The symptoms associated with menstruation are frequently lacking. The endometrium is postmenstrual in character. On the contrary, a unilateral oöphorectomy containing the corpus luteum (hence done in the second half of the cycle) hastens the occurrence of the menstrual period. The bleeding is from a premenstrual endometrium and the symptoms are similar to those of a normal period.

2. The administration of follicular hormone. Allen (1927) has shown that the administration of follicular hormone to an adult castrated

Macacus Rhesus Monkey until growth of the endometrium has been induced, followed by cessation of injections or decrease in the dosage, results in non-menstrual bleeding. Werner and Collier (1933) have obtained a similar result by injecting theelin into castrated women, even when a uniform level of daily injections has been maintained. Kurzrok, Wilson and Cassidy (1935) have observed that when cases of primary amenorrhea (due to the absence of functioning gonads) are *constantly* treated with large doses of follicular hormone cyclical bleeding occurs with great regularity, the intervals of bleeding approximating four weeks. There is no withdrawal or decrease in the dosage, and yet bleeding breaks in with great regularity. (In view of the complete absence of secondary sex characteristics, the presence of large quantities of follicle stimulating hormone and the absence of follicular hormone in the urine, a functioning ovary is definitely excluded in their cases.) This leads to the probability that the stimulus to bleed is extra-gonadal in character, and in all probability emanates from the anterior pituitary gland or the uterus proper. Smith, Tyndale and Engle (1936) were able to induce bleeding in hypophysectomized monkeys by the injection of estrone. Hence neither the hypophysis nor the ovaries are essential for uterine bleeding. Just how this clocklike mechanism functions is at present unknown. Bleeding is an additional factor added to the endometrium, for there are numerous cases on record of women who have never menstruated but who became pregnant without difficulty.

3. Section of the nervous connections to the ovaries. This was carried out in the Macacus Rhesus Monkey peripherally (Zuckerman, 1934), and in the spinal cord (Van Wegenen, 1932). The result is uterine bleeding within a few days. Disturbances of the autonomic nervous system in women may give rise to uterine hemorrhage in the absence of any local pathology, or may suddenly stop a normal menstrual period. The modus operandi is in all probability vascular, vasoconstriction or vasodilatation.

The etiology of the bleeding designated as menstruation is unknown. An opinion, vague at the present time, is that there occurs a decrease in the level of follicular hormone at the end of the corpus luteum phase and that as a result of this hormone withdrawal menstruation sets in. Our studies (Kurzrok and Creelman) of the urinary excretion of estrin fail to show any such loss of hormone at the time of menstruation. Figure 100 shows the excretion in one such case. The post- and premenstrual values are about the same. The peak of excretion corresponds to the time of ovulation. The blood values of estrin during the cycle

are parallel to those found in the urine. If there is a sudden withdrawal of hormone it is not by way of loss through the urine as estrone, but possibly through conversion into a form not demonstrated by the usual technique of estrin determination in the urine (Kurzrok and Ratner, 1932). While the evidence of Cohen, Marrian and Watson (1935) points to the presence of such a fraction in pregnancy urine, its variation during the estrous cycle has not been demonstrated.

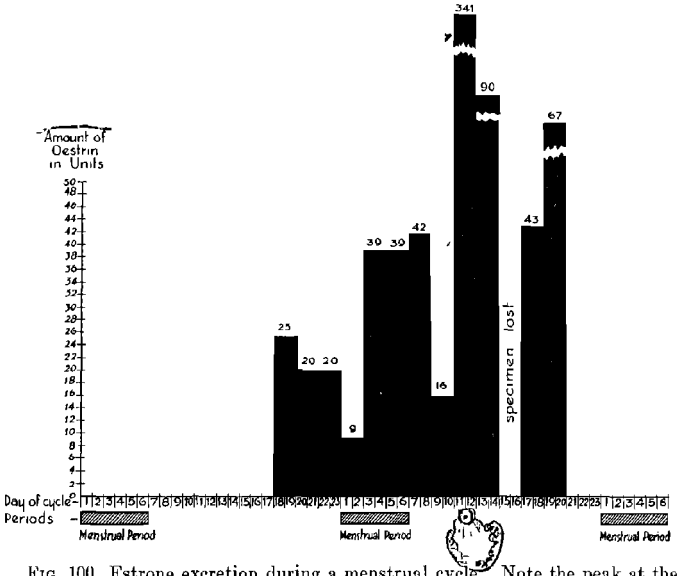

FIG. 100. Estrone excretion during a menstrual cycle. Note the peak at the time of ovulation. There appears to be no significant drop in hormone excretion preceding the period.

Etiology of Sexual Rhythmicity

The menstrual cycle having once begun at puberty is from then on maintained rhythmically up to the menopause, except during periods of pregnancy and dysfunction. What then maintains this rhythm? Is it entirely regulated from without by a special center in the hypothalamus, or does the regulatory mechanism function within the cycle? Whatever evidence has accumulated to date favors the latter concept. The following theory, based on the following facts, is offered:

1. Four distinct hormones, grouped in twos, are involved.

Follicle stimulating and follicular hormone. Luteinizing hormone and progesterone.

These hormones react functionally upon one another.

2. The gonad stimulating hormones vary at different times of the estrous cycle (Smith, 1929).

3. Removal of the ovaries increases the amount of gonadotropic hormone in the anterior pituitary gland (Engle, 1928). Follicular hormone therefore inhibits the gonadotropic hormones.

4. Injections of follicular hormone decrease the gonadotropic potency of the anterior pituitary gland (Moore and Price, 1932).

5. Injections of large quantities of follicular hormone induce changes in the anterior pituitary gland, resulting in luteinization of the ovaries (Hohlweg, 1934).

6. Injections of progesterone are effective in converting a castration hypophysis into a normal one (Clauberg and Breipohl, 1935).

7. This hormone system is in unstable equilibrium.

At the onset of the post-menstrual phase the estrone concentration is low (about the same as at the end of the pre-menstrual phase), depleted in addition by the loss via the menstrual discharges. The inhibition upon the anterior pituitary gland is removed and follicle stimulating hormone is produced in increasing amounts. This increased stimulus acts upon the ovary, and the latter produces increasing amounts of estrone, the peak being reached at about the time of ovulation. The great concentration of follicular hormone in turn depresses the follicle stimulating hormone and stimulates the production of the luteinizing hormone. Follicle growth is inhibited while a corpus luteum forms. The progestin thus formed in increasing amounts (as seen from the increasing effect upon the endometrium) finally reacts upon the anterior pituitary gland, depressing the luteinizing factor and allowing the follicle stimulating hormone to renew its activity. At this point the premenstrual endometrium breaks down due to some other impetus (bleeding factor?) which arises from some source. The rate of this "turn over" may be influenced by changes from within this system of hormones proper, or from without (sexual center or other glands of internal secretion).

Considering the complexity of the factors involved it is of little wonder that aberrations frequently occur. Therapeutically we take advantage of this hormonal interplay and move one hormone against another. And, not only must the right hormone be chosen for the right place, but the amount of hormone exhibited is of great importance.

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CHAPTER XV

OVULATION

The most important event in the menstrual or estrous cycle is ovulation. The term ovulation implies the separation of the ovum from the ovary and its expulsion from the follicular cavity.

Ovulation is associated with other phenomena, namely, follicle growth and then corpus luteum formation. In view of the fact that the ovary has no inherent drive, for it is maintained in a state of function by the anterior pituitary gland, it is not surprising to find that the impetus to ovulate also depends upon the adenohypophysis. The position of ovulation in a given menstrual cycle, as well as in the cycles that follow, is of utmost importance from the viewpoint of sterility and fertility. Furthermore, it is essential to know whether or not ovulation accompanies every menstrual cycle, and whether the two phenomena are dependent or independent of one another. Again, are we always dealing with spontaneous ovulation, or may it be induced out of turn.

The Follicle and the Gonadotropic Hormones

Only mature follicles can ovulate. Immature follicles must first reach a state of maturity. Follicle ripening, according to Lane (1935), involves activity on the part of both gonadotropic hormones. Beginning with the primary oöcyte, the follicle stimulating hormone is apparently responsible for the formation of a granulosa about the ovum. The luteinizing hormone promotes the formation of an antrum folliculi, into which the granulosa cells are made to secrete follicular hormone by the follicle stimulating hormone, or, by a combination of follicle stimulating and luteinizing hormones.

It is a well established fact that the removal of one ovary induces hypertrophy of the remaining one. Arai (1920) has shown that this compensatory hypertrophy is not due to an increased number of ova. Hence doubling the amount of pituitary hormones for one ovary by the removal of the other, will not influence the number of ova in the surviving gonad. The ovogenetic rate of hypophysectomized adult rats is increased when compared with the total eggs, follicles and corpora in the control animals (Swezy and Pencharz, 1933). The rôle of the gonadotropic hormones begins after the formation of the oöcyte.

The first task of the follicle stimulating hormone is to convert the pregranulosa cells into a granulosa layer and thus form the primary follicle.

The Stimulus to Ovulation

The stimulus that induces ovulation has its origin in the anterior pituitary gland. Smith and Engle (1927) produced ovulation in immature mice by successive implants of anterior pituitary glands from adult rats. Similar implants in adult animals rarely, if ever, produced ovulation. Bellerby (1929) induced ovulation in the unmated estrus rabbit by the administration of anterior pituitary extracts. This was also obtained by Friedman (1929) with urine of pregnancy. Hill and Parkes (1931) found that in the absence of the hypophysis, extracts of the anterior lobe and pregnancy urine will induce ovulation. Wolfe (1931) made some interesting observations on ovulation by means of saline extracts of sow pituitaries taken at different stages of the cycle. One milligram of pituitary tissue taken from a sow with large growing follicles (6–8 mm. in diameter) and degenerate corpora was needed to induce ovulation, but 10 mgm. were needed when the donors had larger corpora and 40 mgm. when the ovaries of the donor contained active corpora lutea plus small follicles. Wolfe and Cleveland (1931) report that it takes equal amounts of anterior pituitary tissue from either immature or adult rabbits to induce ovulation.

The Nature of the Hormones (or Hormone) that Induce Ovulation

Is ovulation due to a separate and distinct hormone or to the follicle stimulating and luteinizing hormones? To date there has been no evidence produced to show that a distinct ovulating hormone exists. On the contrary, there is evidence to show that ovulation requires the presence of both the follicle stimulating and luteinizing hormones (Smith and Leonard, 1934). These workers utilized immature hypophysectomized rats in their experiments. The injection of follicle stimulating hormone from castrate urine produced large, normal and uniform follicles. No ovulation was seen. Each follicle contained an ovum. This form of ovarian response was also noted by Kurzrok (Riley, Brickner and Kurzrok, 1933). Leonard and Smith followed their treatment by a single intra-peritoneal injection of pregnancy urine (antuitrin-S) and obtained ovulation. Since pregnancy urine contains large quantities of luteinizing hormone, it appears that the addition of this hormone to a properly prepared follicle induces ovulation. The steps leading up to ovulation are several, namely:

1. The conversion of a primary oöcyte into a primary follicle by the follicle stimulating hormone, followed by a further growth of the granulosa due to the same hormone.

2. The formation of an antrum due to the luteinizing hormone.

3. A growth of the follicle which terminates in the mature Graafian follicle (due to F.S.H.).

4. Ovulation upon the addition of the luteinizing hormone. In the human there is a sudden addition of follicle stimulating hormone at this point (Kurzrok, Kirkman, and Creelman, 1934).

It would seem, therefore, that ovulation is induced by a correct admixture of both gonadotropic hormones acting upon a mature follicle.

The Mechanism Causing Ovulation

The exact etiological factors involved in the expulsion of the ovum from the follicular cavity are unknown. Numerous theories have been offered in explanation. They are as follows:

Increase in Intrafollicular Pressure. This increased intrafollicular pressure has been ascribed to increased secretion of liquor folliculi, hyperemia and blood pressure. C. Hartman (1932), in a recent review of this subject, discusses the various forms of evidence brought forth in support of this explanation. A plausible explanation of the sudden rise of intrafollicular pressure is the presence of smooth musculature about and within the ovary, specifically that about the Graafian follicle. Aeby (1859–1861) described smooth muscle fibers in the theca externa of follicles, as well as heavier strands in the hilus of the mammalian ovary. He attributed the rupture of the follicle to the contractions of these muscle fibers. He was confirmed in these observations by Thomson (1919) and Winiwarter and Sainmont (1908–1909). Guttmacher and Guttmacher (1921) found nerve endings terminating in the muscle fibers of the sow ovary. They observed and recorded contractions of these muscle fibers, as well as the existence of sympathetic (epinephrine caused relaxation) and parasympathetic fibers (physostigmine caused contraction). The theory that ovulation is caused by a contraction of these muscle fibers, especially those about the theca externa, induced by either hormonal or nervous stimuli, is extremely attractive. The point of rupture, or stigma, becomes markedly thinned out and avascular at the time of rupture (Oertel, 1924). Schocket demonstrated proteolytic enzymes in the liquor folliculi and attributed follicular rupture to this factor. Muscular contractions of the ovary aided by proteolytic digestion of an avascular, devitalized stigma offer

the most reasonable explanation for the actual extrusion of the ovum (fig. 101).

Supposing the follicle is artificially ruptured *prematurely*, will ovulation and corpus luteum formation occur? Friedman (1931) reported that artificially ruptured follicles in the rabbit usually retain their ova

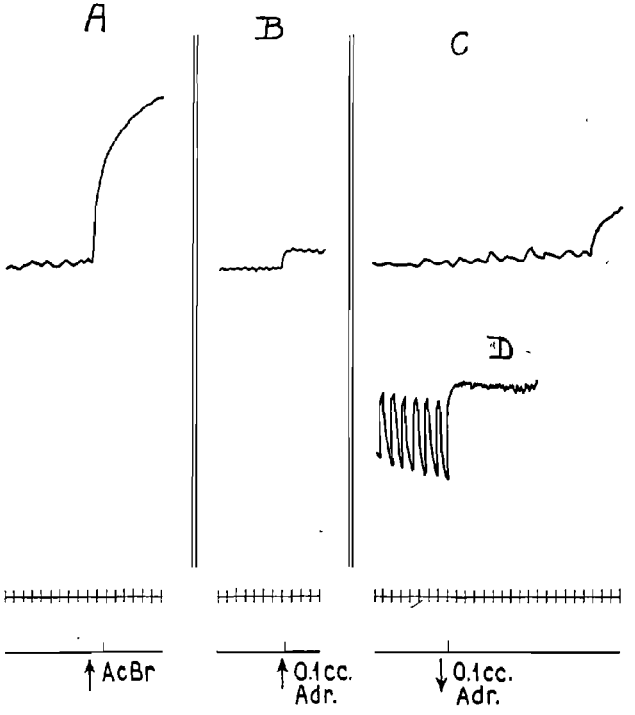

FIG. 101. Mechanism of ovulation. Reactions of muscle fibers about mature follicles. The follicle was carefully dissected out, freed from visible blood vessels and punctured. A section of the wall was suspended in modified Ringer solution and the contractions recorded. Figure *D* represents the uterus of the ovary used in *C*. In figure *A* the action of the hydrobromide of acetylcholine was tested and in figures *B*, *C* and *D* the action of 1:1000 adrenaline hydrochloride.

and do not metamorphose into functional corpora lutea without the stimulus of anterior pituitary substance or pregnancy urine extract.

Supposing a follicle once formed fails to rupture because of hormonal imbalance, or because of enveloping pelvic inflammatory disease, will a corpus luteum form in the follicle? Tietze (1934) has shown that

a persistent follicular cyst fails to luteinize and thereby induces cystic and glandular hyperplasia in the endometrium. Frankl (1934) maintains that the same condition occurs in pelvic inflammatory disease. Friedman (1931) occasionally encountered cystic follicles in rabbits when he injected graded doses of pregnancy urine. Reynolds (1931) obtained the same effect, and the subsequent addition of large doses of luteinizing hormones failed to rupture these large follicles. It would seem that when the normal mechanism is disturbed, the righting of this mechanism is a difficult task for the organism.

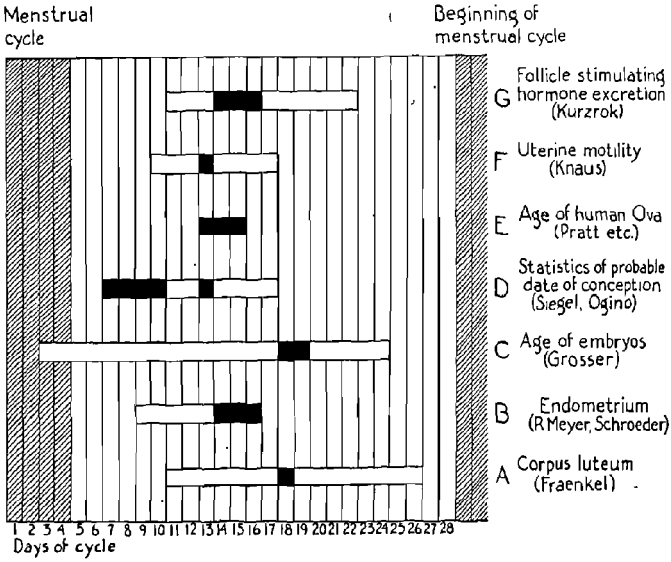

FIG. 102. Methods of studying human ovulation. The unshaded blocks show the limits of ovulation within the cycle; the solid black areas show the greatest probabilities.

Time of Human Ovulation in the Menstrual Cycle (fig. 102)

Kurzrok, Kirkman and Creelman (1934) have recently reviewed the evidence brought forth to determine the time of ovulation in the human menstrual cycle. The problem has been studied by six different methods and to this they add a seventh. The methods are as follows:

a. The macroscopic observation of the ovary in situ.

b. The microscopic examination of removed ovaries and uterine curettings.

c. The determination of the age of early embryos, taken together with the probable time of conception.

d. The maximum probability for ovulation.

e. The recovery of human ova from the uterine tubes.

f. The reversal in the reaction of the uterine muscle to pituitrin.

g. The sudden appearance of follicle stimulating hormone in the urine during the intermenstrum.

a. Based on the macroscopic examination of the ovaries in situ Fraenkel and his school (Ancel and Villemin, 1907; Tschirdewahn, 1921; Derck, 1923) place the limits of ovulation between the tenth and twenty-sixth days of the cycle, with a maximum probability at about the eighteenth day. Shaw (1925, 1932) concluded that ovulation occurs not long before the 17th day, while Newell (1930) places the phenomenon a few days earlier. Corner (1923), Allen (1928) and Hartman (1932) made similar observations on the ovaries of the Macacus Rhesus and placed the limits of ovulation between the 9th and 17th days of the menstrual cycle.

b. Robert Meyer, employing the histologic technic, concluded that the limits of ovulation were from the eighth to the fourteenth day of the cycle (Meyer and Ruge, 1913). Using the same technic, Marcotty (1914) placed the most probable time of ovulation on the fifteenth day, and Schroeder (1918) from the fourteenth to the sixteenth day.

c. The third method of approach to this problem was the estimation of the age of early embryos, when considered together with the history and probable date of conception. Using such evidence Triepel (1914, 1915) placed the greatest frequency of ovulation at the eighteenth to the nineteenth day. Volkmann (1926) based his figures on the examination of eighteen early embryos and favored an earlier ovulation time. On the basis of similar studies (Grosser, 1932) placed ovulation between the second and twenty-fourth days after the onset of the menses. He thought that the early ovulation may have been induced by coitus, drugs, violent exercise, etc., as suggested by Chazan (1911).

d. The fourth method was by means of a study of the most probable time of conception (Siegel, 1917; Jaeger, 1917; Zangemeister, 1917). This presupposes that coitus, ovulation and conception occur within an interval of hours, and that the ovum and sperm remain fertilizable not more than thirty-six hours (Hartman, 1932, review and literature). This method places the ovulation time between the eighth and tenth days. Ogino (1932) holds the favorable period of conception to be between the tenth and the seventeenth day.

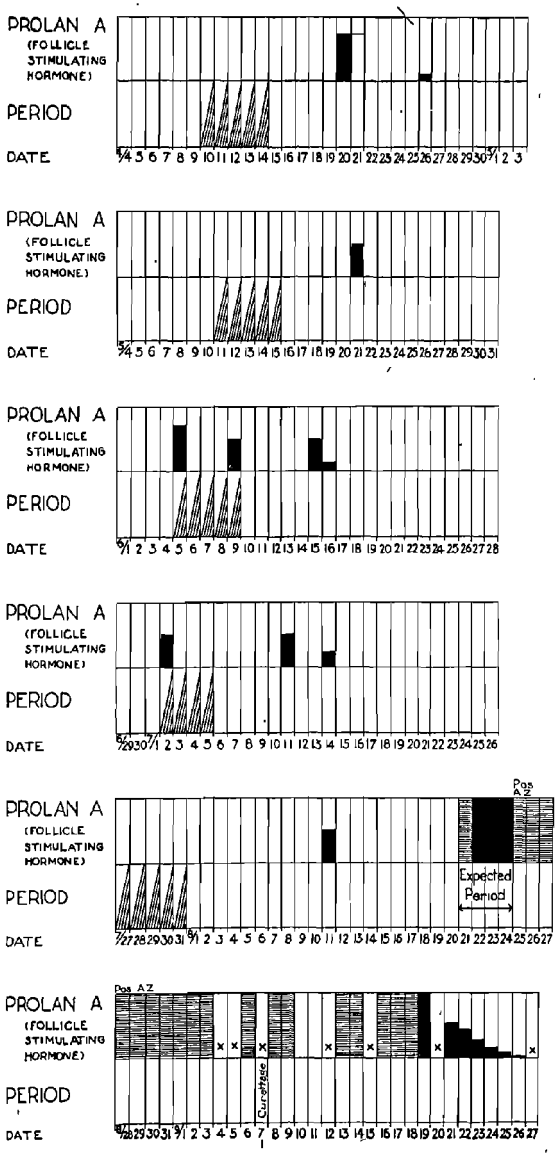
PROLAN A
(FOLLICLE STIMULATING HORMONE)
PERIOD
DATE
Pos A Z
Expected Period
Curettage

Fig. 103

e. It has now been definitely demonstrated by the recovery of ova from the uterine tubes that in regular cycles of average length ovulation usually occurs during the intermenstrum. The estimated ovulation time for five human ova recovered from uterine tubes when possible stimuli of mating were excluded, was between the twelfth and fourteenth days following the first appearance of the menses (Allen, Pratt, Newell, and Bland, 1930). The probable time of ovulation of another human tubal ovum recovered by Lewis (1931) was placed on the eighteenth day following the onset of menstruation, after a previous cycle of thirty-five days' duration (E. Allen, 1932).

f. Another recent approach to this problem was suggested by Knaus (1930). He observed that in the rabbit prior to ovulation, the uterus reacts to pituitrin by a contraction. Twenty-four hours after ovulation the uterus no longer reacts to pituitrin, and this reversal Knaus attributes to the corpus luteum. Knowing the date of reversal he places ovulation one day back (in the rabbit). Knaus found the same reversal in the human being and placed ovulation from two days back, i.e., between the fourteenth and sixteenth days (of a twenty-eight day cycle), with a possible physiologic shift of two days to either side. Wittenbeck (1930), on the basis of experiments in vivo, agrees with Knaus, but adds that ovulation may occur on the ninth day of a twenty-eight day cycle. Knaus' method is not a test for ovulation but is really a test for corpus luteum hormone, because a corpus luteum may form without ovulation (corpus luteum atreticum). Hermstein (1931) agrees with Knaus' findings. Kurzrok et al. (1937) have not substantiated Knaus' findings (Chapter XVI).

g. Kurzrok et al. (1934) observed that if consecutive morning specimens of urine are tested for the presence of follicle stimulating hormone, no hormone will be found except on one day during the intermenstrum, and occasionally at the beginning and at the end of a menstrual period. Excretion of the follicle stimulating hormone occurred on the day preceding the "Mittelschmerz" and also on the day following the onset of ovulation bleeding. Where the excretion was followed in any one case over several consecutive menstrual cycles, it was found that the limits

FIG. 103. Follicle stimulating hormone excretion during six months. *Every* overnight specimen was examined. Note the positive F.S.H. at the time of ovulation, and on two occasions at the beginning and at the end of menstruation. During the first four months ovulation occurred successively on the 10th, 11th, 10th and 9th days. It was therefore believed that her safe period was from the 14th day on. But during the fifth month ovulation occurred on the 15th day and patient became pregnant. Note that the positive A-Z Test terminated after the curettage as a positive F.S.H.

of hormone excretion were between the eleventh and sixteenth day (fig. 103). The limits of all cases studied were between the ninth and twenty-first days, with the greatest number occurring between the eleventh and thirteenth days. The sudden excretion of follicle stimulating hormone means sudden pituitary activity. In all probability this is accompanied by a sudden production of luteinizing hormone. No trace of this hormone was detected in the urine, possibly because a sufficient quantity did not pass over, or the method employed was not sensitive enough to detect it. Kurzrok et al., believe that ovulation occurs on the day following the sudden hormone excretion (fig. 104).

Clinical Signs of Ovulation

Two clinical signs of ovulation in the human being have been recognized by gynecologists, namely, the intermenstrual pain or "Mittelschmerz" and intermenstrual spotting. The former is probably due to the greatest distention of the follicle just preceding rupture. It is not common. It may be perfectly regular, occurring on one side one month, and on the opposite side the next. Or only one side may be involved, distention of the ovary on the opposite side causing no pain. Some abnormality in innervation of the ovary is probably responsible for the phenomenon's reaching consciousness (fig. 105).

We have recently studied a number of cases of ovulation bleeding. Some were referred to us with the diagnosis of polymenorrhea, in other words, the patients were supposedly menstruating every two weeks. Careful questioning of the patients brought out the facts that even though two consecutive bleedings lasted the same number of days, they never were of the same amount, and that one period of bleeding (ovulation) was accompanied by less discomfort than the one that followed (menstruation). By removing fragments of endometrium by means of the suction curette we were readily able to differentiate the two types of bleeding. Intermenstrual spotting occurs quite infrequently. It may last from one hour to forty-eight or more hours, and the bleeding may be very slight or moderate. It has also been observed in the monkey (Hartman). Schroeder (1924) studied 20 cases, and curetted 5 during this period of abnormal bleeding. He found an endometrium that was in the process of changing from a proliferative to a premenstrual type, hence corresponding to the period of ovulation.

Figure 106 represents a case of "Mittelschmerz" that was seen by Dr. Milton Robinson. The patient was a young woman, 24 years of age. Her periods were regular, every 26 to 29 days, moderate in

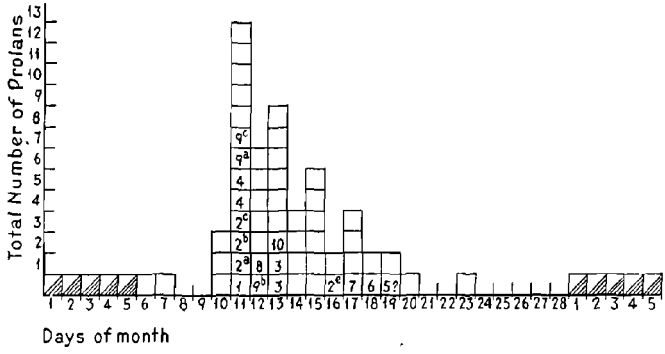

FIG. 104. Human ovulation. Results of studies based on the excretion of follicle stimulating hormone. Each square represents the single excretion of F.S.H. during the cycle. Ovulation probably occurs about 24 hours after the excretion of the hormone. The letters represent successive months in the same patient; the numbers, the cases studied in our first series. (Am. Jour. Obs. & Gyn., **28**, 319, 1934.)

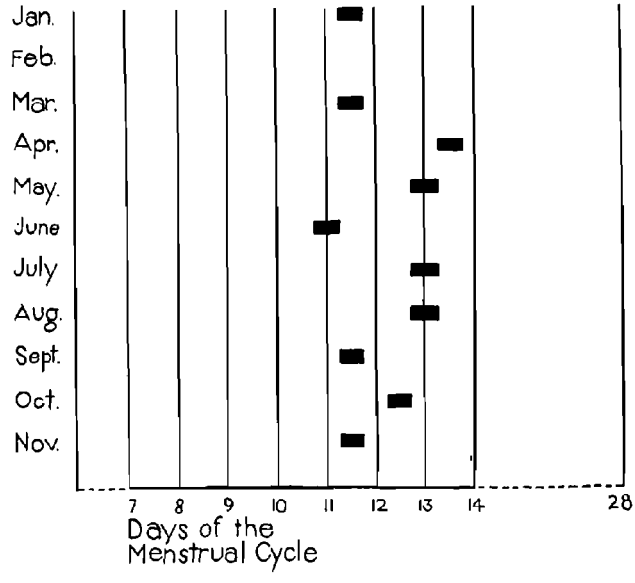

FIG. 105. Case of Mittelschmerz. The black blocks show the date and duration of the pain. (Courtesy of Dr. Milton Robinson.)

amount and without pain. The pain lasted four hours, and moderately incapacitated the patient. The pain never began before the ninth day nor after the thirteenth. We believe that ovulation occurs within one day after the onset of the pain. This case shows the limits of ovu-

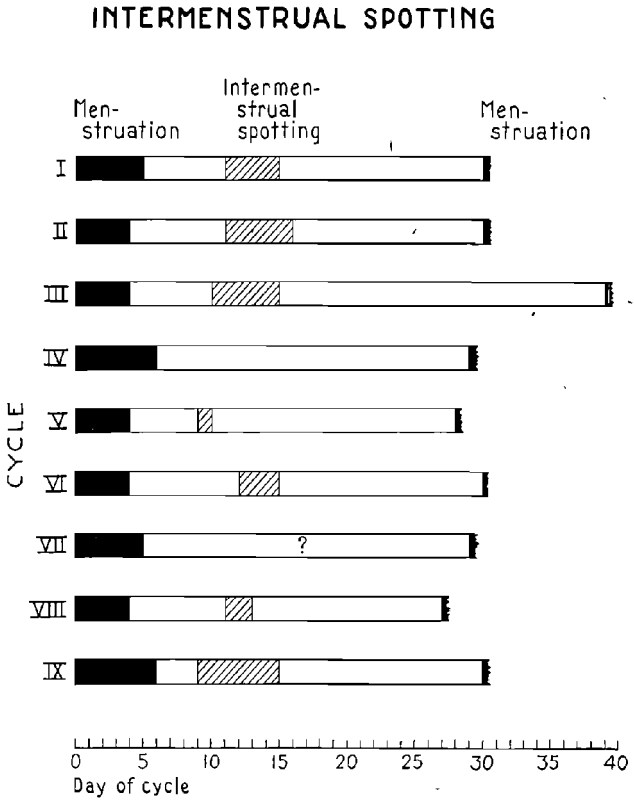

FIG. 106. Case of intermenstrual spotting. The patient did not spot during the fifth period, but did so at about the middle of the seventh. Biopsy during the spotting of the first cycle showed diapedesis through vessel walls, but no desquamation of tissue. (Courtesy of Dr. N. Kopeloff.)

lation during a period of one year. There was absolutely no pain during February. Does this mean that ovulation did not occur in February?

Seguy and Simonnet (1933) have described three other signs of ovulation, namely, the appearance of a glassy, translucent and extremely

fluid secretion in the cervical canal. They believe that the purpose of this secretion is to render the cervix temporarily permeable to spermatozoa.

The second sign of ovulation is the occurrence of increased sexual excitement which lasts throughout the period of glassy secretion in the cervix.

The third sign appears a few days after the glassy cervical discharge and consists of desquamation of the vaginal epithelium. This finding is not completely in accord with the studies of Papanicolaou on the cyclical changes in the human vaginal epithelium. He (Papanicolaou) finds that smears taken during the ovulative phase show a sudden increase in the number of leucocytes. This is particularly noticeable after a typical stage of leucopenia. The epithelial cells are chiefly of the intermediate or superficial type. They greatly resemble the cells of the previous phase, though their contours are not sharp. They form characteristic aggregations mixed with leucocytes. Many cells are penetrated by leucocytes. Cornification is pronounced with most of the cornified cells nucleated. Mucus is on the decrease.

The Period of Fertility in the Human

The period of fertility in the human has formed the subject of much discussion during recent years. The problem has been viewed from two angles, namely, to explain certain types of sterility, and secondly, to determine the so-called safe period for coitus during the cycle. It is with the latter topic that we will concern ourselves at this point.

The factors which determine when during a given cycle fertilization may occur, are threefold:—

1. The duration of life of spermatozoa in the female genital canal.
2. The duration of the life of the ovum after ovulation.
3. The actual time of ovulation in the cycle.

The Duration of Life of Spermatazoa in Female Genital Tract. It may be stated at the very outset that we are of opinion that only experiments in the human are of any value in the solution of the problem. Whatever the duration of life of the rat, guinea pig or even monkey spermatozoa may be in their respective females, it can only have a very indirect bearing on the problem as it pertains to women. Some broad principles may apply, but the specific circumstances are different in each. Another point—the fact that a sperm is motile is no proof that it has maintained its fertilizing power. Fertilization and motility may

be parallel throughout, but it is also conceivable that a sperm may remain motile, but may have lost its fertilizing power.

A. Duration of Life of Spermatozoa in the Vagina, and Their Transport. The duration of life of sperm in the vagina depends upon the following conditions within the vagina:

1. The buffer action of semen and its pH.
2. The hydrogen ion concentration of the vagina.
3. The amount of seminal fluid retained within the vagina.
4. The bacterial flora of the vagina.

Semen is a buffered solution with a pH ranging between 7.8 and 8.4 (Kurzrok and Miller, 1928). This buffering action is limited. Too high concentration of either acid or alkali will overcome this buffering activity and thus become inimical to the life of the spermatozoa. Under normal conditions there is a constant infiltration of the seminal pool by the lactic acid from the vaginal walls. It, therefore, becomes essential for the spermatozoa to move out of the seminal pool, into the alkaline cervical mucus. This movement into the cervical canal is not hit or miss, but is a directed and oriented movement controlled by electrokinetic phenomena across phase boundaries set up by the acid vaginal secretions (lactic acid), the neutral (or slightly alkaline) semen and the alkaline cervical mucus (Miller and Kurzrok, 1932).

At the end of two hours post-coitum ninety per cent of all spermatozoa in the vagina are dead. The writer has never seen living sperm in the vagina later than ten hours post-coitum. Dead sperm may be found after twenty-four hours but rarely after thirty-six hours.

Cary (1935) has not found sperm cell activity in the vagina later than two hours after coitus. Runge (1909) failed to find sperm in the vagina on the third day post-coitum. Haussmann (1879) found them absent at the end of the second day post-coitum. Hoehne and Behne (1914) examined the vaginal canal of a young primipara in her sixth month of pregnancy for spermatozoa at various intervals post-coitum. At the end of three quarters of an hour only an occasional sperm remained motile. Sperm removed seven hours post-coitum showed marked degenerative changes. No spermatozoa remained motile at the end of an hour. The very short life of sperm in the vaginal secretions of a pregnant woman is due to the high vaginal acidity, which in a short time overcomes the buffer action of semen. Furthermore, spermatozoa mixed in vitro with the vaginal secretions of normal women, remained motile up to four hours. All evidence at hand shows that the spermatozoa that eventually reach the upper portions of the genital canal have left the seminal pool within two hours post-coitum.

The hydrogen ion concentration of the vagina is maintained in part by the activity of the Döderlein bacilli. The glycogen of the vaginal walls is split up directly by the Döderlein bacilli as follows (Schultheiss, 1929):

Glycogen → polysaccharides → glucose → lactic acid

The acidity of the vagina is hence a function of its lactic acid content. In addition, there is present in the vaginal secretions a diastatic ferment that is capable of splitting glycogen into glucose (Kessler, 1935). The Döderlein bacilli can then split the glucose further into lactic acid. When the vaginal canal becomes contaminated with other organisms the hydrogen ion concentration decreases (pH goes up). The acidity of the vaginal secretions, pH 4.0-4.7, finally overcomes the buffering action of the semen and renders the seminal pool acid. Acidity destroys the activity of the spermatozoa.

The amount of seminal fluid retained in the vagina plays a distinct rôle in the viability of the spermatozoa. Usually the amount retained is about 1.0–2.00 c.c. All other factors being equal, the greater the amount of buffer the longer will it take to overcome its buffering activity. Cases of genital hypoplasia that have a shallow posterior cul-de-sac retain very little semen, hence motility ceases in a very short time.

Rosenthal (1931) made the interesting observation that cultures of B. coli agglutinate spermatozoa. Motility is lost within a few minutes, and the reaction is not reversible. The clumps formed could not be disintegrated. The phenomenon was entirely independent of the pH and clumping occurred with dead sperm as well. The active principle is contained within the body of the bacteria. Attempts at agglutination with strains of streptococci, staphylococci, or gonococci gave negative results. Considering the fact that the B. coli commonly contaminates the vaginal canal, this agglutinating phenomenon can be very detrimental to fertilization.

B. Duration of Life of Sperm in the Cervix, and their Transport. The duration of life of spermatozoa in the cervix is longer than in the vagina. The writer has seen living sperm in cervical mucus as late as eleven hours post-coitum, whereas no living sperm were found in the vagina. Sperm may be found *within* the cervical mucus in contact with the seminal pool fifteen minutes post-coitum. In about one hour the spermatozoa may be found in the cervical mucus in the region of the internal os. Motion through the cervical os is fairly rapid. This is aided by two known factors, namely, the lytic action of semen upon

cervical mucus, and the increased activity of spermatozoa within the cervix. Kurzrok and Miller (1928) have described this lytic activity which appears to be due to an enzyme. Cary and Hotchkiss (1934) have recently demonstrated by means of a moving picture the marked activity of spermatozoa within the cervical mucus. The motion of the spermatozoa is much more rapid and their motility is of a different character than that seen in the seminal pool. Such an increase in activity may be expected, for it is doubtful whether the energy necessary for the sperm to travel from the seminal pool to the outer end of the Fallopian tube is all stored within the sperm head. It is more than likely that energy is obtained from the surrounding secretions. Cary (1935) has found motile sperm in the cervical mucus six to twelve hours post-coitum, and in one case sixty hours later.

Many believe that the uterus exhibits a sucking action during orgasm which draws the semen into its cavity (like a rubber ball with a small hole in it), or that by the same action the cervical plug is extruded, dipped into the seminal pool, and then sucked back into the cervix. The objections to this are many. Firstly, orgasm is not necessary for conception. Frigid women conceive as easily as those with orgasm (Dickinson, 1927, 1931). It is debatable whether orgasm in the human is of any aid in conception. Many patients are aware of an orgasm during bimanual or speculum examination. Motions on the part of the cervix are not felt or seen, though there may occur a slight descencus, probably due to the increased vascularity of the uterus. There is no opening or closing of the cervical os. Fertilization may occur in the presence of an intact hymen. The ascent of sperm from the seminal pool to the outer end of the Fallopian tubes is made by the movements of the spermatozoa themselves.

C. Duration of Life of Sperm in the Uterus, and Their Transport. The rapidity of the passage of sperm through the cervix determines the time of their appearance in the uterine cavity. Schuwarski (1894) reported finding sperm in the uterine cavity one-half hour post-coitum. The further progress of the spermatozoa through the uterine cavity is here again determined by their own motility, but in addition against an opposing ciliated stream. Both Hoehne (1911) and Roth (1904) are of the opinion that the ciliated stream towards the cervix serves as an orienting mechanism to the upward motion of the spermatozoa. The rate of motion of sperm in a straight line is 3 mm. per minute, hence the distance of 16 cm. between the internal os and ostium abdominalis tubae would take about an hour. This is on the assumption that the

sperm travelled in a straight line and without the resistance offered by an opposing ciliated stream.

Runge studied the survival of spermatozoa in the uterine cavity of 17 normal multipara, making in all 32 observations. Six hours post-coitum all uteri showed living sperm, while twelve hours post-coitum no living sperm were found in one-sixth of the uteri. At the end of 36 hours living sperm were found in only one-fourth of his cases. Beginning with the third day no living spermatozoa were found.

Haussmann (1879) made seventeen observations in eleven women and found actively motile sperm in the uterine cavity on the third day post-coitum. He also observed some motility of weak character as late as seven days post-coitum. It is extremely doubtful whether spermatozoa whose motility was so markedly diminished were capable of fertilization.

D. Duration of Life of Sperm in the Tubes and their Transport. There are only two observations on record describing the finding of living spermatozoa in the tubes. Zweifel (1892) describes the finding of living spermatozoa by Birch-Hirschfeld in the tubes of a prostitute who died in actu with a man (from carbon monoxide poisoning). When found the body had been dead several hours and the autopsy was performed about 14 to 16 hours later. Dührssen (1893) describes finding living spermatozoa in the uterine ends of closed tubes three and a half weeks post-coitum. The woman had been a patient in the hospital for nine days before the operation. Dührssen subsequently tried to demonstrate spermatozoa in the tubes of other patients but never succeeded. Nürnberger (1920) believed that sperm survived in the tubes as long as fourteen days. The writer believes that these findings are exceptional. The fact that spermatozoa have been found in the tubes only on such extremely rare occasions implies that the survival of sperm in the tubes is extremely short. Those in active motion pass out through the fimbriated end of the tube into the peritoneal cavity, while the sluggishly motile sperm are swept back by the ciliated stream into the uterine cavity.

From the evidence at hand the writer concludes that the fertilizing power of sperm is usually less than forty-eight hours, though motility may remain for a somewhat longer interval. All in all, considering the importance of these topics in the problems of fertility and sterility, the inadequacy of the work done on the viability of spermatozoa in the human uterus and tubes is very startling.

Viability of the Ovum. The human ovum, like the animal ovum, is

short-lived. The evidence for the human is still meagre, for human ova were not obtained for detailed study until recently. Allen, Pratt, Newell and Bland (1930) recovered five human tubal ova by a method of washing out the tubes in situ. Two of the ova recovered were twins, one from each ovary. They were the most nearly normal specimens obtained. One ovum was recovered on the fourteenth, three on the fifteenth, and one on the sixteenth day, after the onset of the previous menses. By correlating the stage of development of the corpora lutea with the condition of the ova, ovulation time was estimated at between the twelfth and fourteenth days of the menstrual cycle. Although the ova were obtained within two, or, at the most, three days from the date of ovulation, three of the ova showed histological signs of degeneration. This is the most direct proof as yet offered for the demonstration of the limited life of the human ovum.

Indirect evidence demonstrating the short life of the human ovum was offered by the writer in 1928. Two clinical histories were offered in support of this view. Both patients were young orthodox Jewesses. They observed the Talmudical law of Niddah, in that they waited two weeks from the onset of the period before coitus was attempted. Because their menstrual cycles were of the three week type it was felt that by the time coitus took place the ovum of that cycle was already dead. Both patients were advised to have intercourse on the seventh day of the period and conception promptly took place. The fertility of the orthodox Jewesses was known to ancient medical writers. The reason for their fertility is that under normal circumstances coitus on the fourteenth day of the cycle is probably the most favorable time for conception.

Spontaneous and Induced Ovulation

In contradistinction to spontaneous ovulation, induced ovulation occurs in the cat and in the rabbit. The impetus to ovulate is coitus. The idea that induced ovulation may occur in women was first suggested by Chazan (1911). He offered no evidence to support his claim. The idea is interesting and is worthy of detailed consideration.

Statistical studies dealing with pregnancies resulting from but a single coitus during the entire cycle were first brought forward by Siegel (1917). His figures show that pregnancy can result from coitus very early or very late in the cycle. These pregnancies may be explained by assuming that ovulation may occur much earlier or later than is supposed or that the viability of sperm in the genital canal is

longer than we assume. The assumption of induced ovulation early in the cycle gains support from the following considerations. The rate of development of the Graafian follicles does not always seem to be a constant one. It is usually taken for granted that the follicle develops in a crescendo manner from its primordial state to maturity, the peak being reached at the time of ovulation. But it is conceivable that follicular growth may proceed to a certain stage and plateau off for a number of days. Ovulation may occur anywhere along this plateau depending upon the point where the impulse to ovulate strikes. It is not an uncommon thing for a gynecologist to find at operation, very early in the cycle (fourth to seventh day), a follicle that grossly appears ripe for rupture. Experimentally, Lane (1935) finds that in the rat the follicle increases abruptly in diameter soon after the first appearance of the antrum and then remains at a relatively constant diameter until ovulation.

The author believes that ovulation may also occur very late in the cycle, specifically, following the so-called anovulatory or sterile cycle. In the normal menstrual cycle follicular growth is prevented during the premenstrual phase by the presence of a functioning corpus luteum, and it is only when the corpus luteum begins to degenerate that a primary follicle begins to mature. In the sterile cycle ovulation does not occur. Because of this absence of regular ovulation a corpus luteum fails to appear and the bleeding which subsequently occurs is cyclical bleeding and not true menstruation. But at the end of such a sterile cycle the inhibiting influence upon the growth of a new follicle is absent because of the absence of a corpus luteum. Therefore, a follicle may develop here and mature, be uninfluenced by the intervening cyclical bleeding, and eventually rupture in due course (or degenerate) early during the next menstrual cycle. Such an unusual follicle, matured just before or during the bleeding of a sterile cycle, may rupture under the stimulus of coitus just before or during or immediately after the advent of the cyclical bleeding. A corpus luteum would form as a result of this induced ovulation, and then continue functioning as the corpus luteum of pregnancy. This concept would explain the undoubted occurrence of a pregnancy as the result of a single exposure very late in the cycle.

The most plausible explanation for the actual rupture of the follicle in the human is a contraction of the smooth muscle fibers in the ovary. Kurzrok et al. (1934) have demonstrated that the human ovary undergoes rhythmical contractions and that adrenalin induces a marked con-

traction followed by a considerable period of spasm. If the ovum is expelled by a contraction of the muscle fibers, then the substance actually causing the contraction of the muscle fibers may originate from some other source, such as the posterior pituitary or the medulla of the adrenal, for the adenohypophysis does not secrete a hormone capable of contracting smooth muscle. The excitement of coitus causes a rise in blood pressure, and the adrenalin produced as a result of the stimulation may be sufficient to cause the rupture of the follicle, premature as to time, but not premature as to state of development.

The So-called "Safe Period"

Within the past five years a large body of popular literature has grown up about this subject. The safe period is advocated by many individuals and groups, who do not always fully understand the basic physiology involved. As stated previously, the period of fertility depends on three factors, all variable, and not as yet completely determined. It may be true that a woman has a safe period every month, but how are we to determine it with our present state of knowledge? If ovulation occurs on a given day this month, will it occur on the same day next month, or the one after? What are the limits of ovulation within the menstrual cycle in any woman observed over a long period of time? Are the spermatozoa of an individual always endowed with the same capacity to carry on in the female genital canal, or are there variations in this capacity, thus limiting or increasing their viability? What activating effects have the secretions of the female genital canal upon spermatozoa? That such activation occurs in the cervix is beautifully illustrated by the motion picture film of Cary and Hotchkiss. Is this activation a variable factor in the same and different individuals? If in addition to the above complex factors there is added the possibility of induced ovulation, then the safe period becomes neither safe nor periodic.

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CHAPTER XVI

THE CONTROL OF THE UTERUS

Motility of the uterus depends on three factors, namely:

1. Those factors that are inherent within *smooth muscle* itself.

2. Nervous control through the autonomic nervous system. There is both an extrinsic and an intrinsic innervation.

3. Control through chemical substances circulating in the blood, especially hormones.

These factors are not of equal importance. There may exist special circumstances which determine the factor that is to play the most important and controlling rôle.

Inherent Properties of Smooth Muscle

The uterus is an involuntary, smooth, unstriated muscle. It possesses the spontaneous rhythmic activity common to all such muscle. Like all hollow viscera it adapts itself to its contents, maintaining at all times a distinct tonus. It can be distended, thus increasing the tension within the organ. The contractions resulting from the introduction of a foreign body into the uterine cavity may not be the same as the spontaneous contractions of the uterus at rest. This is of importance, especially in view of the recent studies of uterine motility by means of a hydrostatic intrauterine bag. It is questionable whether such contractions exemplify the spontaneous movements that occur in the uterus. They may merely represent expulsive efforts on the part of that organ to rid itself of a foreign body. There is ordinarily very little space between the uterine walls and the introduction of a bag results in the formation of a distinct cavum uteri.

Studies of uterine motility have therefore been made both in vivo and in vitro. Reynolds has confined his work chiefly to the rabbit, while Robson, Moir, Knaus, Miller, Kurzrok and Cockrill have utilized the human uterus. Kurzrok et al. (1936) have investigated the human uterine strip, and more than six hundred specimens have been set up and their reactions recorded. We believe that the reactions of the human uterine strip in vitro are similar, if not identical, to those observed in the intact human uterus in vivo. Knaus, Moir, Kurzrok et al. (1937) and others have studied the contractions of the human uterus

in vivo by means of an elastic intrauterine bag. The question remains open as to whether the contractions observed are not abnormal, in that they represent the expulsive efforts on the part of the uterus to rid itself of a foreign body. It might be argued that the uterine strip is also not a true indicator of the contractions that go on in the human uterus in situ. The strip is severed of all nervous control. It is true that in the body the uterus is subject to both motor (sympathetic) and inhibitory (parasympathetic) nervous influences. But, at the same time, facts have accumulated showing independence of this nervous control. Thus, in the rabbit, Rein (1902) reported the spontaneous birth of young following section of all extrinsic nerves to the uterus, while Kaminester and Reynolds (1935) demonstrated that the transplanted uterus is subject to hormonal control and the extrinsic and intrinsic innervation are nonessential. Cannon et al. (1930) reported spontaneous parturition in cats and dogs following complete extirpation of the sympathetic trunks. J. Novak (1928) in discussing the effect of lesions of the central and peripheral nervous systems quotes numerous cases from the literature showing that even very extensive lesions may have no effect on ovulation, pregnancy or labor. Parturition following complete paralysis of the lower half of the body by reason of lesions or transection of the spinal cord, proceeds very frequently with abnormal rapidity.

The Hormonal Control of the Rabbit Uterus

In order to understand the hormonal control of the human uterus it is helpful to study the same phenomenon in the rabbit. The human and the rabbit uterus may show some similar reactions, but on the other hand they need not necessarily do so. As we shall subsequently show, one cannot draw a conclusion from one species and then apply it, without modification, to another.

The following facts have been demonstrated for the rabbit uterus:

I. Reaction to Sex Hormones. *A. In Vivo.* 1. Estrone is necessary for the initiation and maintenance of rhythmic motility of the normal uterus.

2. Progesterone exerts a vary strong inhibiting effect on such contractions.

B. In Vitro. 1. Estrin added to the perfusion bath in any concentration will not initiate spontaneous rhythmic motility in a quiescent uterus.

2. Progesterone is only very slightly soluble in water, hence the

failure to inhibit contractions may be due to the low concentration of the hormone in the perfusion fluid or to the necessity of a prolonged action of progesterone on uterine muscle.

II. Reaction to Pituitrin (Oxytocin). When pituitrin is added to the perfusion fluid or is injected into the rabbit, the reaction obtained depends upon the influence of the predominating hormone.

1. When the uterus is sensitized to estrone the result is a contraction.
2. When sensitized to progesterone the effect is absent—no contraction. (There is no "reversal", that is, relaxation.)

During pregnancy pituitrin produces no effect during the early stages, but an increasingly stronger effect as the period of labor approaches. Several days following parturition the reactivity of the uterine muscle to oxytocin decreases markedly, and is independent of whether the animals are suckling or not (Robson, 1933).

The Hormonal Control of the Human Non-Pregnant Uterus

Before we subject the human uterine strip to the action of any chemical substance, it is essential to determine whether the strip is "alive" or not, that is, whether it shows spontaneous contractions. Strips that fail to show spontaneous contractions at the end of one hour in the perfusion fluid (Ringer solution) are discarded. Why some strips will not show any spontaneous contractions is, at present, unknown to us. It does *not depend* upon the phase of the uterine cycle. It may be due to excessive handling at time of operation or to the anesthetic (gas oxygen and ether in almost all cases). As a rule adjacent muscle strips show a similar lack of activity. Nor does it depend upon the length of time the uterus has remained outside of the body, for the strips are set up within a few minutes after the hysterectomy has been performed. Nor does the failure to exhibit spontaneous contractions depend on any pathology within the uterus, for only uteri free from pathology are used, barring the presence of one or two *small* fibroids. Only uteri within the child-bearing age are utilized.

Spontaneous Uterine Contractions. The spontaneous rhythmic motility of the uterine strip varies considerably. There are variations of tempo, rhythm, and extent of contractions in the different strips. Parallel strips from the same uterus behave similarly. When two strips from different uteri are set up in the same chamber each one maintains its own type of spontaneous motility independent of the other. The reasons for these variations are unknown. They are independent of the menstrual phase during which the uteri were removed. Nor are

they functions of the various muscle coats. We are not justified in our present state of knowledge in reading into these variations any special functional significance. Figure 107 shows some of the various types of contractions that we have encountered. Furthermore, one must be careful in the interpretation of the results obtained when various sub-

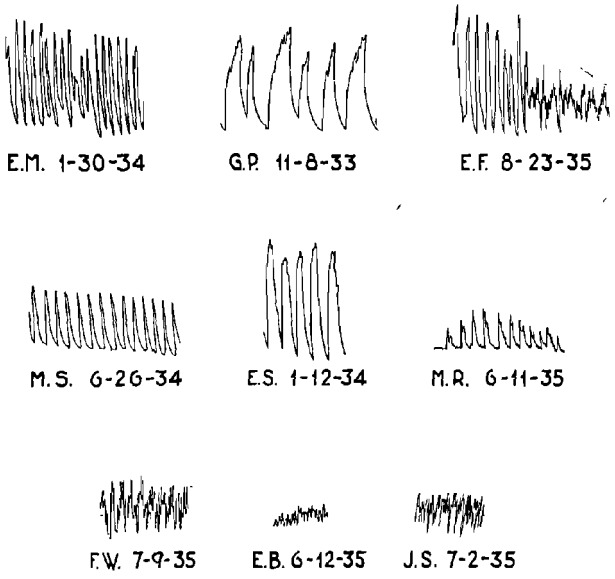

FIG. 107. Spontaneous contractions of the human uterine strips in vitro: E. M., sixth day of cycle; G. P., guinea pig uterus during estrus; E. F., at end of six weeks bleeding. Note spontaneous change of rhythm and character of contractions; M. S., early in cycle (before tenth day); E. S., early pregnancy (fortieth day). It is expected that the corpus luteum of pregnancy would inhibit such contractions; M. R., during menstrual period; F. W., twenty-fifth day of cycle; E. B., twenty-fifth day of cycle; J. S. twenty-first day of cycle.

stances are added to the perfusion fluid. Minor variations in the course of the graph are without significance for they may occur spontaneously. Only definite and unmistakable changes should be accepted as pharmacological reactions.

Reaction to Hormones. Is estrone essential for contractility of the human uterus? For the demonstration of such an action it is essential

to test (in vivo or in vitro) a uterus that has not been subject to recent ovarian influence, namely, one secured after a previous bilateral oöphorectomy, or a uterus from a patient long after the menopause. In the latter case it is essential that the ovaries be atrophic and present no maturing follicles. The following case is of interest. The patient was a woman aged 60. Menopause occurred twenty years previously and upon examination the entire genital tract was found senile. The contractions were similar to those seen in uteri of patients well within the menopause age. Furthermore, the strip reacted promptly and vigorously to pituitrin. Two interpretations are possible. Either estrin is not necessary for spontaneous contractility and a positive reaction to pituitrin, or that a senile ovary produced enough estrin to sensitize the uterus. The latter explanation is the less probable, for the entire genital tract was senile, and furthermore our clinical experience with cases of primary amenorrhea has taught us that the uterus is the last organ to respond (in growth) when *large* doses of follicular hormone are administered. There remains one other remote possibility, and that is that while the hormone estrin was no longer present in the circulation, other estrogenic substances, such as derivatives of cholesterol or cholic acid were present in concentrations large enough to sensitize the uterus.

Change in the Reactivity of the Human Uterus. Knaus (1929 and later) stated that when the human (and also the animal) uterus is under the influence of the corpus luteum pituitrin will no longer produce an increase in tonus. In a twenty-eight day cycle this reversal first appeared on the sixteenth day and lasted to the twenty-seventh day. Since a certain stage of development of the corpus luteum was essential before enough progesterone could be produced, Knaus placed the beginning of corpus luteum formation, or ovulation, on the fourteenth day. Knaus employed first a 10 per cent iodopin and later a small intrauterine bag. He allowed the uterus to contract spontaneously for 30 minutes so as to establish a base line, and then injected 1 Vogtlein unit of pituitrin intravenously. Manzi and Luigi (1931) demonstrated that the human uterine tube in vitro showed increased motility during the follicular phase of the cycle and diminished activity during the corpus luteum phase. The former action favored the transport of the ovum and the latter favored nidation in the endometrium. This explanation is not quite satisfactory, for during the period of greatest motility there is no ovum to transport. It is necessary to assume that this period of greatest motility of the tube extended for some time beyond the period of ovulation.

Knaus aimed to establish by his method the exact date of ovulation. This was an important observation and led to considerable literature. Schultze (1931) repeated Knaus' early experiments and concluded that early in the cycle there is little reaction to pituitrin, but the reaction increases as the cycle advances, the maximum effect being obtained just before menstruation. Wittenbeck (1930) also disagreed with Knaus. He checked his curves by subsequent laparotomies. He found spontaneous contractions and a rise in tonus from pituitrin in the presence of well developed corpora lutea. Hermstein (1931) was more in agreement with Knaus but added that inflammatory conditions in the pelvis can inhibit the motility of the uterus in the absence of a corpus luteum. Tachezy (1934) did not substantiate Knaus. The greatest increase in tonus as the result of pituitrin occurred during the second half of the cycle, namely, during the corpus luteum phase. Five cases of early pregnancy, ranging from the second to the fifth

Action of Pituitrin on the Human Uterine Strip

Day of menstrual cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Beyond 29
Pit contr.	1	0	0	0	0	2	4	3	4	2	2	3	5	3	2	4	4	5	3	4	4	1	1	2	2	3	3	3	2	8
Pit no effect								1	2								1		2			1				1				1
Pit relaxed										1																				

FIG. 108. Number of uteri examined during various days of a regular menstrual cycle. None were obtained during the second, third, fourth, and fifth days of menstruation; we usually do not operate during menstrual flow.

month reacted to pituitrin by an increase in tonus. The last observation is interesting, for Hoehne (1925) suggested the intravenous injection of a drop of pituitrin as a test for pregnancy. An immediate contraction signified pregnancy. This is in direct opposition to the concept that the corpus luteum of pregnancy (or the placenta) protects the uterus against the effect of pituitrin.

These observations throw considerable doubt on Knaus' dictum that the time of ovulation can be ascertained by the reaction of the human uterus to pituitrin.

This problem was also studied by Kurzrok, Cockrill and Miller (1936) on the *human uterine strip* (fig. 108). The date of the cycle on which the operation (hysterectomy) took place was known, and in addition the endometrium was carefully studied to determine the menstrual phase. When the ovaries were removed additional information was obtained. More than one hundred uteri have been investigated in

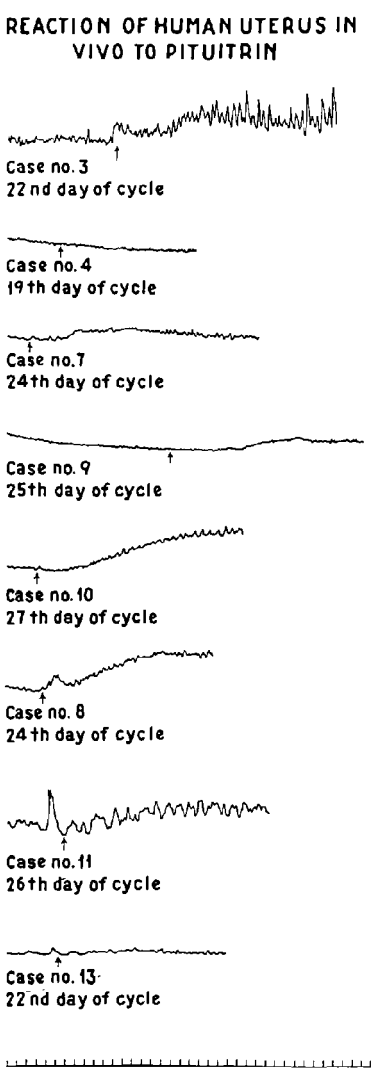

FIG. 109. Reaction of human uterus in vivo to pituitrin. In *all* the graphs the arrow has been placed from one to two minutes *after* the time of injection. It was due to the technical set-up. All graphs are greatly reduced for purposes of reproduction. Illustration of case 4 was included to show our conception of a negative response. There is some increase of rate noticeable in the graph. In case 9 the reaction was delayed, possibly because the injection was made into fatty tissue. Time 1 minute.

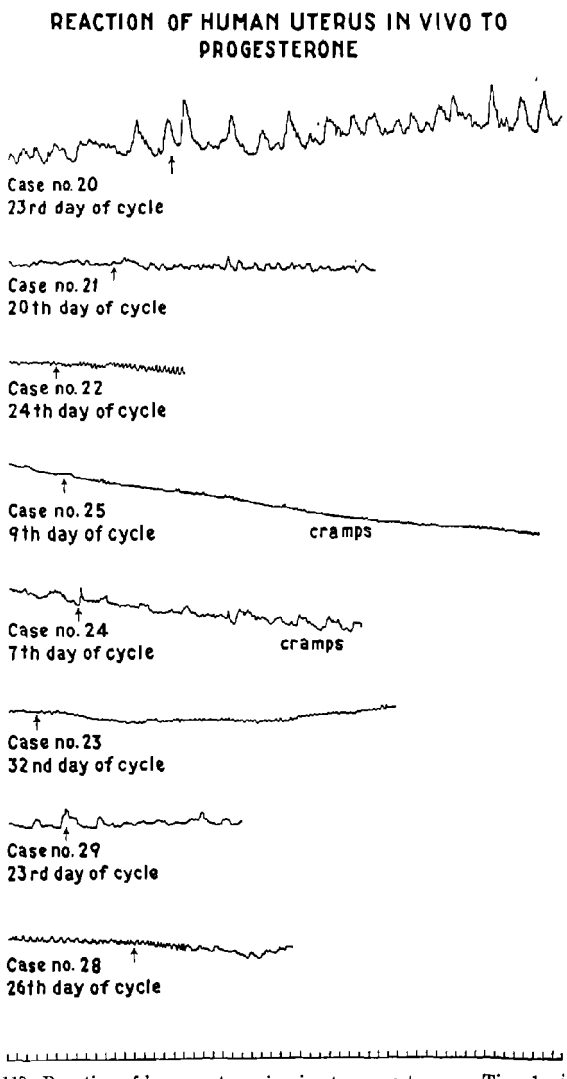

FIG. 110. Reaction of human uterus in vivo to progesterone. Time 1 minute

this manner. The uterus showed spontaneous activity during all phases of the menstrual cycle. There was no inhibition of spontaneous motility during the corpus luteum phase. The uterus reacted to pituitrin during all phases of the cycle. In nine per cent of the cases the uterus did not react to pituitrin, and it was not due to corpus luteum inhibition, for cases occurring on the eighth and ninth day of the cycle showed a typical postmenstrual endometrium.

Still further doubt was thrown on the current concept of the action of corpus luteum on the human uterus by the recent observations of Kurzrok, Wiesbader, Mulinos and Watson (1937). They studied the

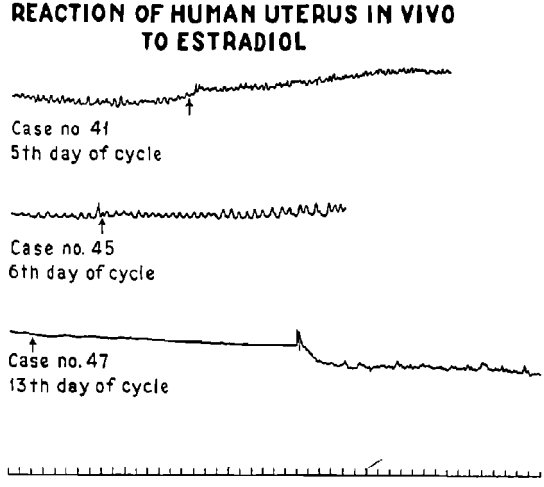

FIG. 111. Reaction of human uterus in vivo to estradiol. Time 1 minute

reaction of the human uterus by means of the intrauterine bag. Their technique resembled that of Knaus, and in addition they studied the action of estradiol and progesterone in vivo (figs. 109, 110, and 111). The results of their experiments led them to conclude that:

1. The uterus shows spontaneous contractions during all phases of the menstrual cycle.

2. The uterus reacts to pituitrin in a positive manner during all phases of the cycle. There is no corpus luteum inhibition, hence the reaction offers no evidence as to the time of ovulation.

3. Progesterone may occasionally produce an increase in tonus and amplitude of contraction.

4. Progesterone produces uterine cramps during the postmenstrual phase of the cycle, but not during the premenstrual phase.

5. The cramps are not associated with significant changes in the graph nor with a previous history of dysmenorrhea.

6. Estradiol (benzoate) occasionally produces a rise in amplitude and tonus.

7. Estradiol (benzoate) may produce cramps during the first half of the cycle, without significant changes in the tracing.

8. Oily solutions of progesterone and estradiol benzoate are rapidly absorbed from the local site of injection, the effects being observed within thirty minutes.

These findings throw still greater doubt on Knaus' test for ovulation. Furthermore, the above findings show that the corpus luteum in the human does not inhibit spontaneous uterine contractions nor inhibit the reaction to pituitrin. The hormonal control of the human uterus is not identical with that of the rabbit.

The Mechanism of Parturition

The cause of the onset of labor has received considerable attention in the hands of various workers. Several theories have been suggested, namely,—

1. The regression of the corpus luteum.

2. The action of estrin upon the uterus, alone, and in association with oxytocin.

3. The production of a particular substance which induces labor, a substance similar in action but not identical with pituitrin.

I. The Regression of the Corpus Luteum. In view of the fact that the corpus luteum protects the fertilized ovum against early miscarriage the idea was extended to include the concept that labor sets in when the corpus luteum of pregnancy begins to degenerate. It was soon demonstrated however, that bilateral oöphorectomy after the sixth week of pregnancy does not induce abortion. The suggestion was therefore made that the placenta takes on the function of the corpus luteum and that labor sets in when the inhibitory placental influence upon the uterus is removed. In other words, as the placenta reaches term ("ages") its ability to produce inhibitory substances lessens. As long ago as 1903, Halban suggested that the placenta was a gland of internal secretion. There are no objections from a histological viewpoint to this concept. Loewe and Voss (1934) transplanted a fragment of human placenta into a mouse, and noted the appearance of rut. The

cells of the transplant were found alive three months after operation. Waldstein (1929) performed a bilateral oöphorectomy on a woman on the 34th day of gestation and noted that the blood and urine contained follicular hormone in the subsequent months of pregnancy. The patient went to term, and one week after term no estrone could be detected in the urine. The estrone content of the placenta was normal. That the foetus does not influence the onset of labor is evidenced by the well known clinical fact that the placenta will maintain function several months after the foetus dies, and that labor will set in at about the expected time. The placenta produces prolan A and B, as well as estrin, and recently (1934) Ehrhardt found corpus luteum hormone as well. De Snoo (1922) reported the successful treatment of repeated abortion with placental extract. Damon (1936) and Kurzrok (1936, unpublished) have confirmed his results. Whether the inhibition of the onset of labor by means of placental extract is due to the presence of corpus luteum hormone or some other unknown factor is still a debatable question.

II. The Action of Estrin upon the Uterus, Alone, and in Association with Pituitrin. It was stated previously that estrin sensitizes the animal uterus. Whether this is true of the human uterus, and to what extent, is still an open question. The uterus is subjected throughout pregnancy to large concentrations of follicular hormone that are present in blood. The purpose of excess of estrin is not known. It is present in increasing concentration as pregnancy progresses and rapidly disappears from the blood stream about 10 days postpartum. The injection of estrin into rats and mice causes abortion (M. Smith, 1926; Parkes, 1930; Hain, 1935). The amount of estrin required to interrupt pregnancy rapidly increases as pregnancy advances (M. Smith). It is occasionally possible to induce abortion clinically during the second month of pregnancy. It has been our experience that when moderate doses (2,000 R.U.) fail, very large doses (40,000 to 80,000 R.U. in one week) are also without effect. The mechanism of induction of abortion by means of follicular hormone is not clear. It may be due to hemorrhage resulting from the increased blood supply to the uterus, for it has been shown by means of ocular implants in the rabbit that follicular hormone has a vasodilator effect on the capillaries of the endometrium (Markee, 1932). The separation of the ovum from its bed causes the uterus to expel it as a foreign body.

That placental and ovarian extracts exert a synergistic action in

sensitizing the uterine muscle to the secretion of the posterior pituitary has been known for some time. Dixon (1923), and Marshall (1924) and Fenn (1924) observed that extracts of the whole ovary, but *not* the corpus luteum increased the uterus contracting substance of the cerebrospinal fluid. Friedman and Friedman (1933) found less oxytocin in the cerebrospinal fluid than has been claimed by Dixon and Marshall. The identity of the uterus contracting substance in the cerebrospinal liquor with oxytocin has been established by Dixon, and by Miura (1926). The closer the animal from which the ovaries were removed was to term, the greater was the stimulating effect. They concluded that, associated with the regression of the corpus luteum of pregnancy, the ovary produced increasing amounts of a substance that stimulated the posterior pituitary to increased secretion of oxytocin. Mayer noted an increased amount of oxytocin in the cerebrospinal fluid of women in labor. Siegert (1927) and Van Dyke and Kraft (1927) could not confirm Mayer's observation. Brdichka (1924) noted an increased concentration of oxytocin in the blood serum of women in labor. Thus a theory for the etiology of the onset of labor was proposed, namely, that as the corpus luteum regressed, the ovary produced increasing amounts of substances (estrone?) that stimulated the posterior pituitary. An immediate objection can be raised to this theory. Bilateral oöphorectomy during pregnancy does not prolong pregnancy, and labor occurs in the usual manner. Furthermore, it has been demonstrated that parturition can occur in the absence of the posterior pituitary (Smith, 1932, rat; Allan and Wiles, 1932, cat). The question arises whether the placenta with its multitude of functions during pregnancy does not also replace or supplement the oxytocic function of the posterior pituitary gland.

The rôle of the anterior pituitary gland in parturition has now received considerable attention. In the rat the removal of the anterior pituitary gland after mid-pregnancy causes gestation to be prolonged. The mother dies for she is unable to litter (Pencharz and Long, 1931; Selye, Collip and Thomson, 1933). Hain (1932, 1934) noted that extracts of pregnancy urine caused gestation to be prolonged. On the contrary very large doses of Antuitrin "S" administered later in pregnancy caused abortion.

The Synergistic Action between Estrin and Oxytocin during Pregnancy. Parkes (1930) demonstrated that abortion can be induced in mice by the synergistic action of estrin and oxytocin. Hain (1935) was unable to confirm this in the rat. The synergism in the human uterus

is not so readily demonstrated. It has been observed clinically that the rhythmic contractions of the pregnant uterus increase both in frequency and intensity during the course of pregnancy. This is by no means universal. We have observed uteri during the latter part of pregnancy and have not noted any increased frequency of contractions. Some uteri are extremely responsive to the examining hand. On the contrary the writer has frequently done an external version during the eighth or ninth month without eliciting any, or at most very few contractions. Bourne (1933) recorded the uterine contractions at the thirty-sixth week of pregnancy and noted that they were similar to those recorded during the early stages of labor. Robson (1933) found no evidence to show that the degree of spontaneous rhythmic activity exhibited by strips of human uterine muscle removed at different stages of pregnancy and at parturition, increased progressively as term and labor approached. The experience of Miller, Kurzrok, and Cockrill has been similar (unpublished data). Robson (1933) studied the behavior of human uterine muscle to oxytocin during the various phases of gestation and noted that the minimal dose of hormone required to produce contractions *decreased* as term approached; the limits being more than 2 oxytocin units during the twelfth week and 0.003 unit during labor.

In view of the fact that in some animals labor may proceed in the absence of the posterior pituitary the question has arisen as to whether pituitrin is the only oxytocic substance and whether the posterior pituitary is the only source of oxytocic substances. Certainly, the hypothalamic region and the placenta cannot be overlooked. Recently, Cockrill, Miller and Kurzrok (1934) studied the urine of patients in *active labor* as a source of oxytocic substances. It was previously demonstrated (Guerin-Valmale, Loriot and Verdeuil, 1931; Barjaktrovic, 1933) that labor could be induced by administering labor urine by proctoclysis. Labor could only be induced in the second half of pregnancy. Amounts up to 4,000 gm. were necessary, and some patients were delivered 12 hours after instillation.

Cockrill et al. (1934) collected the urine from patients during *active labor* and combined the specimens until a total volume of 10 or 12 liters was obtained. (Pituitrin was not given to any case.) Thirty such collections were made, and each was worked up separately. The samples were acidified with acetic acid and concentrated in vacuo at a temperature of 30° to 40°C. The method then used to obtain the oxytocic substance was that described by Kamm and his co-workers (1928) for isolation of the active principles of the posterior lobe of the pituitary gland.

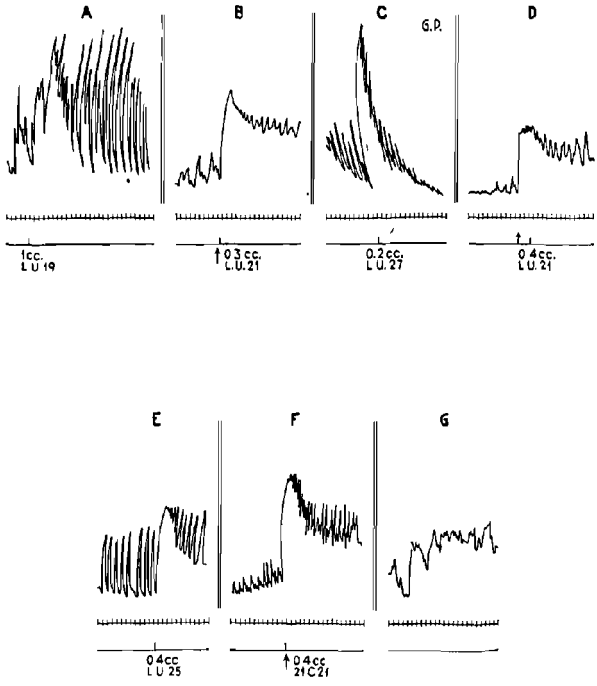

FIG. 112. Action of labor urine extract on the human uterine strip. All strips were obtained from sections at term. Note the variations in the spontaneous contractions and the extent and type of response. Figure *G* shows the irregular contractions of a strip from the lower uterine segment. Figure *C* is the response of a guinea pig.

The final product was a crude material of a gummy character and colored with urinary pigments, 10 liters of labor urine yielding an average of 0.5 gram. This dried material was taken up in distilled water and dialyzed over night in a refrigerator in a parchment bag. The solution from the bag was then made neutral to litmus with sodium carbonate. The solution was tested, using about 0.4 cc. from a total volume of 25 cc. representing the original 0.5 gram, on human uterine muscle obtained from Caesarian sections at term. Twenty-five strips of muscle were used. The muscle was set up according to the technique described by Kurzrok and Lieb (1930). The extract obtained from these fractions caused a prompt and sustained contraction (fig. 112). When the solutions were tested on non-pregnant human uterine muscle (55 strips, obtained at various times of the menstrual cycle), there was no effect or a slight increase of tonus causing the normal contractions to occur with slightly more vigor. Control experiments were made, using material prepared by the same procedure from 2 10-liter samples of urine of male patients and 3 from non-pregnant women. These control materials, tested on parallel strips of muscle from the same pregnant (25) and non-pregnant (55) uteri as were used for the labor urine extracts, gave negative results, although a few gave slightly increased tonus.

The active material was tested by Dr. Mulinos, of the Department of Pharmacology, for its action on the water balance of the frog, and the result indicated that the substance has a pitocin-like action. The controls when tested in the same way had no significant effect.

The Action of Human Semen upon the Human Uterus

The action of human semen upon the human uterus has proven to be a striking phenomenon, one that is involved in the process of fertilization, and again in certain types of sterility. Furthermore, one of the substances, acetyl choline, which partakes in the reaction has been held by some to be a hormone. These considerations have led us to include this work in the chapter on the hormonal control of the uterus (also in Chapter XXVIII).

Kurzrok and Lieb (1930) reported that human semen caused a relaxation of the human uterine muscle. There occurred a marked loss of tonus and a loss of contractions over a period of one-half to one hour or more. In certain cases, depending apparently on differences in the semen, or in the uterus, or in both, the effect was contraction. These effects have been demonstrated both in vitro and in vivo. When a cer-

tain semen specimen relaxed most uteri, an occasional uterus would respond by contraction. On the contrary, some semen specimens (about 15 per cent) always caused contraction. Cockrill, Miller and Kurzrok (1935) studied these phenomena further and observed the following:

1. The pregnant human uterus always responded to human semen by contraction.
2. The phase of the menstrual cycle had no effect upon the uterine response to semen.
3. *Non*-human uteri (rat, rabbit, guinea pig) respond to human semen by *marked* contraction. (A human uterine strip in the same chamber relaxes.)
4. The contracting substance in semen is probably acetyl choline, or some very similar compound.
5. There is suggestive evidence that the relaxing substance is also acetyl choline, but in *different* concentration.
6. The effects of human semen upon the human uterus are independent of the presence or absence of sperm.
7. Crystalline spermine has no effect on the human uterus.

The Action of Ergot and Its Derivatives upon the Human Uterus

The fungus ergot contains a mixture of alkaloidal and non-alkaloidal substances having various pharmacological activities. Tauret (1875) isolated the first pure substance, ergotinine, but it was inert. Barger and Dale (1907) isolated a second alkaloid, ergotoxine, which possessed all the experimental pharmacological actions of ergot. Stoll (1921) extracted two further alkaloids, ergotamine and ergotaminine. The latter is inactive. Ergotamine and ergotoxine are equivalent pharmacologically although slightly different chemically. Recently Adair et al. (1935) have isolated a crystalline substance from ergot having the empirical formula $C_{21}H_{27}N_3O_3$, which they named ergotocin. The same substance (in all probability) has been isolated by other workers; ergometrine (Dudley and Moir, 1935), ergobasine (Stoll, 1921), and ergostetrine (Thompson, 1935). A lively discussion is at present in progress as to which name is to be adopted (Dale, 1935).

The method used for the study of ergotocin was first described by Bourne and Burn (1930) and subsequently employed also by Moir (1932). It consisted of the introduction of an hydrostatic bag into the uterine cavity of a woman on the fifth or sixth day post-partum. The contractions were then recorded on a suitable kymograph. A

similar apparatus was previously used by Pierce Rucker (1930) who introduced a Voorhees bag during labor, and thus studied the reaction of the uterus subpartum to various drugs. Adair et al. obtained the following effects with ergotocin. It is active in doses of 0.2 mg. by mouth. Its effect manifests itself in six to fifteen minutes and produces an increase in tonus and frequent uterine contractions. Ergotocin has a considerable margin of safety and has no apparent detrimental effect upon respiration, pulse, blood pressure and urinary output.

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CHAPTER XVII

TESTS FOR PREGNANCY

It has been the aim of gynecologists to make the diagnosis of pregnancy without the aid of a physical examination. The physicians of antiquity concerned themselves with this problem. The earliest mention of the subject is to be found in the Papyrus-Kahun. This manuscript dates from 2000 B.C. and was discovered by Sir Flenders Petrie in 1889. Therein is a consideration of the problems whether a woman *can become* pregnant or not, and also whether she *is* pregnant or not. The basis for the diagnosis of pregnancy is as follows (the text is here incomplete): the physical condition of the breast, the readiness with which vomiting can be induced, the testing of muscular irritability and the color of the face. It is rather startling to find that this papyrus reports on a method of prevention of conception, and a method of determining the sex of the foetus in utero.

The twentieth century—a space of 4000 years—finds us again deeply interested in a biologic test for pregnancy as evidenced by the Abderhalden test, the phlorizin reaction (Kammitzer and Joseph, 1921), the adrenalin reaction (Roubitschek, 1922), the glucose tolerance test (Frank and Nothmann, 1920). The last three depend on abnormalities of the renal threshold for glucose which occur during pregnancy (and in other related states). The tests demonstrating renal glycosuria are not reliable for pregnancy. Of recent years the Abderhalden test has had few followers, mainly because of the difficulty in technique. Its accuracy has been disputed by many. In 1928 Professors Selmar Aschheim and Bernard Zondek, then of the University of Berlin, announced their biologic test for pregnancy. The Aschheim-Zondek Test, as it is now known, is now a standard laboratory method the world over.

The Biological Basis for the Aschheim-Zondek Test

The test is based on the following two biological principles.

1. The injections of extracts (or implants) of the anterior pituitary gland into *infantile* mice (or rats) induces in these animals precocious sexual maturity. This manifests itself primarily by changes in the ovaries, and secondarily by changes in the tubes, uterus and vagina (Smith and Engle, 1927).

2. Substances having similar gonadotropic properties are found in the blood and urine of women who are pregnant (Aschheim and Zondek, 1928).

It is important to remember that there are no hormones specific for pregnancy, and that the gonadotropic hormones, here prolan A and B may be found in other conditions. The Aschheim-Zondek Test is merely quantitative and not qualitative. This being the case, the first appearance of a positive Aschheim-Zondek Test will vary with different patients, for the rate of increase of hormone production would not be the same in all patients, and their renal thresholds will not be identical.

The Hormones Involved in the Test and Their Actions

There are two hormones involved in the Aschheim-Zondek Test, namely prolan A and B. As stated in Chapter VII the name prolan is used only when the source of the hormones is pregnancy urine. Prolan *is similar but not identical* with the gonadotropic hormones of the anterior pituitary gland. For prolan to be effective the anterior pituitary gland is essential, hence the test could not be carried out on a hypophysectomized mouse (Evans, Meyer and Simpson, 1932).

The action of these hormones on the *immature* ovary is to produce three types of response (Aschheim and Zondek, 1928).

Reaction I. Follicle maturation, with the formation of large follicles. This is due to prolan A. It is *not* specific for pregnancy for it may be found after castration (also spontaneous menopause) and in cases of genital carcinoma. This reaction may precede a typical Aschheim-Zondek Test, and may also terminate it (Kurzrok, Kirkman and Creelman, 1934) (fig. 113).

As a result of the stimulation of the ovary by means of prolan A, the follicles respond by the production of follicular hormone. A secondary reaction is thus set up, namely stimulation of the uterus so that it becomes distended with fluid, and, in addition, the vaginal orifice opens and a positive smear results. The secondary reaction has no bearing on the Aschheim-Zondek Test and is to be disregarded.

Reaction II. Hemorrhage into the follicles. This describes the "Blutpunkte" of the German writers. This reaction *is pathognomonic* of pregnancy and is probably due to the action of both Prolan A and B.

Reaction III. Formation of corpora lutea with imprisonment of the ovum (corpora lutea atretica). This reaction is due to prolan A and B. The normal rhythm of follicle growth—rupture—corpus luteum forma-

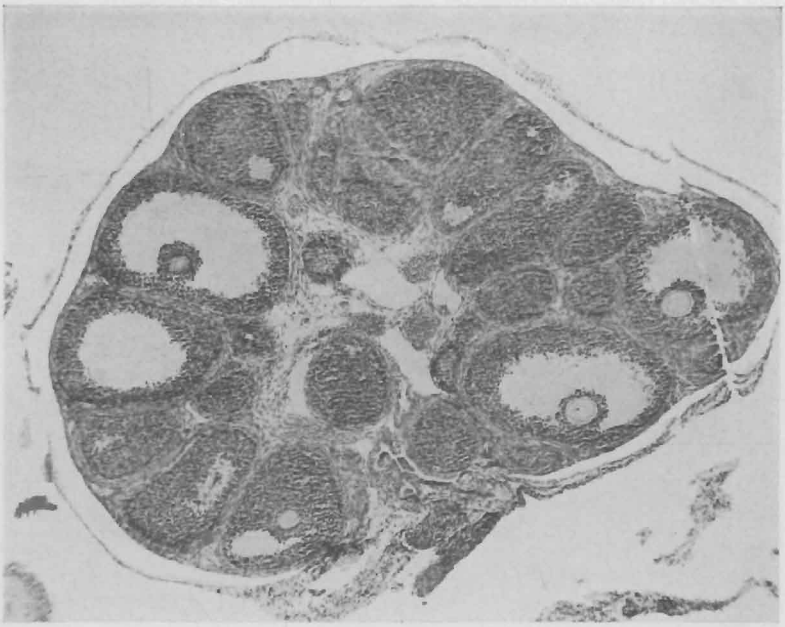

FIG. 113A. Positive follicle stimulating hormone reaction (F.S.H.). Infantile mouse. Follicles only. There are no corpora. There is beginning antrum formation in the growing follicles. This is *not* a positive Aschheim-Zondek test.

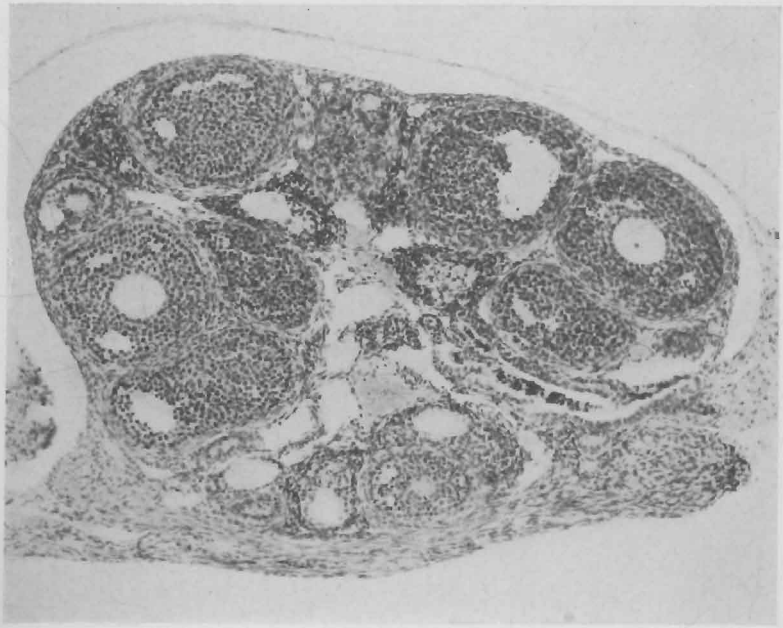

FIG. 113B. Negative Aschheim-Zondek test. Infantile mouse. A negative F.S.H. reaction would appear the same. No large follicles, or corpora lutea.

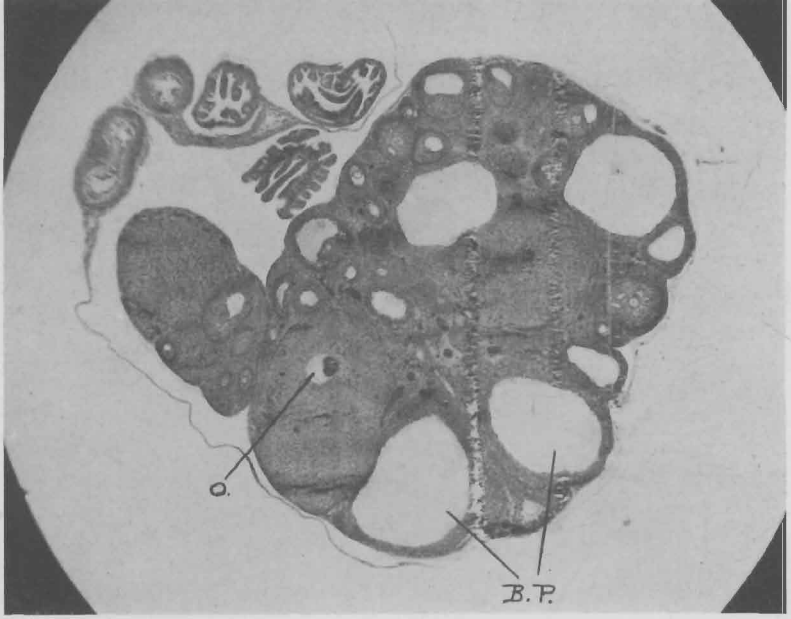

FIG. 114A. Positive Aschheim-Zondek test. Immature rat. *B.P.*, blood points ("Blutpunkte") or hemorrhages into follicles. *O*, ovum within a corpus luteum.

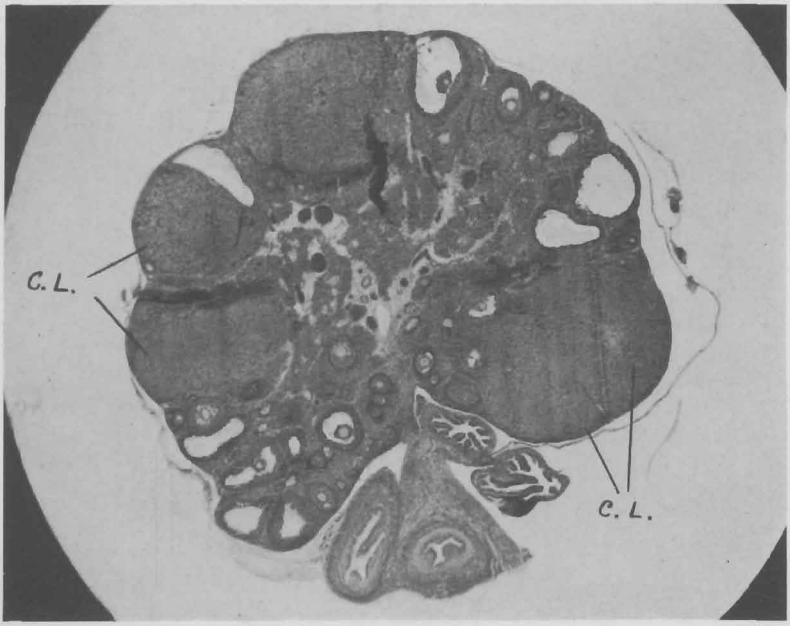

FIG. 114B. Positive Aschheim-Zondek test. Immature rat. *C.L.*, corpora lutea.

tion is disturbed and the follicle luteinizes before rupture occurs. The formation of corpora lutea in an immature ovary *is pathognomonic of pregnancy*. Prolan B, alone, does not stimulate the ovary, unless it has previously been stimulated to form follicles by prolan A, for we could not have a corpus luteum unless there was a follicle.

The Source of Prolan A and B

In view of the fact that prolan A and B have a similar action to the gonadotropic hormones of the anterior pituitary gland, it was assumed that the origin of prolan was this gland. This concept seemed to gain additional support from the fact that the anterior pituitary gland enlarges during pregnancy (Erdheim and Stumme, 1909), while the neurohypophysis seems compressed. The enlargement is due to the formation of pregnancy cells, while the basophiles and acidophiles are definitely decreased. But several investigators soon showed that not one of the three reactions could be produced by implanting the anterior lobe from pregnant women (Philipp, 1930; Ehrhardt and Mayes, 1930). On the contrary, Aschheim was able to reproduce the reaction with transplants of human placenta (also decidua, corpus luteum of pregnancy). The evidence points to the chorionic epithelium as the source of prolan B.[1] Two reasons could explain the absence of the hormones in the anterior lobe: (1) the rate of secretion of the hormone is very slow; and (2) the rate of excretion of the hormone is so rapid that none is found in the implant. The first reason does not seem plausible because of the large amounts of prolan A and B that are found in the blood and urine. Zondek favors the latter hypothesis, pointing out the fact that the thyroid in Graves' disease contains less thyroxin than the normal thyroid.

The Technique of the Test

The technique as described by Aschheim and Zondek calls for six infantile mice, weighing from 6–8 grams each. A *first morning* specimen of urine is collected, acidified slightly with dilute acetic acid, and the *same* specimen is utilized for injections throughout the duration of the test. Each animal is injected twice a day with 0.25 cc. of urine subcutaneously under the skin of the back, for three successive days. Thus each animal will receive a total of 1.5 cc. of pregnancy urine. No injections are given on the fourth day, and the animals are sacrificed on the fifth day. The test, therefore, takes about 100 hours.

[1] Evidence is accumulating to show that prolan A is derived from the anterior pituitary.

The ovaries are then examined in situ, in a good light (preferably daylight), and if necessary with the aid of a lens. If the test is positive the ovaries will show corpora lutea, or punctate hemorrhages, or both. A single corpus luteum or punctate hemorrhage in any of the twelve ovaries is sufficient to make a diagnosis of pregnancy. The number of corpora lutea or punctate hemorrhages varies from one to several per ovary. Punctate hemorrhages seem more common with some specimens than with others. The presence of follicles is *not* pathognomonic of pregnancy. The condition of the uterus and vagina has no bearing on the test.

The reason why infantile animals are used is that adult animals may have corpora lutea in their ovaries, and therefore confuse the test. At the same time the animals should not be too small or too young (less than 6 grams and less than 3 weeks old) for the urine may prove lethal to them after one or more injections, and secondly, very young animals will not respond to any stimulation of the ovaries.

The reason why six animals are used is that inexperienced technicians may kill one or more animals while doing the test, because of faulty technique. Even under the best conditions some animals may die because the urine may be toxic for them. Again, not all animals react with the same intensity to a given stimulus. It is not uncommon to find one (out of five or six) animal in a given test entirely negative, so that if that animal were the only one examined the test would be read as negative, while in reality the others might show a strong positive reaction. Hence, a *negative* reading in a single mouse is never conclusive. So irregular is the response in some animals, that the writer has seen a positive reaction in one ovary and a negative reaction in the other ovary of the same animal. Doubtful reactions should not be considered, but the test should be repeated with a fresh morning specimen of urine. When in doubt it is advisable to section and stain the ovaries and thus obtain a correct diagnosis. The choice of a morning specimen for the test is important. It is preferable that the patient should not have voided for about 6 hours previous to the collection of the specimen. It appears that the concentration of urine that occurs overnight holds also for the hormones. The writer has seen on a number of occasions a negative reaction with a casual specimen, and a positive reaction with the morning specimen of the next day.

The Accuracy of the Test

The reason why the previous tests for pregnancy were discarded was because they had an accuracy of little more than 50 per centum. In

the hands of experienced workers the accuracy of the Aschheim-Zondek Test ranges from 98 to 99 per cent. Aschheim (1933, 1935) recently reviewed his own results in 2000 cases, and obtained an accuracy of 99 per cent. Our own results are based on 1800 cases and show an accuracy of slightly less than 98 per cent. Clauberg (1933) in a recent review of the data on the test reached somewhat similar results.

The reasons for the errors in diagnosis were frequently hard to detect. Aside from conditions other than pregnancy that might give a positive test there are on record definite pregnancies that present a negative reaction. Robson (1934) mentions one such case. The writer had one case of pregnancy complicated by large fibroids wherein a negative test appeared in the fourth and fifth months of pregnancy and a positive test in the seventh month. The patient went to term and delivered a normal male child. Unfortunately the placenta was not examined. It must not be forgotten that the test represents a quantitative reaction and the amount of hormone excreted in the urine depends upon the amount produced and the level of the renal threshold. The amount produced depends upon the metabolic activity of the chorionic epithelium and the interchange of products between the syncicytium and the maternal circulation. The collection of the morning (over-night) specimen is of importance. Often it merely means that the specimen has been collected at about 7 a.m., forgetting or taking no account of the fact that the patient may have voided two hours before. Where there are clinical reasons to suspect a pregnancy and the test is negative, then the test should be repeated with a fresh specimen of urine. It must always be remembered that the test should be used as an *aid* to clinical diagnosis, and not as a substitute for the physical examination.

The reactivity, the age, the size and the condition of the animals all have an important bearing on the outcome of the test, for reasons already mentioned.

The Time of Appearance and Disappearance of the Test

Any non-clinical test for pregnancy that would be positive only in the second and third trimesters would not be of great aid in diagnosis. On the contrary one of the chief virtues of the Aschheim-Zondek Test is that by means of it a very early diagnosis of pregnancy can be made. With it a diagnosis can occasionally be made even before the patient skips a period (Aschheim, 1935; Posner, 1935). We have seen one such

case (Kurzrok, Kirkman and Creelman, 1934). Reaction I (follicles only!) may precede an early positive test by a day or two, so that the demonstration of Reaction I calls for a repetition of the test a few days later. More than two-thirds of the pregnancies will give a positive test when the patient is a day or two overdue, while more than 90 per cent of pregnancies will give a positive test when the patient is 10 days overdue.

The appearance of a positive test depends upon the establishment of a metabolic interchange between the trophoblast and the maternal circulation, in other words, when nidation of the ovum occurs. This in turn depends upon three factors:

1. The time of ovulation in the cycle.
2. The time of fertilization.
3. The duration of the transport of the fertilized ovum and nidation.

These are three variable factors, hence the establishment of a metabolic interchange between trophoblast and maternal circulation must vary. For this reason the first appearance of a positive Aschheim-Zondek Test must vary in different individuals, and in the same individual during different pregnancies. In a previous communication, the writer (1934) pointed out that the duration of the transport of the fertilized ovum from the outer end of the tube into the depths of the decidua is about 10 days. This is an average figure, for the transport may be faster or slower (ectopic?). In all probability fertilization usually (but not necessarily) occurs within twenty-four hours of ovulation. If these two factors (fertilization and transport) remain constant, the time of nidation will depend on the time of ovulation. But ovulation may occur anywhere from the ninth to the twenty-first day of the cycle, therefore the Aschheim-Zondek Test will become positive anywhere from the twentieth day of the first cycle to the seventh of the next, that is, if we assume a twenty-eight day cycle. Schematically, therefore, the first appearance of the Aschheim-Zondek Test can be calculated:

> First appearance of Aschheim-Zondek Test = Date of ovulation in the cycle + fertilization + transport.

If to the above there are added the variables of an irregular cycle, the amount of prolan produced and the height of the renal threshold for prolan, one can appreciate the irregularity of the first appearance of a positive Aschheim-Zondek Test. But in spite of all these variables it is most remarkable that more than 90 per cent of the pregnancies are positive 10 days after the onset of the skipped period.

The test remains positive throughout pregnancy up to about the tenth day post partum. The writer has seen a number of positive tests two weeks post partum, but never three weeks, that is, following normal pregnancy. The reason for the persistence of the test after the expulsion of the placenta is that patchy areas of trophoblast remain attached to the decidua, and maintain function up to the time when they undergo autolysis and destruction. It is worth remembering that Reaction I (follicles) may terminate the test.

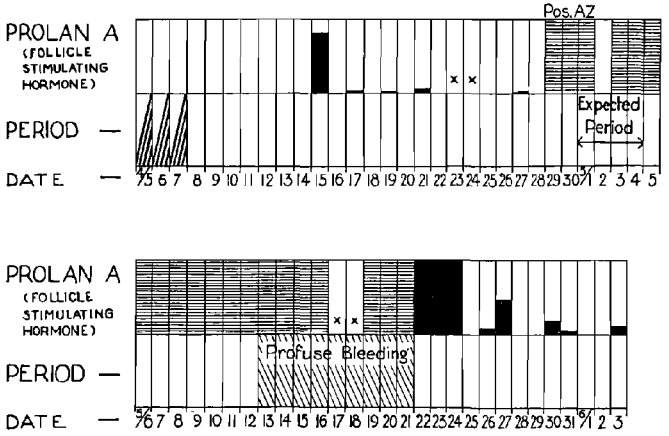

FIG. 115. Early appearance of a positive Aschheim-Zondek test. The F.S.H. excretion was studied every day of the cycle. A positive test was obtained on the eleventh day, hence ovulation occurred on the twelfth day. A positive Aschheim-Zondek test appeared two days before the expected period. Spontaneous miscarriage. At the termination of the Aschheim-Zondek test (horizontal shading) F.S.H. alone was excreted in the urine.

The Intensity of Reaction during Pregnancy

A first-hand familiarity with the test gives one the impression that a more brilliant positive reaction is obtained early in pregnancy than late. The reason for this is to be found in the observation of Aschheim and Zondek that there is a greater amount of hormone excreted at the beginning of pregnancy than at the end. Table 9 after Zondek (1931) shows the excretion of both prolan and estrone throughout pregnancy.

TABLE 9

PERIOD OF PREGNANCY	ESTRONE, MOUSE UNITS PER LITER OF URINE	PROLAN A AND B, MOUSE UNITS PER LITER OF URINE
1- 8 weeks	300- 600	5,000-30,000
3- 7 months	5,000- 7,000	5,000-16,000
7-10 months	6,000-20,000	4,000-20,000

The Relation of the Foetus to the Aschheim-Zondek Test

Patients frequently take it for granted that when the test is positive it means that the foetus is alive. Many physicians seem to be of the same opinion. Nothing could be further from the truth. The Aschheim-Zondek Test is a test for living chorionic epithelium. It merely denotes whether the trophoblast is alive or not. When the placenta dies the foetus succumbs immediately; on the contrary, when the foetus dies the placenta may go on living for months. A positive test means that the placenta is alive, and that the foetus may also be. A negative test (especially when repeated several times) means that both foetus and placenta are dead. Furthermore, in a hydatid mole, where the test is strongly positive, there is no foetus. A pregnancy should not be terminated on the basis of a single negative test, but the test should be repeated. The clinical findings should not be lost sight of. Again it may be repeated, that the test supplements clinical diagnosis but does not supplant it.

The Effect of Uterine Bleeding

Uterine bleeding, per se, has no effect upon the test. In a case of threatened abortion a positive test means that a metabolic interchange is being maintained between mother and chorionic epithelium. No prognostic significance is implied by the positive test. In a threatened abortion a positive Aschheim-Zondek Test followed by several negative tests is excellent evidence that the pregnancy has terminated. Given an early pregnancy in which there is persistent bleeding, the pregnancy should not be terminated as long as a positive Aschheim-Zondek Test persists, unless special circumstances arise. It is essential to remember that the test may remain positive for a week or ten days after the gross products of conception have been expelled.

Extrauterine Pregnancy

The Aschheim-Zondek Test gives no information as to the position of the pregnancy. The test is positive *wherever* there is a living chorionic epithelium. When the clinical evidence is positive for ectopic and the test is positive the procedure is, of course, operation. But where there is an ectopic clinically and a negative Aschheim-Zondek Test (repeated) the treatment may be conservative. The negative reaction implies that the chorionic epithelium is dead, the foetus is dead, and that the villi can no longer invade blood vessels. The writer has not seen a hemorrhage from a ruptured tube in the presence of a persistently nega-

tive test. Many clinicians are disappointed in the results of the Aschheim-Zondek tests when applied to ectopic gestation. When the results are interpreted in terms of living trophoblast, then there will be no grounds for disappointment.

Hydatid Mole and Chorioepithelioma

Aschheim and Zondek first observed that in the above conditions there is an increased excretion of prolan A and B. So great is this excretion that they have adopted it as a point in the differential diagnosis between hydatid mole, chorioepithelioma and normal pregnancy. In the hydatid mole the hormone titer may reach more than 250,000 M.U. per liter (normal pregnancy 30,000 M.U.). The amount of hormone depends upon the size of the mole, the extent of its degeneration and its association with the maternal circulation. Where the interchange of metabolic products is impeded or absent then a negative test will be obtained. Values of 100,000 M.U. per liter point to the probable presence of a hydatid mole, and the diagnosis becomes certain in the presence of more than 250,000 M.U. per liter. On the contrary, low or negative hormone findings do not exclude the presence of hydatid. After the expulsion of the mole a positive reaction may persist for as long as six weeks.

The intensity of prolan A and B excretion is greatest in the chorioepithelioma. We have observed one case (courtesy of Dr. Smiley) which excreted one million mouse units per liter of urine. Figures of more than 200,000 M.U. are common findings. This type of tumor frequently presents difficulties in histological diagnosis, especially when the tissue is removed by curettage and there is no history of recent pregnancy. A high hormone titer in the urine (more than 100,000 M.U.) definitely favors a diagnosis of chorioepithelioma. Since these tumors frequently follow hydatid moles, repeated Aschheim-Zondek Tests should be due after the expulsion or removal of the mole using serial dilutions of urine. The persistence of a positive test for more than six weeks, especially when the concentration of the hormones in the urine is high, should arouse our suspicions to the presence of a chorioepithelioma. If after the expulsion of the mole the Aschheim-Zondek Test becomes negative, and then after two or three weeks becomes positive again, malignant degeneration should be suspected, and all clinical and laboratory procedure should be employed to check the diagnosis. After the removal of a chorioepithelioma the test becomes negative within a few weeks. Metastases produce a recurrence

of a positive reaction, in fact, the reaction becomes positive before the clinical diagnosis of metastases can be made. Chorionic epithelial tissue may be found in other tumors of both men and women, as in teratomas, especially in the generative organs. A positive reaction is obtained in these cases. Heiderich, Fels and Matthias (1930) described a case of teratoma of the testes containing chorionic epithelium.

Modifications of the Test

The Aschheim-Zondek Test has one distinct disadvantage, namely, that it takes 100 hours to complete the test. Speed in diagnosis is

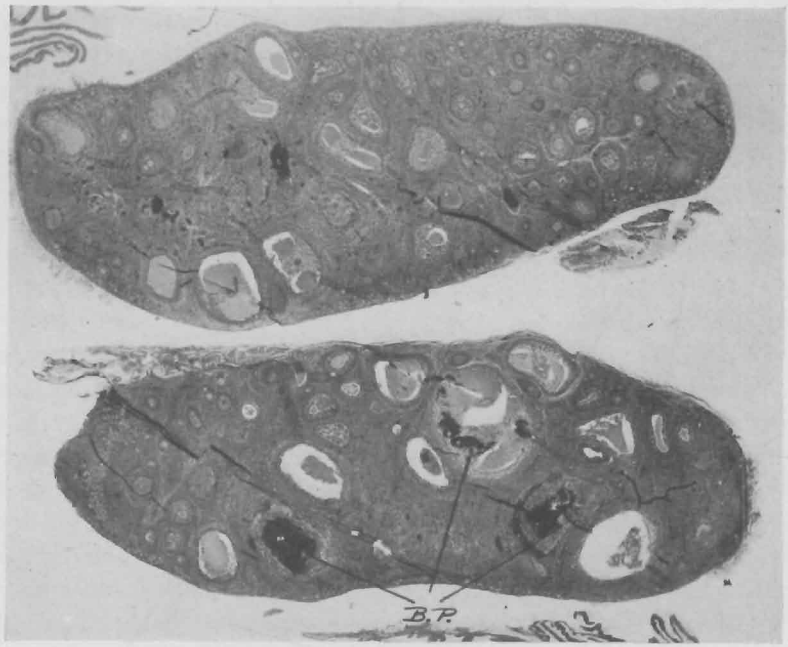

FIG. 116. Positive Aschheim-Zondek test. Immature rabbit. *B.P.*, blood points ("Blutpunkte") or hemorrhages into follicles. The opposite ovary (above) shows less reaction.

essential in certain cases. This is accomplished by the use of the rabbit instead of mice.

A. The Friedman Test (1929). The basis for this test is that a rabbit ovulates only after copulation or after the administration of gonadotropic hormones. If the injection is made intravenously then ovulation occurs in twenty-four hours. This occurs both in adults and in sexu-

ally immature animals (usually a little older than 12 weeks). A positive result is read when one or both ovaries show (fig. 116):

1. One or more ovulated follicles; or
2. One or more hemorrhages in the follicles.

The uterus is usually markedly enlarged and engorged, but it is not considered in the reading of the test.

Immature rabbits of about 12 weeks are obtained and separated. The reason for this separation is that females often mount one another, thereby causing ovulation and pseudopregnancy. The animals remain separated at least two weeks before they are used, to prevent the possibility of such ovulation. Ten cubic centimeters of morning urine are slowly injected into the ear vein of the rabbit. Twenty-four to forty-eight hours after injection the rabbit is sacrificed and the results read. We always wait 48 hours. Some workers do a laparotomy under anesthesia after 24 hours, and if the result is doubtful reoperate twenty-four hours later. Because of the expense of the test some laboratories use the same animal repeatedly. If the test is negative the animal can be used again in two weeks; if positive in four weeks.

Our results appear less accurate when the rabbit is used as a modification, namely, 94.5 per cent. The reason is twofold. The reactivity of animals varies. Secondly, small blood spots are occasionally found in the ovaries of normal rabbits. At the present time we usually do both tests, i.e., rabbit and mice. If the test is strongly positive we discontinue further work with the mice (and do not use them for the Aschheim-Zondek Test again). If negative, the injections are continued and the mice are then sacrificed at the usual 100 hours. To offset the factor of variability in response in rabbits some laboratories use two animals for every test. Greater accuracy is claimed for this procedure.

B. Use of Male Animals—Brouha and Simmonet (1930). This test depends upon the growth of the seminal vesicles after injection with gonadotropic hormone. The effect is primarily upon the testes, which in turn, secrete testicular hormone and the latter then acts upon the seminal vesicles. Morning urine is injected into six infantile male mice for 10 days. The animals are then destroyed and the vesiculae observed. If they are definitely enlarged the test is positive. We tried out this test (injections for one week) in 1929 (simultaneously with the above authors) but did not find the results reliable. The different members of our group could not agree upon the readings. The time required is too long. It is of scientific interest but of little assistance to the clinician.

C. The Growth of the Ovipositor in the Bitterling. Kanter, Bauer and Klawans (1934) have described the elongation of the ovipositor in the female bitterling as a result of injections of pregnancy urine. The accuracy of their results is somewhat less than for the Friedman test. It is too early to form a definite opinion as to the value of the test.

The Excretion of Follicular Hormone during Pregnancy

Aschheim and Zondek (1927) and Margaret Smith (1927) independently discovered that large amounts of follicular hormone are excreted in the urine during pregnancy. Beginning at about the middle of the second month of pregnancy the estrin titer constantly increases up to the end of pregnancy (from 200 to 1000 M.U. per liter). This increased excretion has been suggested as a test for pregnancy. It is not reliable for several reasons. (1) The increase may not come until the end of the second month, hence the value of early diagnosis is lost. (2) A form of amenorrhea known as the hyperhormonal type gives rise to increased estrin excretion, and clinically presents the same signs as an early pregnancy. Zondek (1931).

The source of follicular hormone during pregnancy is the placenta, i.e., the chorionic epithelium. The appearance of estrin in the urine would depend upon:

a. Whether the trophoblast is alive or dead.

b. Whether there is an interchange of metabolic products between the maternal and foetal tissues.

c. The renal threshold for estrone.

d. The form in which estrone appears. Spielman, Goldberger and Frank (1933) utilize the presence of *estrin* in the blood as an indicator whether the foetus is alive or not, obtaining a reduction of the blood estrogenic factor within 24 hours after foetal death. We have not been able to confirm this in all cases. The trophoblast is the primary factor, the foetus the secondary and wholly dependent upon the trophoblast for life. The chorionic epithelium seems to maintain its function in as far as gonadotropic and follicular hormone production is concerned, entirely independent of the foetus.

Cohen, Marrian and Watson (1935) observed that during the greater part of pregnancy over 99 per cent of the total estrogenic material excreted in the urine is in the "combined," ether-insoluble form, which possesses only a low physiological potency. Estrone in the urine, occurs therefore in two forms. The "free" estrone can be extracted from fresh untreated urine with ether. The "combined" estrone is that

which can only be extracted from the urine with ether *after* treatment with acid. Estriol also occurs in the free and combined form. It is conceivable that the great concentration of estrone during pregnancy does no harm to the mother or foetus because the hormone exists there almost entirely in the combined form.

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CHAPTER XVIII

MAMMARY DEVELOPMENT AND LACTATION

An examination of the processes of lactation and mammary development reveals a hormonal interrelationship involving the anterior pituitary gland, the ovary, the placenta and the mammary glands. This interrelationship is complex for it involves qualitative and quantitative factors, as well as stimulations and inhibitions on the part of the hormones.

To date the following facts appear to be established in mammary growth and lactation.

1. Preliminary mammary development is essential for lactation.
2. Growth of the duct system is a function of follicular hormone.
3. The mammary lobules in all probability are stimulated to further growth by corpus luteum hormone.
4. The immediate stimulus to lactation is provided by the lactation hormone of the anterior pituitary.
5. This lactation hormone can only act after previous action by the gonadal hormones (a view not entirely supported by Corner's experiments).
6. The lactation hormone has been separated from growth, gonadotropic and thyrotropic factors.
7. Prolactin—as the lactation hormone is known—has been used clinically with success in women in whom lactation had failed to develop adequately by the sixth day postpartum.

The following discussion will elucidate these facts and attempt to show how they were derived.

In view of the changes that the breast undergoes during pregnancy and lactation some coördinating mechanism between the ovaries, uterus, foetus, placenta and mammary glands has long been suspected and sought. It was supposed that the synchronizing mechanism was nervous in character. In 1894 Mironoff demonstrated that a breast severed from its nervous connections during pregnancy secreted milk after parturition. This observation was further supported by Goltz and Ewald (1896) who removed the lumbar cord from a bitch. The animal became pregnant, gave birth and lactated normally. Transplantation of the mammary gland was first accomplished by Ribbert

(1898). He transplanted the mammary gland of a guinea pig into the skin of the ear. Following pregnancy lactation occurred in the transplanted gland.

The Gonads and Mammary Growth

Clinically it is an established fact that if the gonads are removed during adolescence, or if there is a failure of a functioning gonad to develop, the mammary glands remain infantile. The nipple is small and flat. The same facts have been demonstrated experimentally. The sudden impetus to growth which the breasts receive at puberty links the glands still further to the follicles and corpora lutea.

The Relation of Menstruation to the Mammary Glands

The mammary glands undergo cyclical changes with each menstrual period (Rosenburg, 1922). Often at a point midway between two menstrual periods, the breasts begin to show definite fullness and enlargement. This increases in intensity as the menstrual period approaches and usually ceases abruptly with the onset of the flow. On palpation the glands may give a distinct lobular feel. Occasionally this premenstrual engorgement becomes very painful and distressing so that the patient may seek surgical aid. Histologically, the following changes are noted during the menstrual cycle, according to Rosenburg.

1. During the premenstrual phase—the glandular areas seem fairly numerous. The glands show many bud-like processes, some solid and others hollow, thus forming typical areoli. There is a distinct membrana propria about each gland.

The picture resembles that of early pregnancy.

2. During menstruation—degenerative changes begin. The nuclei stain poorly, the glands become fewer in number, the membrana propria is less distinct so that the glands are not distinctly set off from the surrounding connective tissue.

3. During the (early) postmenstrual phase the same degenerative processes continue. At the end of this phase the glands have disappeared, and only large and middle sized ducts remain. Alveoli are completely absent.

The Mammary Glands during Pregnancy

At the end of the second month of pregnancy the breasts begin to enlarge. Occasionally the patient is aware of fullness of the breasts some time before this. The enlargement involves the fatty capsule (corpus adiposum), the connective tissue (corpus fibrosum) and the

parenchyma. There occurs marked sprouting of the tubules, which become finer as they pass away from the main ducts. Each tubule finally terminates in an alveolus. The new tubules are at first solid cords, but become canalized during the second half of pregnancy (v. Jaschke, 1926). The connective tissue is seemingly almost obliterated by the growth of the parenchyma, but there is at the same time a definite increase in the elastic tissue fibrils surrounding the ducts. These fibrils play a definite rôle in the emptying of the breast during lactation.

Experimental Development of the Mammary Glands

1. Early Experiments. Lane-Claypon and Starling (1906), Foa (1908), Aschner and Grigoriu (1911) and many others prepared aqueous extracts of the fetus, placenta and ovary and injected them into female rabbits and guinea pigs. Some parenchymal growth was obtained, but their experiments are open to the objection that they failed to castrate the animals prior to the experiment. Thus the spontaneous growth of the duct system has not been ruled out. Lipoidal extracts of the ovary, corpus luteum and placenta were used by Iscovesco (1912), Fellner (1913) and Herrmann (1913, 1915) and growth of the mammary glands was obtained in both castrate and normal male and female rabbits and guinea pigs. Milk secretion did not occur. The use of a lipoidal extract, and the castrate animal were a distinct step forward.

Stimulation of Mammary Duct Growth with Estrone. The rapid growth of knowledge with regard to the source, mode of extraction and standardization of estrone renewed our interest in the problem of mammary growth and lactation. Vintemberger, (1925) injected liquor folliculi from cows' ovaries into young castrated male and female rabbits and obtained growth of the ducts of the mammary glands. Allen (1927) injected estrone into a spayed monkey and obtained marked growth of the ducts and some additional growth of the alveoli. Turner and his co-workers (1931) after an extensive experiment concluded that development of the duct system and lobule growth (depending on the species) may be produced experimentally by injections of the estrus-producing hormones (theelin and theelol) in spayed animals. Furthermore, the same stimulation can be produced in the mammary gland of the castrate male where the growth is usually very slight. In view of the fact that growth obtained by follicular hormone is mainly in the duct system, and because during pregnancy there occurs in addition rapid and extensive hyperplasia of the lobules, a second hormone is apparently necessary.

Stimulation of Lobule Growth with Corpus Luteum Hormone. During pregnancy or pseudo-pregnancy rapid lobule hyperplasia occurs. This is usually associated with active corpus luteum function. Ancel and Bouin (1911), Loeb and Hesselberg (1917) and many others utilized aqueous lutein extracts without notable success. Corner (1930) showed that the injection of progestin *alone* into spayed rabbits did not induce lobule growth or lactation. Turner and Frank (1931) demonstrated that the injection of follicular hormone into spayed male rabbits *followed by* the injection of progestin, failed to obtain the type of growth seen during pseudopregnancy or early pregnancy. On the contrary, the *simultaneous* injection of follicular hormone and progestin into castrated male rabbits caused the development of both ducts and lobules in a manner strikingly similar to that seen in early pregnancy. On the contrary Nelson and Pfiffner (1930–1931) produced marked hypertrophy of the glands and nipples of immature male and female gonadectomized guinea pigs with daily injections of a lipoid extract of sows' corpora lutea believed to be practically free of theelin. The results are different from those obtained by other workers, and that difference may be due to the possible small amounts of follicular hormone present in the lipoid extracts of corpora lutea.

Turner and Schultze (1931) implanted each day the pituitaries from castrated male and female rats into normal and castrated young female rats. When these implants were continued for nine days, growth equal to that of the mammary gland in early pregnancy was induced in the *normal* female. The implants were not effective in the castrates. The mammary growth produced in the normal animal is due to the luteinizing effects of the implants upon the follicles in the ovary.

Stimulation of Mammary Growth with Anterior Pituitary Extracts. Corner (1930) injected alkaline extracts of pituitaries of sheep into ovariectomized rabbits which had never ovulated. Milk was produced and mammary proliferation resulted which could not be distinguished from that observed at the very end of pregnancy. Since these animals had never ovulated, there could not have been any lutein effects on the mammary glands. At the same time, it is more than probable that the glands had been subjected to the action of estrone produced by the growing gonads prior to ovulation. Corner's experiments make it seem possible that the anterior pituitary gland produces a specific hormone which is the direct cause of mammary growth during pregnancy.

Stimulation of Mammary Growth in the Human. During the treatment of primary amenorrhea associated with an *absence of secondary*

sex characteristics by means of large doses of follicular hormone we (Kurzrok, Wilson and Cassidy, 1935) noticed definite growth of the breasts. In some cases the growth was great enough, so that after several months such breasts could not be differentiated from normal breasts, insofar as size and texture were concerned. We have not had the opportunity of examining such an artificially developed breast microscopically. During the early stages of growth the breasts had a pointed appearance, similar to that seen in young girls. This leads us to believe that the growth we obtained was limited to the duct system. Secretion was not seen in any of our cases.

The Relation of the Anterior Pituitary Gland to Lactation

The relationship between the pituitary and lactation may be studied by the effect of hypophysectomy and by the use of extracts of the gland. Pencharz and Long (1933) and Selye, Collip and Thompson (1933) showed that mammary growth is diminished following hypophysectomy during pregnancy, and lactation fails to develop after parturition. If rats are injected with gonad-stimulating extracts mammary gland development occurs. Oöphorectomy is not followed by lactation.

Stricker and Greuter (1929) were the first to produce lactation by means of anterior pituitary extracts. They utilized rabbits in which pseudo-pregnancy was produced through mating with vasectomized bucks. During pseudo-pregnancy the breasts develop in a manner similar to normal pregnancy. They then injected the pseudo-pregnant rabbits (both intact and castrated) with alkaline extracts of the anterior pituitary. Lactation resulted. Their results were confirmed and extended by Corner (1930), Evans and Simpson (1929), Nelson and Pfiffner (1931), Turner and Gardner (1931), Asdell (1932), Nelson (1934), and especially by Riddle and his co-workers (1932, 1933, 1934). Riddle, Bates and Dykshorn (1932, 1933) have isolated the lactogenic factor, and have shown it to be different from the growth, gonadotropic and thyreotropic factors. They have named it prolactin. Gardner and Turner (1933) have also purified this hormone and named it galactin. Prolactin as prepared by Riddle et al. involves initial acid or alkaline extraction, iso-electric precipitation in an aqueous medium, and repeated washing of this precipitate. As now prepared, prolactin contains some inert protein (Riddle, 1935), since about 0.1 mg. is required to give a detectable response. There is little loss of potency upon boiling salt free preparations at pH 8.0. It is completely destroyed by tryptic digestion, and is soluble at pH 8.0. Prolactin cannot be obtained from any other gland.

Several methods for the bio-assay of prolactin have been proposed. Riddle has shown that the growth of crop-glands in pigeons is a reliable and practicable method of assay. For accurate assay five nonincubating adult pigeons are injected once daily for four days and killed ninety-six hours after the first injection. The weight of the two crops is proportionate to the amount of lactogenic factor injected. Gardner and Turner (1933) proposed the lactation response in pseudo-pregnant rabbits as a rough method of bio-assay of the lactogenic factor.

A very interesting problem in the physiology of lactation is what mechanism releases prolactin during the puerperal period and what keeps it in check during pregnancy. A body of evidence is accumulating which shows that the lactogenic factor of the pituitary is suppressed during pregnancy by estrone, and that the sudden drop in estrone production during the puerperium releases prolactin from the pituitary. The evidence is as follows:

1. It is a well established clinical fact that whenever a placenta is retained within the uterine cavity and remains *attached* to it, lactation will not set in as long as the attachment persists (Halban, 1905). The same holds true in an abdominal pregnancy wherein the placenta is attached to the abdominal contents, and is allowed to come away piecemeal.

2. Frankl (1923) implanted placental tissue into rats near term and observed that lactation did not occur following delivery as long as the grafts remained alive.

3. There is a constant increase of estrone in the blood and urine with the progress of pregnancy and a marked drop immediately post-partum (Aschheim and Zondek, 1927).

4. The source of estrone during pregnancy is the placenta.

5. De Jongh and Laqueur (1930), using guinea pigs as experimental animals, injected large quantities of menformon (estrone). Upon sudden cessation of administration of estrone lactation set in.

6. Nelson (1934) showed that in the guinea pig the surgical removal of the uterus and its contents plus the ovaries resulted in lactation. Removal of the ovaries alone did not induce lactation until after the expulsion of the foeti whether abortive or at term. Lactation did not occur during the period of placental retention when the ovaries and the embryos were removed (leaving the placentae intact), but did occur following the expulsion of the placentae. Injections of estrone at parturition and during the early puerperium inhibited lactation.

Failure to lactate following parturition may therefore be due to an excess of estrone in the system or to an insufficiency of prolactin.

Many factors in lactation physiology are still unsolved, namely; the stimulation of lactation by continued suckling or emptying of the milk ducts; the maintenance of secretion in the breast that is being emptied but not in the other; and the mechanism of the uterine contractions induced by suckling.

The Clinical Use of Prolactin

Kurzrok et al. (1934) reported on the clinical use of prolactin. The hormone was prepared and assayed by Bates and Riddle in sterile solution of pH 8.0–8.4, and was injected intramuscularly in the buttocks. The dose varied from 2.5–10.0 cc. (75–400 units). When the dose was divided the second injection followed within 12–24 hours.

The subjects were 37 women delivered of normal children at the Sloane Hospital for Women (table 10). All were afebrile except one who had a moderately severe sapremia. Twenty-nine cases were chosen in which lactation had failed to develop adequately by the sixth day (fifth in two cases) or later postpartum.[1] These were cases which, in the clinical experience of the staff, would in almost every instance have failed further to improve in milk production. In 21 of these cases there was a gain greater than 100 grams of breast milk daily when comparison is made between the day preceding prolactin dosage and the date of the patient's discharge 3 to 9 days later (good result). The amount of increase in these 25 cases ranged between 50 and 400 grams. In four other cases (failures) there was either loss or a gain of less than 50 grams; in two of these failures the dosage was purposely reduced to one-half the recommended dosage; in the other two the cause is less evident but may be due either to insufficient dosage (only 100 units) or to an inherent deficiency of mammary tissue. The amount of milk was determined by careful weighing of the babies before and after nursing, and the amounts recorded for each day. Growth curves of the babies usually ran parallel with the increase in the amount of breast milk, and as a rule there was a definite weight increase beginning on the date of treatment.

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[1] Werner (1935) criticizes our work by stating that we used no controls, and quotes Hirst on the amount of milk to be found on the seventh day. Our control is the clinical experience of a staff dating back many years, and which has as its background the very large material of the Sloane Hospital for Women. We have made the statement that if spontaneous lactation does not set in by the seventh day post-partum, the chances are against its setting in later. We have rechecked this statement during the past two years and believe it correct.

TABLE 10

Clinical Data on Sub-normal and Normal Lactation in Women

CASE NUMBER	DOSAGE		GRAMS MILK IN DAY		DAYS AFTER PROLACTIN (DATE OF DISCHARGE)
	Prolactin units	On day postpartum	Day before prolactin	Day of discharge	
1	100	6	200	395	7
2	100	5	330	495	9
3	100	6	210	360	6
5	100 + 50 + 50	6	230	405	8
6	50 + 50	8	295	50	3
7	50 + 50	9	100	260	4
8	50 + 50	10	140	220	3
9	50 + 50	7	225	280	7
11	75 + 35	8	330	170	4
12	75 + 35	8	200	380	5
13	75 + 35	8	170	320	4
14	40 + 35	21	200	310	4
15	100 + 50	8	170	315	2
16	100 + 50	6	170	375	5
17	75	8	200	310	3
18	100 + 50	6	50	90	6
19	160	7	310	450	6
20	250	10	210	360	3
21	250	7	350	470	7
22	100 + 100	9	45	280	8
23	100 + 100	8	310	460	4
24	100 + 100	8	170	570	3
25	100 + 100	9	180	260	2
26	100 + 100	7	35	225	7
27	200 + 200	6	310	480	9
33	200 + 200	6	40	150	3
34	100 + 100	6	210	410	8
36	100	7	120	110	5
37	100	5	20	90	6
4	100	8	420	575	5
10	75 + 35	15	520	550	4
35	100 + 100	7	380	300	5
28	200 + 200	3	220	350	8
29	200 + 200	2	70	190	10
30	200	3	190	400	8
31	200	2	90	300	11
32	200	1	70	600	11

In a small group of three cases (4, 10, and 35; at 7 to 15 days) the amount of breast milk was probably at its maximum level, and further

stimulation produced no consistent effect. In five presumably normal cases (28–32) prolactin was used on the first, second or third day postpartum. The data are insufficient to show whether such dosage increased the lactation beyond the normal.

In three cases of premature births (not tabulated) the dosage was also effective.

Prolactin injections produced neither local nor general reactions nor postpartum complications.

There were no caked breasts.

On the basis of these results the clinical dose of prolactin is provisionally considered as an initial 150 units usually followed within 12–24 hours by 100 units.

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CHAPTER XIX

PRIMARY AMENORRHEA

It may be stated at the onset that it is an error to class a girl of sixteen as a case of primary amenorrhea simply because she has not menstruated. The mere absence of menstruation at this age is insufficient to establish the diagnosis. Other significant physical findings must be present. Some, otherwise normal girls, begin to menstruate rather late, and such factors as race, climate, familial tendency and general health should receive consideration.

It is very essential that an attempt be made to establish the etiological factor in this symptom complex. It will serve as an index of therapy and prognosis.

Primary amenorrhea can be due to any one of three major etiological factors:

I. Failure of the anterior pituitary gland.
II. Failure of the ovary.
III. Failure of the Müllerian duct system.

It is not uncommon to find two or all three factors associated.

Failure of the Anterior Pituitary Gland

When primary amenorrhea is due to hypofunction of the anterior hypophysis then disturbances of growth of the individual as a whole are also present. The earlier in life the hypophyseal inadequacy begins the greater will be the abnormalities of growth. In view of the fact that the anterior pituitary gland is the driving power (motor) behind the entire genital tract there are always found inadequacies of form and function in the ovaries and Müllerian duct system.

The following two cases are typical examples of this group.

Case R. D. P. Age 25. Referred by Dr. Louis M. Wager of New York City.
Chief Complaint. Never menstruated.

The patient is the oldest of six children. Four younger sisters are all well, and menstruate regularly. None is married. Patient weighed 8 pounds at birth; walked at 14 months. Talked when "very young." Graduated public school at 15. Had "double pneumonia" twice, but is uncertain as to dates. Mumps and measles during childhood. Stopped growing at 16 years. She is six inches shorter than her shortest sister. She is steadily employed as a saleslady. She "keeps company" for the past two years and contemplates marriage in six months.

Mother first menstruated at 16 years. The patient likes the company of boys. No definite attitude towards sex and sex life could be ascertained, but one gained the impression that she desired marriage because of the protection that is offered.

Physical Examination. The patient is a young, short and stocky Italian girl. She appears in good health. Weight: 113 pounds. Height: 55 inches. Span 56 inches; upper border of symphysis to soles of feet: 28.5 inches; upper border of symphysis to top of head: 26.5 inches.

Head: negative.

Eyes: moderate squint. Pupils react to light and accommodation. No eye signs of thyrotoxicosis.

Hair: moderate on head, but bleached. No axillary hair. Sparse pubic hair. No excessive hair distribution.

Dentition: irregular, crowded, many cavities.

Neck: no thyroid enlargement.

Heart and lungs: negative. Pulse: 140—regular; of good quality. Blood pressure: 140/80.

Breasts: completely absent. Areoli: 1 cm. in diameter. Nipples: rudimentary.

Abdomen: negative, stout.

External genitalia: hypoplastic. No enlargement of the clitoris.

Vagina: of moderate depth, narrow, conical.

Cervix: very small, movable, pin point os.

Uterus: could not be palpated by either rectal or vaginal examination; possibly due to stoutness of the abdomen.

Adnexa: not felt.

X-ray of Pelvis and Long Bones. A.P. diameter: 9 cm. Transverse: 11.5 cm. The inlet is typical android in both segments. The sacrosciatic notch is the narrow male type. The side walls converge slightly. The subpubic arch is narrow with convex male type of pubic rami.

Type of pelvis: extreme android, characteristic of the pelvis associated with primary amenorrhea. In addition to the type of pelvis, films of the long bones show delay in epiphyseal fusion; and, apparently, this tendency of under-development is general throughout the skeleton. Dr. Swenson may make special comment on this point because of Dr. Kurzrok's interest. (Dr. Howard Moloy.)

General skeleton films show a diffuse decalcification of the bones, suggesting a nutritional change. There is also a definite delay in epiphyseal fusion, as pointed out by Dr. Moloy, in all the long bones. We also note exostoses on the medial margins of the tibiae, just below the knee joints, of the type usually associated with a hereditary familial chondrodystrophy. This is considered to be on an endocrine basis. The general osteoporosis could be due to a disturbed calcium metabolism. It might be well, also, to call attention to the fact that the first metatarsals are much shorter than usual, throwing the weight on the head of the second metatarsal. This had produced some hypertrophy of the shaft of the second metatarsal. (Dr. P. C. Swenson.)

B. M. R.: plus 28 to 30 per cent. Repeated five times.

Hormone findings in the urine: (1) Follicular hormone—not found. (2) Follicle stimulating hormone—present in some tests and not in others. (3) Thyrotropic hormone—present.

Diagnosis. (1) Hypopituitarism. (2) Hypogonadism. (3) Primary amenorrhea. (4) Hyperthyroidism (?).

Progress. The patient has been treated with estradiol benzoate (progynon-B), 50,000 I.U. per week for the past year. There has been definite growth of the breasts, mainly in the subareolar area. The external genitalia have increased in size. A small nodule can now be palpated at the upper border of the broad ligaments, possibly a growing uterus. No menstruation. Pulse has slowed down to 100, but patient would not repeat B.M.R.

Case 2. R. Z. Age 20. First seen in 1932.

Chief Complaint. Never menstruated.

Patient is the second of four children. She was in fairly good health up to the age of 12. An attack of "influenza" left her partially deaf. This deafness has been progressive so that at the present time it is necessary to read all questions. Similarly, speech has grown progressively poorer. She stopped growing at the age of 12, and is shorter than her sisters. Patient's mother began to menstruate at the age of 18, patient's 24 year old sister at 18, and her 20 year old sister at 14. She had measles, diphtheria, scarlet fever, mumps and whooping cough in childhood, without any noticeable sequellae.

Physical Examination. The patient is a short, stocky girl in apparent good health. She is rather nervous and apprehensive during the entire examination.

Head: essentially negative. Eyes react to light and accommodation. No nystagmus; no exophthalmus.

Mouth: teeth crowded. Tonsils removed.

Neck: no enlargement of the thyroid, but is just palpable; no substernal extension.

Skin: fairly dry, not flushed.

Hair: normal on head, somewhat scant in axillae and over pubic region. Excessive distribution of hair on forearms and lower extremities.

Extremities: fine digital tremor. The legs are definitely masculine. No convergence of the knees.

Trunk: wide shoulders; inner border of scapula concave, hips narrow.

Breasts: absent. Nipples and areoli markedly hypoplastic.

Chest: heart and lungs negative.

Pulse: 90. Blood pressure: 118/80.

Abdomen: negative.

External genitalia: hypoplastic, no enlargement of the clitoris.

Vagina: short, shallow, 2 inches in depth.

Cervix: very small, pin point os.

Uterus: small, movable, about 2 cm. in diameter, firm.

Adnexa: not felt.

Basal metabolic rate: from plus 21 to plus 36 per cent—repeated on numerous occasions. Dropped to plus 13 per cent after 240,000 R.U. of Progynon-B.

Hormone studies on the urine: Follicle stimulating hormone—always positive. Estrin—4 rat units found at the first examination but repeated subsequent tests failed to find it again.

Wassermann: negative with all antigens.

Blood and urine: no significant findings.

X-ray of the sella: the sella is clearly outlined. The dorsum is heavy and inclines forward. The antero-posterior diameter of the sella is but 7 mm. The capacity is small. The basal angle is normal, as is the development of the air cells. (Dr. C. W. Schwartz.)

X-ray of the pelvis: films of the pelvis show it be of the extreme male type in all respects. The inlet is blunt heart shape. The lower ilium is short and the sacrum flat. The notches are of the extreme male type. The anterior segment is narrow. The transverse of the inlet is close to the sacral promontory. The sacrum is flat, has 5 segments, is straight. The pubis is typical wedge shape male. There are prominent tuberosities as found in the male. The subpubic angle is very narrow. The rami are straight and arise from the lower border of the symphysis in a typical male manner. (Report of Dr. Swenson.)

Psychological Studies. On the Terman Revision of the Binet-Simon Test this girl now measures 13 years, 3 months with an intelligence quotient of 83.

Sexually she appears to be very immature. She had no curiosity about sex and was told about menstruation and intercourse by her sister two years ago. The sister has recently given her books on the sexual problems of women. She has definite erotic sensations when reading them but her day dreams are concerned with having a husband in order to be protected and taken care of after her parents are dead. She has been fond of children since she was little and would like to have them. She has gone out with boys once or twice but did not particularly enjoy it, and has no wish to go again. She would prefer to have girl friends. She is anxious to menstruate so that she may be normal like other people. Report of Dr. Florence Powdermaker.

Diagnosis. Pituitary hypofunction. Ovarian hypofunction. Hyperthyroidism?

An examination of both cases reveals the following common factors.

1. Growth—distinct retardation. Epiphysis—union is late, and consequently the bone age of the patient is less than the actual age.

Extremities—the relation of the span to the height is not out of proportion, so that distinct eunichoid-growth is not evident. Similarly, the relation of the lower extremities to the trunk is not disproportionate. Pelvis—extreme male types.

2. Dentition—crowded and irregular.

3. Sex characteristics: Both patients are *genetically* female. The *hormonal augmentation of sex in the same direction* is absent in both cases for neither patient has developed functioning ovaries. The outcropping of characteristics of the *opposite* sex was not marked in case R. D. P. but distinctly so in case R. Z. Patient R. Z. had square shoulders and narrow hips. The pelvis in both was android in type. We have found this pelvic type in all cases of primary amenorrhea associated with a complete absence of ovarian function. Whether the android pelvis represents a type which may be modified into the gynecoid by the action of the ovarian hormones, or modified into the typical

male type by the accentuation of its characteristics through the medium of the testicular hormone is at present undecided. The reasons for the ovarian failure in these cases may be twofold. One is the absence of an ovarian anlage, and the other is an absence of gonadotropic hormone during intrauterine life. The other pituitary dysfunctions in these cases make the latter explanation the more probable. When at a later date, sufficient amounts of gonadotropic hormone did become available, the ovary was far too compromised to respond adequately. It may well be that the gonads are very susceptible to gonadotropic stimulation during intrauterine life, less so during the years preceding puberty, again susceptible between puberty and the menopause, and finally insusceptible after the menopause. To produce a desired hormonal effect both the hormone *and* a susceptible end organ must be present.

Secondary sex characteristics were absent in both cases. This was expected for their development is entirely dependent upon both estrone and progesterone.

Müllerian Duct development—no internal genitalia could be palpated in R. D. P. when first seen, but a small nodule was originally felt in R. Z. Apparently the ovarian failure occurred very early. In fact, the lack of development of the Müllerian ducts leads one to suspect that in R. D. P. the ovaries never functioned at all. On the other hand, the slight development in R. Z. offers some evidence that very inadequate ovarian function was present for a short time. The significance of these points for therapy will be discussed later.

Hormonal findings were as follows:

a. Persistent absence of estrone.

b. Occasional presence of follicle stimulating hormone.

c. High basal metabolic rate with accompanying presence of thyrotropic hormone in the urine.

The persistent absence of estrone is due to the absence of a functioning gonad. (The morphological absence of both gonads is rare.) Repeated urine examinations on these and other patients were always negative for estrone.

The follicle stimulating hormone is occasionally found in some cases and constantly in others. It must be remembered that when the ovary is absent (morphologically or physiologically) the follicle stimulating hormone appears in the urine. The sporadic appearance of follicle stimulating hormone implies either failure or irregular production of this hormone. This finding in some of our cases is suggestive of

an inadequate production of the gonadotropic hormone on the part of the anterior pituitary gland. The finding of a high basal metabolic rate in these cases, especially in the absence of clinical hyperthyroidism, is of distinct interest. In order to find some explanation for this we tested the urine of R. D. P. for the presence of thyrotropic hormone. This was done by means of a technique by Loeser (1932). We have found that the daily output of normal urine did not contain sufficient hormone to stimulate the thyroid of a guinea pig. On the contrary, one-half of the daily output of case R. D. P. was sufficient to induce definite stimulation of a guinea pig thyroid. A litter mate control was injected with the extract made from the total 24 hour urine of a patient of the same age. There was no evidence of stimulation. The possibility suggests itself that the gonadotropic and thyrotropic molecules have a common nucleus (compare the gonadal hormones) and that this common nucleus may be utilized to form one or the other, or both.

Therapy. The usual treatment for these cases is that applied to case R. Z. This patient was treated with small doses of estrin for about 1 year without any effect. Since January, 1934, the patient has received progynon-B. The injections are given intramuscularly in the buttocks, and each dose contains 10,000 R.U. Usually, two injections are given per week. On several occasions the treatment was stopped in order to study the regressions that took place when treatment was discontinued. To date (December, 1936) the patient has received 1,290,000 R. U. of progynon-B.

The **results of therapy** have been as follows:

Toxicity—no toxic effects of any kind were noted. During the middle of the second year of therapy the patient complained of loose bowels for several weeks. The condition was alleviated by diet. Whether loose stools had any relation to the treatment is problematical. It must be remembered that occasionally normal patients suffer from diarrhea when menstruating and that constipated patients may have normal bowel movements during their menses.

Bleeding. Twenty *cyclical bleedings* occurred from January, 1934, to December, 1936. It is understood that this is *not menstruation* for no corpus luteum hormone has been given (except during 1 month) and we feel that the patient is incapable of producing this hormone herself. The dates of bleeding in relation to the amount of hormone required to produce the bleeding are given below. It required 70,000 R.U. to bring on the first period, 50,000 R.U. the second, etc.

Dosage in Rat Units	*Dates of Bleeding*	*Amount*
	1934	
70,000	1. Feb. 24 (first period)	Scant
50,000 plus 10 rabbit units of C.L.H.	2. Mar. 29–Apr. 6	Moderate
40,000	3. May 5–6 (Breast developing)	Moderate
10,000	4. May 20–23	Moderate
70,000	5. July 5–11	Moderate
40,000	6. Aug. 16–17	Fair
	Treatment discontinued for 5 weeks	
	Regression noted	
150,000	7. Nov. 20–28	Moderate
	1935	
50,000	8. Jan. 3–8	Moderate
70,000	9. Mar. 7–11	Moderate
30,000	10. Apr. 6–9	Moderate
20,000	11. May 2–4	Moderate
40,000	12. June 6–9	Moderate
40,000	13. July 10–13	Moderate
80,000	14. Sept. 28	Scant
	Attendance irregular	
70,000	15. Nov. 14–22	Moderate
60,000	16. January 1–4 (1936)	Moderate

As soon as treatment became irregular or stopped completely the cyclical bleeding ceased. The occurrence of the bleeding had a profound psychic effect upon the patients. A significant change occurred in their dress and appearance. It became less infantile and very much more ladylike. (We noticed that as a rule these patients did not use cosmetics when they first came to the clinic.) The use of cosmetics began soon after the first few periods were obtained. One patient with an infantile habitus, and immature sexual desires and emotions, became engaged when her sexual development progressed and periods occurred. She subsequently married and is leading an apparently normal sex life. Sporadic treatment is being maintained.

The bleeding was not due to withdrawal of hormone, for it occurred during the *constant* administration of progynon-B. It was not associated with dysmenorrhea except in one instance. It was never profuse, usually moderate and often scant. The bleeding was accompanied by the normal premenstrual molymina seen in normal women. The

patients were aware of its impending occurrence for some days before the onset of the flow. Once a fairly regular cycle was established the patients became distinctly perturbed when an expected flow failed to appear. The generalized non-pelvic symptoms of menstruation such as increased or diminished activity, mental irritability or fatigue were also present in our cases. Subjectively it was impossible to differentiate such cyclical periods from normal menstruation.

Breast Development. Breast development has occurred in all of our cases of primary amenorrhea. It *is usually the earliest response to treatment.* The extent of development varies. In some cases the breasts reached a size comparable to those of normal women of the same age. Lactation was never observed. The nipples grew at a corresponding rate. Growth began in the subareolar area, so that this zone usually projected beyond the level of the breast. The breast felt smooth and even on palpation. The only expected growth was in the duct system, though we never had the opportunity to take a biopsy so as to substantiate this. When treatment stopped definite regression occurred. Whether the regression would proceed as far as complete disappearance of the breast we do not know, for the patients usually insisted that sufficient treatment be maintained so that a maximum amount of development would be gained.

Genital Development. Growth of the genital tract occurred.

1. The external genitalia—showed distinct growth. Where the labia minora were originally barely indicated, their growth progressed slowly but definitely. The amount of pubic hair increased.

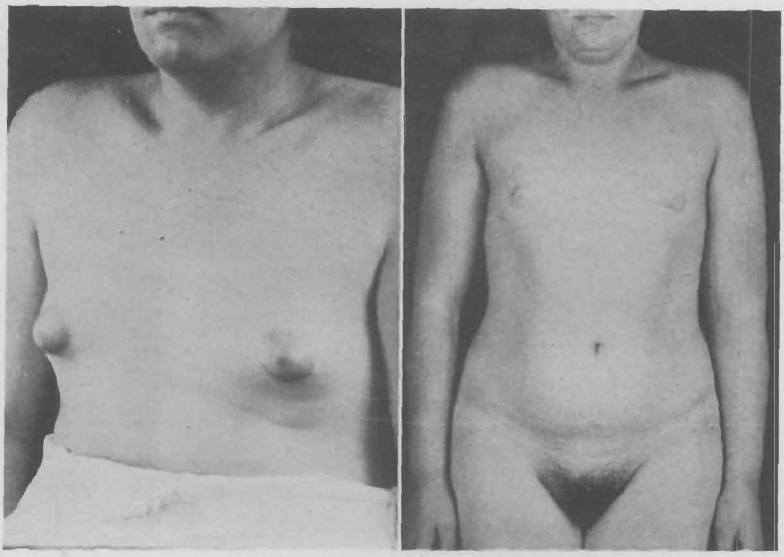
2. The vagina—grew in width and depth. This was distinctly surprising to us for it involved growth of a hollow tube. In one case where the original depth was two inches, a depth of 3.5 inches was reached in six months. The fornices remained shallow.

3. The uterus—whenever bleeding occurred it was associated with definite growth of the uterus (body and cervix). Beginning with a nodule about 2 cm. in diameter, a uterus approaching the lower limits of normality can be obtained. The uterus was always very firm at the beginning of treatment, but as growth progressed a distinct general softening was noticeable. Growth involved the entire uterus. When treatment was stopped-regression occurred. The bleeding was the first to cease, the breasts receded next, and the uterus last.

The endometrium—at the beginning of treatment the endometrium was thin and showed few glands. Mitotic figures were absent. It represented what may really be called a resting state of the proliferative phase. Some thickening occurred as treatment progressed. A

premenstrual phase was never seen. Endometrial biopsies removed just before or during bleeding always showed the proliferative phase. Desquamation of tissue was not a noticeable feature. The bleeding resembled more the diapedesis seen in ovulation bleeding rather than that associated with a sterile cycle.

Psychic Changes. The changes after therapy in the mental status of these patients is often very striking. Once cyclical bleeding has begun and definite growth of the breasts has occurred the patients insist that therapy be maintained. They are told before treatment is begun



A B

FIG. 117A. Primary amenorrhea group I. B. R. P., aged 19 years. Marked hypodevelopment of all secondary sex characteristics.

FIG. 117B. B. R. P. Growth of breasts from 100,000 R.U. progynon. Has had 5 successive periods under treatment. Distinct change in psychic outlook.

that the changes that will be produced are only temporary, that is, they will last as long as treatment is continued. The changed mental attitude of the patient frequently manifests itself in greater neatness in attire and in the use of cosmetics. Greater interest is taken in the opposite sex. Whether these changes are due to the fact that their body contours now resemble other women, and that they bleed at regular intervals—visible manifestations that they are women—or whether follicular hormone has a more profound and direct deep-seated action upon the nervous system, is a very interesting consideration. It is remarkable that the psychic changes are the slowest to regress once treatment is stopped.

Failure of the Ovary

In this group will be considered patients who show no growth disturbances, other than those due to an absence of ovarian function. These patients constitute the eunuchoid type. The growth disturbance manifests itself in:

1. Late union of the epiphyses.
2. Overgrowth of the long bones.
3. Slender build.

The short and flat bones are not involved for they develop from their osseous centers. In the hypogonad the epiphyses close much later than their normal time of union. This allows excessive growth of the long bones, providing of course that the function of the growth hormone of the anterior hypophysis remains normal. Because of this late closure of the epiphysis the span exceeds the height, and the lower measurement exceeds the upper. The reverse is true of *hypergonadism*. In this syndrome the epiphyses close before their normal time of union. This results in a short individual whose height exceeds his span, and whose upper measurement exceeds the lower.

Just how the gonadal hormones influence epiphyseal closure is not clearly understood. The fact that castration in some animals leads to a delay in the epiphyseal union in the ends of the long bones was described by Sellheim (1899) and Tandler and Grosz (1910). Silberberg (1935, 1936) observed that acid extracts of anterior pituitary of cattle have a stimulating effect on the growth of cartilage and bone of growing guinea pigs. The various layers of cartilage cells become hypertrophic and hyperplastic, and are subsequently quickly calcified and replaced by bone. The epiphyseal line undergoes, therefore, premature closure under the influence of the extract. Thyroidectomized guinea pigs respond in a similar fashion to the extract.

The hypoplasia of the genitalia and the absence of secondary sex characteristics is common to both the pituitary and ovarian types of amenorrhea.

M. P. Age 20, No. 448759. First seen in April, 1935.

Chief Complaint. Never menstruated, and absence of breasts.

The patient is the second of four children, all girls. They are all taller than the patient. The oldest is 24 years of age and 66 inches in height, the third is 18 and 68 inches in height, and the youngest is 11 years and 60 inches. The patient had the usual childhood diseases. She was fairly bright at school. The two older sisters are menstruating regularly having begun at 13 and 14 respectively. Her mother began to menstruate at 14.

Patient was delivered normally at full term. She thinks that she grew nor-

mally up to the age of thirteen, but since then her rate of growth was definitely less than her sisters! There are no menstrual molymina. Physical examination—the patient is a healthy looking young woman, but appears younger than her actual age.

Height: 57¼ inches.

Span: 61 inches.

Lower measurement: 31 inches.

Head: negative.

Neck: negative.

Chest: rather heavy and seems foreshortened when compared to her legs.

Heart and lungs negative. Blood pressure: 110/70. Pulse: 88.

Skin: fine texture: soft.

Hair: absent in pubic and axillary regions. Hair on head is thick, not coarse (eunuchs are rarely bald).

Breasts: slight elevation in nipple area. No glands palpable. Nipples and areoli rudimentary.

Abdomen: round, smooth.

External genitalia: labia very small and hypoplastic. Clitoris very small.

Vagina: sound passes in 4 cm.

Uterus and cervix: by rectal examination reveals a body about 2 cm. in diameter, movable, not tender. Cervix cannot be distinguished from fundus.

Adnexa: not felt.

Neurological examination: negative.

Basal metabolic rate: minus 13 and minus 16 per cent.

X-ray of the sella: a lateral film of the skull shows a rather small pituitary fossa, with heavy posterior clinoids which bend forward at rather an acute angle. (Dr. Swenson.)

X-ray of long bones and pelvis: films of the wrists, shoulder girdles, elbows, ankles and knees show the epiphyses still ununited in the wrists, ankles, shoulder girdles and knees. This shows definite retardation of development and corresponds to an epiphyseal development of approximately 17 to 18 years. (Dr. Swenson.)

Films of the pelvis show an extremely small, but very typical android type, with marked convergence of the side walls, very narrow subpubic angle with straight rami and narrow male type of sacrosciatic notch. In fact, this pelvis reveals no female sex characteristics. (Dr. H. Moloy.)

Hormone studies: June 11, 1935; Follicular hormone—none. June 12, 1935: Follicle stimulating hormone—negative.

No other significant findings.

Wassermann: negative.

Diagnosis. (1) Hypogonad. (2) Eunuch.

Treatment and Results. Beginning with June 25 the patient has received a total of 400,000 R.U. of progynon-B. It required 150,000 R.U. of progynon-B to bring on the first period on September 26–31, 1935; 70,000 R.U. for the second on November 5–9, 1935; 50,000 R.U. for the third, on December 5–7, 1935; 20,000 R.U. for the fourth on December 25–28. The last two periods were scantier than the first.

On December 30, 1935 the following was noted—there is a definite growth of the

breasts especially in the subareolar areas. Both areoli and nipples are pigmented. Pubic hair has appeared.

The cases in this group are similar to one another. They are either tall or short, but show at the same time an overgrowth of the long bones. The short patients had the potentialities of becoming tall, but possibly an insufficient amount of growth hormone to accomplish this. It is noticeable that the cases responded in a similar fashion, and that the state of development reached was generally the same. Estrone did not seem to hasten the closure of the epiphyses.

Failure of the Müllerian Duct System

The failure of development of the Müllerian duct system may be partial or complete. There may be a complete absence of the vagina, cervix and uterus, or there may be merely a marked hypoplasia of the genital tract. Various gradations between these two extremes may be found. The *secondary sex characteristics are normal.* We are therefore dealing with an *inherent* defect in the Müllerian duct system, in spite of the presence of estrone. For even if estrone is not demonstrable in the blood or urine, the presence of well developed secondary sex characteristics speak for its presence and action at some time during development. Why did not the Müllerian duct system respond? The reason is that it is not sufficient to have a stimulus to produce a certain reaction. The end organ acted upon must be responsive to the stimulus. This will be borne out by one of the cases described below.

S. B. Age 18, No. 419042. First seen June 26, 1934.

Chief Complaint. Failure to menstruate.

History not significant. Mother and sisters began to menstruate at about 13 years.

Physical Examination. The patient is a well developed young girl with marked scoliosis.

Head: negative.

Neck: negative.

Heart and lungs: negative. Blood pressure: 104/74. Pulse: 86.

Skin: normal texture.

Hair: normal distribution—no excess.

Breasts: well developed, a little asymmetrical.

Measurements: no disproportion.

Abdomen: negative.

External genitalia, well developed. No hypertrophy of the clitoris.

Vagina: cannot be felt. There is an indentation or depression below the urethra measuring 1 cm. in depth.

Cervix and uterus: cannot be felt by rectal examination. A slight transverse thickening may be felt high up, probably the top of the broad ligament.

Adnexa: not felt.

Neurological examination: negative.

Psychological examination: The outlook of the patient is entirely feminine. She "keeps company" and desires to marry.

B.M.R.: plus 8 per cent.

Hormone studies on urine: Follicle stimulating hormone—negative. Follicular hormone—negative. Both studies repeated several times.

X-ray of pelvis: April 29, 1935: Large anthropoid with gynecoid tendency. Prognosis: Pelvis should offer no difficulty. If a transverse or posterior engagement occurs rotation should be easily brought about.

Diagnosis. Absence of Müllerian duct system.

Treatment and Results. Between June 12, 1934 and October 29, 1935 the patient received 580,000 R.U. of progynon-B, between January and September (1936) an additional 600,000 R.U. The results were as follows.

1. The breasts showed no additional growth.

2. The external genitalia showed no change. The indentation described above did not grow deeper.

3. There was no definite evidence of any growth of the Müllerian duct system. During the course of treatment a small mass about 3 cm. in diameter was occasionally felt. It did not change its size during the treatment, and from its position was evidently an ovary.

H. E. Age 25, No. 412358. First seen April 3, 1934.

Chief Complaint. Never menstruated. Primary sterility.

The patient is one of four children. One sister began to menstruate at 12 and another at 14 years. Was seriously ill at one year, but the nature of the illness is unknown. Appendectomy at 14. Usual childhood diseases, without complications.

Patient has been married for 3½ years. She comes to the clinic because she wishes to have children, but realizes that this is impossible (?) since she has never menstruated. No menstrual molymina. Leads a normal sex life and usually has orgasm. Has gained 22 pounds since marriage in spite of decreased food and increased exercise.

Physical Examination. The patient is a well developed white woman.

Head: negative.

Neck: negative.

Heart and lungs: negative. Blood pressure: 122/84. Pulse: 78.

Breasts: well developed. Nipples are small.

Hair: normal distribution. No hirsutism.

Extremities: negative. No disproportion in length.

Skin: normal texture.

Abdomen: negative. Healed scar in right lower quadrant (appendectomy).

External genitalia: fairly well developed.

Pelvic floor: intact.

Vagina: short and shallow, fornices about equal in depth and very shallow.

Cervix: small, hypoplastic, "pin-point" os.

Uterus: anterior, very small, movable, smooth, fundus somewhat larger than cervix.

Adnexa: negative.

Neurological examination: negative.

Basal metabolic rate: minus 16 per cent.

Follicle stimulating hormone (urine): slightly positive.

Follicular hormone (urine): negative.

X-ray of pelvis: January 31, 1935. Lipiodol x-ray of no consequenee. Repeated June 18, 1936. A.P. stereos of the pelvis, with the uterine cannula in place and following injection of lipiodol show the lipiodol to be outlining the Fallopian tubes which do not suggest abnormality. The uterus is not filled for study. The pelvimetry films taken later still show some lipiodol in the distal ends of the tubes. Incidentally there are some asymmetrical facets at the lumbosacral junction.

No other significant findings.

Therapy and Results. The patient was given progynon-B in doses of 10,000 R.U. each, intramuscularly in the buttocks. In addition ½ grain of thyroid extract, t. i. d.

A total of 1,300,000 R.U. of progynon-B was given between April 24, 1934 and December, 1936.

On September 18, 1935 after an amenorrhea of two months the patient was re-examined with the question of possible pregnancy. The following are the findings:—

Vagina: very moist, slight cyanosis.

Cervix: softened, slightly bluish.

Uterus: anterior, antiflexed, normal in size, a little softened.

Adnexa: negative.

Diagnosis. Premenstrual uterus? Early pregnancy?

An Aschheim-Zondek Test was negative on September 25, 1935 and another endometrial biopsy was removed.

The results obtained in these two cases are of interest. (1) The secondary sex characteristics were not overstimulated in either patient. They had apparently reached their limits of growth. (2) The response of the genital tracts varied a great deal. Patient *S. B.* did not respond at all. To the best of our knowledge there was a complete failure of development of Müllerian ducts. The anlage may have been present, but its subsequent growth and differentiation were absent. It is probable that for the development of the Müllerian ducts a functioning embryonal ovary is necessary. If we assume that such a functioning ovary was absent during intrauterine life (or at least during the first half) but developed later, then in the interim the Müllerian duct might have lost its ability to respond to the gonadal hormones. They (the ducts) may have a different rate (or ability) of response (early) in intrauterine life than later during extrauterine existence. We know that secondary sex characteristics are capable of development later in life, providing an adequate supply of hormone is present. Furthermore, more estrone is required to stimulate growth of the uterus and vagina than the amount required to stimulate the growth of secondary sex characteristics.

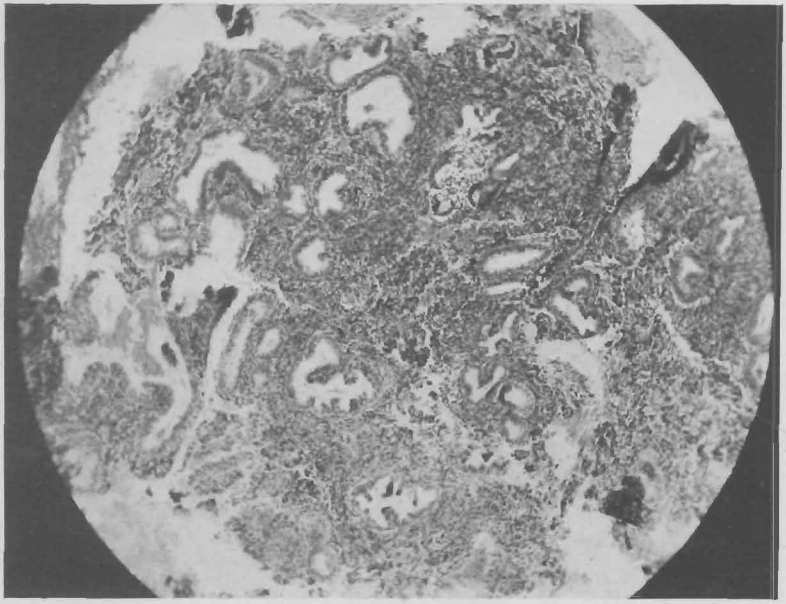


FIG. 118A. Primary amenorrhea. Case H. E., aged 25. Endometrial biopsy on second day of first period. Followed the administration of 30,000 R.U. of progynon-B. Endometrium in general is postmenstrual in character. In several areas the glands show papillae and suggestive secretion. ×75.

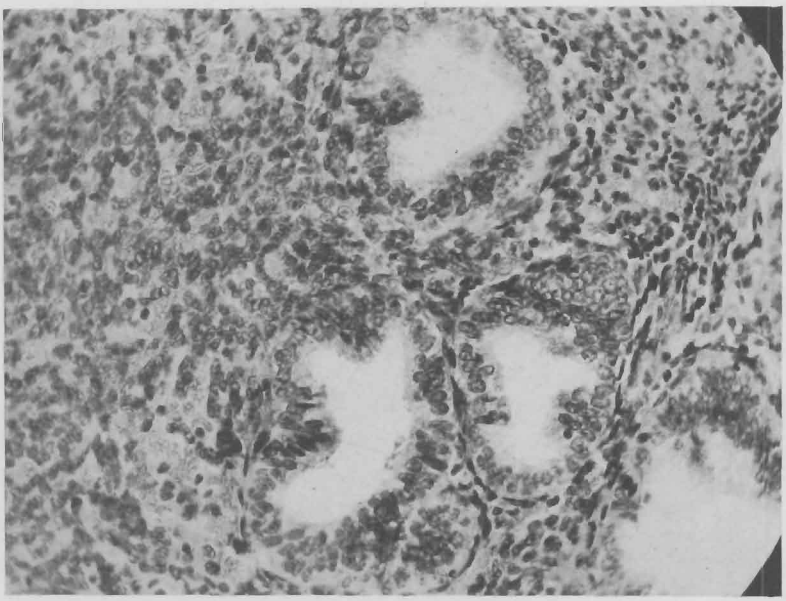


FIG. 118B. Primary amenorrhea. Case H. E., aged 25. Note the density of the stroma, and the pseudostratification of the glandular epithelium. ×750.

Hence the concept, that the failure of the growth of a uterus and vagina may be due to the possibility that the ovaries began their function after birth (or shortly before), acquires some validity.

Are foetal hormones similar to those of later life?

The second case (H. E.) is also of considerable interest. Growth of the uterus and vagina was secured by means of progynon-B. In fact, the growth was extensive enough to suggest the possibility of pregnancy. Cyclical bleeding occurred. The subsequent history of this patient brought out other interesting points. The patient stopped treatment for several months. When she was seen again the uterus had regressed from its maximum growth, to a size somewhat larger than that found at the beginning of treatment. If the patient had functioning ovaries, why then was the growth not maintained? When first seen by us the hormonal findings suggested ovarian hypofunction. It seems therefore that not only were we dealing with an inherent genital hypoplasia but also with an added (later) ovarian hypofunction. This brings up the question of a reciprocal stimulation between ovary and uterus.

As to the etiology of the genital hypoplasia of case H. E. the same explanation as that offered for case S. B. may be suggested. Ovarian function might have started a little later than normal, late enough for the Müllerian duct development to be so compromised as not to be able to approach normal growth and differentiation.

Prognosis of Cases of Primary Amenorrhea

The foregoing description of several typical cases suggests what can be accomplished with our present therapeutic armamentarium. Something has been achieved with these cases that was not possible a few years ago. We know that the results that we have obtained are reversible, that is, the patients revert to their original status of sexual development upon cessation of treatment. This may be unsatisfactory to some clinicians, but we feel that a beginning must be made. The results obtained point in the right direction. The next few years will see the production of more potent gonadotropic hormones, possibly in the form that will allow successful medication by mouth, or in such solvents as will permit few injections, but with prolonged and constant action. To-day we are in a position to inject 100,000 R.U. of estrin at a dose. Ten years ago the combined laboratories in the world did not possess that amount of hormone.

In considering all our cases of primary amenorrhea as a whole, one is impressed by the following. It usually takes a large dose of estrone to

produce the first period and less as time goes on. Eventually a dose of 20,000 to 40,000 R.U. is reached that maintains fairly regular flow. But as time progresses this maintenance dose becomes inadequate and more and more hormone is needed to produce the desired effect. At the same time growth of all secondary sex characteristics is at first rapid but then slows up considerably. Eventually a stage is reached where even very large doses of estrone produce only infrequent and very scanty periods. This demonstrates a resistance on the part of the patient to the medication. Is this resistance due to the development of an antibody against estrone or a modification of the molecule, or is it due to some process of inactivation?

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CHAPTER XX

SECONDARY AMENORRHEA

By secondary amenorrhea we mean the cessation of menstruation at any time after the menstrual rhythm has begun, up to the time of menopause. It is a very common gynecological *symptom.* Secondary amenorrhea is not a disease, but usually implies a dysfunction on the part of the organism. The etiological factors are numerous, and one occasionally sees cases in which an etiological factor cannot be determined. Many syndromes are associated under this heading, the reason being that the cases present themselves not as syndromes but as patients with the significant symptom, secondary amenorrhea.

Secondary amenorrhea may be divided into two types in so far as etiology is concerned, namely:

A. Physiological amenorrhea.

B. Pathological amenorrhea.

Physiological Amenorrhea

Physiological amenorrhea occurs during:

1. Pregnancy.
2. Lactation.

Pregnancy. While by far the greatest number of patients show no evidence of menstruation during pregnancy there are some women who bleed to a greater or lesser extent at the expected menstrual time. This occurs only during the first four months, that is, up to the time of fusion of the decidua capsularis and parietalis. On the other hand there are patients who present their usual menstrual molymina throughout pregnancy without any evidence of bleeding. The bleeding, when it occurs, is truly menstrual in character, for, according to our definition it occurs from a secretory endometrium. Actual desquamation of tissue is rare, the process seems to be more of a diapedesis.

What is the etiology of the amenorrhea of pregnancy? It is not due to the foetus, for the amenorrhea persists after the death of the foetus. On the contrary, in ectopic gestation, the bleeding is often initiated by the death of the embryo. Its death may be due to destruction of the greater part of its chorionic epithelium. The ovarian factors necessary for menstruation are present, for both follicular and corpus luteum hor-

mones are available. The reason for the huge estrin excretion during pregnancy is not as yet clear. Progesterone is present in the corpus luteum verum and in the placenta (Ehrhardt and Fischer-Wassels, 1936). Wilson and Kurzrok (1936) suggest that the pituitary like hormones (prolan A and B) produced during pregnancy by the placenta, inhibit the production of the bleeding factor. As to the origin of the bleeding factor Smith (Smith, Tyndale and Engle, 1936) demonstrated that the pituitary is not essential for bleeding in the Macacus Rhesus Monkey. Any hemorrhage is of potential danger to the foetus, hence some mechanism for the prevention of its occurrence is expected. The problem has not been settled. It is conceded by many that the follicles do show some growth during pregnancy but they evidently undergo atresia. A follicle may ripen and ovulate but it must be a relatively rare occurrence. Injection of prolan A and B does not induce ovulation (Hamblen and Ross, 1936).

Lactation. It is well known that many women do not menstruate during lactation. Others menstruate throughout lactation, while still others do not menstruate for the first few months but do so during the latter. Furthermore, some women conceive during the lactation amenorrhea, while others, equally fertile, do not. This implies that ovulation occurs in some but not in others. What then is the status of anterior pituitary and ovarian function, hormone production, and the endometrial cycle during lactation? Kurzrok, Lass and Smelser (1937) studied a group of lactating women who were having regular cycles. They observed that between 55 and 70 per cent of the women showed anovulatory cycles. The bleeding, therefore, was not true menstruation for it did not come from a premenstrual endometrium. Several cases stopped having cycles during this period of observation. There was no evidence of cyclical endometrial change during the period of amenorrhea. Hormonal studies are now being carried out on this group.

Pathological Amenorrhea

Amenorrhea is an important symptom. While it is true that it occasionally implies merely a cessation of bleeding, it more often implies a hypofunction of the gonads. Hypofunction of the ovaries leads to secondary atrophic changes in the genital tract. The uterus, cervix and vagina must constantly be stimulated, otherwise their morphological and functional status regresses. It is well known to gynecologists of experience that during the period of amenorrhea patients complain of a host of symptoms which are relieved by the menstrual flow. It

might be argued that the etiological factor causing the amenorrhea is also responsible for the other symptoms. This is often true, but not always. A flow may be established without influencing the etiological factor, and the patient experiences considerable relief. There may be more to menstruation than the mere desquamation of a pregestational endometrium. The idea of a menstrual "toxin" comes back frequently to plague the conscience of the gynecologist (Schick, 1920: Macht and Lubin, 1924; Mommsen, 1934). The importance of amenorrhea in sterility is not to be underestimated.

Pathological amenorrhea is due to numerous causes. Of these the following are the most important.

Disturbances of the Genital System.

1. The uterus—genital hypoplasia.
2. The ovaries.

Genital hypoplasia—underdevelopment of the genital tract is one of the commonest causes of amenorrhea. As stated previously, hypofunction of the ovaries may lead to secondary atrophy of the genital tract but the cases under consideration at this point show no evidence of ovarian failure. In fact, the hypoplasia is present in spite of normal ovarian function (as evidenced by hormone titration).

Genital hypoplasia may be part of a *general* or *universal infantilism* (Aschner, 1924), or a *partial infantilism* which is limited to the genital tract. Universal infantilism forms the commonest and most important constitutional anomaly in women. It may be defined as a condition in which the complete development of the whole organism (or its component organ systems), insofar as morphology and function are concerned, does not occur at all, or occurs very late. The syndrome implies immaturity, both somatic and psychic. Of particular interest to us is the problem of *local* genital infantilism or hypoplasia. Genital hypoplasia may be internal or external or both. A hypoplasia involving the external genitalia *alone* is rare. Usually it is associated with an internal hypoplasia and forms part of a general infantilism. On the contrary internal genital hypoplasia is fairly common and in the discussion below this is the form under consideration.

Physical Signs of Genital Hypoplasia. External Genitalia: Labia majora—are small, and sparsely covered by hair.

Labia minora—small, occasionally just barely indicated.

Perineum—short, often with an indentation just above the anus. Occasionally a median fold crosses the perineum from the fourchette to the anterior anal wall, representing the fusion of each lateral anlage.

Vagina—either short and shallow, or narrow, conical and long. Occasionally prominent transverse ridges may be felt, resembling the condition found in the foetal vagina. The fornices are poorly developed, are shallow, and are usually of equal depth. Occasionally there may be felt in the anterior fornix a curved linear thickening in the vaginal wall, extending forward from the cervix. Saenger described it as the "crista cervicalis mediana," and considers it as a vestige of the fused Müllerian ducts.

Cervix—small and may point in any direction. The os externum is usually "pinpoint." Occasionally the cervix has a lobulated appearance with a ridge-like prominence along the lowest circumference.

Uterus—is most commonly involved, and may show variations in size, length, form and position. The total length is shortened to a greater or lesser extent. The cervix may be as long as, or longer than the fundus. Thus there are two types of hypoplastic uteri. One, in which the foetal form is maintained, that is, the cervix is longer than the fundus. Such a uterus may grow to normal length, but there remains always this *inhibition of differentiation*. The second type presents the adult differentiation, but is smaller in *size* than the normal uterus. The latter type occurs more frequently. There is either acute retroflexion or antiflexion, with accompanying acute angulation of the canal. The reason for the acute flexions is not clear. It has been suggested that the acute antiflexion is due to short utero-sacral ligaments and their insertion higher up on the fundus. On the other hand, the ligaments may be short because the uterus lies well back. There may be lateral flexions of the uterus to the right or left due to shortness of the broad ligament on the same side. The narrowing at the internal os may be due to the acute angulation or to excessively prominent folds of the arbor vitae, the latter a persistent foetal condition.

The ovaries—they may fail to descend into the pelvis and therefore remain situated above the linea terminalis. The pull of the abnormally situated ovarian ligaments may be sufficient to overcome the pull of the round ligaments and thus result in a retroversion or flexion. The ovary proper may be small, its surface smooth due to inadequate follicle growth.

Tubes—may be tortuous. This is due to the persistence of a foetal condition, for in descending from their original position along the lumbar spine to the pelvis a certain amount of spiral twisting occurs. Normally when reaching the pelvis the tubes lose their spiral twist (Mayer, 1927).

Etiology. The damage that eventually gives rise to genital hypoplasia (as well as general infantilism) may occur in:

1. The germ plasm.
2. The foetus.
3. The child.
4. The adult.

It is very difficult and frequently impossible to state when the damage occurred. It is well known that several cases may occur in one family. We have at present under our care two sisters with an identical genital hypoplasia, one suffers from amenorrhea and the other from hypermenorrhea. Aschner points out that conditions which injure germ plasm such as chronic infections (lues, tuberculosis) and intoxications (morphine, alcohol) may cause infantilism, either general, or involving one organ system. He also believes that advanced age of the parents has an unfavorable effect on the germ plasm. Similarly, malnutrition and disease may exercise the same influence. The ultimate effect of the x-ray on the germ plasm is still under discussion. In addition, Aschner adds prematurity. He points out that the mother with genital hypoplasia tends to premature delivery. She may thus transmit to the child infantilism as a dominant characteristic.

The development of the uterus during foetal life is a progressive condition. The factors that enter into play at this time are fourfold, namely:

A. The genetic factor of sex.
B. The Müllerian duct system.
C. The ovaries.
D. The anterior pituitary gland.

The sex of the individual is determined at the moment of fertilization. This also predetermines the character of the gonad. Thus we have the sex of the individual determined by genetic and hormonal factors. The Müllerian ducts develop very early in embryonic life. Their subsequent growth and development depend upon the genetic impulse, and the ovarian hormones, which in turn depend upon a hormonal drive from the anterior pituitary gland. An inadequacy of any of these factors would lead to a retardation in the rate of growth of the Müllerian ducts. Thus the defect might be corrected to a greater or lesser extent in later life, or the defect may be permanent. The greatest impetus to growth comes during intra-uterine life, either from more potent growth promoting factors or from a greater response of the growing tissues.

During childhood the genital canal is stimulated to further progressive growth by gonadotropic and ovarian hormones (leaving out for the

moment the other hormones). Should the stimulus be insufficient or the genital tract non-responsive, then genital hypoplasia will result.

Furthermore, in adult life conditions causing prolonged secondary amenorrhea may lead to resultant uterine atrophy. The atrophy may lead to a small uterus, but other distinguishing features of a true genital hypoplasia are usually absent. A normal uterus undergoing secondary atrophy retains its adult proportions. It must be remembered (v. i.) that genital hypoplasia, per se, may lead to secondary amenorrhea.

Symptoms. The effects of genital hypoplasia may manifest themselves in several ways, namely, in:

1. Disorders of menstruation.
2. Sterility.
3. Repeated miscarriages.
4. Difficulties of labor.
5. Menopause precox.

Before discussing these topics it is advisable to call attention to the hormone findings in genital hypoplasia. Kurzrok and Rätner (1932) pointed out that many cases show a normal estrone excretion in the urine. Other cases, fewer in number, show an absence of estrin on repeated examinations. The follicle stimulating hormone shows no abnormal variation, in that, with the original Zondek technique none is found in a morning specimen of urine. The above findings have since been confirmed by us many times.

Menstruation. Genital hypoplasia may be associated with all abnormalities of menstruation. Puberty may be retarded. If the defect is ovarian in origin then the secondary sex characteristics will develop late. The menses, having begun, may continue with greater or lesser regularity, and then gradually become more infrequent and scanty and finally cease altogether. This may occur in the twenties or early thirties. When the periods are regular the hormonal findings may offer a clue to the etiology of the hypoplasia in any particular case. The hormonal findings may be normal. This implies a normal production of estrone on the part of the ovaries. Why then the genital hypoplasia? The conclusion seems justified that even with a normal hormonal supply the Müllerian duct system failed to respond. An inherent defect in the genital tract seems to be basically responsible for the condition. Again, the hormonal findings may show an insufficiency of estrin. This is most frequently seen in women who present the various types of pituitary adiposity. The associated adiposity, genital hypoplasia and low estrin values, speak for a pituitary hypofunction.

The menstrual flow may show abnormalities ranging from hypo- to hypermenorrhea. The same patient may have scanty menstruation at one time and profuse at another. This is understandable when one considers that the hypoplasia involves the myometrium, endometrium, vascular supply and probably the local nerve mechanism. Whether patients with genital hypoplasia have a greater number of anovulatory cycles than normal women is not known. A compromised myometrium may exhibit inadequate contractions for the control of menstrual bleeding.

Dysmenorrhea is very common in this syndrome. It may be due to some defect in uterine innervation. A hypoplastic uterus may also exhibit irregular uterine contractions. We feel that it is the irregularity of the contractions more than their intensity and tempo that predisposes to dysmenorrhea. It has been our experience that the administration of estrin in large doses, especially during the first half of the cycle leads to relief of this type of dysmenorrhea. Apparently growth of the uterus plays a part in determining the type of contractions. The acute angulation present in such uteri may offer an obstruction to blood and tissue fragments coming from the fundus. As growth of the uterus progresses the angulations become less marked.

Genital hypoplasia is one of the commonest inherent causes of *sterility* in women. There are several possibilities as to the mechanism. The anovulatory cycle may be a factor. The endometrium may be insufficiently developed to harbor a fertilized ovum. The secretions from a hypoplastic endometrium may be inimical to both sperm and fertilized ovum. Considering the importance of this condition (hypoplasia) there is woefully little exact work on the aspect that touches upon sterility.

A further difficulty is encountered during *pregnancy*. Repeated early miscarriage is not uncommon. Whether this is due to the inability of the uterus to expand proportionately with the growth of the foetus, or to irregular uterine contractions, or to changes within the foetus as the result of inadequate blood supply is not known at present. Mayer points out that women with genital hypoplasia are apt to suffer from considerable abdominal discomfort throughout pregnancy due to the inadequate expansion of an infantile uterus. Premature labor often occurs. When pregnancy does go to term the pains are inadequate and the labor long. Postpartum hemorrhage may occur.

Premature menopause is another important consequence. Why should the ovary cease to function earlier than usual? For we have

not only cessation of bleeding but also cessation of ovarian function. The latter is evidenced by the hormonal findings (Chapter XXIII). Two intriguing explanations present themselves. First, the ovary has a limited life and is capable of producing just so much hormone. That amount is expended upon a hypoplastic genital tract, which requires for its function a greater hormonal drive than the normal uterus. Second, the uterus exerts some stimulating influence upon the ovaries. The removal of the uterus (no matter how careful one is to preserve the blood supply) leads to cessation of ovarian function, much sooner than otherwise. An infantile uterus when it ceases to respond to ovarian hormones may also lose its stimulating effect upon the ovaries. The concept that the stimulus, ovary to uterus, also functions in the opposite direction, has much in its favor, though experimental proof is lacking.

Aschner states that the hypoplastic genital tract has a tendency to favor ectopic gestation, due to increased tortuosity of the tubes. Furthermore, pelvic infections, especially tubercular, run a more stormy and protracted course.

Treatment. Two broad methods of treatment are potentially available, namely, to stimulate genital growth by means of gonadotropic hormones, or by means of ovarian hormones. The exhibition of gonadotropic hormones should be the method of choice. But large doses of these hormones, especially the follicle stimulating hormone, are not as yet available therapeutically. Pregnancy urine extracts containing prolan A and B do not stimulate the follicular apparatus to any extent (Hamblen and Ross, 1936). The gonadotropic effect of pregnant mares' serum on the human ovary is very marked but the use of this material has not as yet entered the clinical phase.

The substances available for therapy are therefore estrone and progesterone. Up to the present time the high cost of the latter and its scarcity have prohibited its clinical use. The main therapeutic agent has been estrone in one of its commercial forms. We have used amniotin or theelin when smaller dosage was required and progynon-B when large dosage was essential. It might be inquired, why not use very large doses of estrone at all times, and thus secure the greatest therapeutic effect. Our results in a large series of cases of genital hypoplasia have shown us that while large dosage of progynon-B frequently brought about an immediate result, specifically where bleeding was desired, its persistent use in the more moderate cases was not necessary, and occasionally after some time, detrimental. We occasionally noted moderate but persistent headache as the result of continuous injections of progy-

non-B. The dosage necessary to produce headache was 20,000 R.U. per week for six to eight weeks. Not all patients showed this, and what is very striking, the patients with primary amenorrhea showed it very rarely, or not at all. The headache usually cleared up within four or five days after the hormone was discontinued. The cause of the headache was probably the enlargement of the anterior hypophysis which results from the administration of large doses of estrin (Halpern and D'Amour, 1936; Kurzrok and Kirkman, unpublished results).

There is an additional theoretical objection to the use of such large dosage here. It has been demonstrated experimentally that large doses of estrin depress the production of the follicle stimulating hormone. This in turn would be followed by the ripening of fewer follicles and secondarily by diminished estrone production. Such therapy would therefore defeat our purpose, which is to exhibit estrone, and also to maintain ovarian function.

The age of the patient with genital hypoplasia is of considerable importance in the response to therapy. All other factors being equal, patients under twenty-five respond best, while those above thirty-five respond least. As a general rule it may be stated that the larger the uterus at the onset of treatment the greater will be the response to therapy. At the same time there are considerable individual variations. The first response to estrone treatment is an increased vascularity of the genital tract. This manifests itself in a distinct leucorrheal discharge, cyanosis of the vagina and cervix, and softening of the uterus. The entire picture resembles an early pregnancy, except that the total size of the uterus is smaller. When a pregnancy does occur the uterus lags behind in growth when compared with the normal uterus. Our impression is that they tend to approximate in size at about the fourth month. The enlargement due to increased vascularity is temporary, for when treatment is discontinued the original size pertains.

When actual growth occurs the result may be due to hypertrophy or hyperplasia, or to both. In mild cases hypertrophy of tissue alone would seem to be adequate. Where growth is extensive hyperplasia and hypertrophy must occur. We have seen patients whose vagina was about 1 cm. in diameter before treatment, but at the end admitted a medium speculum with ease. In such cases hypertrophy alone seems inadequate.

An extremely important question is: How permanent are the results? We are unable to judge this from our present-day standpoint. Our experience is still too limited, for the treatment in vogue at the present

has not yet weathered the test of time. Our oldest cases do not exceed five years. It is our impression that the genital hypoplasia due to pituitary (hence ovarian) insufficiency (that is, when associated with inadequate development of the secondary sex characteristics and absence of follicular hormone from the urine on repeated examinations) reverts back to the original hypoplastic condition, sooner or later, when therapy is discontinued. On the contrary, when the hypoplasia is due to an inherent defect in the Müllerian duct system, associated with normal ovarian function at the time when the patient is first seen, then the growth which results from therapy is maintained.

The Ovary (and the Anterior Hypophysis). In view of the intimate relationship between the ovary and the anterior pituitary gland the discussion must include both glands. As stated previously the ovary will not function alone but is stimulated to do so by the anterior hypophysis. Hence the demonstration of one or the other of the ovarian hormones implies pituitary function.

Four hormones (at least) are involved in the process of menstruation, namely two ovarian, estrone and progesterone, and two gonadotropic—the follicle stimulating (F.S.H.) and luteinizing hormones (L.H.). Only estrone and F.S.H. have been demonstrated in the blood or urine in the non-pregnant state. The remaining hormones have not been found, because our technique is not sufficiently fine enough to demonstrate them in minute quantities. We will therefore consider the pituitary-ovarian relationship to secondary amenorrhea from the viewpoint of the two demonstrable hormones.

Considering, therefore, estrone and F.S.H. alone, four types of excretion are present (Kurzrok, 1932), namely:

I—F.S.H. absent	Estrone—present
II—F.S.H. absent	Estrone—absent
III—F.S.H. present	Estrone—absent
IV—F.S.H. present	Estrone—present

With Kurzrok and Ratner's (1932) method for estrone determination the hormone has practically always been demonstrated in the urine of normally menstruating women. F.S.H. as determined by Zondek's method is usually absent except just preceding ovulation (Kurzrok, Kirkman and Creelman, 1935). Hence the normal (F.S.H.) is always a negative test, and the presence of too much F.S.H. is abnormal. Albright, Halsted and Cloney (1935) are in general agreement with the above findings.

Type III Excretion. Estrone Negative; F.S.H. Positive. Given a

case of secondary amenorrhea in which the homone excretion *on repeated tests* (at least two, taken a week apart) is of Type III (F.S.H. present, estrin absent) the diagnosis of cessation of ovarian function may be made. It signifies menopause (whether spontaneous, surgical or roentgen irradiation). It might be added in passing that during *temporary* x-ray castration the same hormonal excretion occurs, but as the effect of the rays wears off the F.S.H. becomes negative and the estrin positive. In other words type A excretion is not always an irreversible reaction. The importance of *repeated* hormone examination must be stressed. Ovulation may occur during a period of amenorrhea. Ovulation during a normal cycle would usually be represented by F.S.H. positive and estrin positive, but during amenorrhea may be represented by type III excretion. A repetition of the test one week later will determine the exact state of events. For if reaction Type III recurs then we are dealing with a cessation of ovarian function. The sudden appearance of F.S.H. in the urine just preceding ovulation is "explosive" in character and disappears within twenty-four hours.

Case. M. D., age 20, No. 499748. (Fig. 119.)

Chief Complaint. (1) Amenorrhea—18 months. (2) Headaches, dizziness and backache—18 months. (3) Hot flushes 2 to 3 per day—18 months. (4) Gain of 100 pounds in past two years.

Present Illness. Eighteen months ago without any preceding menstrual irregularity there was sudden onset of amenorrhea. There has been no vaginal bleeding since that time. Gradual onset of headaches, dizziness and backache. Hot flushes began gradually and have increased in frequency. There has been a steady, gradual increase in weight amounting to 100 pounds. No increase in appetite or thirst. Slight increase in urinary frequency. Chronic constipation and piles. Dyspnea and palpitation on two flights since gain in weight. Has been suffering increased "nervousness" irritability and depression for the past year.

Menses: onset 11 years; 28 day type; 3 days in duration; moderate in amount and without pain.

L. M. P. {May 9, 1935.
{April 9, 1935.

Marital: married three years. No children. Was overdue a week on two or three occasions. Took castor oil and quinine and brought period around.

Operation: Appendectomy in 1930, followed by "rectal" abscess.

Significant Physical Findings. The patient is a very obese young married woman of twenty years whose fat distribution is mainly about the pelvic girdle. Breasts are very pendulous and large with small nipples—gland being composed mainly of adipose tissue. Hands small, ankles rather moderate. Skin of normal texture. No abnormal distribution of hair. Abdominal stria present.

Abdomen: obese. No masses or viscera palpable. Deep suprapubic scar. No hernia.

Pelvic: External genitalia: negative, no hypertrophy of clitoris.
Pelvic floor: intact.
Cervix: nulliparous, small.
Vagina: short, shallow, narrow.
Uterus: small, anterior, movable, firm.
Adnexa: not felt.

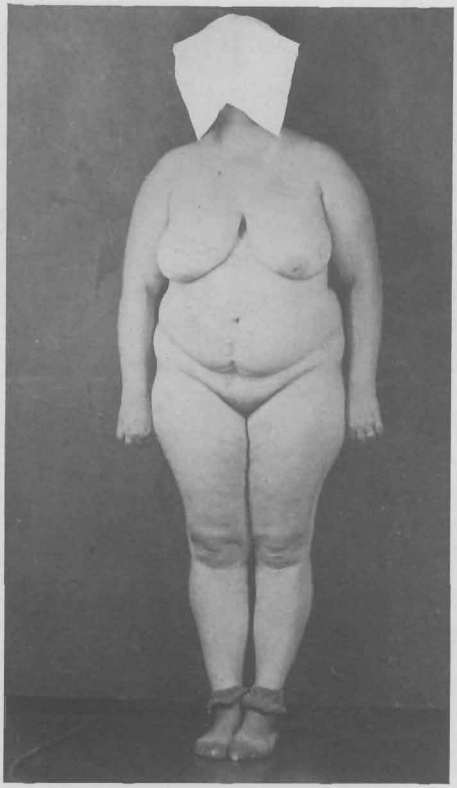


FIG. 119. Premature menopause at 19 years of age. Note trunk adiposity

Diagnosis. (1) Genital hypoplasia. (2) Fröhlich's syndrome—secondary. (3) Menopause precox.

Endometrial Biopsy. Endometrium very thin. Insufficient quantity removed for exact diagnosis.

B.M.R.: plus 6 per cent. Weight 105.7 kgm.

Blood count: Hemoglobin: 90 per cent. R.B.C.: 4,630,000. W.B.C.: 8,600. Polymorphonuclears: 52 per cent.

X-ray of skull (Dr. Friedman): A lateral film of the skull shows no definite evidence of enlargement of the sella turcica. There is no evidence of erosion or atrophy suggestive of intracellar tumor.

Hormone determinations—urine: estrone and follicle stimulating hormone.

October 22, 1936 { Estrone—negative / F.S.H.—positive }

October 26, 1936 { Estrone—negative / F.S.H.—positive } Reaction III

November 4, 1936 { Estrone—negative / F.S.H.—positive }

Visual fields: normal.

Discussion. The amenorrhea, flushes, sweats and irritability are very suggestive of premature menopause. This diagnosis is confirmed by the repeated finding of large quantities of follicle stimulating hormone and an absence of estrone in the urine. It seems reasonable to believe that the primary defect resides within the ovaries. They "aged" prematurely. They can no longer respond to the gonadotropic hormones present in the system. Considering the genital hypoplasia it is doubtful whether their function was ever complete. The adiposity might have resulted from a pituitary (hypothalamic?) dysfunction secondary to the loss of estrone. In view of the extensive genital hypoplasia it is rather doubtful whether the patient was ever pregnant. The prognosis is uncertain as to whether ovarian function will ever return. The youth of the patient is slightly in her favor. Under similar circumstances, we have not seen a return of function when the patient has passed thirty. As to therapy we believe that large doses of estrone are contraindicated, for fear of further depressing the function of the anterior pituitary gland. An ovarian transplant from a young woman will be attempted.

Type IV Excretion. F.S.H. Present; Estrone Positive. This is the usual finding just preceding ovulation. It implies the pituitary stimulus and the ovarian response. It is an unusual finding during secondary amenorrhea though it is occasionally found at the beginning of menopause. The finding may signify a certain imbalance between the anterior hypophysis and the ovary. Furthermore, Type IV excretion, may be the first biological evidence of pregnancy. It may precede a positive Aschheim-Zondek Test (Kurzrok, Kirkman and Creelman, 1935). A repetition of the test a few days later would establish the correct diagnosis.

Type I Excretion. F.S.H. Absent; Estrone Present. This is the normal finding. It may also be found during secondary amenorrhea, and denotes both hypophyseal and ovarian function. Type I excretion, amenorrhea and genital hypoplasia are frequently found together.

That is, when the genital hypoplasia is accompanied by normal secondary sex characteristics. When the uterus is normal in size and there are no other significant physical findings then the question arises whether the amenorrhea in the case at point is not due to an absence of the much debated bleeding factor. It might be argued that possibly the corpus luteum phase of the cycle is incomplete, hence the patient does not menstruate. This is true when menstruation alone is considered, but cyclical bleeding may occur when the estrin phase *alone* is present. An endometrial biopsy taken just before the expected period will immediately orient us as to the presence of a pregestational phase.

Type II Excretion (F.S.H. Absent; Estrin Absent). This is only infrequently found in the normal patient, especially on repeated examination. It is usually found associated with secondary amenorrhea, hypo- or oligomenorrhea. It implies, in the main, pituitary hypofunction in as far as gonadotropic hormone is concerned. For if the anterior hypophysis were functioning and the ovary was not responsive, then we would expect to find F.S.H. in the urine.

Disturbances of the Endocrine System

The Pituitary Gland. One of the commonest conditions associated with secondary amenorrhea is obesity. One frequently encounters discussions as to which symptom leads to which. In all probability both are due to a common endocrine disturbance, most often in the anterior pituitary gland, less often in the thyroid, and very rarely primarily in the other glands of internal secretion.

In order to understand the nature of the metabolic processes involved in obesity and its relation to the pituitary and ovary, a short résumé of lipoid metabolism will be given. For a more comprehensive survey the reader is referred to the work of Peters and Van Slyke (1931). By lipoids we mean substances in the organism which resemble fats in their chemical and physical properties. The lipoids may be subdivided into the following three classes.

a. True fats, or triglycerides of fatty acids.

b. Lipins, substances of fat-like nature which (on hydrolysis) yield fatty acids or their derivatives, and in addition contain either nitrogen or phosphorus, or both.

c. Sterols, complex chemical substances which do not resemble fats chemically, but whose importance is constantly increasing. The gonadal hormones are sterols.

Fats are triglycerides of fatty acids, that is, esters of fatty acids and

glycerine. Palmitic, stearic and oleic acids are the commonest in the animal organism. The latter is unsaturated, and all contain an even number of carbon atoms. The fat in the body resembles the ingested fat, but when an animal is fed a fat-free diet, it develops a body fat of constant composition, which is characteristic for that animal. It is known as "physiological fat." Both types of fat are utilized by the animal organism interchangeably. Distinction has also been made between depot (storage) and tissue fat, the former implying reserve fuel and the latter is an integral part of the tissue protoplasm. Tissue fats are probably phospholipins. The fat depots are the subcutaneous tissues, mammary glands, perirenal tissues, retroperitoneal tissues, etc. The size of the fat depots depends upon hereditary, sexual, metabolic, endocrine and dietary factors.

The ingested fat is digested in the intestine by the lipase of the pancreatic juice and the succus entericus into fatty acids and glycerine. This reaction is assisted by the bile which aids the emulsification of fat and promotes the action of the lipase. The fatty acids are converted into soaps by the alkali of the intestines and are absorbed as soaps. They are then reconverted into fatty acids (possibly by the aid of the phosphatides), absorbed by the lymphatics, reach the blood stream and then the lymphatic duct. The systemic blood fat begins to rise in from one to three hours after a fat meal, and the lipemia reaches its peak in six to seven hours, returning then to its original level. In all probability the free fat enters the fat cells of the depots without undergoing any change. In the process of catabolism the first step may be desaturation of the fatty acids in the liver. The next step is oxidation which takes place at the β carbon atom, so that two carbon atoms are split off at a time. This reaction yields a fatty acid with two carbon atoms less, plus acetic acid. The process continues until a 4 carbon acid is produced, butyric acid, which in turn is oxidized to acetoacetic acid. The latter substance is a ketone and can only be oxidized in conjunction with carbohydrate. If we begin with a fatty acid with an odd number of carbon atoms ketone bodies cannot be produced, for a 4 carbon acid does not result.

The excessive accumulation of fat is known as obesity. There are differences in the pattern of fat distribution in both sexes. Castration and hypopituitarism in the male cause approximation to the female type. Adiposity may be generalized or localized to particular areas. Sudden increases of weight may occur in adolescence, after pregnancy, and at the menopause.

Are there any differences in metabolism that differentiate the obese from the normal or the lean? To date no such differences have been found. It must be true that the obese ingest more food than their immediate needs, but many obese people are not heavy eaters. Nor is it a matter of muscular activity, for many stout people lead very active lives, and at the same time it is also true that a stout person will expend more energy than a thin one. Apparently the metabolic processes are more economical in a fat person than in a thin one. The basal metabolic rate is usually within normal limits. Hagedorn, Holton and Johansen (1927) found that if obese persons were fed a high carbohydrate diet for several days and then the basal respiratory quotient taken, it was lower than that of normal people under similar conditions. They interpreted this to mean that the obese people converted their carbohydrate to fat and then burned the latter. Gardner-Hill, Smith and Jones (1925) showed that adolescents presenting pituitary adiposity and increased sugar tolerance, exhibited less than the normal rise of respiratory quotient after sugar. Adrenalin produces a rise in the basal metabolic rate and in the respiratory quotient, the elevation remaining as long as the effect of the drug persists. The reasons for this are probably multiple. Kranz and Means (1927) found a diminution in the respiratory quotient in the obese following adrenalin, and believe that more fat than carbohydrate was utilized.

The relationship of the ovary to the oxidation of ketogenic material has recently been studied by Butts and Deuel (1933) and Butts, Cutler and Deuel (1934). They showed that when the sodium salt of acetoacetic acid (ketogenic material) was administered to fasting rats large amounts of acetone bodies were excreted in the urine (ketonuria) due to failure of complete oxidation. The surprising finding was that the urine of the females contained twice as much unoxidized material as the male. The same holds true for the human. The administration of carbohydrates results in the oxidation of these ketogenic substances, hence the conclusion is drawn that there exists a fundamental difference in carbohydrate metabolism between the sexes (since carbohydrates are required to oxidize these ketone bodies). Furthermore, when the ovaries are removed, the oxidation of the diacetic acid is increased, so that the ketonuria is about one fourth that found in normal females and about one half that obtained with normal male rats. The injection of theelin does not significantly increase the ketone body excretion in either the castrated males or females. It would seem that the ovary contains some other substance (or hormone) which influences fat me-

tabolism and produces a ketonuria. This brings up the question—whether we are through with the ovary when both estrone and progesterone are removed.

We have previously mentioned that extracts of the anterior lobe of the hypophysis contain a ketogenic principle, and thereby produce a ketosis. Acetone bodies are produced whenever there is a disturbance in the relative proportions of fat and carbohydrate that are being coincidentally oxidized in the tissue. If there is a decrease in the available carbohydrate, oxidation of fat is correspondingly accelerated, the acetone bodies arising in consequence of an incomplete oxidation of the latter. Mirsky (1936) studied the source of these acetone bodies in the liver and found that the ketogenic principle acts directly on the liver. He points out that only glycogenic substances are antiketogenic and that a low liver glycogen is found in all conditions which are associated with ketonuria. Deuel (1934) noted that the ketogenic principle caused a diminution of liver glycogen. Mirsky concludes that the deprivation of glycogen in the liver cell and the associated increased catabolism of fat leads to the formation of ketone bodies.

There are abundant clinical and pathological data which permit us to assume that the diencephalon also contains a center which exerts an important influence on the deposition, distribution and consumption of fat, especially in the subcutaneous tissue. Fulton and Bailey (1929) observed that in the "hypothalamic syndrome" produced by very early tumors arising in the infundibulum of the hypothalamus adiposity is a characteristic symptom. Smith (1927) demonstrated that injury to the infundibulum of rats produced adiposity which was not repaired by pituitary implants. Furthermore, local nerve lesions may be a basis for the "partial" or "local obesity" that one frequently encounters. Mansfeld and Müller (1913) observed that section of one sciatic nerve in an overfed dog produced a greater accumulation of fat in the corresponding limb. A somewhat similar finding is reported by Lostat and Vitry (1909).

Distrophia-Adiposo-Genitalis. This condition was first described by Fröhlich (1901). It may be primary or secondary, that is, it may begin before adolescence or after growth and sex function have reached adult proportion and function. When dealing with pre- or adolescent types three cardinal signs are present, namely,

1. Adiposity.
2. Genital hypoplasia (and hypofunction).

3. Disturbances of growth.

On the contrary, when the dystrophy is secondary and adult growth has been attained, the signs are twofold:

1. Adiposity.

2. Genital hypoplasia and hypofunction.

Etiology. It has not been established whether the genesis of dystrophia adiposogenitalis is hypophyseal or cerebral (diencephalon). In all probability there is an involvement of both, and the lesions may be neoplastic, destructive or benign hyperplasia. Occasionally a lesion in a remote area of the brain, or a hydrocephalus may lead to adiposogenital dystrophy because of secondary pressure changes. The Fröhlich Syndrome may best be considered as a hormonal (pituitary) dysfunction plus a disturbance of a center in the floor of the third ventricle which exerts a regulatory influence on the deposition and consumption of fat. Whether the action of this center is direct or by way of a hypophyseal hormone is unknown. It is of great importance to realize that when the adiposity becomes pronounced in the adult, genital hypofunction may set in. Just how this interrelationship comes about is uncertain. May there exist a chemical interrelationship between the two hormones of such a character that an overproduction of one leads to an underproduction of the other? More likely, a disturbance of the function of one leads to dysfunction of the other. But the fact that the symptoms of adiposity and genital hypofunction occur very frequently together makes the chemical interrelationship between the two hormone systems a distinct probability. There must exist under normal conditions a chemical balance between the pituitary hormones, so that a disturbance of one in the form of over or underproduction, will lead to an upsetting of the chemical equilibrium of the others. Whether such dissociation will lead to an excessive or diminished production of the other hypophyseal hormones will depend on factors that are as yet wholly unknown. Therefore, beginning with the symptoms of adiposity and genital hypofunction, the other symptoms and signs that may develop will depend upon which (and how) other hormones will be secondarily involved. The exact nature of the secondary symptoms will depend upon the type of dysfunction of the other involved hormones. For example, some cases of dystrophia adiposogenitalis present symptoms and signs of hyperthyroidism, others of hypothyroidism, while many show no thyroid symptoms at all. This is best explained by assuming that thyrotropic hormone has been involved in the first two groups, but not in the last. But in any

case, whether the thyrotropic hormone will or will not become involved is unpredictable.

Symptomatology. a. Adiposity. The sites usually involved are the trunk, the mammary regions and the mons veneris. The obesity most commonly involves the lower girdle and extends from the level of the navel to the lower portion of the thigh. In severe cases the fat pad over the mons may project downward in form of an apron hanging between the thighs. The breasts may become very large and pendulous. When the adiposity develops during adolescence, the increase in size is due solely to fat and not to duct or alveolar hyperplasia. If the dystrophy develops during the early pre-adolescent period the breasts may fail to grow and be indicated merely by fat pads and rudimentary nipples. Some have maintained that this adiposity is not amenable to fat cures, but our experience has been contrary to this.

b. Gonadal Hypofunction and Hypoplasia. In view of the hypofunction of the gonadotropic hormones, hypofunction of the ovaries follows. This in turn results in hypoplasia of the genital tract and in the other secondary sex characteristics. The earlier the gonadotropic failure occurs the more profound is the underdevelopment of the genital tract. When the dystrophy is secondary and menstruation ceases the endometrium retains its postmenstrual character and shows no cyclical change. The uterus becomes smaller than normal because insufficient follicular hormone is produced to maintain it in normal function. The regression in size rarely reaches the proportions of the infantile uteri seen in the pre-adolescent dystrophies.

The ovaries show no mature Graafian follicles and hence no corpora lutea. Sterility is very common. Libido is usually diminished. The menses may cease suddenly, or, what is more common, gradually diminish in amount. The intervals between periods becomes longer. After the menses have ceased, menstrual molymina may be maintained for some time but even these soon cease. Occasionally slight bleeding occurs every few months, but it bears no resemblance to a normal period. Patients frequently consider themselves pregnant during these periods of amenorrhea because it is associated with increasing girth.

Statural Growth. When the dystrophy occurs during the pre- or adolescent state stunted growth is the rule. In the secondary form growth is already complete, hence no growth abnormalities are to be expected. The stunting of growth implies a hypoactivity on the part of the growth hormone. We are assuming for the moment that thyroid function (thyrotropic hormone) is normal, because when it is subnormal then the added effect of the thyroid on growth must be

considered. It is interesting to compare this with the eunuch where the skeletal growth usually exceeds the normal, because of the late closure of the epiphyses. In the eunuch, therefore, the growth hormone maintains its function and growth ensues. This brings us to a point previously made in the discussion of the fat hormone in relation to the gonadotropic hormones. There probably exists some chemical interrelationship between the three hormones, possibly a common nucleus or origin (in the sense that the cholesterols bear to follicular, corpus luteum and testicular hormones). Much interesting speculation is possible, but the facts at hand are as yet inadequate to indicate the proper direction of thought.

The other signs and symptoms that have been observed in the Fröhlich Syndrome depend on the secondary involvement of the remaining pituitary hormones, and the hormones produced in other glands as a result of pituitary activity.

Carbohydrate Metabolism—may be involved, resulting in an increased sugar tolerance. By this we mean that after glucose feeding (1.75 grams per kilogram of body weight) the blood sugar instead of rising to about 160 to 180 mgm. per cent after one hour, rises only to 130 mgm. per cent. If we consider the level of the blood sugar to depend upon (amongst other factors) insulin and the contrainsular hormone of the anterior hypophysis, then the preponderant insulin effect in dystrophia adiposogenitalia becomes more understandable. Glycosuria and hyperglycemia have been reported.

Protein Metabolism. After the ingestion of a mixed meal the heat production of the body rises. This rise in heat production which has been called "specific dynamic action" of foods, varies with the nature of the food given. It is greatest for protein. It is caused by the stimulating effect of the absorbed digestion products, or of substances formed from them, upon the cellular metabolic processes. Wang, Strause, and Saunders (1924) have demonstrated a low specific dynamic action of protein in patients with endogenous obesity. This has not been confirmed by others, and a subnormal specific dynamic action has been found in Addison's disease, myxoedema, in pregnancy and in normal healthy subjects. Even if the lowered specific dynamic action held for endogenous obesity, it would be inadequate to account for the adiposity. Protein represents no more than fifteen per cent of the total calories of the food. Hence, the calories saved by this lowered oxidative stimulus from proteins would not suffice to explain the development of obesity.

Nervous System. Headache is the chief nervous reaction. Whether it signifies a compensatory change in the hypophysis is at present undecided.

The *muscular system* has a low tonus. Hyperextensibility of the joints is frequently seen. The fingers are long and tapering.

Cardio-vascular System. Dyspnea, tachycardia, palpitation and syncopal attacks are frequently encountered. Whether they are due to fatty infiltration of the myocardium or an instability of the vegetative nerves is not known. These distressing cardiac symptoms are frequently brought out by small doses of thyroid extract.

The *gastro-intestinal system* is often involved. Distention, constipation, nausea, vomiting, and disturbances of gastric secretion are not uncommon. They may be due to secondary disturbances of the posterior pituitary or to abnormal function of the vegetative nerves.

The *renal symptoms* may consist of polyuria, and, secondarily to that, polydipsia and polyphagia. This may be due to an involvement of the basal nuclei in the hypothalamus that control water (and salt) metabolism or to disturbed pituitrin secretion.

The *skin* may vary considerably in texture depending on the state of the thyroid and to the water retention. The hair is usually scanty. Hypertrichosis is occasionally seen and implies adrenal involvement.

Hormonal Findings. We have studied the presence of estrone and follicle stimulating hormone (F.S.H.) in the urine in a number of cases. The results were as follows:

Adolescent Group:

a { Estrone—none found.
F.S.H.—none found.

or

b { Estrone—none found.
F.S.H.—present.

Type *a* excretion signifies primarily a pituitary hypofunction, and a secondary failure on the part of the ovary. Type *b* implies pituitary function in so far as the gonadotropic hormones are concerned but a lack of response on the part of the ovary. It is our impression that Type *b* is an attempt on the part of the body to correct the dystrophy.

Adult Group (secondary).
Estrone—none found.
F.S.H.—none found.

As above we believe this to mean pituitary-ovary hypofunction. When

estrone reappears in the urine, menstruation, as a rule, soon follows. This may be used as an index of prognosis.

Case Histories:

Case 1. J. C., age 16. First seen March 6, 1936.

Chief Complaint. Failure to grow. Failure to develop. Absence of menstruation. Adiposity.

Past History. The patient is the second of two children. The older brother is normal. She was three weeks premature (?), though she weighed 7 pounds. Walked at one year. Dentition normal. Chicken pox, measles, and whooping cough. Tonsillectomy at 4 years. Her growth has always been slow. Normal appetite, except a great desire for ice cream and cake. Progress in school moderate. Always a little overweight and short.

Physical examination. The patient is a short, stocky, young girl. There are definite rolls of fat about the region of the breasts, hips and mons.

Height: 54¾ inches.

Weight: 119½ pounds.

Span: 58 inches.

Lower measurements: 26 inches.

Upper measurements: 28¾ inches.

Head: negative.

Mouth: dentition normal, large incisors.

Neck: negative.

Skin: smooth, no pubic or axillary hair. No hirsutism.

Chest: negative.

Heart: negative.

Blood pressure: 130/90.

Pulse: 88.

Breasts: absent. No gland tissue palpable. Areoli and nipples rudimentary.

Abdomen: negative.

External genitalia: Labia majora: markedly hypoplastic. Labia minora: barely indicated. Clitoris: very small, barely palpable. Perineum: very short.

Vagina: has a depth of 1.5 inches, as measured by sound.

Uterus: *neither cervix nor uterus can be felt by rectal examination.*

Adnexa: not felt.

Basal metabolic rate: (March 17, 1936): minus 3 per cent. Sex hormones in urine: Follicular hormone—absent. Follicle stimulating hormone—present.

X-ray (March 10, 1936): Skull: the skull is of moderate size. The bones are of usual thickness. No intracranial calcifications. The sella turcica is well formed, its floor is regular, concave and shows no erosion of either clinoid. (Dr. W. T. Robinson.)

Right elbow, right wrist and pelvis March 10, 1936. (Dr. R. Golden.) A film of the right elbow in the A.P. position shows an apparently normal epiphyseal closure. A film of the right wrist in the A.P. position shows the epiphyseal lines at the lower end of both bones to be still open. At the age of 17 they should be closed as they are at the elbow. The epiphyses at the end of the metacarpals are still open and also those at the proximal end of the proximal phalanges. Those of

the little finger curiously enough are closed. The above described findings suggest a rather irregular epiphyseal development.

Routine pelvimetry films show a distinctly male type of inlet with narrow, high sacrosciatic notches. The ischial spines are prominent. The subpubic arch is narrow and the side walls converge moderately. The sacrum is curved with a very moderate inclination. The pelvis as a whole is quite small. The centers of ossification of the epiphyses of the iliac crests have not yet appeared. The books say that these should appear at the age of 17 but I have seen a number of instances in which they have been present at the age of 13 and 14. The epiphyses of the lower two of three vertebral bodies have apparently developed and fused normally. This examination still further suggests a disturbance and irregularity in the epiphyseal development. Calcified nodes are present in the right lower quadrant.

Treatment and Results. Progynon-B, 10,000 R.U. twice a week was begun on April 16, 1936. After the first three doses the patient complained of soreness in the breasts and pelvis. After 50,000 R.U. of progynon-B were injected 200 R.U. of Antophysin (Pregnancy urine extract—Winthrop Chemical Co.) were substituted for one Progynon-B injection. The purpose of this was to prevent possible secondary ovarian atrophy due to the depressing action of large doses of follicular hormone on follicle stimulating hormone production. Six weeks after the onset of treatment the growth of breasts was definite and vaginal discharge was noted. Thyroid (B. W. and Co.) gr. I, t. i. d. was added. Vaginal bleeding on June 6 and 7 after a total of 120,000 R.U. of progynon-B and 1,200 R.U. of Antophysin. Re-examination of the uterus on July 3, 1936 showed it to be the size of a small walnut. (It was not palpable at the first examination!) Vaginal bleeding (cyclical) occurred. Up to the end of December, 1936 seven periods of cyclical bleeding have occurred. It now takes 20,000 R.U. of estradiol benzoate (progynon-B) to maintain regular cycles. The uterus corresponds in size to one seen in a normal girl nine years of age. The breasts resemble those of a young adolescent. A sound introduced into the vagina shows a depth of two and a half inches. There has been no growth in height.

Opinion: The results obtained in this case (and in other similar cases) are satisfactory in that the patient feels like any other normal woman, in that she has breasts and "menstruates." To us the treatment leaves much to be desired, first because the effects obtained are temporary, and second, the treatment is solely replacement therapy for there has been no permanent stimulation of the gonads. The general contour of the patient is now distinctly feminine. Growth has not been obtained. A really potent growth hormone for the human is not as yet available. In spite of these shortcomings the treatment is worth while from every viewpoint.

Therapy. Several methods of therapy are *potentially* available, namely,

I. To stimulate the hypophysis.
II. To substitute for the hypophysis, which is
III. To stimulate the other endocrine glands.
IV. To substitute for the other endocrine glands.

The Roentgen ray has been used to stimulate the hypophysis, with the purpose of stimulating the ovaries. What, therefore, is the action of the x-ray on brain tissue in general, and on the pituitary in particular?

Scholz (1935) reported on the action of the x-ray and radium on brain tissue. His experimental work (as well as that of his collaborators) is confined to dogs. Applications ranging between 1 and 2 skin erythema doses given to young animals produced severe damage in the brain tissue. There occurred multiple circumscribed necrotic areas in the immediate vicinity of the blood vessels. Scars and cysts subsequently followed. Experiments on older dogs demonstrated that the brain tissue is *not* radiosensitive, but at the same time not as radioresistant as some suspect. Both immediate and remote effects were produced. The former resulted in lymphocytic infiltration of the intracerebral blood vessels with a mild reaction of the perivascular glia. The conditions were fully developed after 5 weeks, but clinical signs and symptoms were absent. But after 3 to 6 months a secondary reaction set in. Multiple necroses occurred with marked degenerative reactions in the intracerebral blood vessels. It required a minimum of 8 S.E.O. to produce these effects in the blood vessels, the brain changes being considered secondary. Marked clinical changes occurred. Scholz quotes several cases from literature in which such results occurred in the human in whom the radiation was used for therapeutic purposes. Here, as in the experimental animals the clinical picture became evident after a certain latent period.

Lacassagne (1935) reports a study of the effect of radium on the hypophysis of the rabbit. His method consisted of the introduction of radon needles into the pituitary. He was able thereby to destroy the pituitary in toto or in part. The destruction was very rapid during the first few days, but became progressively slower. He observed that: (1) the cells of the adenohypophysis were radioresistant, and (2) that all morphological types of cells showed the *same* radioresistance. The entire (or part of the) pituitary is eventually replaced by a network of collagen tissue.

Within a few days from the onset of the radon implantation and the resulting total destruction of the pituitary, the rabbits show evidence of heat. They accept the male but not a single one (out of 150) became pregnant. All sexual activity disappears at the end of a week and the breasts and vulva begin to atrophy. Beginning with the eighth day all cells of the mature and growing follicles undergo cytolysis and atresia. The ovary grows gradually smaller, due mainly to resorp-

tion, and destruction of the interstitial tissue. The primordial follicles do not seem to be involved in the destruction. Beginning with the 12th to 15th day the uterus, tubes and vagina undergo atrophy, and this reaches a maximum at the end of a month.

If the radon destroyed the posterior pituitary, the intermediate lobe and two-thirds of the anterior lobe, then the remaining one-third is sufficient to carry on full function. The animals seem to function normally. The animals can become pregnant, go to term, deliver and rear the young. This confirms the work of P. E. Smith (1930) who did a partial hypophysectomy, leaving only 30 per cent of the anterior lobe in situ. Lacassagne concludes that all portions of the adenohypophysis are equally capable of producing all the hormones. Furthermore, he does not believe that therapeutic *radium* treatment of the pituitary has an effect upon the ovary and that it is impossible by this radiation to produce a so-called "hypophyseal castration."

Lacassagne then studied the effect of x-ray radiation on the hypophysis. He noted that the pituitary was resistant to the x-ray. Repeated fractional doses, totalling at the end a large amount, produced no effect on the pituitary or ovaries. The animal receiving the largest dose subsequently became pregnant. When a very large dose was given in a single sitting the animals lost weight. The hypophysis remained normal but the ovary showed some degenerative changes. He believes the changes in the ovaries to be due to inanition. Lacassagne concludes that it is doubtful whether radiation of the hypophysis has any effect on the gonads.

Another way of potentially stimulating the hypophysis, and in turn the ovaries, is possible. That is by small doses of estrone. In the consideration of the physiology of estrogenic substances it was stated that large doses of estrone inhibit and small doses stimulate the adenopituitary. The administration of small doses of estrone has occasionally led to the clearing up of a secondary amenorrhea. We believe such small dosage to be inadequate for stimulating the uterus directly, hence the effect was probably by way of the anterior pituitary gland. It must be stressed that large doses of estrone depress the gonadotropic function of the pituitary, hence the constant administration of large quantities of hormone defeats the purpose of the therapy. The writer has noticed the frequency of headache in patients with pituitary dystrophy in whom large amounts of hormone (greater than 100,000 R.U.) were injected in a short time. Experimental evidence (on rats) has led us to believe that this headache is due to a temporary enlargement of the pituitary.

Can we replace a pituitary in a state of partial hypofunction by means of potent pituitary extracts? We probably could if we had extracts potent enough. The pituitary extracts that we have available, namely, antuitrin (Parke-Davis and Co.) and Anterior Pituitary Extract (Squibb) are, in our opinion, a step in the right direction. From their mode of preparations these extracts should contain:

a. Growth hormone.

b. Thyrotropic hormone.

c. Gonadotropic hormone.

We have never succeeded in satisfying ourselves that these extracts have *clinically* produced demonstrable body growth. We were very careful to use the extract only *in cases where the epiphyses were open.* Nor have we observed any noticeable thyrotropic effect. It required 1 to 2 cc. to stimulate the thyroid of a guinea pig. It is quite probable that the dosage given to our patients was inadequate. The extracts contain gonadotropic hormones, for 0.25 cc. gives a positive Friedman test. Another question is whether these anterior pituitary extracts will augment the action of pregnancy urine extracts (Follutein or Antuitrin-S) clinically, in the same manner that they do experimentally. Leonard (1932) demonstrated that when a pregnancy urine extract is injected concurrently with a gonadotropic hypophyseal extract containing a follicle stimulating substance in normal immature rats, the increase in the weights of the ovaries is greater than that which would be predicted by adding the increases produced by each of the extracts injected individually.

We are as yet undecided whether such augmentation exists clinically. Several factors are involved in this indecision. (1) Adequate follicle production has not been demonstrated for the human. Neither pregnancy urine extract nor the gonadotropic factor from the serum of the pregnant mare exhibit true follicles in the human (Kurzrok, Smith and Watson, 1937). (2) The clinical dosage of gonadotropic hormone has not yet been determined. (3) A given physiological response in one species is often not applicable in another.

The sole available method of therapy is substitution. The administration of estrone is indicated. It has been our custom to begin with 10,000 R.U. every fourth day, till 40,000 R.U. are given. This is followed by a rest of two weeks and if no bleeding occurs then 40,000 R.U. are again administered. If bleeding appears then the subsequent dose is now 30,000 R.U. In this manner the dosage is stepped-down until the minimal dose is found that will maintain menstruation. For

it is interesting to note that while the periods obtained with 40,000 R.U. are cyclical bleedings, those resulting from 10,000 R.U. or less are frequently true menstruation. The total duration of treatment varies from case to case. Many cases will resume spontaneous cycles. Others will show occasional lapses. Very few cases are wholly resistant to treatment. We have emphasized here the production of periodic bleeding, but there occurs, at the same time, a definite growth of the genital tract.

The reduction of weight is important. A diet of 900 calories is of great benefit. The greater the reduction in weight the more apt are we to obtain a satisfactory therapeutic effect. The association of weight loss and return of genital function is frequently so parallel, that the association of the hormones involved must be more than a mere coincidence. It speaks for a definite relationship.

Thyroid extract is of great benefit in the cases of Fröhlich Syndrome associated with hypothyroidism. Even when the basal metabolic rate is normal there is a tendency to prescribe thyroid extract, for the impression is current that thyroid extract somehow "activates" estrone given at the same time. We are not willing to subscribe to this notion in the cases where the metabolic rate is normal. On the contrary, we advise against the indiscriminate use of thyroid extract in these cases, for symptoms of thyrotoxicosis are only too frequently produced.

Until a definite method for stimulating the pituitary (and the ovaries) becomes available, we must utilize those procedures which are second best.

The Adrenals

It has been known for some time that anatomical changes of the adrenals are associated with abnormalities of the reproductive system. Bullock and Sequeira (1905) and Gallais (1912) collected and reviewed the existing cases and as a result the *adreno-genital syndrome* has become a clinical entity.

The Androgenic Adrenal Tissue. It has been recognized for some time that the adrenal cortex may be subdivided into two zones, each having an entirely different function. The outer zone produces cortin, while the inner zone may be concerned with certain phases of the reproductive system. It is this inner zone that concerns us here. Grollman (1936) has recently reviewed the evidence in support of this inner zone and has designated the tissue as androgenic. On the other hand, androgenic tissue may be found as part of the accessory adrenal tissue.

At birth the adrenal is one-third the size of the kidney, while during embryonic life it exceeds the kidney in size. The thickness of the androgenic tissue is responsible for the large size of the adrenal. At birth the true cortex divides into the glomerular, fascicular and reticular zones. During the next two years the androgenic zone undergoes rapid involution. In the adult human adrenal the androgenic zone forms a thin juxta-medullary zone composed of osmophilic and pigmented cells.

Symptoms of Adreno-genital Syndrome. The symptoms depend upon the age and sex of the individual. In the main it involves a masculinization of the female, although feminization of the male has been described (Holl, 1930; Lisser, 1936).

The cardinal symptoms are as follows:

1. Hypertrichosis of the male type.
2. Hypertrophy of the clitoris (or penis).
3. Deepening of the voice.
4. Dysfunction of the genital tract.
5. Change in body contour.
6. Change in psychic outlook and behavior.

It can be seen from the above symptomatology that we are dealing with aberrations of the secondary sex characteristics, hence with abnormalities in the action of either estrone or testosterone. When estrone, in excess of the usual amount, acts upon the female we get sexual precocity in the young, and hyperfeminization in the adult. Pregnancy may in a sense be considered a form of hyperfeminization. Precocious sexual maturity in a girl is hyperfeminization. On the contrary, when estrone (in excess of the normal amount) acts upon the male it will stimulate in him those homologous secondary sex characteristics which it stimulates normally in the female. For instance, one of the forms of feminization in the male is gynecomastia. This stigma may be present in the absence of any other feminizing characteristics. In our work with cases of primary amenorrhea (Kurzrok, Cassidy and Wilson, 1935) we have been impressed with the fact that the breast is the *first* tissue or organ to respond to the action of estrone. Whether it denotes a special disposition of the part of the breast anlage, or whether estrone is the key that particularly fits the cells composing the rudimentary breast, we do not know. For it must be remembered that there are many estrogenic molecules and one may "fit" a given organ better than another. Burrows (1935) studied the changes produced by pure estrogenic compounds in the mammae of mice. He concluded that estrone, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-

1:2:5:6-dibenzanthracene, equilenin, and estrone methyl ether, caused an extension of the mammary duct system with little development of acini. Equilin and estradiol, in the doses given, caused relatively little

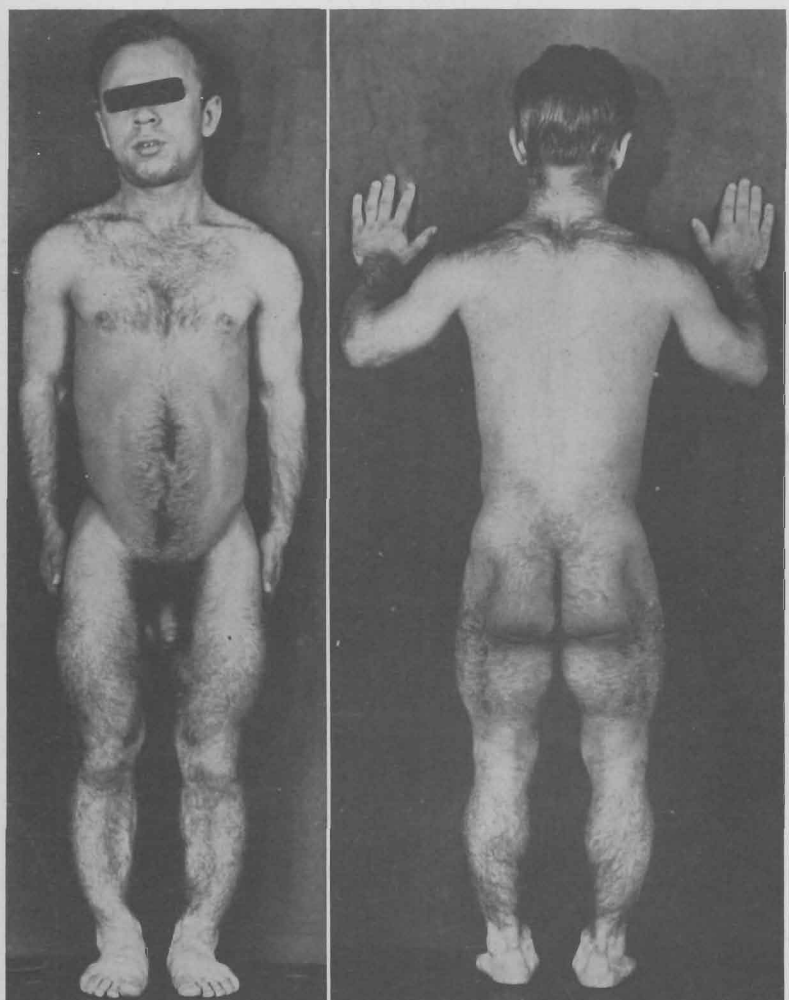


FIG. 120. Case 1—a boy of 11 years of age. Shows extreme hirsutism and precocious masculinity. Note the relatively long trunk due to premature epiphyseal closure of the long bones.

extension of the mammary duct system, but induced a copious development of acini. A very interesting observation was made by Nelson and Gallagher (1936). They noted that androstane-diol and andro-

stene-dione produced remarkable proliferation of the ducts and acini. Androsterone exerted no detectable influence on the mammary glands. These observations add to our understanding of the syndrome of gynecomastia.

Furthermore, the earlier the estrogenic action begins in the male, the more profound will be the induced changes.

The same reasoning is applicable to the action of testosterone on the male and female. When a young boy is involved the result is precocious masculinity [Case 1, Cahill et al. (1936)] (fig. 120).

Case 6. L. S. (chart No. 379627), boy, aged 11 years, was admitted to the hospital May 29, 1933, complaining of dwarfism, excessive hairiness and sexual maturity. Father and mother were alive and well. There was no history of family abnormalities. Patient was the first born to his mother and considered normal up to 22 months of age. At that time he began to show evidence of structural changes. He began to become hairy at the age of 5 years, and at 7 or 8 developed adult genitalia. He was backward at school; was apt to be moody and although at times was good natured, he was likely to be sensitive about his condition. He was proud of his strength and adult characteristics, and was fond of cigars. No sexual history could be obtained from him as he refused to answer all questions.

On examination he resembled a muscular achondroplastic dwarf with profuse growth of hair on the face, chest, shoulders, arms, back and abdomen and legs. His genitalia were adult. Red blood corpuscle count was 4,880,000; hemoglobin, 92 per cent; white blood corpuscle count was 11,500 with polymorphonuclears, 80 per cent; lymphocytes, 16 per cent, miscellaneous 4, and basal metabolism plus 10. There was no hypertension. X-ray examinations of his skull showed no change from normal. Repeated Wassermann tests were negative.

	Hormone Studies	
Date	*F.S.H.*	*Estrone*
May 30, 1933	Negative	Negative
May 31, 1933	Negative	4 R.U. per liter
June 1, 1933	Negative	4 R.U. per liter
June 2, 1933	Negative	4 R.U. per liter
June 3, 1933	Negative	4 R.U. per liter
June 4, 1933	Negative	4 R.U. per liter
June 5, 1933	Negative	2 R.U. per liter
June 6, 1933	Negative	No R.U. per liter
June 8, 1933	Negative	4 R.U. per liter

Analysis of his blood serum, fasting, was: Protein, 7.1; carbon dioxide, 68.2; sugar, 0.90 gram per liter; chlorides (as NaCl), 5.79; non-protein nitrogen, 25.0 milligrams per liter; phosphorus, 3.4; sodium, 136.2 milli-equivalents per liter; potassium, 4.8.

X-ray examination of the chest showed no evidence of a thymus. Air injections of the adrenals showed the left adrenal to be larger than the right but of normal shape, and not sufficiently changed so that a diagnosis of tumor could be made.

The patient was taken home by his mother and no further study was possible in this case.

The amount of estrogenic substance found in the urine is high. It may not be estrone, but some derivative of testosterone that has gained estrogenic properties due to the change in molecular structure. On the contrary it could be estrone, for whenever large amounts of male sex hormone are produced, there is an accompanying production of female sex hormone (in the stallion). Unfortunately, when this patient was in the Hospital we were not doing male hormone determinations.

Possibly the so-called "he-man" may be a similar condition, but one which started after growth was completed. When such masculinization involves the female the result is the classical adrenogenital syndrome, virilism. Here the change is due to the action of *testosterone* (or related male hormone) in the female. The effect upon her will be the stimulation of these homologous secondary sex characteristics, that are ordinarily stimulated in the male by testosterone. One expects, and obtains, hirsutism of the male type, hypertrophy of the clitoris and deepening of the voice. We have actually been able to demonstrate very large quantities of male sex hormone in our cases (2 and 3).

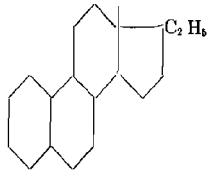
Male hirsutism in women is particularly important. In some it presents the sole masculinizing effect. We believe, but have not proved, that it presents the *first* effect of the action of testosterone (or related compound) on a woman. The hair follicles of the skin may form the most sensitive human tissue response to male sex hormone (compare estrone and the breast!) hence this symptom may be the first to appear, and may be the only one if the process of masculinization does not go too far. Whether the hair follicle has a particular affinity for male sex hormone, or whether a molecular variation in the male hormone is involved, we do not know. The future work with *adrenosteron* (v. i.) will be watched with great interest from this angle.

That hirsutism is a local skin manifestation of specific male hormone activity is strongly supported by the work of Greenwood and Blythe (1935) on the brown leghorn capon. They showed that by means of intradermal and subcutaneous injections of relatively small amounts of estrone, they were able to induce a *local* feminizing effect in the feathers of the injected area. The effect produced was most marked in feathers near the point of injection and waned as the distance from the injected site increased. Effects other than local were not found. Darby, Miller and Kurzrok (1936, unpublished) have confirmed and extended this work in the rooster.

The syndrome of virilism has been found in the following conditions:

1. Tumors of adrenal which involve the cortex (the androgenic tissue?).
2. Tumors of the pituitary—Cushing syndrome.
3. Tumors of the ovary—arrhenoblastoma.

We suggest that the masculinizing effects of the Cushing syndrome are due to stimulation of the androgenic tissue, and that the other symptoms observed (glycosuria, obesity, etc.) are due to secondary involvement of the other hypophyseal hormones. The male sex hormone that may be active in the arrhenoblastoma can arise from the tumor cells or from the medulla of the ovary, for that has potential masculinizing powers, or from the androgenic tissue. An excess of male sex hormone in arrhenoblastomas has as yet not been demonstrated.



Pregnan skeleton

FIG. 121

Chemistry. The organic chemist has again assisted the physiologists and clinicians to a better understanding of their problems. Recent work on the chemistry of substances found in the adrenal cortex has greatly helped our understanding the problem of virilism. In attempting to isolate crystalline cortin Wintersteiner and Pfiffner (1935) and Reichstein (1936) isolated a group of crystalline substances that were inactive inasfar as any lifesaving properties of cortin were concerned. Reichstein called these substances A, B, C, and D. He believed that they belonged to the sterols, and have as their basis the pregnant skeleton (fig. 121). Further investigation of substance A, its reaction with lead acetate in acetic acid, as well as its reaction with periodic acid, led him to believe that the side chain was glycerin. Oxidation with periodic acid led to the formation of a monoketone. Furthermore, oxidation with chromic acid yielded a diketone. He proposed the hypothetical formulae shown in figures 122 to 124.

Substances C and D gave the same diketone, thus showing their chemical similarity. The position of the third oxygen atom is unknown and has merely been indicated by a bracket. The resemblance of the monoketone to a substituted androsteron led him to study its action on the capon. The administration of 20 gamma daily led to a positive test. Its activity is only about $\frac{1}{30}$ of androsteron where 0.7 gamma

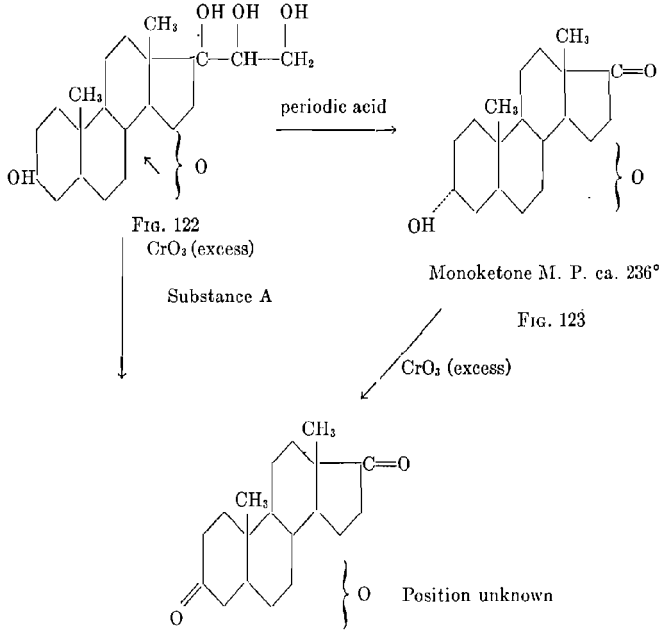


FIG. 122

Monoketone M. P. ca. 236°

FIG. 123

Diketone M.P. 178°

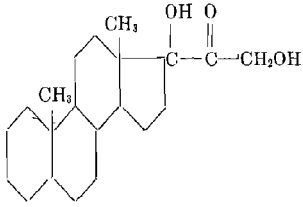
FIG. 124

daily gives comb growth. The weakening of the physiological activity may be due to the third-oxygen atom, or to the position of the hydroxyl group on C-atom 3 (cis or trans). Reichstein places the OH group in the trans position, similarly to trans-androsteron.

Substances C and D have marked reducing properties and he suggests the constitutional formula given in figure 125.

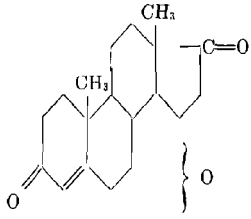
In addition Reichstein isolated another compound G, an unsaturated diketone. The substance did not possess estrogenic activity but was $\frac{1}{5}$ as active as androsteron. He called this substance *adrenosteron* and suggested the formula shown in figure 126.

There is thus demonstrated in the adrenal gland a substance having male sex hormone properties. Its amount may be increased by a tumor or hyperplasia (of the androgenic zone?), or by some deviation in the normal metabolism of the cortex.



Substance C and D

FIG. 125



Substance G = *adrenosteron*

FIG. 126

Case 2. A. D., No. 338313, Age 32, Autopsy 12,044. (Figs. 127A, 127B, 127C, 127D.) First admission April 25, 1932.

Chief Complaint. Amenorrhea, 3 years. Furunculosis, 3 years.

Present Illness. About $3\frac{1}{2}$ years ago patient was told by local doctor that she was pregnant. Patient does not know what he did but says he performed an operation so that she would not have a child. No anesthesia. For 3 or 4 months after operation her periods were perfectly regular and normal. They then stopped suddenly and she has not menstruated since that time. No bleeding. No discharge. No pain.

For past 3 years has had "one boil after another." Went to local medical doctor who told her she had diabetic symptoms and put her on a diet. Boils have persisted. She drinks a great deal of water. No loss of weight.

Menses: began 13–14; regular; 26 days; 4–5 days in duration.

Family history: negative.

Physical Examination. Patient is a large stocky young colored woman. Face hairy and bloated. Hair follicles are very prominent. Male hair distribution throughout body. Breasts well developed. Chest negative. Abdomen negative. Clitoris resembles a small penis in size and shape. External genitalia otherwise negative. Pelvic floor intact. Vagina short, narrow, shallow. Cervix: very small. Uterus very small, retroverted, retroflexed, to the left of the midline. Adnexa not felt. Weight: 170 pounds. Blood pressure: 180/130.

Wassermann: negative.

Basal metabolic rate: minus 18 per cent.

Blood sugar: 257 mgm. per 100 cc.

Estrone (in urine): negative.

Follicle stimulating hormone: negative.

X-ray of sella (No. 96825. Dr. Kasabach): Film of the skull in the lateral projection shows a large sized sella turcica, the walls and the floor of which appear eroded. The dorsum sella is thinned out and lacks a normal amount of calcification. There is no definite destruction of bone comprising the sella. The picture is that of a macrosella, probably due to increase in the intrapituitary pressure from a growth, probably au adenoma.

Diagnosis. Adenoma of pituitary. Diabetes.

Progress Note. An attempt was made to treat this patient with amniotin and follutein, and she was in addition referred to the diabetes clinic. The patient soon ceased coming to the clinic. The social service finally located her in November, 1932. She appeared subsequently several times and in May, 1933 an abscess of the right thigh was incised. Blood sugar 261 mgm. The patient consented to a limitation of diet and insulin so that the blood sugar in August, 1933 was 98 mgm.

X-ray of spine (June 16, 1933. Dr. Dyke (No. 25326)): Anterior pituitary stereos of the entire spine show some spur formation on the lateral margins of the bodies of the midthoracic vertebrae. No evidence of atrophy of any of the bones.

X-ray treatment of the pituitary was started at the Neurological Institute.

The visits again became irregular and the patient was admitted to the Presbyterian Hospital on May 17, 1934. She complained of poor vision, slowing of mentality, some dyspnea on climbing, and edema of the ankles at the end of the day.

Physical Examination. Essentially the same as above.

Blood sugar: 254 mgm.

Blood count: Hemoglobin: 100 per cent. R.B.C.: 5,600,000. W.B.C.: 13,300. Polymorphonuclears: 71. Lymphocytes: 14. Monocytes 4. Eosinophiles: 1. Smear normal.

May 18, 1934. X-ray of the skull (No. 96825. Dr. Swenson): Additional films of the skull show the signs of pituitary tumor as previously described. There is an additional interesting feature—an osteoporosis of the calvarium with a grainy appearance, much like that seen in hyperparathyroidism. This x-ray appearance has been described by Sossman and others as accompanying basophilic adenoma, probably due to a secondary stimulation of the parathyroid.

Ophthalmological examination (May 22, 1934. Dr. Perera): There are several

small superficial hemorrhages around both macula regions. The retinal arteries show slight sclerosis.

Visual fields show suggestive contraction of left upper temporal field and a definite superior wedge-shaped contraction of right upper field, which does not appear to be due to the small area of old chorioretinitis beneath a vessel under the right disc. Discs normal. No atrophy or edema.

Color fields with red and blue show some contraction of the field for blue with indentation of both in upper temporal field. These findings are consistent with pressure on the chiasm.

Blood Ca: 11.3 mgm. per cent.

Blood P: 2.4 mgm. per cent.

Blood phosphatase: 3.3 units per 100 cc. serum.

Blood sugar (all fasting): 124 mgm. per cent.

Basal metabolic rate (May 26, 1934): minus 26 per cent.

Follicle stimulating hormone (May 27 and May 28): negative.

The patient was given 100,000 R.U. of progynon-B over a period of 5 days to study its effect on the blood sugar. It appeared to have no effect. Her diet was 60–60–60 and her urine was almost sugar-free, although fasting blood sugar was still up a bit. No insulin.

June 16, 1934: Vaginal bleeding on June 15 and 16, two weeks after the last progynon injection.

July 17, 1934: Endometrial biopsy.

No tissue.

July 30, 1934: Endometrial biopsy.

Post-menstrual.

The patient received 70,000 R.U. of progynon-B between July 24 and October 4, 1934. She had a period in mid-August and another October 6–9. She was working at this time. Her visits again became irregular until she was admitted to the hospital on January 27, 1936 with a history of unconsciousness of 6 hours duration. She died February 7, 1936. On admission she was comatose, respirations heavy and rapid. Striking exophthalmos. Fundi showed marked retinal arteritis, discs clear, slight stiffness of neck. Lumbar puncture: yielded pink fluid containing 11,000 R.B.C. and 46 W.B.C.; globulin 2+; initial pressure 400 mm. dropped to 96 on withdrawal of about 5 cc. No evidence of block. Her diabetes was partially controlled by conservative measures, i.e., small amounts of insulin to cover known amounts of carbohydrates. This was in the main successful, though she did spill on and off. A rapidly spreading fatal lobar pneumonia developed.

Autopsy. The body is that of a well developed, obese young negress measuring 158 cm. The fat is fairly uniformly distributed over the body and is not confined to the pelvic region. The legs are large. The pigmentation of the skin is everywhere of a uniform shade and very dark. There is no abnormal pigmentation of mucous membranes or nail beds. There is rigor mortis of the jaw, neck and extremities, but no detectable livor. The calvarium is negative externally and covered with an abundance of black, kinky hair. There is a beard of considerable size, composed of black, kinky hair over the cheeks and chin. No distinct hair is present on the chest but the follicles are prominent over the breasts and suprasternal region. There is a male distribution of the pubic hair. An unusual amount of hair, uniformly dark, is present over the arms, legs. The eyes are moderately prominent. The sclerae are slightly congested, but there are no petechia. There

is no icteric tint. The pupils are round, regular and equal, measuring about 4 mm. in diameter. The muscles of the face are symmetrical. There is no discharge from the ears, nose or mouth. Small caries are observed in several of the front teeth. The others cannot be seen. The thyroid is palpable, but not enlarged. The trachea is in the midline. The thorax is symmetrical. The breasts are large, equal and rounded. The nipples are brown and everted. No masses are felt. The costal angle measures about 110°. The abdomen is smoothly rounded. No stria are seen. There are no scars. The external genitalia are negative except for enlargement of the clitoris which is approximately the size of a thumb. There is no pitting edema of the extremities. Venipuncture wounds are evident in both antecubital fossae. The fingers are not clubbed. (Only significant findings are given below.)

Pancreas: Measures 10 cm. in length but is somewhat flattened. On section it, too, appears somewhat browner than usual. The lobules are clearly seen. There is no increase in fibrous tissue and no necrosis. The islands of Langerhans cannot be detected.

Adrenals: Are of normal size, shape and consistency. They are embedded in an abundance of adipose tissue, but upon removing the latter the entire adrenal is found to be present in the specimen. A series of cuts were made approximately 2 or 3 cm. apart, and no tumor was found. The cortex is of the usual depth and uniformity. It contains an abundance of fat. *The intermediate zone is everywhere dark brown and somewhat broader than usual.* There is no disintegration of the medulla, but only a small amount of medullary tissue appears to be present. The periadrenal and perirenal adipose tissue are searched for additional adrenal tissue, but none was found.

Pelvic organs: The *bladder* is somewhat dilated and filled with black viscid fluid, apparently due to installation of argyrol. Upon removing the latter, the mucosa is found to be irregularly roughened and red, but no areas of ulceration are found. The *vagina* is rather smooth and congested. The external os of the *cervix* is round and patent. A quantity of thick mucoid material is present in the cervical canal. There are no erosions. The *uterus* is very small for an individual of her age, but is symmetrical and no masses are present. The *endometrium* is slightly congested and very thin. The *Fallopian tubes* appear normal. Both *ovaries* are small and appear to be composed largely of fibrous tissue, although the corrugated surface seen in the senile fibrotic ovary is not present here. No corpora albicantia can be seen and there are no hemorrhages or corpora lutea.

Neck organs: The *thyroid* is of normal size and symmetrical. On section there is less colloid than is usually present and the tissue has a somewhat fleshy appearance. There are no nodules. Only the left *parathyroids* were found. Weights: LU. 42.4 mgm.; LL. 45.9 mgm. The *uvula* and *pharyngeal mucosa* are deeply congested and occasionally slightly granular, but no definite exudate is present. The *trachea* contains mucoid material similar to that in the larger bronchi.

Pituitary: The sella turcica appears slightly larger than usual, and the pituitary protrudes into the cranial cavity, approximately 2 mm. above the level of the clinoid processes. The posterior processes are very easily broken. The pituitary is symmetrical, but appears slightly enlarged. On section through the midline, no tumor is noted.

Brain (Dr. Wolf): Weight: 1320 grams. The cerebrum is small. The cerebral hemispheres are symmetrical. The gyri are well rounded and the sulci of normal

width. In the frontal regions the latter are filled with fluid. The pia-arachnoid is thin and translucent throughout. There is a mild pressure cone on the interior surface of the cerebellum. The stem appears normal externally. There are small subarachnoid hemorrhages over the posterior aspects of the lateral margins of the cerebellar hemispheres. The basal vessels present no abnormalities.

On section of the cerebrum a large recent hemorrhage is found occupying the head and body of the right caudate nucleus, and dorsal half of the right thalamus. It has ruptured into the right lateral ventricle distending and distorting it. There is hemorrhagic staining of the wall of the left ventricle (lateral) and, to a slight degree, the lining of the third ventricle. The foramen of Monro, anterior portion of the third ventricle, and the left lateral ventricle are displaced towards the left. The lateral angle of the latter is rounded. The blood clot is browner in the posterior portion of the thalamus than it is anteriorly in the head of the caudate. Section of the cerebellum and stem reveals no abnormality.

Microscopic Examination. Pancreas: Many of the islets of Langerhans show a reduction in the cellular content, and partial replacement by homogeneous, eosin-staining material. They are either enlarged or of the normal size. There is considerable postmortem change evident in the acini, but the latter are otherwise normal. There is no fibrosis. The arterioles and the small and medium sized arteries are prominently thickened and a few are hyalinized.

Elastic tissue and Van Gieson stains: There is a slight hyalinization of the small and medium sized arteries, but no reduplication of the internal elastica. A few hyalinized arterioles are seen. The hyalinized portion of the islets of Langerhans does not take the Van Gieson stain. Rib: The trabeculae are reduced in size and number. The marrow cells are poorly preserved, but myelocytes can be identified and there is an occasional megakaryocyte, as well as the other normal elements. There is no fibrosis.

Adrenal: Fig. 127A: There is a moderate amount of protein precipitate in the periadrenal adipose tissue. A few small hyalinized arterioles are present in the capsule. The cortex is broad. The cells show the usual arrangement, except for a few small intracortical adenomata. There is a moderate amount of lipoid in the cortical cells. There is some postmortem change in the intermediate zone and it is congested. A small circumscribed nodule of medullary cells is present. Fig. 127B: Is similar, except for the presence of an extra-cortical adenoma in which the cells are large and contain very little fat.

Uterus: The endometrium is of the usual depth, and includes many glands which are straight, and are lined by high columnar epithelium. The superficial portion of the endometrium is deeply congested. The myometrium appears normal.

Ovary: There are rare Graafian follicles. The connective tissue of the cortex is highly cellular. There is only a single corpus albicans. The blood vessels are thickened and hyalinized. L. A rare primitive Graafian follicle is present in this section, and there are several small corpora albicanta. Again the interstitial tissue is cellular.

Thyroid: Is composed of acini of average size, containing considerable colloid. There is no increase in interstitial tissue. The acini are lined by flattened or cuboidal epithelium. There is no infiltration of lymphocytes.

Parathyroid: The two left glands are present. There is considerable infiltra-

tion of large fat cells throughout the gland. All types of cells are present; small basophilic cells seem to predominate.

Breast: R. The tissue is that of a normal resting gland. Only small duct-like alveolar structures are present. L. Is similar.

Brain: Basal ganglia—right: There is a large recent hemorrhage into the thalamus. In the hemorrhagic area, the parenchyma has disappeared completely. At its margins the parenchyma is somewhat congested and markedly edematous. There is an occasional perivascular hemorrhage and some of the vessels have small numbers of lymphocytes and monocytes in their walls. Many of the nerve cells contain an excess of lipochrome and some appear to be undergoing disintegration.

Phosphotungstic acid stain: Negative.

Cerebellum: There is a large recent hemorrhage into the subarachnoid space and pia. There is some loss of Purkinje and mild edema of the granular layer in the cerebellar lobules.

Phosphotungstic stain: negative.

Frontal lobe: right: The parenchyma is moderately edematous. There are no other significant changes in the gray or white matter.

Midbrain: The parenchyma is quite edematous. This is most marked in the region of the substantia nigra. Many of the ganglion cells contain an excess of lipoid. There are ependymal granulations of the lining of the aqueduct of Sylvius.

Phosphotungstic acid stain: Negative.

Medulla: The findings are essentially similar to those in the midbrain. There is some amorphous orange material on the ependymal surface of the floor of the fourth ventricle.

Pituitary (fig. 127C): Serial sections were made and every tenth section mounted and stained with hematoxylin and eosin. In most of the sections, no abnormality of anterior or posterior lobe is noted. In about one-third of the sections there is a distinctly outlined nodule, without encapsulation, composed of a fairly uniform type of cell. There is exceedingly little stroma, and the tissue is fragmented. A few delicate capillaries are present. The cells show no alveolar arrangement and occur in small clumps or singly. The type of cell cannot be determined with this stain.

Masson's trichrome stain: The majority of the cells are slightly smaller than normal basophiles. The cytoplasm is homogeneous and blue. The nucleus is round or ovoid and either solidly red or slightly vesicular.

Anatomical Diagnosis (in Part). Adenoma of pituitary: basophilic. Cortical adenoma of adrenals. Adenoma of medulla of adrenal. Hirsutism. Hypertrophy of clitoris. Atrophy of ovaries. Atrophy of uterus. Osteoporosis of ribs. Hyalinization of Islands of Langerhans. Diabetes mellitus (clinical).

Discussion. We are dealing here with a masculinizing syndrome in an adult woman. What is the mechanism involved? Where is the source of the male sex hormone? We can assume in general that the adenoma of the pituitary was the starting point. It stimulated some other gland to produce large quantities of male sex hormone, whether androsterone, testosterone or adrenosterone, or some other related compound is as yet not known. The source of the male sex hormone

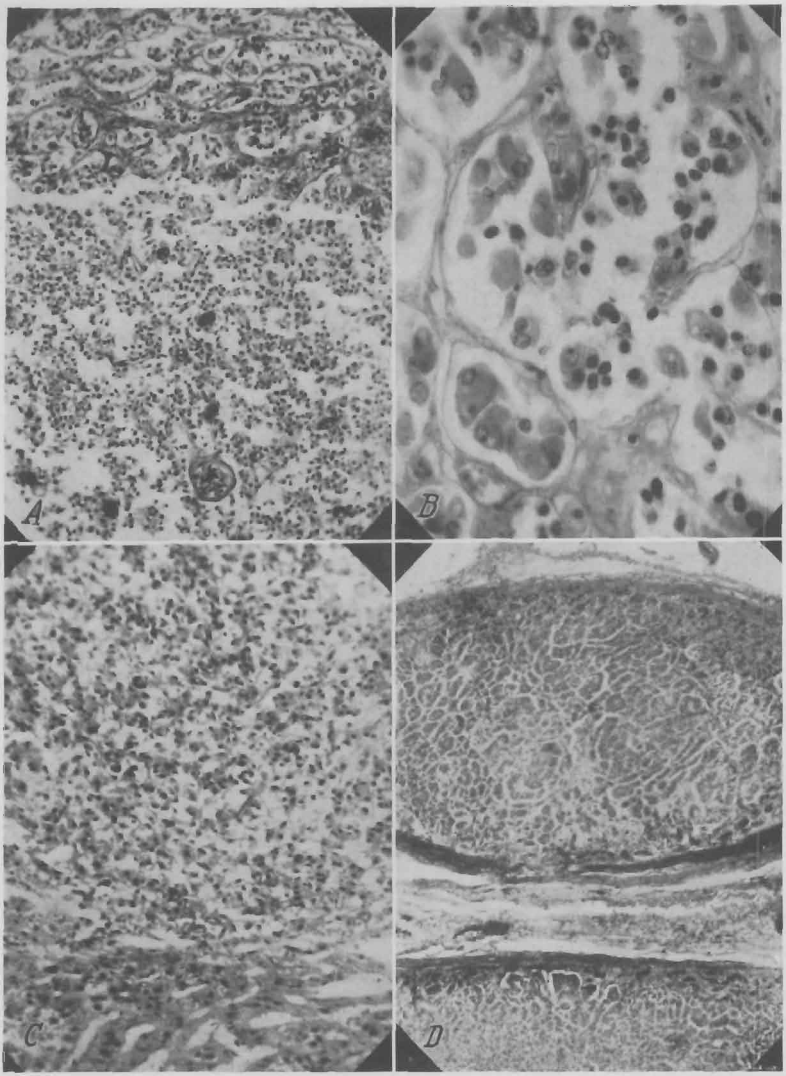


FIG. 127A. Case 2. Basophilic adenoma. Adenoma of anterior pituitary. L. P. (Courtesy of Department of Pathology.)

FIG. 127B. Case 2. Basophilic adenoma. Adenoma of anterior pituitary. H. P. (Courtesy of Department of Pathology.)

FIG. 127C. Case 2. Basophilic adenoma. Adenoma of adrenal medulla. (Courtesy of Department of Pathology.)

FIG. 127D. Case 2. Basophilic adenoma. Adenoma of adrenal cortex. (Courtesy of Department of Pathology.)

could be the adrenal cortex or the medulla of the ovary. The nature of the hormonal stimulus from the pituitary adenoma could not be the follicle stimulating hormone, for it was not found on repeated examination. It could potentially be an excess of adrenotropic hormone, if by that we imply that the adrenal cortex responded by an increased production of adrenosterone (or similar compound). The status of adrenal adenomata is difficult to evaluate in this case. They are known to occur with relative frequency without the production of any clinical signs. The other source of the male sex hormone could be the medulla of the ovary. Against this contention may be stated that the medulla as well as the entire ovary showed no signs of being actively stimulated, on the contrary, both ovaries appeared atrophic. The ovaries did not show mature follicles, the sign of follicle stimulating hormone activity. A conceivable theory is that the pituitary adenoma produced an *altered* tropic hormone which acted on adrenal cortex (adenomata or androgenic cells?) with the resultant production of an excess of male sex hormone. It must not be forgotten that a cell may undergo changes in function without showing any histological change.

In addition, the patient presented other hormonal dysfunctions, namely, diabetes, amenorrhea, hypothyroidism, and osteoporosis. These are explicable on the basis that other pituitary hormones were involved, some being produced in excess and others in insufficient amount. Just what factors govern such quantitative variation is as yet problematical.

Case 3.[1] (Chart No. 445624.) G. C., 16 years old, student admitted to the hospital March 14, 1935, complaining of hair on face and body, a deep voice, and no menstruation. The patient's father and mother are alive and well. The father was born 39 years ago in Italy. The mother was born 33 years ago in the United States, of Italian parentage. Patient, the second oldest of the 4 children, has 3 sisters living and well. One brother died in childhood of an unknown cause. One sister died 2 years ago from a hip infection. There is no history of any family illnesses. The patient was born in the United States. She had measles and chicken pox in early childhood. At the age of 12 years she had mumps. She began her schooling at 5½ years of age and is now in her third year in high school, which is normal for her age. She is considered bright, receives good marks, and is considered talented in music. She began to menstruate at the age of 13 and had 2 periods a month apart. Her hair then was brown, her form and voice girlish. After the second period her hair became thicker, and thick dark hair developed on her face, chest, arms, body and legs. This has gradually grown heavier. She has not menstruated since the beginning of this hairy growth. Her voice has changed; has become cracked and deep and resembles a boy's "bass."

[1] Taken from Cahill, Loeb, Kurzrok, Stout and Smith, 1936.

She developed pimples on her face, chest and back. She has not grown in stature since the change in her hair. She has had no headaches and no disturbances of her eyes except myopia.

The patient states that she is very good in athletics and is strong as a boy, although she is slight. She has no change in her affections and is sensitive concerning her present condition and wishes to be like other girls as soon as possible. She shaves her face daily (fig. 128A).

Examination reveals a small, wiry girl with a very heavy black bushy mop of hair, a "deep blue" partially grown mustache and beard, even though shaven. She had hair on chest, arms, and abdomen. Her thighs, legs, pubis, and genitalia were excessively hairy. Her labia were hypertrophied. She had a long clitoris almost 2 inches in length that stood erect like a penis. The urine was negative for albumin, sugar and pathological sediment. The red blood cell count was 4,900,000; hemoglobin, 90 per cent; white blood count, 11,480, polymorphonuclears, 73; lymphocytes, 23; eosinophilia, 3; transitionals, 1. The blood urea was 16.1; blood sugar, 105; blood pressure 138/76.

On March 18, 1935 her blood showed: carbon dioxide content, 70.1; Cl (as NaCl), 5.9 grams per liter; inorganic products, 3.2; protein, 7.6; sodium, 141.5 milligrams per liter; potassium, 4.7; calcium, 10.2; non-protein nitrogen, 27. The pulse was 68; temperature, 97.4 degrees; weight 47.3; height, 152; body surface, 1.41; basal metabolism minus 8 per cent.

On March 20, 1935, pulse was 72; temperature 97.4 degrees; weight, 47.3; height, 152; basal metabolism minus 1 per cent.

Eye examination showed a myopia of right 20/100; left 20/70. Her eye grounds were normal as were her perimeter fields.

On March 21, 1935, pulse was 68; temperature, 97 degrees; weight, 47; height, 152; body surface, 1.41; basal metabolism minus 5 per cent.

X-ray films taken of her chest were normal; of her abdomen showed the left kidney low, as if pushed down. Her right kidney was normal. Cystoscopy showed a normal bladder and normally functioning kidneys. Pyelograms showed kidneys to all appearances normal with the left low but not rotated. Roentgenograms taken after the injection of 250 cc. of air into the region of the left kidney showed a large tumor mass above the left kidney, rounded in shape and with a slight notch at the upper pole, and pushing the kidney downward. X-ray film taken after injection of 250 cc. of air into the region of the right kidney showed what appeared to be a normal right kidney and an apparently normal adrenal. X-ray film of the sella turcica appeared normal. X-ray film of the pelvis and extremities showed that the epiphyses had fused and no cartilage was present. Laryngoscopic examination revealed a male voice with the thyroid cartilage more prominent and with the vocal cords longer than in a girl.

April 9, 1935: sex hormone determination of urine showed: (1) follicle stimulating hormone, negative; (2) follicular hormone 8 rat units per liter. (This is approximately normal for an adult woman.)

A glucose tolerance test was made March 15, 1935, and showed at 8 a.m. blood sugar, 95; at 8:30 a.m., 133; at 9 a.m., 133; at 10 a.m., 87; with the urine negative at all times.

The patient's bleeding time was 5½ minutes, and the coagulation time 4 minutes.

Chemical analysis of serum on 4 day excess salt diet showed; carbon dioxide content, 70.1; Cl (as NaCl), 5.9 grams per liter; inorganic products, 3.2; protein,

7.6; sodium, 141.5; potassium, 4.7; calcium, 10.2; non-protein nitrogen, 27 mgm.

Pelvic examination showed the pelvis to be android with gynecoid characters and moderate sized outlet.

April 10, 1935, a left oblique subcostal transperitoneal incision was made and the abdomen was explored. The ovaries were very small. The uterus was small. The right adrenal area was palpated but no certainty was felt concerning its presence. There was a large round tumor mass above the left kidney and retro-

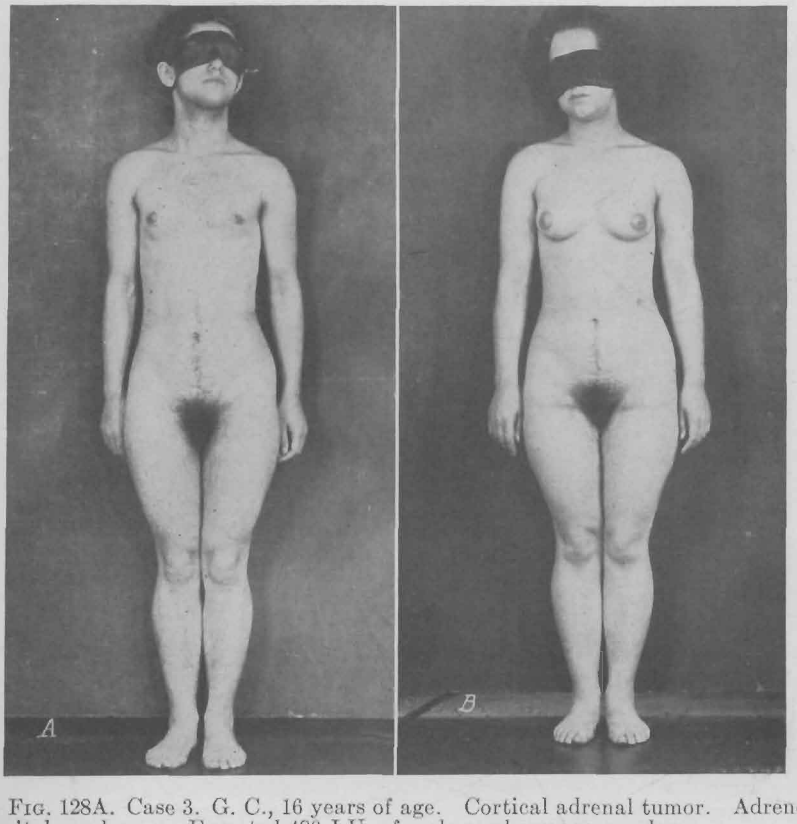


FIG. 128A. Case 3. G. C., 16 years of age. Cortical adrenal tumor. Adrenogenital syndrome. Excreted 480 I.U. of male sex hormone per day.

FIG. 128B. Case 3. G. C., ten weeks after operation. Note the change in distribution of hair, mammary development and distribution of body fat.

peritoneal. Approach to this was made through the posterior parietal peritoneum and the colon was displaced downward. The kidney and the tumor were exposed. The tumor was covered with large veins. These were ligated, as well as the adrenal artery, and the tumor was then delivered with its fascial planes, and removed. There was no injury to the adjacent structures and the tumor was handled very little. The posterior peritoneum was closed without drainage as was the incision in the anterior wall. Clysis of 2,500 cc. normal saline solution was given afterward.

April 11, 1935, the temperature was 103; pulse, 130; blood pressure 158/28. A clysis of 3,000 cc. normal saline solution was given.

April 12, the condition was excellent, she takes fluid well, her pulse is 100, and blood pressure 140/70.

April 14, temperature was normal; condition splendid; blood pressure 120/60.

She made an excellent recovery and the wound healed by primary union. April 27, injection of air into the right side showed apparently no increase in size of the right adrenal as compared with the former x-ray films. She was discharged from the hospital. May 8, she began to menstruate, the first time in 3 years. It was quite profuse and lasted 4 days. Her voice has become more soprano. Her hair has been coming off her body when bathing. She menstruated the second time, at an interval of 29 days after the first period, which also lasted 4 days.

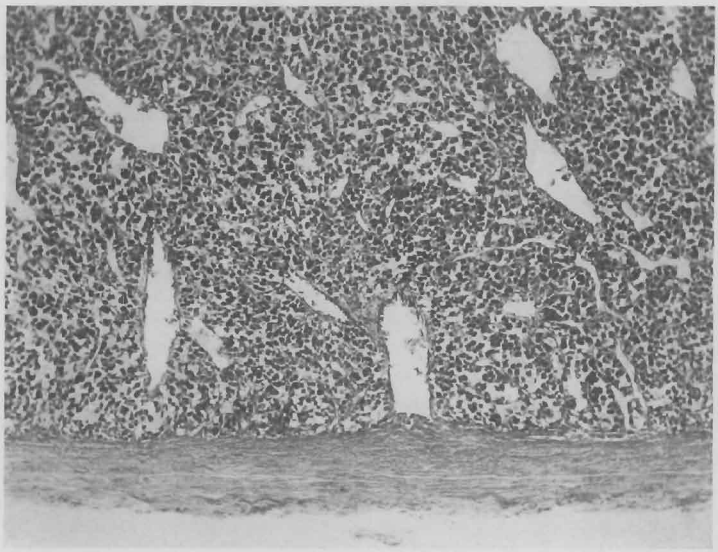


FIG. 128C. Case 3. Photomicrograph of tumor near the capsule. Shows areas somewhat resembling the reticular layer of the adrenal.

She is very happy. The hair is less and she shaves now every fifth day. She menstruated the third time at an interval of 27 days after the preceding period. She was photographed for comparison. She is attending dances and enjoys her status (fig. 128B).

Pathological report (56596 Urol. No. 445624, J. C.). The specimen consists of paraffin blocks from the Squier Laboratory No. 2701. Microscopic examination of sections shows a very cellular tumor which appears to be partially encapsulated and which contains numerous areas of necrosis and some hemorrhage. The tumor cells are exceedingly numerous, rather oval or rounded and somewhat loosely arranged in medullary masses with delicate strands of connective tissue running through them and dividing them into small nests, and occasionally in small, cord-like formations. In other areas the cells form rather broad sheets. The cells are

fairly uniform in size, shape and staining quality; have round or oval nuclei with a fine chromatin network, and show occasional nucleoli. The nuclei are located centrally within the cells. The cytoplasm is moderate in amount and rather deeply eosinophilic. No mitoses are seen. Small blood vessels are also seen coursing through the tumor. Some of these blood vessels appear to be containing collections of tumor cells. In many areas the tumor resembles the adrenal cortex, particularly the reticular zone, and to only a slight extent the glomerular zone. Owing to the cellularity, the large areas of necrosis and the presence of tumor cells in blood vessels, I believe that this tumor should be considered a carcinoma in spite of the fact that no definite capsular invasions can be made out. Sections prepared with the trichrome stain (Fuchsinophil) show the cytoplasm of the tumor cells to be bright red. The iron, mucicarmine, Fontana, and phosphotungstic acid stains reveal nothing of note.

Diagnosis. Carcinoma of suprarenal gland (cortical type) (fig. 128C).

A fraction from the tissues was prepared and assayed on the adrenalectomized dog for the cortical hormone (life maintenance factor). I was unable to demonstrate the presence of the life maintenance hormone. The quantity of tissue available in work of this character is always a limiting factor. We can conclude from our data, however, that the concentration of the cortical hormone in this adenoma was less than 20 per cent of the concentration of the hormone in the normal beef adrenal cortex. No comparison can be made with the normal adrenal since no data on this point are available. It seems to me that these observations speak against the possibility of an excessive secretion of the cortical hormone (life maintenance factor) occurring in this case of virilism. In the course of separating the cortical hormone fraction from the adenoma, fractions were obtained which should have contained the sex hormones if they were present. These fractions were turned over to Dr. Kurzrok. (Dr. Pfiffner.)

None of the fractions examined by me contained any estrone. Unfortunately they were not examined for male sex hormone.

The urine collected prior to operation was sent to Dr. T. F. Gallagher of the University of Chicago for determination of male sex hormone. The entire output of one week was evaporated under reduced pressure to 1.0 liter. *The sample showed the presence of 3,350 International Units of male hormone, or 480 International Units per day. This is by far the greatest amount of male hormone ever found in any urine, whether male or female.* The average value for normal males is 40 International Units per day; for females about 10.

Discussion. This case presents the masculinization of a young girl. It is the first recorded case in which the adreno-genital syndrome was shown to be due to an excessive production of male sex hormone. Unfortunately the tumor was not examined for male sex hormone. Its demonstration there would have been tremendously interesting, but even if the male hormone were not found it would not disprove its production by the tumor. The symptoms and signs evidenced by this patient were the result of the action of male sex hormone on those tissues that it normally stimulates to growth in the male. Coincidental with the production of huge amounts of male hormone, a normal amount

of estrone was produced (estrone 8 R.U. per liter; F.S.H. negative). The physiological *action* of estrone was entirely "checked" by the high concentration of male hormone. Immediately upon the removal of the tumor—the source of the male hormone—the physiological action of estrone manifested itself. Menstruation began. The breasts grew. We do not imply that the estrone was neutralized by the male sex hormone in a chemical sense. We shall report (Darby, Miller and Kurzrok) that when two hormones, such as estrone and testosterone, are injected into an animal, then the animal will choose that hormone which is present in greatest concentration irrespective of the sex of the animal.

It is interesting to note that hirsutism was the first symptom to appear.

Case 4. I. F., No. 479144, age 22 (figs. 129A and 129B). First seen February 13, 1936.

Chief Complaint. Hirsutism—past 10½ years. Fingers cold and cyanotic. Menses irregular. Dysmenorrhea.

Present Illness. For the past 10½ years the patient has noticed an increase in amount of body hair. It has been a gradual process increasing in amount on her legs, about the umbilicus, nipples and chest, sides of face, chin, neck and upper lip. For the past five years she has shaved once a week. Her fingers have been cold and cyanotic as long as she can remember. Her feet have also been cold. She perspires considerably and her skin is constantly moist. She has always been thin, though there has been no loss of weight. No blotches or other neurocirculatory manifestations.

Menses: onset at 11½ years, but very irregular from onset, about every 3 months. Periods sometimes scanty and sometimes very profuse, associated with pain, lasting from 10 to 14 days. Has had considerable endocrine therapy in past 4 or 5 years with relief only from pain. Since above treatments have been stopped the dysmenorrhea has returned.

P.M.P.: September 21–25, 1935.

L.M.P.: January 12–18, 1936.

Physical Examination. Patient is a young girl.

Weight 88 pounds.

Height 158 cm.

Head and neck: negative.

Teeth: well preserved.

Chest: negative. Blood pressure 118/60. Pulse 88.

Skin: hot, moist. There is a heavy growth of black and coarse hair on chest, abdomen and extremities. Its distribution is characteristically male. Hair on face has been removed by electrolysis.

Breasts: very slightly developed. Nipples of fair size.

Voice: normal pitch and timber.

Abdomen: right kidney palpable.

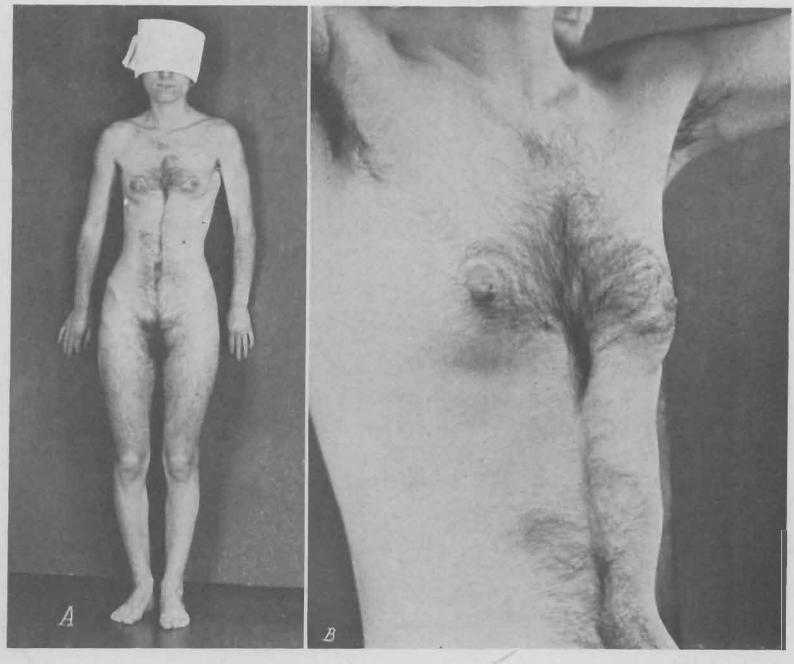
External genitalia: slight hypertrophy of clitoris. Labia majora and minora distinctly underdeveloped.

Rectal examination: uterus is small, to the left of the midline, freely movable, anterior. Adnexa: negative.

Provisional Diagnosis. Cortico-adrenal tumor or hyperplasia. The patient was admitted to the Squier Urological Clinic on February 26, 1936. Discharged April 20, 1936.

Airogram (March 26, 1936. Dr. Fish): The right kidney is well outlined by air and appears normal. The air has not surrounded the left kidney. The kidney shadow itself appears normal. The adrenal is not clearly outlined.

Twenty-four- and 48-hour films show the right kidney well outlined by air. It is normal in size, shape and position. The right adrenal appears normal. The



FIGS. 129A AND 129B. I.F., aged 22. Hirsutism and irregular menstruation. Patient excreted 74 I.U. of male sex hormone per day.

left kidney is normal in size, shape and position. The spleen is fairly well outlined by air. The adrenal shadow seems larger than normal, but there is no definite tumor mass outlined by air on this picture.

X-ray of skull: negative. (Dr. Fish.)

X-ray of pelvis (Dr. H. Molloy): This is a small female or gynecoid with slight narrowing of the subpubic arch. There are a great many normal, female sex characteristics on this pelvis.

Basal metabolic rate: February 28, 1936: plus 3 per cent. March 4, 1936: minus 9 per cent.

Blood count: February 27, 1936: Hb.: 80 per cent. R.B.C.: 4,380,000. W.B.C.: 19,800. (Polymorphonuclears, 85; Lymphocytes, 12; Monocytes, 2.)

Urine: Specific gravity: 1.012–1.025. Albumin: v. f. h. to one plus; sugar, acetone and diacetic—negative. Microscopic: occasional W.B.C. mucus threads.

Blood chemistry: April 1, 1936. Serum CO_2 content: 68.2 vols. per cent. Sodium 140.8 mgm. per l. Cl (as NaCl): 5.97 mgm. per cent. Potassium 3.3 mgm. Inorg. P.: 2.3 mgm. per cent. Calcium 8.9 mgm. per cent. Protein: 7.21 mgm. per cent. Sugar 101 mgm. per cent. N.P.N.: 24 mgm. per cent. Cholesterol 227 mgm. per cent.

Hormone determinations—urine: February 28, 1936: follicular hormone—no R.U. Follicle stimulating hormone—negative.

February 29, 1936: Follicular hormone—no R.U. Follicle stimulating hormone—negative.

March 1, 1936: Follicular hormone—no R.U. Follicle stimulating hormone—negative.

Male sex hormone (Dr. T. F. Gallagher): March 2, 1936: 74 International Units per day.

March 3, 1936: 74 International Units per day.

March 4, 1936: 74 International Units per day.

March 5, 1936: 74 International Units per day.

During the next five days (March 6–10) the patient was given Progynon, 10,000 R.U. per day, a total of 50,000 R.U. The following change in the hormone titer was noted:

March 6, 1936: 48 International Units per day.

March 7, 1936: 63 International Units per day.

March 8, 1936: 9 International Units per day.

March 9, 1936: 26 International Units per day.

March 10, 1936: 32 International Units per day.

The average value for normal females is 10 International Units per day.

Operation. (April 1, 1936. Dr. G. F. Cahill.) The left adrenal appeared normal and felt normal; the right was normal to palpation. The left ovary contained several firm nodular areas. The right as well as the left felt slightly smaller than normal.

A left oblique subcostal incision was made down to the peritoneum. The anterior peritoneum was opened and the transverse colon and splenic flexure packed medialward. The phrenico-colic ligament was divided and by blunt dissection the posterior peritoneum was then opened and the left adrenal exposed. A portion of it was excised for biopsy—.

Pathological report. (Dr. Melicow.) Gross: the specimen consists of two small fragments of adrenal tissue which measure 7 x 3 x 3 and 10 x 4 x 3 mm. respectively.

Microscopic: The sections show tissue characteristic of the adrenal gland. The zona glomerulosa is very scanty and often not seen. The cells are small and packed in small irregular groups. The nuclei stain deeply. The cytoplasm is scanty but compact. The zona fasciculata is the widest portion and its groups of large "foam cells" because of spongy vacuolated cytoplasm stand out from the rest. The cells are polyhedral, contain round to oval nuclei and in places their grouping and general appearance recall the microscopic picture of the so-called hypernephroma of the kidney. In two places the cells look ragged and their nuclei stain deeply. Mitoses are rare. It is difficult to identify the reticular layer.

Diagnosis. Adrenal gland tissue.

Subsequent history. (1) Menstrual—the periods occurred as follows: March 17–22, 1936 (one week after last dose of progynon). April 4–10, 1936 (operation April 1, 1936). May 10–18, 1936. June 9–24, 1936. July 14-20, 1936. Since the operation the periods have become more regular and are of longer duration. (2) Hirsutism: there has been no perceptible change to date (September, 1936).

Discussion. The report presents the first case of partial masculinization—hirsutism—in which a large amount of male sex hormone has been demonstrated, i.e., about twice the amount found in the normal male and eight times the amount found in the normal female. Other masculinizing characteristics were present to a lesser degree. Estrone was not found. It may be stated, as in the previous case, that the excess of male hormone was responsible for the masculinizing process. Again, the skin seemed to be the first involved. What is the source of the male sex hormone? We are not implying that the hormone was either testosterone, androsteron or adrenosterone, but could also be some derivative of these compounds. The source is probably the cortex of the adrenal, though the medulla of the ovary furnishes a potential possibility. If the cortex of the adrenal, then which side? Or are both sides involved? The histological picture of the left adrenal is normal, but conclusions as to its function are not justified. What, then, was the nature of the stimulus acting upon the adrenals that caused this excessive production of male hormone? To these questions there is no satisfactory answer.

Estrone was absent upon repeated examination, though the infrequent periods speak for occasional follicular hormone production. It is conceivable that the excess of male hormone could depress the estrone *production.* A little light on this notion is furnished by the following observation. The male hormone content of the urine from March 2 to 5 was 74 I.U. per day. The administration of 50,000 R.U. progynon-B during the next 5 days produced a sharp drop in the male hormone output. This is very suggestive of a suppression of male hormone production by estrone. Is there a therapeutic advantage in the estrone administration? Further work is extremely essential before this question can be answered.

A very interesting therapeutic approach to hirsutism presents itself. It is based on the following considerations.

1. Greenwood and Blythe (1935) have shown that the local cutaneous administration of estrin governs the type of feathers that will grow locally. At the site of injection the feathers are those of the hen, elsewhere those of the capon.

2. Our work suggests that the skin is very susceptible to the action of male hormone, and may respond by hirsutism in the absence of any other masculinizing features. Whether this is due to an inherent property of the skin or due to a special male sex hormone molecule is not known.

3. Zondek (1935) has shown that estrone is rapidly absorbed through the skin.

4. Male hormone production may be suppressed by the injection of estrone.

These considerations suggest the administration of large doses of estrone (or its derivatives) directly to the skin, both by inunction and intracutaneous injection. We are beginning such therapy in a group of cases.

The Thyroid

The relationship of the thyroid to disturbances of menstruation has been known to clinicians for a long time. Taking cases of hypothyroidism as an example, we know that some are associated with normal menstruation, some with excessive bleeding, but the majority with amenorrhea. A similar relationship is true for hyperthyroidism. This clinical interrelationship between the thyroid and the gonads was usually explained on the basis of a direct effect of the thyroid upon the metabolism of the gonads. With the extension of knowledge, the problem has become still more complicated. The anterior hypophysis with its numerous tropic hormones, especially the thyrotropic, must be an important cog in the mechanism. The relationship between thyroid and ovary may therefore be direct, and indirect through the adenohypophysis.

The effect of estrone upon the thyroid is evidenced from the following observations. Starr and Patton (1935) studied the effect of antuitrin-S and theelin upon hyperthyroidism. They noted remission of the disease coincident with treatment. In the earliest successful cases the remission had continued for eighteen months, and in the others no recurrence had been observed to date. The women in whom the treatment was successful were all below the menopause and had no history of ovarian disease. The failures included one boy, two women at or past the menopause, and one who had had an ovary removed. Evidently induction of remission by pregnancy urine extract is dependent on normal ovarian function.

The writer and Dr. Alexander Goldman (1935–36 unpublished) have studied the effect of estradiol benzoate and estrone upon hyperthyroidism. They administered intramuscularly 40,000 to 80,000 R.U. within

two weeks. In several of the cases a distinct drop in the basal metabolic rate was noted. There occurred a coincident remission of the disease in all its clinical manifestations. A rather startling effect was the diminution in the intensity of the exophthalmus. On the other hand, several cases failed to show any response to treatment. We are unable to prognosticate which cases will respond and which will not. One is tempted to offer a theory that the cases which responded were those whose hyperthyroidism was due to an excessive amount of thyrotropic hormone. The effect of the large doses of estrone was to depress the function of the anterior pituitary gland.

The following case presents an interesting interrelationship between hypothyroidism and amenorrhea.

L. S., No. 430652, age 32. July 16, 1935.

Chief Complaint. (1) Amenorrhea 18 months. (2) Sluggishness 9 months.

Menses began at 15 years—with 30 to 90 day intervals, 3 to 4 days in duration with moderate pain on first day. Single, no pregnancies.

Noted increasing sluggishness, with failing memory and inability to perform usual duties. There has been marked sensitivity to cold weather for the past two years, troublesome dryness of skin and hair for the same period. No obesity or fat pads. Best weight 150 pounds some years ago, gradual loss to 127 pounds.

Operation (1934): left iridectomy for secondary glaucoma due to post-operative iridocyclitis.

Physical Examination. Face is bloated, features immobile, skin coarse, dry and scaly, complexion waxy. Hair not particularly dry. No fat pads. No goiter. Speech quiet, deliberate and slow.

Breasts: small, hypoplastic.

No hirsutism or obesity.

Abdomen: negative.

External genitalia: negative.

Pelvic floor: intact.

Rectal: uterus at lower limits of normality, anterior, regular, movable.

Adnexa: not felt.

Diagnosis. Hypothyroidism. Genital hypoplasia. Secondary amenorrhea.

Laboratory findings: July 16, 1935: Blood count: Hgb.: 84 per cent. R.B.C.: 3,920,000.' W.B.C.: 7,300. Polymorphonuclears: 55 per cent.

July 22, 1935: B.M.R.: minus 40 per cent. Pulse: 64. Temperature: 97.4. Weight: 56.1 kgm.

July 21, 1935: Estrone (urine): none found. Follicle stimulating hormone (urine): none found. Urine: negative. Wassermann: negative, all antigens.

October 28, 1935: Between September 9, 1935 and October 22, 1935 the patient was given 12 doses of thyrotropic hormone, with definite clinical improvement. B.M.R. now: minus 31 per cent.

November 26, 1935: No further thyrotropic hormone being available patient was given 2 cc. of anterior pituitary extract (Squibb) twice a week without further improvement.

Progynon-B, 50,000 I.U. per dose was started

December 19, 1935: Serum chemistry: CO_2 content: 68.4 vols. per cent. Cl (as NaCl): 6.08 grams per liter. Inorg. P.: 3.2 mgm. per cent. Protein: 6.40 per cent. N.P.N.: 28 mgm. per cent. Sodium: 141.4 mgm. per liter. Calcium: 9.8 mgm. per cent. Sugar: 0.80 gram per liter. Cholesterol: 200 mgm. per cent.

December 21, 1935: Slight staining for one day, with definite menstrual molymina.

January 7, 1936: Has received 400,000 I.U. progynon-b. P.D. Thyroid extract gr. I t. i. d. started on December 10, 1935.

January 18, 1936: B.M.R.: minus 3 per cent.

February 8, 1936: B.M.R.: plus 15 per cent. Definite clinical improvement. No symptoms of overdosage.

March 6, 1936: B.M.R.: plus 1 per cent.

March 27, 1936: B.M.R.: plus 2 per cent.

April 2, 1936: Stained March 27–29.

April 16, 1936: Regular period April 12–14, profuse flow with typical menstrual molymina: 650,000 I.U. of progynon-B given since January 1, 1936.

It is worthy of note that 400,000 I.U. of progynon-B were inadequate to produce a "menstrual" flow. Only staining was obtained. This, when the basal metabolic rate remained low. But when the basal metabolic rate reached the plus side, 650,000 I.U. were sufficient to produce a profuse menstrual period. Since then constantly decreasing amounts of estrone are necessary for the production of bleeding, providing the basal metabolic rate is within normal limits. When the basal metabolic rate becomes too low it is necessary to increase the amounts of estrone.

Nutritional (?) Hypothyroidism and Amenorrhea. During the past few years the writer had the privilege of seeing about 50 cases that presented certain features common to all of them. All the patients were young girls in their teens. The history almost always began with the fact that the patients considered themselves too fat (120–130 pounds) and hence dieted. The ultimate goal was to reach about 100 pounds. The diet was usually of a high protein character, and was extremely poor in fats and carbohydrates. When the desired loss of weight was attained the patients noted that the loss of weight continued and that they could not eat, even when they wanted to. Coincidental with this loss of weight there was a diminution in their physical energy, which manifested itself in various ways. They were either constantly tired, or appeared excessively fatigued after a moderate amount of work. Mentally they apparently were able to carry on as usual, but close questioning brought out the fact that they occasionally had to exert considerable mental drive to maintain their high grades in either high school or college. The following three cases are representative of this group.

Case 1. J. V., age 18, single. December 1936.

Chief Complaint. Amenorrhea—14 months. Loss of weight—16 months. Fatigue—1 year.

Menses: 13 years; 28 ± 2; 5–6 days; moderate; without pain. In June, 1935 patient skipped a period. Was given small amounts of thyroid extract and had a normal period in August and September. L.M.P. October, 1935.

During the spring of 1935 patient began to diet. This consisted of leaving out all possible fats and carbohydrates. Weight when diet began: 125 pounds. Present weight: 88 pounds. At the present time she has no appetite, but when she does eat it nauseates her. No vomiting or diarrhea. No abdominal discomfort.

The physician at the college which the patient attends informs me that the patient becomes exhausted very easily. She does excellent work and is rated as one of the best students in school.

B.M.R. studies: November 2, 1935: minus 11 per cent. October 9, 1936: minus 16 per cent. November 6, 1936: minus 20 per cent.

There have been no menstrual molymina except possibly on one occasion.

Hormone studies (urine): April, 1936: Estrone—negative. Follicle stimulating hormone—present.

Usual childhood diseases. No operations. No other significant symptoms.

Physical Examination. Height: 63¼ inches.

Weight: 88½ pounds.

Pulse: 88.

Temperature: 98.

Patient is a young girl. Very bright and intelligent.

She is very thin and angular.

Head: negative.

Neck: thyroid not enlarged.

Skin: dry. No excessive hair.

Extremities: hands cold. No tremor.

Breasts: very small, hypoplastic.

Heart and lungs: negative.

Blood pressure: 90/68.

Abdomen: scaphoid. Viscera not palpable.

External genitalia: negative.

Rectal examination: reveals a very small hypoplastic uterus, anterior, movable.

Adnexa: not palpable.

B.M.R.: January 2, 1937: minus 27 per cent.

Hormone determination. F.S.H.—negative. Estrone—negative.

Diagnosis. Nutritional (?) hypothyroidism and amenorrhea.

Case 2. L. M., age 16, No. 499542. October 26, 1936.

Chief Complaint. Amenorrhea since August 1, 1936.

Menses began at 11, and have usually been irregular, varying from two to six weeks, lasting four days, moderate flow and without pain. Occasionally gets R.L.Q. dull aches between periods. Has had acne since the age of 12 years.

Has lost 12 pounds in the past year, from 130 to 118 pounds.

Diet was very poor in fats and starches.

Physical Examination. Patient is a young healthy girl with a moderate acne.

Face shows sunburn (treatment), scars, comedos.

Skin: dry, moderate excessive hair distribution on arms and legs.

Breasts: well developed.

Heart and lungs: negative. Blood pressure: 90/64. Pulse: 72.

Abdomen: negative.

External genitalia: negative.

Rectal: uterus is small, movable, anterior.

Adnexa: negative.

B.M.R.: November 27, 1936: minus 30 per cent.

Hormone studies—urine:

November 26, 1936 { Estrone—negative
F.S.H.—positive

December 13, 1936 { Estrone—negative
F.S.H.—negative

Blood chemistry (December 19, 1936. Dr. Alexander Weech); Micro-Kjeldahl determinations on serum: N.P.N.: 28 mgm. Albumin: 6.54 grams per cent Globulin: 3.13 grams per cent. Total protein: 9.67 grams per cent. A/G ratio = 2.09.

Diagnosis. Nutritional (?) secondary amenorrhea.

December 29, 1936 patient given thyroid extract 0.3 gram t. i. d. and a high fat diet was urged.

January 12, 1937: Patient had regular period January 3-7, moderate flow. Pulse 96 when resting. Thyroid was reduced to 0.3 gram per day.

Case 8. L. M., age 17. October 1936.

Chief Complaint. Amenorrhea—18 months.

Menses 13 years; 3 to 5 weeks; 4 to 6 days; moderate; without pain.

L.M.P.—April 1935.

No menstrual molymina.

Patient started dieting in April, 1935. Left out all possible fats and carbohydrates. Weight at onset of diet: 125 pounds. During the past winter her weight dropped to 95 pounds. Since then she has gained some weight and is now about 110 pounds. She does not want to eat because she will get "fat." She can eat, but curbs her appetite.

B.M.R. studies: June, 1935: minus 15 per cent. December, 1935: minus 19 per cent.

There is an occasional tendency to fatigue.

Usual childhood diseases. No operations.

No other complaints.

Physical Examination. The patient is a young girl who does not appear ill. She is very bright and intelligent.

Height: 62 inches.

Weight: 108 pounds.

Temperature: 99.

Head: negative.

Neck: thyroid not enlarged.

Skin: dry, excessive hair on arms and legs.

Breasts: quite large, somewhat pendulous. Small superficial stria on surface. (They have neither grown nor decreased in size during the past year, although previous to that they grew quite rapidly.)

Extremities: negative.

Abdomen: viscera not palpable.

External genitalia: hypertrophy of labia minora.

Rectal examination: reveals a uterus that is anterior, normal in size, shape and consistency.

Adnexa: not palpable.

Hormone determination (urine): F.S.H.—negative. Estrone—negative.

B.M.R.: minus 13–15 per cent.

Diagnosis. Nutritional (?) hypothyroidism and amenorrhea.

Therapy. The patient was put on a high fat and carbohydrate and high caloric diet. She would not eat all of it. The first menstrual period started 2 weeks after the increased diet was begun. A second period occurred one month later.

All of the cases studied so far presented the following characteristics:

1. Loss of weight.
2. Amenorrhea.
3. Low basal metabolic rate.
4. Absence of estrone from the urine.

One is impressed by the study of these cases in that the dieting, resulting in the loss of weight, is of major importance in this syndrome. The other signs and symptoms follow. The low basal metabolic rate and the absence of estrone speak for an involvement of both the thyroid and the ovaries. The relationships of the sex hormones to the sterols, and of tryptophane to thyroxin are of interest in this connection. On the contrary, the relationship of both glands to the hypophysis, most likely brings the latter gland into the picture.

Our treatment at the present time consists of the administration of a high caloric diet, abundant in fats and carbohydrates. We usually add a vitamine preparation, such as a pill containing A, B and D vitamines. Where the youngster has been spoiled by doting parents, we occasionally hospitalize the patient. The diet (when eaten) seems to be all that is necessary to stimulate function again. The basal metabolic rate goes up, the menses begin, and estrone is again found in the urine. The rapidity with which improvement begins is frequently very surprising. We are very doubtful as to efficacy of thyroid extract and estrone in these cases. Our early patients were treated with these two substances alone, and our results were invariably bad. We then used the high caloric diet plus estrone and thyroid extract. The results were good, but not better than when an adequate diet alone is given.

The Pancreas

The association of diabetes with disturbances of genital function is not uncommon. One most frequently encounters amenorrhea and sterility. The former is most often seen in the young diabetic. Excessive menstruation is not common. The relationship between diabetes and pregnancy and labor has been adequately discussed in the various specialized textbooks (Adair and Stieglitz, 1934), and will not be considered in this volume. Other relationships have been discussed in Chapter XI.

The relationship of the anterior hypophysis to sugar metabolism by virtue of the pancreotropic hormone as well as the contrainsular hormone, makes for interrelation with the gonads, by way of the gonadotropic hormones. Thus, we find disturbances of menstruation treated by insulin, such as dysmenorrhea (Altschul, 1936), as well as leukorrhea (Klaften, 1934). We have noted temporary improvement in diabetics when given large doses of estrogenic material. The insulin requirement was lessened. There occurred a simultaneous improvement in the general well-being of the patient. The effects were fleeting and short-lived.

We do not as yet understand the etiology of the amenorrhea so frequently seen in young diabetics. There is a persistent absence of estrone and Follicle Stimulating Hormone from the urine.

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CHAPTER XXI

CYSTIC GLANDULAR HYPERPLASIA OF THE ENDOMETRIUM

Cystic glandular hyperplasia of the endometrium is a functional disturbance of the endometrium, characterized by cystic and glandular hyperplasia of the endometrium often associated with irregular, and usually prolonged periods of bleeding.

History

The older gynecologists believed the disease to be inflammatory in origin, in fact, attempts were made to isolate the causative microorganism. Such names as endometritis fungosa, endometritis chronica, hyperplastica (subdivided further into glandularis, interstitialis or simplex) call to mind the supposed inflammatory origin of the disease. The histological picture which superficially resembles an inflammation is now known to be a phase of the cyclical change which the endometrium undergoes. The newer concept of this functional disturbance arose subsequent to the classical description of the endometrial cycle by Hitschmann and Adler (1908). Cystic glandular hyperplasia is a functional hypertrophy and according to our most recent view, an *exaggeration of the proliferative phase of the menstrual cycle.*

With regard to the etiology of this condition it is historically interesting that Bennecke in 1882 suggested that a correlation between the endometrium and the ovary must exist. He believed that "endometritis fungosa" was due to disturbed ovarian function. The disease was not of local origin, for local treatment had little or no effect. Furthermore, the condition of the endometrium did not resemble a true inflammatory reaction. He suggested that the disease was due to an abnormally functioning ovary, which acted upon the endometrium in a reflex manner.

Pathology

In considering the etiology of cystic glandular hyperplasia of the endometrium we shall discuss it from the viewpoints of the anterior hypophysis, the ovary and the uterus. Hence these structures have been minutely examined for pathological changes.

The Anterior Hypophysis. No histological change has been observed in this gland in the course of the symptom complex.

The Ovary. There are two significant findings in the ovary, namely;

1. The persistence of one or more follicles.
2. The complete absence of luteinization.

Both ovaries are usually involved, and each ovary contains usually one, but occasionally, several cysts. They may reach a diameter of 3 to 5 cm. Each cyst represents a follicle that has not reached maturity, in which ovulation has not occurred and wherein there is hardly a trace of luteinization. Such follicles *persist* beyond their allotted span of life (a single menstrual cycle). Their content is a clear fluid, probably a modified liquor folliculi. The wall of the follicle is composed of a definite granulosa layer, surrounded by a distinct theca interna. In some follicles the walls may show patchy areas of atrophy, due in all probability to pressure within the follicle. Occasionally the granulosa wall may show areas of early luteinization. The cells increase in size, become rounded out and take a lighter stain. This reaction may represent an abortive cure on the part of the organism, an attempt to complete the life cycle of the follicle—(ovulation and) corpus luteum formation. Where the follicles are numerous the granulosa layer of some may show evidence of degeneration. Ova are occasionally demonstrated in serial sections. Recent corpora lutea are never found, though old regressive corpora may very occasionally be seen.

The Uterus. 1. *The Myometrium.* The myometrium is thickened, vascular and softened. The uterus is often slightly larger than usual and strongly resembles one containing an early pregnancy. Occasionally a small hard hypoplastic uterus is encountered. When bleeding has persisted for a long time the uterus frequently is of decided "flabby" consistency.

2. *The Endometrium.* The changes in the endometrium give this functional disturbance its name. Macroscopically the endometrium is thickened and velvety, its surface is studded with small cysts of various sizes. The surface is thrown up into folds and polypoid protrusions, hence the name "endometritis fungosa." Often various areas of the endometrium are covered by blood clots, while elsewhere the surface is distinctly necrotic.

There is no attempt at division into a decidua compacta and spongiosa.

A secretory phase is never developed.

The glands are irregular, coarse, some being long, while many show cystic dilatation. Interspersed between the cystic glands are appar-

ently normal glands, the latter taking a deeper stain than the former. The epithelium is very irregular, the height of the cells varying considerably, though flattening does not occur even in the largest cysts. The nuclei are oval and stain deeply. Mitotic figures are few. In some areas the epithelial cells are heaped up in irregular rows. The evidence for a distinct glandular hyperplasia is frequently lacking (hence the name glandular hyperplasia does not describe the condition).

The stroma is of unequal density. In some areas there is a distinct edema, while in others the stroma cells lie close together forming deeply staining masses of cells. Hemorrhages are present in some areas, especially near the surface, where hemorrhagic and necrotic areas may be seen. In such areas the veins are thrombosed. When such necrotic structures are present, the surface epithelium is absent, while the stumps of the glands and the stroma lie in direct contact with this necrotic area.

Nomenclature

Schröder has proposed the name "metropathia hemorrhagica" for this clinical entity. It must be associated with persistent follicles in the ovaries and cystic dysplasia of the endometrium. Based also on the age incidence, the condition has been called puberty bleeding, maturity bleeding, and climacteric bleeding.

Age Incidence. By far the largest number of cases occur beyond the thirty-fifth year, and according to Tietze (1934) who reported 466 cases, the forty-seventh year presented the greatest number (60). In his series multipara outnumbered the nullipara, two to one.

Symptomatology

The chief symptom is irregular and persistent bleeding. The bleeding may be severe enough to threaten life, or what is more frequent, the bleeding is in the form of a persistent bloody mucoid discharge. Such a discharge may be punctuated by recurring periods of excessive bleeding, resembling somewhat the maintenance of a menstrual-like rhythm. Or, the bleeding may be periodic, that is, a period of bleeding lasting two or more weeks may be followed by one or more weeks of amenorrhea, and then a recurrence of the bleeding. Where the bleeding is profuse clots may be present. Dysmenorrhea is rare. This is of great interest, for if functional dysmenorrhea is considered to be due to an insufficiency of corpus luteum hormone, then in cystic glandular hyperplasia of the endometrium, a condition characterized by the absence of a functioning corpus luteum, dysmenorrhea should be a prominent symptom. The

reverse is the case. In the juvenile form of this syndrome the abnormal bleeding may start with the first period or after a number of normal periods. As a rule, premonitory menstrual symptoms appear before the onset of the bleeding, namely, a short period of amenorrhea, or several irregular periods, irregular as to tempo, duration or intensity of bleeding.

Another interesting feature about the bleeding is the tendency to spontaneous cures and recurrences. We have observed the following types: after a period of bleeding lasting some weeks there is a short pause and the bleeding recurs, but only for a week or two, then another pause which is again followed by a period of bleeding. Thus periodic bleeding is reëstablished, but each period of bleeding lasts too long or is too profuse, or both. Finally, apparently normal cycles are reëstablished. After a period of regular bleeding, regular as to tempo, duration and intensity, the bleeding may begin anew. Another form frequently seen is that of a period of bleeding lasting for a variable time, followed by a period of amenorrhea ranging from 3 months to two or more years. Such periods of amenorrhea have raised the following question: What is the character of the endometrium during this period of apparent cure? Has it returned to normal cyclical variation? We, (Wilson and Kurzrok, 1936) have observed that during the period of amenorrhea or apparent cure, the *endometrium retains its cystic glandular character.* The *morphological picture is the same, but the bleeding factor is absent.* The significance of this observation will be discussed together with the etiological factors of the disease.

Cystic glandular hyperplasia of the endometrium is a benign condition. This is all the more interesting when we consider that cystic hyperplasia is brought about by an excess of follicular hormone, relative or absolute, and because of the recently demonstrated relationship between estrogenic and carcinogenic substances. The conversion of a cystic and glandular hyperplasia of the endometrium into a carcinoma of the fundus uteri has not been definitely demonstrated.

Cases of maturity bleeding frequently follow a pregnancy. No abnormality is noted before the pregnancy but a short time after its termination disturbances in the tempo, intensity or duration of bleeding begin. These are shortly followed by a period of prolonged and irregular bleeding. They do not otherwise differ from the cases of juvenile or climacteric bleeding.

The other symptoms noted in cystic and glandular hyperplasia of the endometrium are entirely due to the secondary anemia. The hemoglobin and red blood corpuscles are low. We have seen values of 15 to

25 per cent for the former and one to two million for the latter. The leucocytes are normal, though a relative lymphocytosis may be present. There is pallor of the skin and mucus membranes, and a haemic murmur may be present over the precordium. The patients are asthenic and constantly tired. It is worth remembering that persistent oozing may be more debilitating than a single severe hemorrhage.

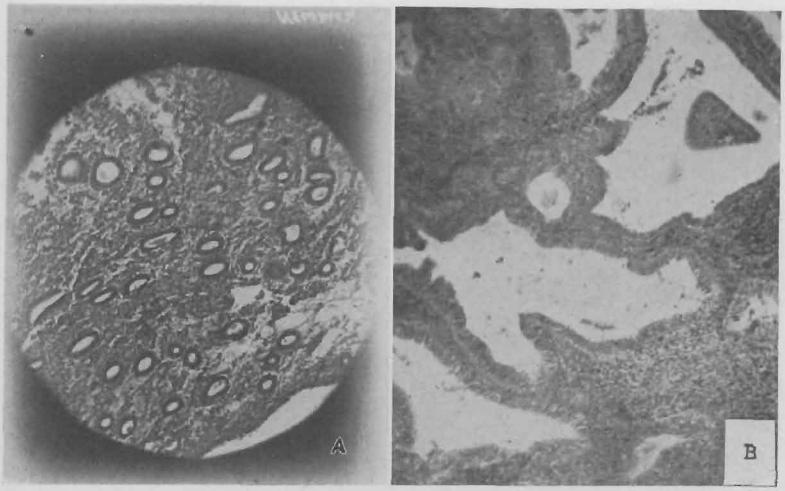


FIG. 130A. Case 1. H. N. Anovulatory bleeding, proliferative endometrium. The previous two periods have occurred after a four-week interval. No premenstrual phase. Proliferative phase present during cyclical bleeding, hence no corpus luteum formation. Biopsy taken two days before a twelve-day flow.

FIG. 130B. Case 1. H. N. Anovulatory bleeding, proliferative endometrium. Biopsy taken while patient was bleeding after an eight-week interval. Cystic dilatation of the glands is explained on the basis of prolonged and unopposed follicular hormone activity.

Significant cases[1]

Case 1. Anovulatory bleeding—proliferative endometrium. H. N., aged thirty-three, para ii, last pregnancy 1925. Menses regular from onset at thirteen years until 1932 when alternating amenorrhea and polymenorrhea ensued. Biopsies during bleeding and amenorrheic stages always showed a proliferative type of endometrium (fig. 130A). An endometrial specimen taken while the patient was bleeding after a two months' period of amenorrhea showed cystic dilatation of the glands (fig. 130B). The latter is explained on the basis of prolonged and unopposed follicular hormone action.

Case 2. Puberty bleeding—cystic and glandular hyperplasia. J. L., aged nineteen, single, onset of menses at fifteen years, cycle always irregular varying

[1] From Wilson and Kurzrok, 1936.

from a two to six weeks' interval with a profuse flow of from four to ten days' duration. Patient bled profusely and continuously from December 5, 1932 to January 10, 1933, and the hemoglobin dropped to 40 per cent. Urine examination showed no follicular or follicle-stimulating hormones. Curettage revealed cystic and glandular hyperplasia of the endometrium (fig. 131). The bleeding stopped after intensive pregnancy urine extract therapy (Follutein-Squibb). Five blood transfusions were necessary to combat the anemia. There was no bleeding for eight months when another short episode of profuse bleeding ensued. This was readily controlled again by the gonadotropic fraction of pregnancy urine (follutein) and the treatment was continued for three months after the bleeding had stopped. There has been no bleeding since (eighteen months). A biopsy taken

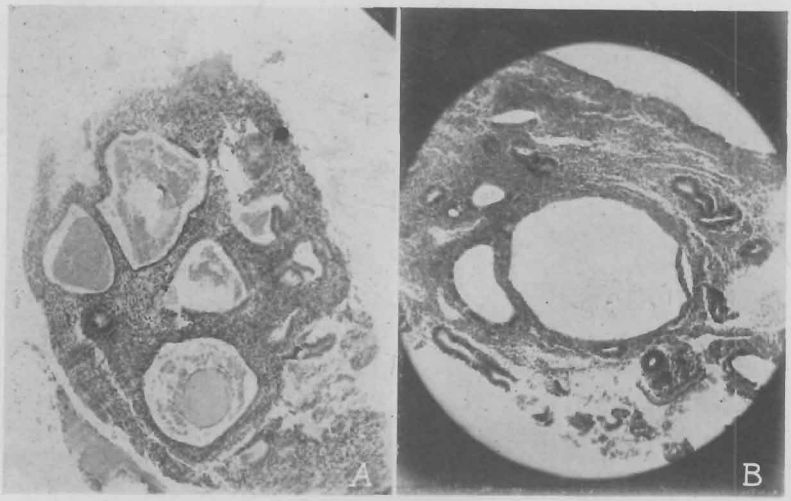


FIG. 131A. Case 2. J. L. Puberty bleeding, cystic and glandular hyperplasia of the endometrium. Cystic and glandular hyperplasia of the endometrium during active bleeding.

FIG. 131B. Case 2. J. L. Persistence of the same type of endometrium after fourteen months of amenorrhea. Treatment with large doses of follutein had stopped the flow. The bleeding has not recurred, and it is now eighteen months since the last flow.

after fourteen months of amenorrhea revealed the persistence of the cystic and glandular hyperplasia. Urine examination at the same time showed no follicular hormone but the presence of follicle-stimulating hormone. Klingler and Burch (1933) and Rock (1935) have noted that a secretory endometrium failed to develop after the bleeding was stopped by the administration of pregnancy urine extract. Thus, the cessation of the bleeding cannot be attributed to luteinization.

Case 3. Continuous bleeding for eleven years—cystic and glandular hyperplasia. C. P., aged twenty-four, menses began at thirteen years, patient bleeding continuously ever since. The bleeding varies from a profuse flow to staining but the patient is never entirely clean. Hemoglobin 65 per cent. No pregnancies

although married three years. Patient was curetted three times in the past four years because of the profuse bleeding. The endometrium has persistently shown marked cystic and glandular hyperplasia. The patient was given daily intravenous injections of prephysin (1 to 2 cc.) for twelve days beginning July 9, 1935. No bleeding since July 23, 1935, except for slight staining from October 12, 1935, to October 17, 1935. Biopsy during the amenorrheic phase (September 17, 1935) shows the persistence of the cystic and glandular hyperplasia (fig. 132). The cessation of the bleeding following intravenous anterior pituitary extract cannot, therefore, be attributed to luteinization. Incidentally, this patient had a severe and persistent facial acne which spontaneously disappeared soon after the bleeding stopped.

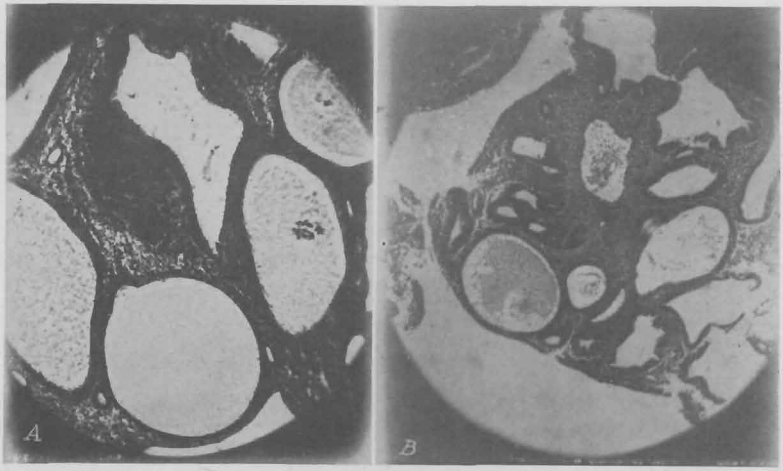


FIG. 132A. Case 3. C. P. Cystic and glandular hyperplasia of the endometrium. Treatment with prephysin. The histologic picture during active bleeding (typical "Swiss cheese" type).

FIG. 132B. C. P. Persistence of the same morphologic characteristics during a two-month period of amenorrhea. More complete absence of premenstrual phase.

Case 4. Maturity bleeding—secretory endometrium. B. B., aged twenty-eight. Hyper- and polymenorrhea past seven months. Menses previously regular since onset at fifteen years. Biopsy on the second day of a ten-day period revealed a well-developed secretory endometrium (fig. 133). Biopsy on the fourth day of the following period (eight days' duration) showed the presence of of an early proliferative endometrium. The regeneration of the endometrium thus appears to take place despite the continuation of the bleeding. Under pregnancy urine extract therapy (600 R.U. per week) there has been a gradual return to a normal cycle with a more moderate flow of five days' duration.

Case 5. Secondary amenorrhea—bleeding from a proliferative endometrium. M. S., aged thirty, amenorrhea six months' duration, menses previously regular since onset at fourteen years. No atrophy of genital organs or breasts. Biopsy

taken three days before the onset of a flow described by the patient as a typical menstrual period, revealed a proliferative endometrium of slight activity ("resting endometrium") (fig. 134).

Case 6. Secondary amenorrhea—cystic and glandular hyperplasia. C. G., aged twenty-six, onset of menses at ten years, regular until seven years ago when

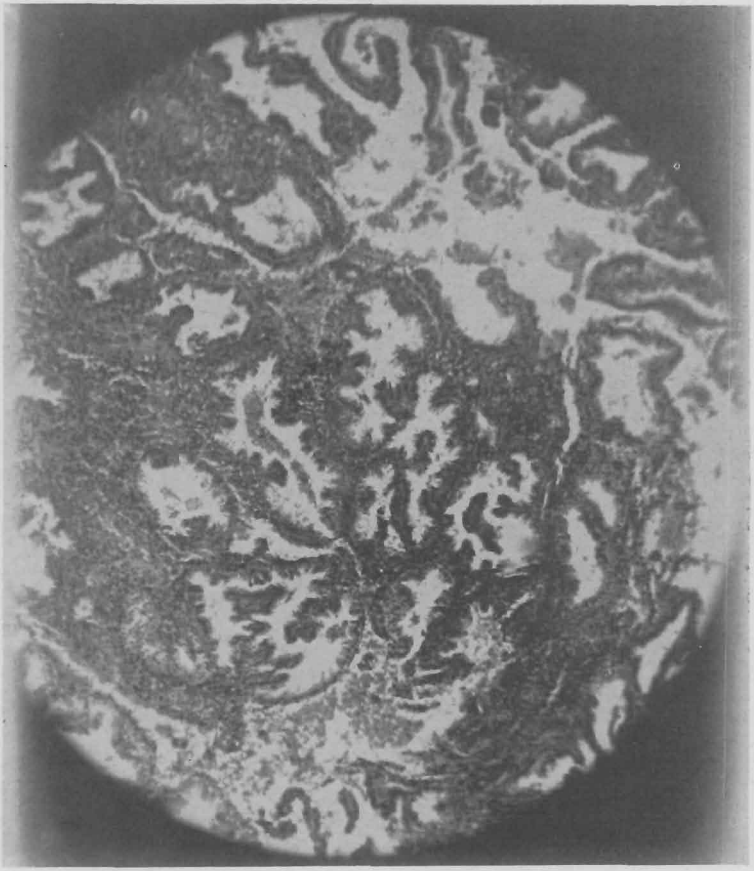


FIG. 133. Case 4. B. B. Maturity bleeding; secretory endometrium. Biopsy on the second day of a ten-day menstrual flow. Prolonged and profuse bleeding from a secretory endometrium.

oligomenorrhea and obesity developed. No period for seven months. Married nine years and never pregnant. Basal metabolic rate: minus 56 per cent (repeated) but no signs of myxedema. Urine examination revealed 9 R.U. follicular hormone per liter and no follicle-stimulating hormone. Biopsy showed marked cystic and glandular hyperplasia of the endometrium (fig. 135).

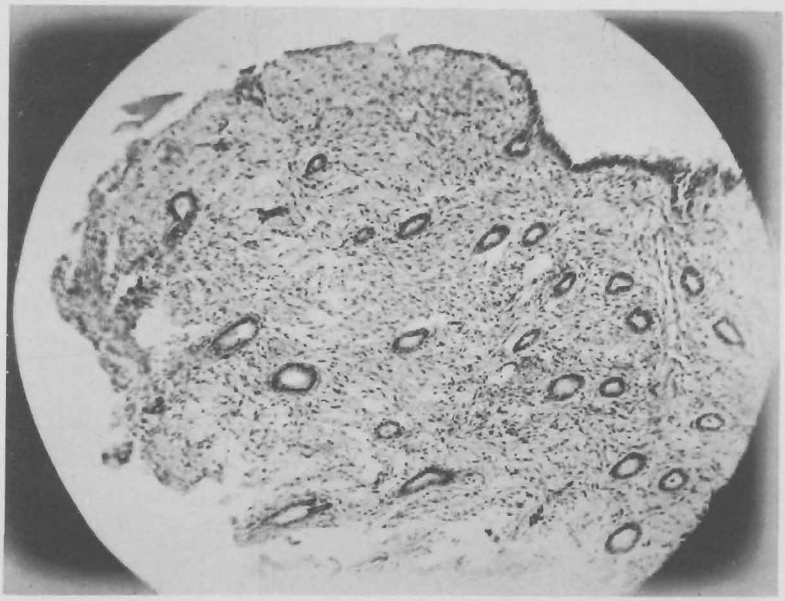


FIG. 134. Case 5. M. S. Secondary amenorrhea; bleeding from proliferative endometrium. Biopsy taken three days before onset of period.

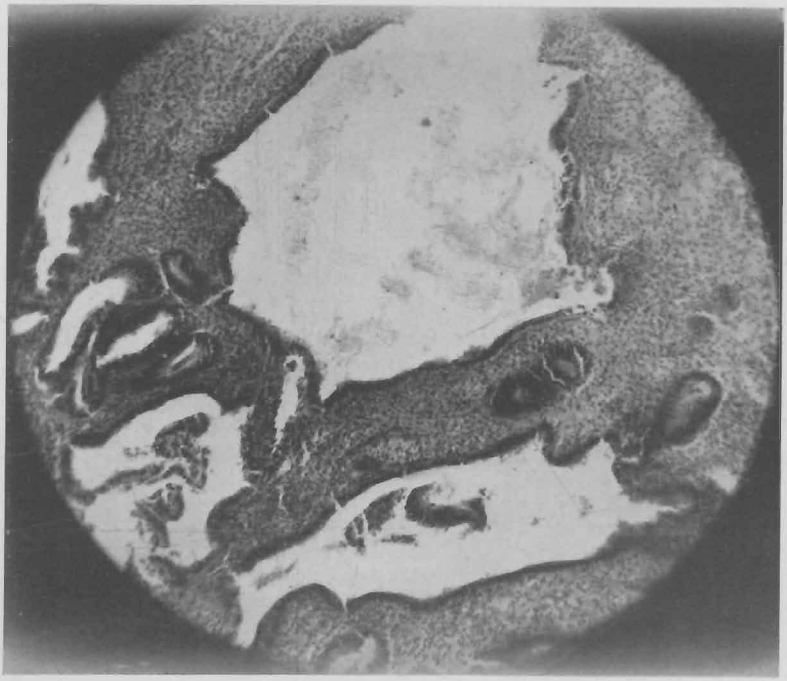


FIG. 135. Case 6. C. G. Secondary amenorrhea. Cystic and glandular hyperplasia of the endometrium. Biopsy taken after amenorrhea of seven months. No previous history of excessive bleeding, but on the contrary, oligomenorrhea.

Case 7. Secondary amenorrhea—secretory endometrium. S. M., aged thirty-five, amenorrhea of two years' duration. Menses always irregular since onset at fourteen years. No genital hypoplasia. Normal hormone findings in urine (follicle-stimulating hormone, negative; 10 to 40 R.U. follicular hormone per liter). Basal metabolic rate minus 4 per cent. Biopsy revealed a secretory endometrium (fig. 136). Scanty periods resulted from the administration of large doses of progynon-B but bleeding stopped after discontinuation of treatment.

Case 8. Secondary amenorrhea (x-ray castration)—experimental production of cystic and glandular hyperplasia of the endometrium. W. U., aged forty-five, diabetes mellitus of nineteen years' duration, x-ray abortion five years ago fol-

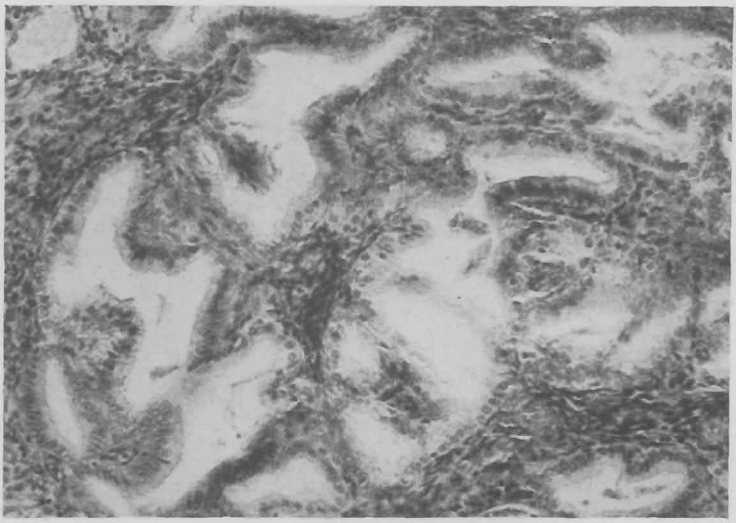


FIG. 136. Case 7. S. M. Secondary amenorrhea. Secretory endometrium. Biopsy taken during amenorrhea reveals secretory endometrium. At other "periods" has very faint staining lasting several hours, from a premenstrual endometrium.

lowed by severe menopause symptoms, genital atrophy and aggravation of diabetes. During the past eighteen months the patient has received a total of 700,000 R.U. of progynon-B. This has afforded complete relief from her menopause symptoms. In addition, there has been considerable improvement in her diabetic condition. The uterus, breasts, and external genitalia have come up to full development and irregular periodic bleeding has occurred. Biopsy of the endometrium reveals cystic and glandular hyperplasia, which has been artificially produced in this case by the administration of huge doses of follicular hormone (fig. 137).

Case 9. Primary amenorrhea—experimental cystic and glandular hyperplasia. H. E., aged twenty-five, never menstruated, infantile uterus and slightly hypo-

plastic breasts and external genitalia. No follicular hormone in urine; follicle-stimulating hormone present. These findings indicate that the amenorrhea was probably due to both partial failure of development of the Müllerian duct system and subsequent cessation of gonadal function. Bleeding was induced by the administration of progynon-B. Endometrial biopsy following 70,000 R.U. over a period of eight weeks showed cystic and glandular hyperplasia (fig. 138).

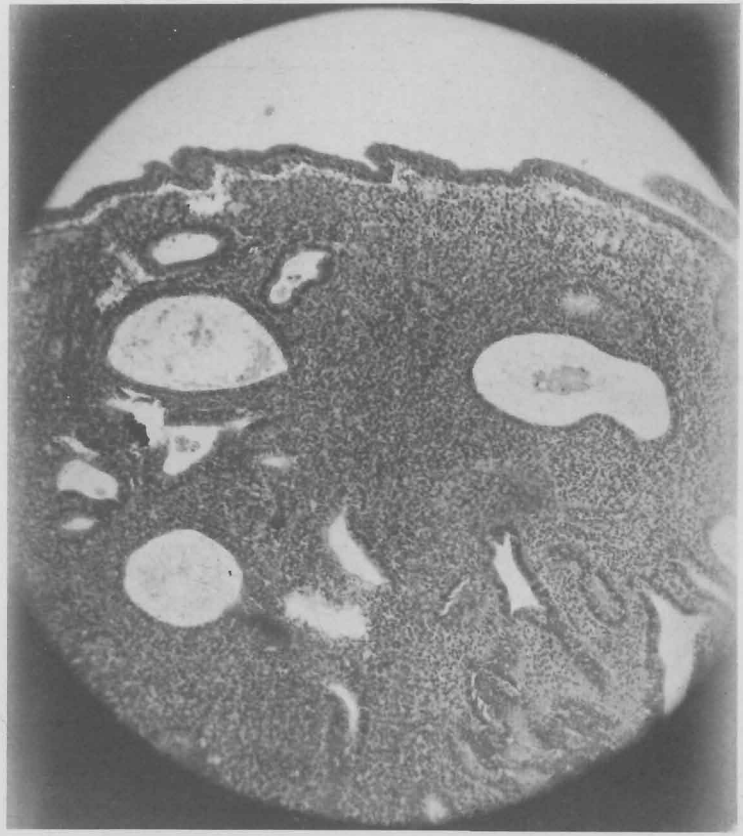


FIG. 137. Case S. W. U. Secondary amenorrhea (x-ray castration). Experimental production of cystic and glandular hyperplasia of the endometrium. 700,000 R.U. of progynon-B given.

Case 10. Ovulation bleeding—transitional endometrium. M. S., aged twenty-six, "menstruated" every two weeks during the past year, previously once in four weeks. Each profuse period is followed with considerable regularity by a scanty flow. Dysmenorrhea occurs only with the profuse period. Biopsy on the twenty-fourth day of the cycle revealed a secretory endometrium (fig. 139A). The ovulation bleeding lasted from the seventeenth to the twenth-third day. The next

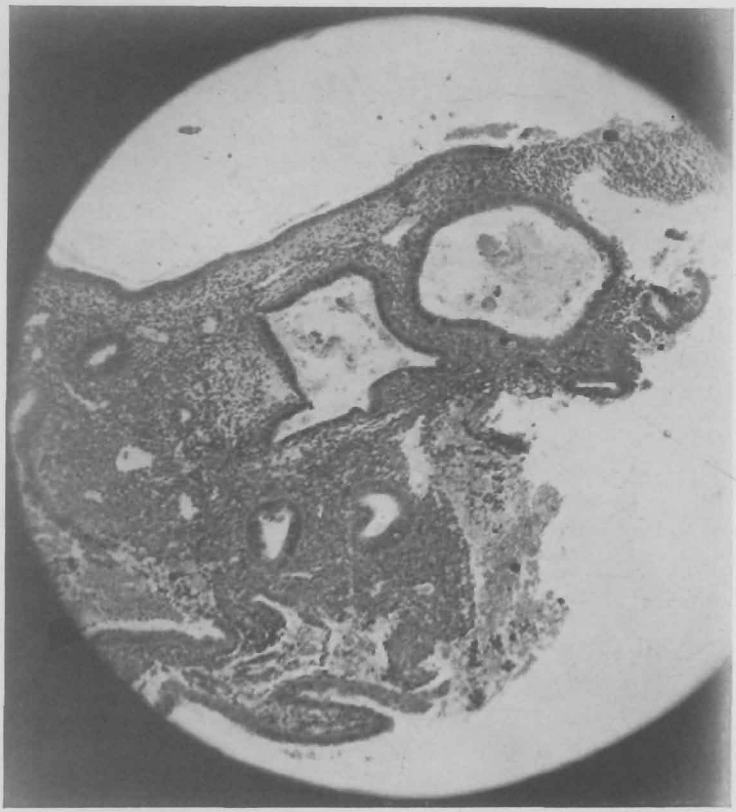
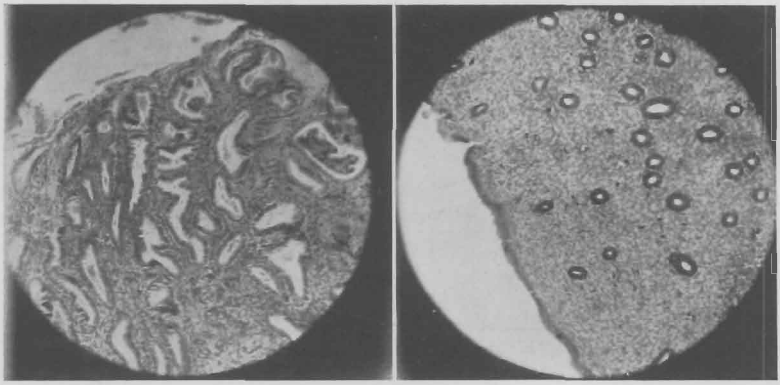


FIG. 138. Case 9. H. E. Primary amenorrhea. Experimental cystic and glandular hyperplasia of endometrium. 70,000 R.U. of progynon-B given.



A B

FIG. 139A. Case 10. M. S. Ovulation bleeding. Ovulation bleeding from the seventeenth to the twenty-third day. Biopsy on the twenty-fourth day. Note premenstrual type of endometrium.

FIG. 139B. Case 10. M. S. Ovulation bleeding from the eleventh to the fourteenth day of the cycle. Biopsy on the eleventh day. Note postmenstrual type of endometrium.

profuse period began twenty-eight days after the onset of the previous profuse flow. Another biopsy taken on the first day of the ovulation period (eleventh day of the cycle) showed a proliferative endometrium (fig. 139B). This patient, therefore, had a normal twenty-eight-day endometrial cycle. The profuse flow represented the true menstrual period and the scanty flow the ovulation bleeding. It will also be seen that the ovulation bleeding did not occur on the same dates on two successive months.

Diagnosis

The most important diagnostic factor is the finding of a cystic glandular hyperplasia of the endometrium. Hence, a curettage or an endometrial biopsy is essential. The latter procedure can be carried out by a modified Klingler and Burch (1932) suction curette (fig. 99). This method of obtaining a specimen is a simple and safe office procedure in the hands of a trained gynecologist. It has the great advantage that it does not require any anesthetic, it does not necessitate a stay in the hospital, and can be repeated at frequent intervals. In cases of very young girls, where neither procedure is feasible the history of the case is of great significance. The preliminary menstrual disturbance is usually amenorrhea. Occasionally abnormalities in the rhythm of the cycle, plus subsequent persistent irregular bleeding in the presence of a grossly negative pelvis, greatly favor the diagnosis of a cystic glandular hyperplasia of the endometrium. The closer the symptoms appear to the menopause the more insistent should be the demand for a curettage.

Hormonal Findings in the Blood and Urine

In view of the fact that cystic glandular hyperplasia is due to an excess of follicular hormone, either absolute or relative, one would expect to find high estrin values in either the blood or urine. Our own studies on the hormone excretion in the urine demonstrate the following types of excretion.

Estrone	*Follicle Stimulating Hormone*
(a) Greater than 20 R.U.	Negative
(b) Less than 10 R. U.	Negative
(c) Negative	Positive

The least frequent form of excretion is type "a". That is, it is unusual to find large estrone values in the urine. It may signify that whatever hormone is produced is utilized mainly by the endometrium. Other tissues that estrone acts upon, such as the breasts and myometrium, do not show any excessive stimulus to grow during the course

of this bleeding syndrome. The enlargement of the uterus that is occasionally found during the active bleeding is due to an increased vascularity of the organ, for when the bleeding stops the uterus becomes decidedly smaller. The most severe hemorrhages can be found associated with small, hard, uteri. We have never noted any undue breast enlargement during the course of the bleeding. This might imply several possibilities, namely:

1. An absolute increase of estrone rarely, if ever, occurs, for if it did certain other tissues would show increased stimulation.

2. There is a change in the distribution of the total estrone produced, so that the endometrium takes up and utilizes more than its usual share. This would necessitate the concept that the first disturbance is within the endometrium proper.

3. Some change in the estrone molecule occurs, and the new compound has a special "affinity" for the endometrium. The large number of modifications that the estrone molecule may undergo, and still remain estrogenic, makes this explanation possible.

The most common type of excretion is represented by type "b". The urinary estrone is low, and the follicle stimulating hormone is absent.

When the bleeding has persisted for a considerable time we have repeatedly found in the urine an absence of estrone and the presence of the follicle stimulating hormone. This combination is usually associated with the loss of ovarian function. It may imply an exhaustion on the part of the ovaries in spite of the fact that the stimulus (follicle stimulating hormone) is still present. In two cases we noted this finding just before bleeding stopped. On the other hand we have not noticed this finding in other untreated cases prior to the cessation of the bleeding.

Differential Diagnosis

The following conditions may give rise to acyclical bleeding:

1. *Polyps*—cervical polyps sooner or later become visible. Bleeding usually occurs immediately after intercourse, or occasionally after severe bodily strain. Intrauterine polyps can occasionally be diagnosed only by means of the curette.

2. *Submucous Myoma*—the history of bleeding dates back a considerable period. The periods are at first prolonged and intensified. Bleeding occurs soon after the period, between the periods, and finally continuous bleeding results. These symptoms correspond to increasing

encroachment of the fibroid upon the cavity of the uterus, the atrophy of the overlying mucosa and the irregularity of the uterine contractions.

3. *Carcinoma of the Uterus*—Cervical carcinomas become palpable and visible very early. The bleeding at the beginning is usually wholly acyclical. Carcinoma of the fundus presents a more difficult problem. The bleeding may closely resemble that of cystic hyperplasia. A history of recurrent attacks of bleeding, separated by regular cycles or amenorrhea greatly favors cystic hyperplasia. The curette is the final arbiter.

4. *Incomplete Abortion*—The history of a period of amenorrhea preceding the bleeding may be common to both conditions. The soft and enlarged post-abortal uterus is a significant sign. A positive Aschheim-Zondek Test is almost conclusive for pregnancy. At the same time it is to be remembered that the urine of patients with cystic hyperplasia may occasionally show a positive follicle stimulating hormone reaction. This is especially true if the bleeding has existed for a long time. The patients are usually in their generative years.

5. *Endometritis*—especially post-abortal. The history is significant. The bleeding is usually associated with the periods. A temperature may be present. The patients are usually young.

Etiology

Two questions are in need of further elucidation, namely, the etiology of the entire symptom complex, and the etiology of the bleeding.

The etiology of the entire symptom complex may be considered from the viewpoint of:

a. The endometrium.

b. The ovary.

c. The anterior hypophysis.

We do not believe that cystic glandular hyperplasia of the endometrium arises solely from a condition inherent in the endometrium proper. The disease never occurs spontaneously in the absence of a functioning ovary. Neither trauma nor inflammation has been known to produce this condition. (There is the possibility that due to some change in the endometrium there occurs a change in the partition of the total amount of estrone produced. The endometrium takes up more than its normal requirement. This concept leads to the following idea. The endometrium, having thus altered its hormonal status, stimulates the ovary in an abnormal way and induces thereby cystic follicles. The latter explains why a curettage occasionally cures this syndrome.) In view

of the fact that the endometrium reflects the happenings in the ovary our attention has naturally shifted to the latter.

We consider cystic glandular hyperplasia of the endometrium to be due to a dysbalance between follicular hormone and progesterone in favor of the former. It can be due to a preponderance of the former whether absolute or relative. Cases 8 and 9 demonstrate the production of a "Swiss cheese" endometrium by means of large doses of the benzoic acid ester of estradiol (progynon-B). The amount of estrone varies considerably in each case but this may have been due to the variation in reactivity of the endometrium. To put this concept in another way, we can say that cystic glandular hyperplasia is merely an extension or exaggeration of the proliferative phase of the endometrial cycle, and that it develops whenever there is an *inadequate* supply of progesterone. Follicles form corpora lutea. But the transformation of a mature follicle is a function of the anterior pituitary gland, hence it is the accepted viewpoint to-day, that the etiology of cystic glandular hyperplasia of the endometrium is a dysfunction of the adenohypophysis.

In considering normal menstruation we stated that the interplay of hormones must occur with clock-like regularity. One paves the way for the action of the other. The follicle stimulating hormone develops the follicle to maturity. When ovulation occurs the follicle is converted into a corpus luteum by the luteinizing hormone. The entire sequence of events may be disturbed in two ways, either the follicles cannot be brought to maturity, and an immature follicle does not luteinize normally; or, the follicle is brought up to maturity but the luteinizing hormone fails to convert the follicle into a corpus luteum (absence of this hormone?). The follicles may then undergo a certain amount of regression, for they usually lack the size of mature follicles.

The etiological factor of the bleeding may reside in the endometrium per se. Neither the ovaries nor the anterior hypophysis are essential for bleeding.

The cases presented above demonstrate the following facts.

1. Bleeding may occur from any type of endometrium—proliferative, transitional or premenstrual.

2. Cystic glandular hyperplasia may occur in the absence of all previous history of bleeding, even during a prolonged period of amenorrhea (case 6).

3. In cases of cystic glandular hyperplasia, the endometrium still retains its identical pathological character long after bleeding has stopped. The bleeding is superimposed upon a cystic glandular hyperplastic endometrium.

4. Cystic glandular hyperplasia of the endometrium may be produced experimentally by means of follicular hormone in the absence of a functioning ovary.

These findings have led us to formulate the concept of a bleeding factor to explain abnormal uterine bleeding of functional origin.

Treatment of Functional Uterine Bleeding

Excessive functional bleeding may be controlled in any of the following ways:

1. *Removal of the Bleeding surface.* Curettage affords only a temporary control of the bleeding. In preclimacteric cases it should be employed routinely as a diagnostic measure in order to definitely exclude malignancy. In puberty bleeding, curettage should be limited because it is usually unnecessary and often produces an unpleasant psychic effect on the patient. Hysterectomy today has no place in the treatment of functional bleeding, except perhaps in some of the preclimacteric cases.

2. *Removal of the Stimulus (Follicular Hormone) Which Produces* the Bleeding Hormone. The stimulus for the secretion of the bleeding hormone may be removed by castration, either by operation or radiation. The latter is ideal for cases of preclimacteric bleeding but should never be used in younger women because it might result in permanent castration. Oöphorectomy also has no place in the treatment of functional bleeding. Resection of a portion of each ovary does not appear to be a rational procedure, for a part of an ovary may produce cystic follicles as well as the whole one. The cystic follicles are the result of abnormal pituitary function. (Furthermore, how much ovarian tissue should be removed?)

3. *Prevention of Bleeding Hormone Production.* This may be accomplished by roentgen ray irradiation of the pituitary gland but is too dangerous a method. The exact effect of a given dosage of radiation on an individual patient is not accurately predictable and permanent damage may result when only temporary suspension of function is intended

4. *Inhibition of the Activity of the Bleeding Hormone.* This offers the best method to date of controlling functional uterine bleeding. The activity of the bleeding hormone may be effectively checked by the administration of extracts of the corpus luteum, pregnancy urine or the anterior lobe of the hypophysis. Potent corpus luteum extracts are not generally available because of their expense and limited supply. Adequate amounts of synthetic progestin should soon become available.

Practically every case of functional uterine bleeding can be controlled by pregnancy urine extract, providing the dosage is adequate. The average daily dose required during the stage of active bleeding is from 200 to 500 R.U. In very severe cases as much as 750 R.U. daily, in 2 or 3 divided doses, may be necessary. The injections are best given intramuscularly in the buttocks. When the bleeding ceases, the patient may be carried along on much smaller doses (200 R.U. once or twice a week) and this should be continued for several weeks or months. Following this treatment the periods may occur at fairly normal intervals. At first they may be prolonged and profuse, but continued treatment both shortens and lessens the flow. This is especially likely if the previously excessive flow was periodic in occurrence. On the other hand, if the bleeding was formerly more or less continuous, a prolonged period of amenorrhea may result. In some instances there has been no bleeding for well over a year. The value of such a prolonged period of amenorrhea is twofold. It relieves the overworked hematopoietic system and gives the endocrine apparatus an opportunity to reëstablish an equilibrium and later resume normal function.

The exact mechanism by which pregnancy urine extract controls functional bleeding has not as yet been definitely established. The absence of effect on the endometrium conclusively shows that the cessation of the bleeding cannot be attributed to luteinization. We believe that pregnancy urine extract acts directly on the tissue producing the bleeding factor, possibly the endometrium.

Similar, although not as effective, results were obtained by the use of anterior pituitary extracts. They contain the gonadotropic hormones. In very severe cases, anterior pituitary extract (prephysin), in daily doses of 1 cc., may be injected intravenously. This usually stops the bleeding promptly but the severe reactions that occur in some patients from this intravenous administration somewhat limit its use.

Certain adjuvants to the treatment of functional uterine bleeding are important. The anemia resulting from prolonged or excessive bleeding demands careful attention. In the milder cases, iron may be given. The severe cases often require one or more blood transfusions. *If a pregnant donor can be obtained, not only are erythrocytes and hemoglobin supplied but also the anterior pituitary-like hormones.* Oxytocics as pituitrin and ergot are occasionally of value especially when the bleeding is associated with uterine atony. Where the uterus is firm they are of no value.

The writer has used the transfusion of human pregnancy blood on

several occasions. All bleeding ceased within 48 hours. Blood was obtained from a healthy donor in her second trimester of pregnancy. The amount transfused was 350 cc. It is of interest to note that neither the blood nor the urine of the recipient gave a positive Aschheim-Zondek Test at any time after the transfusion.

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CHAPTER XXII

FUNCTIONAL DYSMENORRHEA

In the writing of this volume the author left the chapter on functional dysmenorrhea for the last, with the hope that some work would appear that would clarify this subject. No such work has appeared. In fact, the problem is more baffling than ever. Many reports on the subject describe some endocrinological form of treatment. We have followed the suggestions faithfully, only to meet with disappointment time and again. Here and there an excellent result is obtained, but very frequently the repetition of the treatment in a similar case leads to complete or partial failure. Our difficulty lies mainly in the fact that we do not understand the problem, for here, as in other issues, the understanding of the problem is three-fourths of the solution.

The controlling factors of the problem are:

1. The development of the uterus.
2. The hormonal control of the uterus.
3. The nervous control of the uterus.

Other factors also enter in, namely,

(a) The constitutional type of the patient. It is well known that the asthenic type is more prone to dysmenorrhea than the other types. The patient with general infantilism belongs to the same category.

(b) The nervous makeup—of the patient is of great importance. The highly sensitive and nervous patient suffers a great deal more than the phlegmatic type. But even the latter group can be made acutely miserable by dysmenorrhea. The psychic reaction of a woman to her sexual function is important. Undoubtedly these considerations would become clearer if we fully understood the controlling factors.

The Development of the Uterus

The majority of patients with functional dysmenorrhea present a hypoplasia of the uterus. It is equally true that many patients with marked infantilism of the internal genitalia have no dysmenorrhea, while others with normal uteri have. Similarly, an acute antiflexion (or retroflexion) is present in some but not in others. There is no relationship between the extent of the genital hypoplasia and the intensity of the dysmenorrhea. A number of questions with regard to the myo-

metrium are still awaiting a solution. What are the histological variations in the myometrium during the cycle? Are all muscular layers of the uterus equally involved by the hypoplasia? Could a dissociation of function between the various muscular layers be responsible for the pain? Moir (1934) made the interesting suggestion that dysmenorrhea may be due to ischemia of the myometrium brought about by a uterine pressure upon the blood vessels greater than the systolic blood pressure.

The Hormonal Control of the Uterus

The human uterus undergoes contractions and relaxations throughout the cycle. Only infrequently do we find a quiescent uterus, and that may occur during any phase of the cycle. Our own evidence is against any corpus luteum inhibition of uterine motility. One would expect that in the absence of a corpus luteum the menses should be painful. This is not the case, for patients with cystic glandular hyperplasia who bleed cyclically do not have dysmenorrhea. Similarly the anovulatory cycle is not painful. On the contrary, Kurzrok, Wiesbader, Mulinos and Watson (1937) have observed severe cramps in patients who were injected with 2 Rabbit Units of Progesterone during the follicular phase of the cycle, but not during the corpus luteum phase. The patients were being tested with an intrauterine bag in vivo. We have no explanation for this observation. The uterine contractions as evidenced by the kymographic tracing show no significant change. We have repeatedly examined (by means of biopsies), during the premenstrual phase, the endometrium of patients suffering from dysmenorrhea. The endometrium showed adequate premenstrual characteristics and a diagnosis of insufficient luteinization could not be made.

The Nervous Control of the Uterus

The autonomic nervous system that supplies the uterus must have some coördinating function even though uterine motility and function (i.e. endometrial transplants in the eye) seem wholly independent of it. The internal os seems to be closely related to dysmenorrhea. It is a well known clinical fact that in some patients the passing of a sound through the internal os, even though very little obstruction is encountered, leads to immediate severe uterine cramps. In a case recently observed by us, the gentle passage of a sound through the internal os led to severe uterine cramps plus nausea and vomiting. These same symptoms occurred at the usual menstrual periods. It is conceivable that the passage of tissue fragments and blood through the internal

os has the same stimulating effect as the dilator. Whether such observations imply a different type of innervation of the internal os in some patients is entirely undecided. In considering the question of menstrual blood passing through the internal os, the following questions present themselves: What is the character of the decomposition products (through the action of endometrial enzymes) of menstrual blood? What pharmacological effects could such derivatives exercise upon the uterus? Could such derivatives be modified by different uteri? Is acetylcholine (or its derivatives which act as a powerful oxytocic) a possible factor? Miller, Cockrill and Kurzrok (1937) observed that acetylcholine contracted all uteri, during all phases of the cycle, and that the magnitude of response tended to increase from about the twentieth day on.

Another interesting possibility pertains to the function of the Fraukenhäuser ganglion. Branches from this ganglion pass to the sacral nerves and to the hypogastric plexus. Blotevogel (1933) has shown that in the mouse this ganglion undergoes morphologic change (the chromaffin cells) in direct relation to the amount of estrone and progesterone. Whether there is a similar change in this ganglion during the menstrual cycle is unknown. Variations from the normal could have interesting possibilities on the nervous control of the uterus.

One can readily observe from the above discussion how incomplete is our knowledge with regard to the etiology of dysmenorrhea. One thing is certain,—the etiological factors must be multiple.

Therapy

In view of the multiple etiological factors involved the treatment is of necessity varied. We have recently tried out the recommended medical methods. We have frequently succeeded, and often failed. A given method may relieve the pain during one or several periods, and then subsequently fail completely. Occasionally, when one or two periods are rendered free from pain the dysmenorrhea seems to be permanently relieved. Many cases are permanently relieved by pregnancy, while in others there is a definite recurrence of the pain. It is our custom to start with peroral medication and when we do not succeed we resort to parenteral methods.

Medical Measures. *A. The Benzyl Compounds.* In view of the fact that benzyl compounds are to a certain extent antispasmotic we have used them extensively either alone or in conjunction with other forms of treatment. We always exhibit this form of medication first. Benzyl fumarate (in 5 grain tablets) is the drug prescribed. We recommend

10 grains t. i. d., p. c. for the two weeks preceding the flow, and then when the pain is expected we change to 5 grains every one-half hour for ten successive doses. During the remaining days of the menstrual period the original dosage is again recommended. No untoward or toxic symptoms have been observed by us. The treatment may be repeated as often as necessary. This method affords relief in about 10 per cent of the cases.

B. Atropine. This presents our second method of therapy. It has been our custom to exhibit atropine whenever the passage of a sound through the internal os produced severe pain, especially of the type the patient experiences at her menstrual periods. It is based on the action of atropin on the sympathetic nervous system. We have used the official tincture of belladonna in doses ranging from five to ten drops three times a day. In view of the sensitivity of some patients to atropine poisoning it has been our custom to *write down* the toxic symptoms for the patient. They may be a dry mouth, dilated pupils, a flushed face, or a rapid pulse. The treatment is begun 3 days before the onset of the flow and continued throughout the flow. The effective dose is usually well within the limits of the recommended dose. This method affords relief in about 10 per cent of the cases.

C. Moderate Doses of Estrone. The administration of estrone is occasionally of value in those cases in which a genital hypoplasia is present. The milder the degree of hypoplasia the better are the results. The purpose of the treatment is to stimulate growth of the genital tract. The patients are injected with 1000 R.U. (5,000 I.U.) of estrone twice a week, throughout the cycle, except during actual flow. This dosage does not disturb either the rhythm of the cycle or the duration of the flow. No untoward effects are noted. The number of cases benefited amounts to 15 to 20 per cent. Recently we changed to peroral administration of estrone, giving 2,000 I.U. per day. Not enough cases have been studied to date to give a definite opinion as to the value of oral medication.

D. Large Doses of Estrone. This method of treatment was first suggested by Dr. Leo Wilson (1936). It consists of the administration of 20,000 R.U. of estradiol benzoate (progynon-B) during the follicular phase of the cycle. He suggests the intramuscular injection of 10,000 R.U. on the seventh and eleventh day of the cycle. The administration of the same dosage during the latter half of the cycle is without effect. When given as suggested by Wilson, the tempo of the cycle is frequently disturbed. The period may be (about ten days) premature, or late. Often such premature or delayed periods are pain-

less. Some patients are not disturbed by double the suggested dose. The modus operandi of this large dose of estrone is not entirely clear. One would expect a disturbance of luteinization of the uterus. We have previously stated that the effect of the gonadal hormones on the endometrium is a balanced reaction, so that an excess of estrone will inhibit the development of the corpus luteum phase. Judging from our results such failure of luteinization is infrequent, though larger doses (40,000 R.U.) may lead to inadequate (or failure of) transformation of the postmenstrual endometrium into the premenstrual type. A sterile cycle may result. *The relief of pain is independent of the character of the endometrium just before menstruation. In other words, the corpus luteum plays no part in the relief of symptoms.* This method of therapy seems promising. It is applicable to only fifteen or twenty per cent of the cases of dysmenorrhea. We have as yet not devised a method for choosing the proper cases for this form of therapy. While it is true that many cases present a genital hypoplasia, others having a normal uterus react just as favorably.

E. Corpus Luteum Hormone. The demonstration of an inhibitory effect of corpus luteum hormone on the rabbit uterus has naturally led to its application for the same purpose on the human. From experimental evidence the treatment of dysmenorrhea with progesterone would seem justified. As stated in Chapter V our experiments with corpus luteum in vivo have failed to demonstrate any inhibitory action during the corpus luteum phase of the cycle. The injection of progesterone (2 rabbit units) caused no appreciable change in the kymographic tracing, in fact we occasionally felt that the contractions were accentuated. We have used progesterone in the treatment of dysmenorrhea; the dosage given ranged between two and thirty Rabbit Units of hormone (proluton or progestin). Our results were poor. Only an occasional case responded to progesterone. Data are completely lacking on the character of uterine contractions during menstruation in normal women and those suffering from dysmenorrhea. A difference may be expected in some cases. The relief afforded by corpus luteum hormone in some cases may be due to its action on some other factor than uterine motility, possibly on the blood supply or the autonomic nervous system.

F. Anterior Pituitary-like Hormones. Novak (1933) suggested the use of pregnancy urine extract (Follutein or Antuitrin-S) for the relief of dysmenorrhea. Witherspoon (1935) has also reported good results. The basis of the theory is the luteinizating effect upon the ovary. Hamblen (1935, 1936) studied the effect of pregnancy urine extract on the luteinization of the human ovary. He noted the formation of

follicular cysts with active granulosa and fairly well preserved ova, and surrounded by a proliferated theca uterus with prominent theca lutein cells and with increased thecal vascularization. This is most marked in the younger group of patients, though no inference can be drawn as to the function of such luteal tissue. Geist (1933) also noted some increase in theca lutein cells. Assuming then that luteinization does occur when dosages ranging from 800 to 8,200 R.U. are given, it becomes quite questionable whether the administration of several hundred units just prior to and during the flow produces any such change. The absence of corpus luteum effect upon the human myometrium is again commented upon. The good results with this form of therapy must rest upon some other factor. It has been suggested that the pain may be due to a sudden rise in the estrone titer just before menstruation. Our own experience is that the premenstrual estrone level is usually low.

Novak and Witherspoon recommend the daily intramuscular injection of 1 cc. of follutein for three to four days previous to the flow and also for the first two days of the period. Brown (1935) also has noted good results from this form of therapy. We have tried pregnancy-urine extract and our good results do not exceed fifteen per cent.

G. The Injection of other Substances. 1. Calcium—Boynton and Hartley (1934) report the treatment of essential dysmenorrhea with calcium, or calcium and viosterol. Two-thirds of the cases were greatly benefited. They believe that calcium is especially indicated in those cases that bruise easily. We had had no experience with calcium as the sole remedy for dysmenorrhea.

2. Insulin has been recommended by Altschul (1936). He recommends the use of doses up to 10 Units (brand c) per day from three to seven days before or during the period. No deleterious effects have been noted. The patients showed no definite nutritional disturbances. As to the modus operandi of insulin in dysmenorrhea, Altschul cites Abel's (1931) work with insulin on animals, wherein he noted a long estrus pause with a decrease in the number of follicles and an increase in the number of corpora lutea.

Surgical Measures. It has been well known that dilatation, or dilatation and curettage occasionally relieve a functional dysmenorrhea. The relief may be temporary or permanent. Stone (1935) recommends the gradual dilation of the cervix with a Hegar dilator as an office procedure.

Sympathetic neurectomy has recently come to the fore in the treatment of functional dysmenorrhea. It is needless to state that the

operation should only be considered when all conservative methods have failed. Wetherell (1935) recommends the resection of the superior hypogastric plexus of the sympathetic. He notes no deleterious effect on libido or on future pregnancy. Greenhill (1934), Adson and Masson (1934), Keene (1935) and Counseller and Craig (1934) advocate resection of the presacral sympathetic nerves. The latter writers are of the opinion that the primary etiologic factor in dysmenorrhea is dysfunction of the pelvic sympathetic nervous system. When this dysfunction is corrected by resection the benefits are permanent, while the functions of normal menstruation and childbirth are not disturbed. There is no loss of motor control of either bladder or rectum. These operations were first recommended by Cotte (1925).

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CHAPTER XXIII

THE CLIMACTERIC

The factors governing the termination of the sexual epoch are closely associated with those factors concerned in its inception. Hence, if we knew the exact cause of puberty we would know the reason for the menopause. In considering the factors immediately concerned with menstruation we discussed the anterior hypophysis, the ovaries and the uterus. To these glands we must add the adrenals, the thyroid, the pancreas and possibly the thymus, though their contributions are as yet not clearly understood. We have at least three etiological factors causing the climacteric,[1] depending on the organ primarily involved:

1. Anterior pituitary gland.
2. The ovaries.
3. The uterus.

But in the final analysis, the ovary is eventually involved, and about it the syndrome centers.

Anterior Pituitary Gland

This involvement is occasionally seen in cases of secondary Fröhlich Syndrome. The patient may be in her early thirties and the periods may become scantier and less frequent and finally stop completely. As time goes on one of the several symptom complexes may develop. The usual vasomotor symptoms may not appear at any time, they may appear within two or three years with varying degrees of severity, or they may first make their appearance at the usual time, that is, in the late forties. It is the first group that interests us at the moment. Two explanations for this absence of symptoms may be given. First, that the menopause was the result of ovarian failure, but *so gradual* that no noticeable symptoms occurred. Second, that the mechanisms involved in the usual form of menopause (v. i.) were absent.

The hormonal picture in these cases has not been extensively studied. The few cases that have come under our observation presented an ab-

[1] We shall use the term climacteric in the larger sense, meaning all the symptoms in all organ systems arising from permanent cessation of function of the anterior pituitary-ovary-uterus complex. The term menopause is here used in the narrower sense, meaning permanent cessation of menstruation.

sence of both the follicle stimulating and follicular hormones in the urine.

The Ovaries

It has previously been considered that the cessation of ovarian function was the *sole* cause for the climacteric. It might be stated at the outset that we are not in complete agreement with this idea. The reasons are that the ovarian failure may be secondary to that of the hypophysis, and that the symptoms of the climacteric are not solely due to the loss of ovarian function but to a disharmony of the other glands of internal secretion.

The Uterus

The third factor mentioned as pertaining to the menopause is the uterus. This may be encountered in cases of genital hypoplasia, wherein the periods completely stop in the twenties, or before; similarly when a hysterectomy is performed in a young woman. In either case the onset of the climacteric may vary. It may come on soon after cessation of menstruation or be postponed until the expected onset in the late forties. In the early climacteric associated with uterine hypoplasia it is justifiable to assume that the early stopping of ovarian function was in some way due to the loss of uterine function. We have previously (Chapter XX) stated that uterine hypoplasia is frequently due to an inherent defect within the uterus and not to ovarian hypofunction. This brings us back to the unsolved question, as to whether the uterus is merely the receptor of ovarian activity, or a contributor to it. One frequently hears the statement that when the ovaries cease to function soon after a hysterectomy it is due to disruption of the ovarian circulation. That may be so, but need not always be so. We may be dealing with an internal secretion of the uterus.

Symptoms

The symptoms arising at the time of complete cessation of ovarian function vary tremendously with the constitutional makeup, the rapidity with which the function is lost and with the psychic balance of the woman. Aschner (1924) makes the point, and very correctly so, that some women seem to be greatly benefited by the menopause. This is particularly applicable to the thin, anemic, and asthenic types, and especially so to those that suffered from profuse or prolonged menstruation and nervous dyspepsia. He observed that their appearance becomes so striking that they seem rejuvenated.

It is exceedingly difficult to state when the climacteric begins and when it ends. The mere cessation of menstruation—menopause—is not the sole criterion. The beginning of the climacteric may be years before the menopause, that is, some symptoms which are part of the climacteric syndrome begin years before the termination of the flow. Similarly, the end is not a sharp demarcation, for many women continue to show isolated symptoms into old age. The fact that a pregnancy may occur a number of years after the menopause also points to the fact that cessation of menstruation is an important, but not the sole factor that demarcates the climacteric. Wiesel (1924) observes that the climacteric is not a limited syndrome, for many other disturbances, both functional and pathological, give rise to temporary symptoms resembling those of the climacteric.

There are no characteristic pathological (morphological) changes in the organs during the climacteric, except in the ovary and genital tract. Wiesel points out that the adrenal cortex may show a greater amount of lipoids, and the thyroid an excess vascular sclerosis. The changes in the other organs are not significant, though it might be added that exhaustive pathological studies have not been made, for patients do not die of the menopause. Furthermore, our concept of the climacteric is not that of a disease based on pathological anatomy, but rather a functional disturbance of the hormonal and nervous equilibrium, in which the ovary plays the dominant or central rôle. The hormonal findings, so characteristic of the fully developed climacteric (v. i.), do not limit this syndrome, for they are not present in many patients either at the beginning or at the end of this epoch.

It is not essential at this point to enumerate all the symptoms of the menopause. But the mechanism by which they arise is important. The hormones are regulators of the body functions, and in addition, *they regulate one another*. It has definitely been established that one gland of internal secretion may influence another both in respect to morphology and to function. Furthermore, there is an intimate relationship between the hormones and the central nervous system, for instance, adrenalin stimulates the vagus and vasomotor centers, the hypoglycemia following insulin results in nervous manifestations, and the thyroid increases the irritability of the peripheral sympathetic nervous system. One may therefore expect a profound change in the entire regulatory mechanism when the ovary ceases to function. The gonads are an important cog in the machine. Consequently, the effects produced by this disturbance in equilibrium may extend as far as the regulatory mechanism controls, and that is, the entire organism. The

great number of dysfunctions that arise from this disturbed equilibrium is still further increased by the fact that the same hormone can convert a stimulating into an inhibitory reaction by a change in its concentration (adrenalin, thyroxin). Hence, one may expect the climacteric syndrome to include symptoms referable to every tissue and organ system. But which symptoms will develop depends upon the constitution (in the larger sense) of *each particular* patient. That some symptoms occur with greater frequency merely indicates that the mechanism causing these symptoms is closely allied to ovarian function. One frequently encounters discussions whether the menopause syndrome is due to a loss of estrone or to an excess of follicle stimulating hormone. That tries to simplify a problem that will not be so simplified. The climacteric syndrome implies a general disturbance of equilibrium and control, more profound in some systems than in others.

The effect of menstruation on the symptomatology of the climacteric is interesting. Many patients state that the menstrual flow results in a marked improvement of the symptoms. The reasons for this are not quite clear. The normal curve of estrin excretion is lowest at the time of menstruation. Hence, if the symptoms were merely a function of the lack of estrin one would expect a marked aggravation. Two explanations are possible: one, that the estrone excretion curve reaches a peak at the time of menstruation instead of a diminution; two, that the symptoms are in part independent of the estrone titer, but are due to some other undiscovered factor—, a point for further study.

Etiology of Cessation of Ovarian Function

Considering therefore the ovary as the hub (in respect to the climacteric syndrome) about which the hormonal and nervous control radiates, certain questions become pertinent. What causes the ovary to cease functioning? Does this cessation occur at the time of the menopause, or before, or after? Do both ovaries stop their function at the same time? Can such function, once stopped, be revived?

The statement has previously been made that the ovary does not possess the *inherent* ability to function. The ovary must constantly be stimulated to function by the anterior hypophysis, or to put it differently, the ovary needs for its inherent metabolism a substance (gonadotropic hormone) originating in the anterior hypophysis. Without this specific substance, or when the ability to utilize this chemical compound is lost, the ovary ceases to function. Hence, the cessation of ovarian function could be due to a failure of the stimulator or a failure of the gonad proper. The hormonal findings at the time when

the climacterium is fully established give us some insight into this problem. They show an absence of estrone and the presence of large quantities of follicle stimulating hormone. It is believed, on good evidence, that the amount of follicle stimulating hormone produced at this time is in excess of the amount produced during normal ovarian function. The excess is probably due to a loss of inhibitory control that follicular hormone normally exercises upon the anterior pituitary gland. Hence, the loss of ovarian activity is not due to the absence or insufficiency of the gonadotropic hormone necessary to stimulate the follicular apparatus of the ovary. The cessation of ovarian function is then evidently due to a change within the ovary proper, and this manifests itself in a *failure on the part of the ovary to respond to stimulation. That is, the functional life of the ovary is limited.*

This functional limitation is first noted during early infancy. Smith and Engle (1927) studied the effect of anterior lobe implantation on immature female rats. They noted that the maturing of the reproductive system takes place rapidly, but 2 to 5 days are required when treatment is started at weaning (21 days). The response becomes less rapid the earlier the injections are started. In rats fourteen days of age at the beginning of treatment, 8 to 10 days supervene before the full effect of the implants is induced. A similar type of response in immature rats was observed by Saunders and Cole (1936) when using mare gonadotropic hormone. That these observations may apply to the young human ovary can be gathered from the following indirect evidence. Gonadotropic hormone has been demonstrated in the blood and urine of the newborn by many observers (Neuman, 1930). Prolan occurs in large quantities in the maternal circulation from the second month of pregnancy, and yet the ovaries of the new born only rarely show signs of stimulation, in the form of follicular cysts. It might be added at this point that in the treatment of cryptorchidism we have noted that very young boys (under six years) are very refractory to treatment, and that as puberty approaches a lesser amount of prolan is necessary to secure descent of the testes.

In a recent study of the effect of the follicle stimulating hormone from mare-serum (Antex-Leo) on the human ovary by Kurzrok, Smith and Watson, (1937) the following interesting and pertinent facts were noted. When adequate quantities (3600 M.U.) were injected during the first phase of the cycle and the patients were in the third decade of life, then there resulted a *marked increase in size and weight of both ovaries.* Numerous large follicular cysts appeared. On the contrary, when the

hormone was injected into women during the fourth decade the response was only slight; an occasional small follicle appeared. When the injections were given to patients in fifth decade or beyond the ovaries did not show the slightest response, even when several times the dose (9000 M.U.) was injected. Even when a marked response was present in some young patients, the response could be wholly in one ovary, while the other would remain entirely refractive. In the latter cases the stimulated ovary was removed and the refractive ovary was allowed to remain. Menstrual cycles shortly reëstablished themselves. *These findings indicate that the ovaries in the older group of patients were refractive to a dosage which was adequate for the younger group, and furthermore, that while one ovary may lose its ability to respond to a given stimulus the other ovary may still retain that capacity.* The reason why the refractive ovary was able to maintain function after the removal of the stimulated gonad, is that the entire amount of gonadotropic hormone liberated by the patients own pituitary was available to the single remaining ovary. *It is reasonable to assume that the extent of refractiveness increases until no available amount of gonadotropic hormone suffices. The time of onset and extent of refractiveness must vary from patient to patient. Hence the time of onset and intensity of the climacteric would vary as a result of this. The climacteric is basically due to a failure of responsiveness on the part of the ovaries* (figs. 148 and 149).

Whether we are to assume that the functional life of *one* ovary is longer than the other, or that the one began to function earlier and ended sooner, is an entirely debatable question. If ovarian function ceases and results in the climacteric, can such function subsequently be revived? Under normal circumstances it cannot. Certainly the addition of more gonadotropic hormone does not revive the ovary nor does stimulation with the x-ray. The ovary seems to have undergone some irreversible change. This brings up another interesting point. Supposing the ovary originating from a *young* girl is transplanted into a middle-aged woman during her climacteric. The menopause patient produces (and excretes) quantities of gonadotropic hormone greater than normal. Will such an ovary "take," that is, function and produce demonstrable quantities of follicular hormone? The stimulus (gonadotropic hormone) is present and a receptive ovary is provided by the donor. Moricard (1936) states that such grafts will not take. This would imply that the aging of the host is an additional factor in the function of the ovary. This subject is deserving of further intensive study.

Therapy

The chief therapeutic agents for the treatment of the climacteric are estrone, calcium and such therapeutic measures as each case may specifically require. We take cognizance of the fact that the number and intensity of symptoms vary from patient to patient, and that women differ a great deal in temperament. Hence, there can be no cut and died method of treatment. It has been argued in many quarters that the therapeutic results obtained were merely suggestive. We do not deny that in some patients there is a distinct psychic element, but in our experience it does not involve more than 10 or 20 per cent of the cases. It has frequently seemed to us somewhat bizarre that the loudest exponents of the "suggestive results" idea have never themselves treated a patient.

The results of therapy can be verified experimentally by the vaginal smear test as demonstrated by Papanicolaou and Shorr (1936). These investigators found that the vaginal smear test furnishes a simple objective guide for the treatment of menopausal syndrome with follicular hormone. With an adequate amount of estrone, there occurs a transformation of the vaginal smear from the menopausal to the leucopenic type, with the large flat cells, largely cornified, with small pyknotic nuclei. This is the type of smear which is found normally during the high follicular phase of the menstrual cycle just prior to ovulation. This change in the smear is generally associated with relief of the menopausal symptoms (figs. 140 to 147). Biopsies of the vaginal wall show a close correlation between the changes in the vaginal epithelium and in the vaginal contents. As a result of treatment, the vaginal epithelium becomes hypertrophic with the more superficial cells showing cornification and small pyknotic nuclei. The outer basal zone was better differentiated and showed nuclear enlargement and mitosis.

A further effect of follicular hormone in the castrate (spontaneous or induced) is to lessen the secretion and production of the follicle stimulating hormone. This excessive secretion is due to the absence of the inhibiting effect that estrone exercises on the anterior hypophysis (Meyer, Leonard, Hisaw and Martin, 1930). It might be implied that because the follicle stimulating hormone decreases at about the time when the symptoms are alleviated, this hormone is responsible for the menopause symptoms. We do not favor this idea because the injection of follicle stimulating hormone does not produce menopause

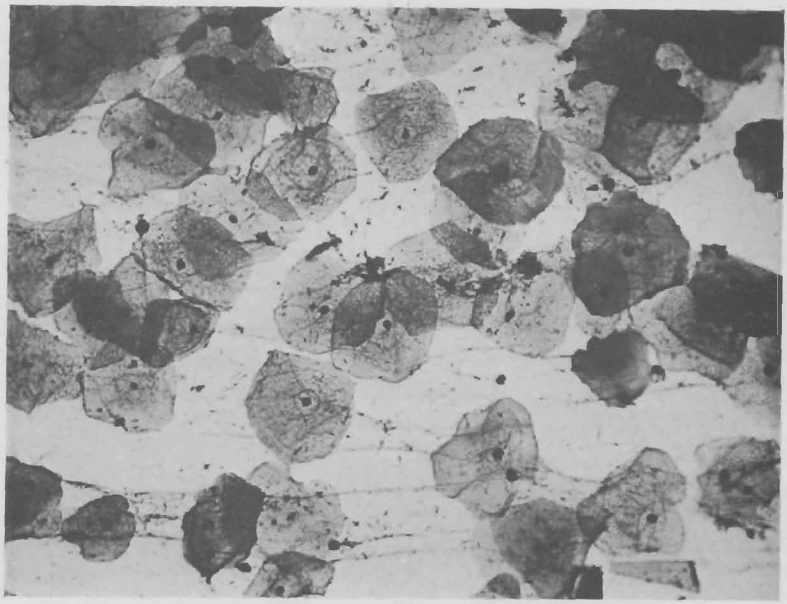

FIG. 140. Human vaginal smears. Follicular (copulative) phase of a normal woman at the eleventh day of the menstrual cycle. Note leucopenia and prevalence of large flat cells with pyknotic nuclei, either isolated or in small groups. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

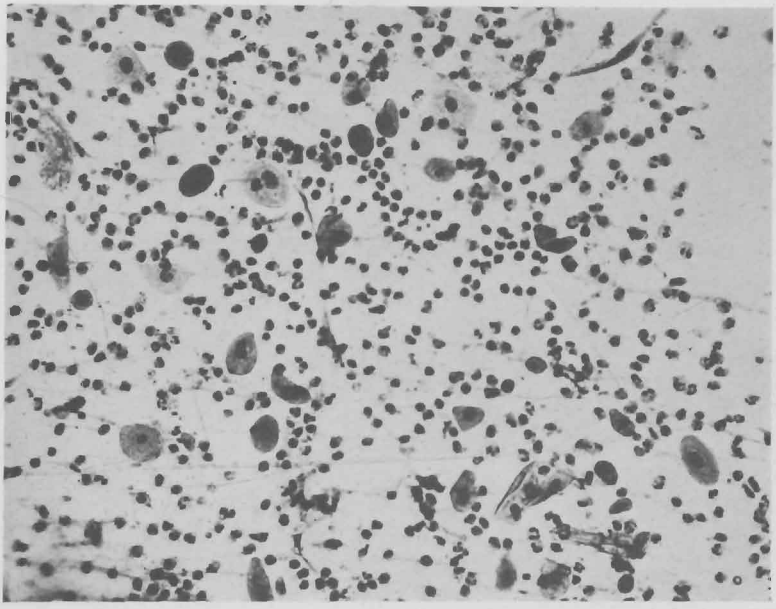

FIG. 141. Human vaginal smears. Case E. G. Surgical menopause before treatment. Note abundance of leucocytes and prevalence of round or oval deep cells. Some erythrocytes are present. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

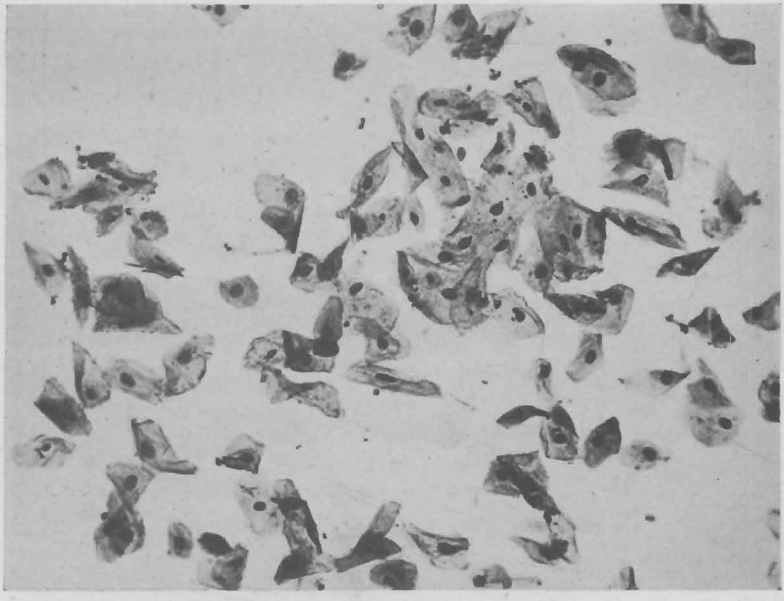

FIG. 142. Human vaginal smears. Case E. G. Shows disappearance of leucocytes and erythrocytes, and clearing of smear on further treatment. Deep and elongate cells prevailing. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

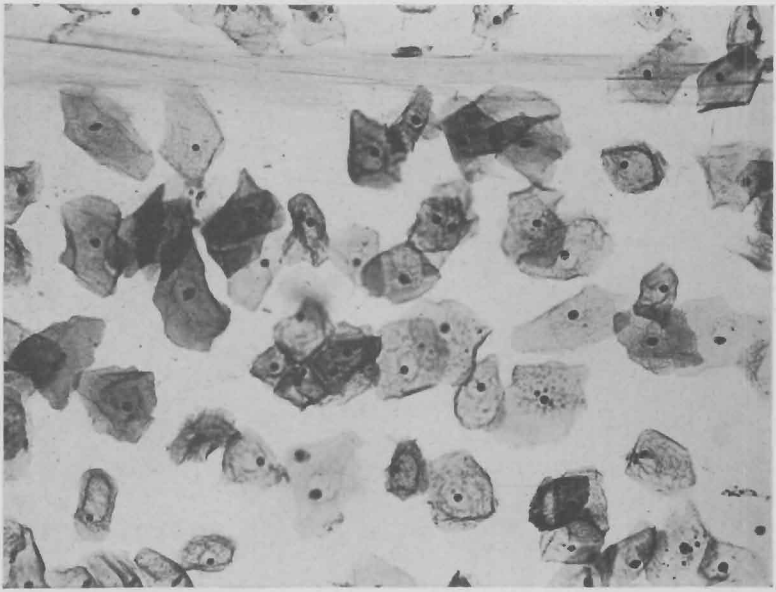

FIG. 143. Human vaginal smears. Case E. G. Typical follicular phase induced by further treatment. Note leucopenia and prevalence of flat cells with pyknotic nuclei. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

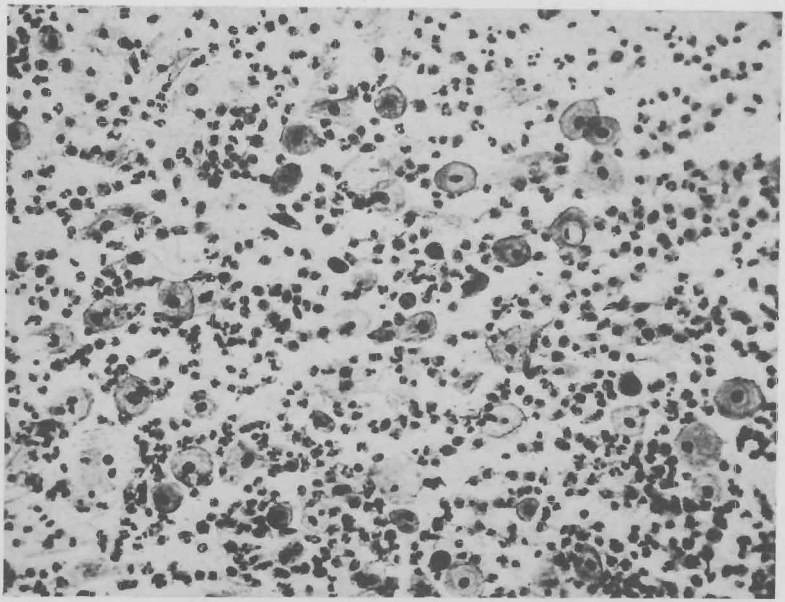

FIG. 144. Human vaginal smears. Case E. G. Almost complete regression to original type after discontinuation of treatment. Note return of many leucocytes and deep cells. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

FIG. 145. Human vaginal smears. Case E. G. Follicular phase following resumption of treatment. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

symptoms, and furthermore such symptoms may occur in the complete absence of follicle stimulating hormone from the urine.

Estrone should be given in large doses. It is best administered hypodermically, though the oral route may be used. Oral administration required from five to twenty times the hypodermic dose. It has been our custom to begin with 50,000 I.U. (1 R.U. = 5 I.U.) of follicular hormone (amniotin or Progynon-B) twice a week. The injections are always given (with a 20 gauge needle) intramuscularly into the buttocks. Subcutaneous injections produce painful lumps. As the vaso-

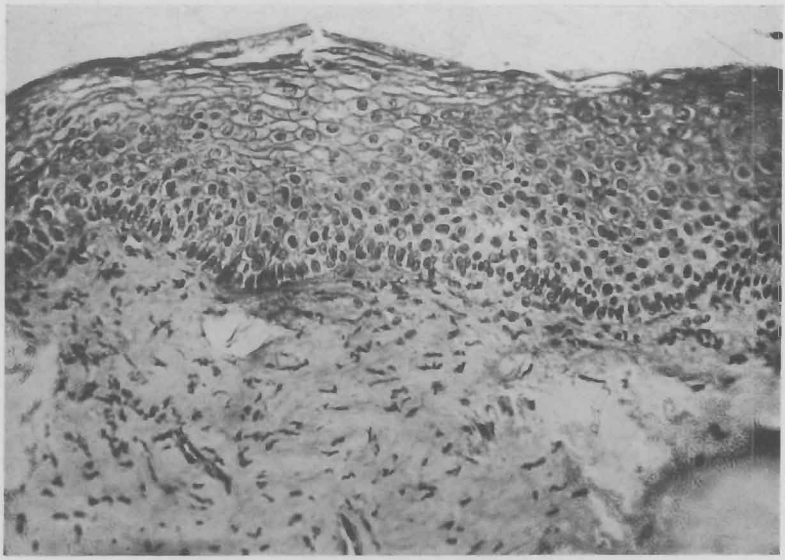

FIG. 146. Human vaginal smears. Case E. G. Regression of vaginal epithelium after discontinuation of treatment. Superficial cornified zone has disappeared. Figure 144 illustrates corresponding smear. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

motor symptoms diminish the number of injections is decreased until one dose every ten days is reached. Maintaining the same frequency of injection an attempt may be made to use less than 50,000 I.U. per dose. Either the patient's symptoms or the vaginal smear may be used as a guide. It is not advisable to increase the intervals between injections beyond two weeks, for there is a recurrence of symptoms. Adequate treatment results in a definite decrease in the number of sweats and flushes per day, namely, from one every half hour to one or two per day. There is a definite increase in energy and sleep becomes

more restful and refreshing. We must stress the importance of adequate and frequent dosage. Insufficient hormone is equivalent to none at all, for it leaves the patient discouraged with the treatment.

During the past few years we have injected in addition to estrone an alcohol-ether extract of the entire ovary. It is commercially available

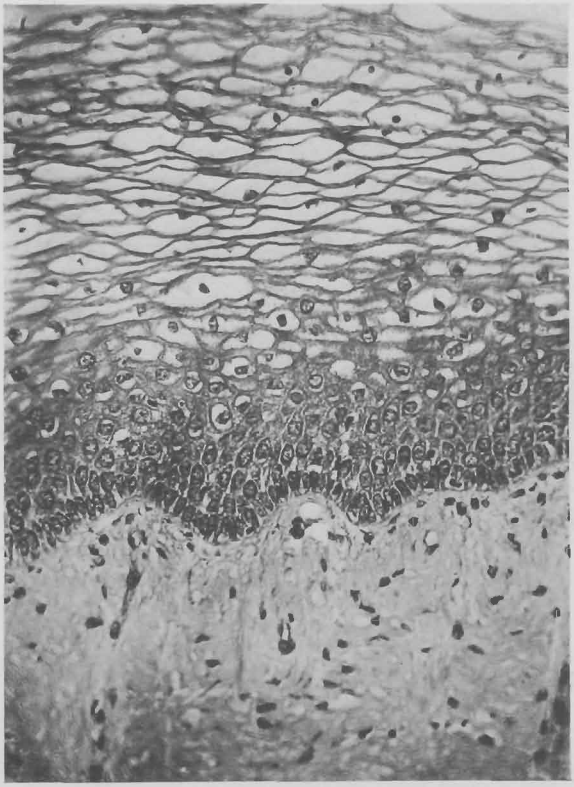

FIG. 147. Human vaginal smears. Case E. G. Hypertrophy of vaginal epithelium after resumption of treatment. Note reappearance of superficial cornified zone and orderly structure. Figure 145 illustrates corresponding smear. (Courtesy of Profs. George N. Papanicolaou and Ephraim Shorr.)

in the form of Sistomensin (Ciba). The basis for the use of this extract is as follows. One is frequently asked whether estrone and progesterone are the only hormones in the ovary. May there not be others whose function is not as yet clearly defined (Kaufmann, Müller and Mühlbach, 1932; Laqueur, 1933) but whose action is on the general metabolic processes, the blood vessels, blood cholesterol, fat metabolism, etc.

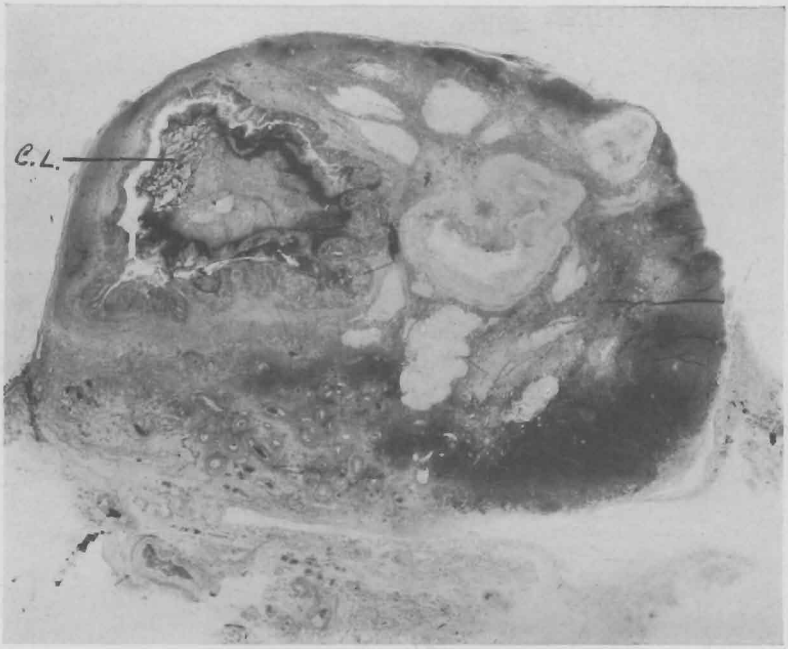

FIG. 148. Human ovary. G. P., No. 9298; aged 43. Mare serum, 3,600 M.U. Only one very young corpus luteum and numerous corpora albicanta.

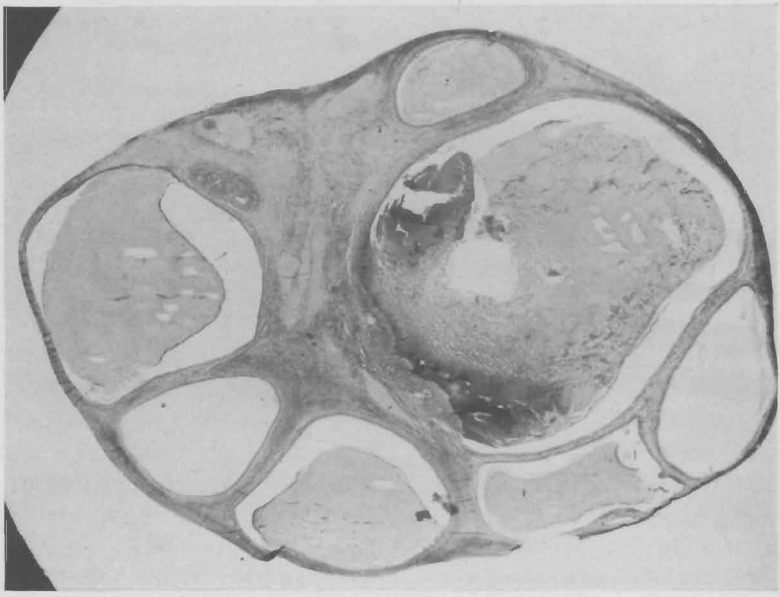

FIG. 149. Human ovary. G. P., No. 9546; aged 25. Treatment with mare serum, 3,000 M.U. Note the large follicular cysts which filled the entire ovary.

(F. Laqueur, 1934). It has been our impression (based on its use for more than 400 cases in 5 years) that when Sistomensin (1 cc.) is added to the injected estrone the therapeutic effect of the latter is enhanced. This applies only to the relief of the vasomotor symptoms.

The third therapeutic agent that we have routinely used is calcium. The reasons for its use are as follows: Many of the symptoms frequently encountered during the climacteric are due to disturbances of the vasomotor system—such as sweats, flushes, palpitations, etc. We believe these to be due to an increased irritability of the vasomotor system. Calcium depresses the irritability of the cell, hence its use in this connection. The salt used is the gluconate in doses ranging from 2.0 to 5.0 grams per diem.

We have occasionally used estrogenic material by mouth with excellent results. Theelol has been especially effective in cases of spontaneous menopause. The recommended dose is 150 R.U. (3 capsules) per day.

A point to be remembered is that the climacteric may begin years before the cessation of bleeding. The symptoms are varied and occur (or are intensified) during or just preceding the menstrual flow. Premenstrual headache, lassitude and asthenia are especially troublesome. For a number of years we treated these symptoms with small or moderate doses of estrone. Our results (Kurzrok, 1932) were uniformly bad. We have recently tried large doses of estradiol (progynon-B, 5,000 to 20,000 R.U.) injected intramuscularly during the ten days preceding the flow. The results are often decidedly better. The effect on the headache is frequently startling. One may include with this therapy premenstrual migraine (Riley, Brickner and Kurzrok, 1935). The estrone should be given several days *preceding* the onset of the headache. If given during the headache the therapeutic effect will be nil. If the headache begins three days before the expected period, then a first dose (10,000 R.U.) is given seven days before the onset of the flow and a second dose (10,000 R.U.) four days before the onset. In the most successful cases the patient will be completely relieved. Some patients experience the sensation of impending headache, but it never materializes. In the smallest group no therapeutic benefit is obtained. Our impression is that the headache is the result of pituitary change (enlargement?) during the premenstrual period, for we occasionally find contractions of the visual fields or a tendency to bitemporal hemianopsia. The suggested doses of estrone apparently inhibit such changes. But it must not be forgotten that very large doses of follicular hormone may increase the size of the pituitary and

thus aggravate the headache. It is understood, of course, that all organic causes of headache have been excluded.

The question is frequently asked: How long should treatment be continued? Does treatment prolong the duration of the climacteric? Our belief is that treatment should continue as long as distressing symptoms are present, whether it takes five weeks or five years. Patients will vary tremendously in their ability to endure uncomfortable sensations. Hence the psychic make-up of the patient must serve as an additional guide in our therapy. Every effort should be made to lessen the frequency of injections, and finally to replace injections by oral medication. We are constantly being supplied with tablets having greater potency (1 tablet = 3,000 to 10,000 R.U.) but it must be remembered that it takes from 5 to 20 times as much follicular hormone to produce an effect when given by mouth as by injection. We feel that treatment neither lengthens nor shortens the duration of the climacteric. Its duration is a function of the individual as an entity. The aim of our present treatment is to alleviate symptoms. What tomorrow will bring we cannot prophesy.

In conclusion, it may be stated that our knowledge of the climacteric is incomplete. Its mechanism is obscure, and the treatment occasionally disappointing.

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CHAPTER XXIV

TOXEMIA OF PREGNANCY

It has been stated, and with a great deal of truth, that eclampsia is a disease of theories. That is not denied. But from this welter of theories some have been discarded immediately, while others have led to further fruitful work and speculation. In general it may be stated that the most recent work has demonstrated that eclampsia is not a renal disease, and that the renal damage, except where it was present prior to the pregnancy, is not of great significance. More recently the rôle of the endocrines in the causation of toxemia of pregnancy has received considerable and much-deserved attention.

Hofbauer (1918) was the first to postulate an endocrine basis for toxemia of pregnancy. He believed it to be a disturbance of the neurohypophyseal-adrenal system, to which concept he added later the theory of intoxication by means of placental biogenous amines. Küstner (1928) involved the pituitary still further when he described in the blood of eclamptic patients the presence of a substance causing expansion of the melanophores in the frog. This reaction is a peripheral one (Krogh, 1926). The significance of Küstner's finding is not clear.

Cushing (1933, 1934) has contributed significant pathological data. He observed a marked infiltration of basophile cells from the anterior lobe into the pars intermedia and into the posterior lobe. Furthermore, he noted within the tissue spaces of the neurohypophysis accumulations of hyaline bodies (Herring, 1908) in which were still present fragments of basophile cells. These hyaline bodies are considered as products of degeneration and secretion of the basophile cells. Cushing presented the general hypothesis that "The extent of basophilic invasion from the pars intermedia is a measure of posterior lobe activity and that extensive infiltration (of the posterior lobe) by these elements represents the histopathological basis of eclampsia." In addition, the number of basophiles in the adenohypophysis was greater than in normal pregnancy. The assumption has been made that the basophile cells are the source of the neurohypophyseal hormones and that normally the posterior pituitary is merely a depot for such hormones. The neurohypophysis then, according to this theory, has no internal secretory activity. Thus the total increase in

basophiles, the infiltration of the pars nervosa by these cells and their products of degeneration, implies an increased production of the pressor and anti-diuretic factors.

These latter concepts are not in accord with the results obtained by tissue cultures of the adult mammalian hypophysis (Geiling and Lewis, 1935). Mouse hypophyses were used. The tissue cultures grew luxuriantly. The material from the cultures of the pars intermedia possessed no blood-pressure-raising property, but had marked melanophore-expanding effect when injected into frogs. Cultures from the anterior lobe were negative for both actions. Cultures from the neural lobe had both pressor and melanophore-expanding actions. Cultures of connective tissue, to which posterior pituitary solution had been added, had no pressor action, indicating that the hormone had been inactivated.

Ählström (1935) studied the basophilic infiltration in the neurohypophysis on thirty-six patients without hypertension and on twenty-five patients with hypertension. He noted that eighteen of the thirty-six patients without hypertension had a basophilic cell infiltration in the neurohypophysis, and that the infiltration was quite severe in eight cases. He concluded that basophilic cell invasion may exist in the absence of hypertension. Of the twenty-five patients with hypertension twenty-four had a basophilic cell infiltration, but it could be considered rather severe in only six cases. Ahlström concluded that the interpretation of basophilic cell invasion is still obscure, for, although the studies on his material corroborated Cushing's claim of an increased neurohypophyseal basophilia in hypertensive conditions, they indicated also that considerable basophilic invasion may exist in patients without hypertension and that in patients with hypertension the basophilic invasion may be only slight. Russel, Evans and Crooke (1934) correlated pituitary basophilic adenoma, but not basophilia of the posterior lobe, with a raised blood pressure. Spark (1935) studied 189 pituitaries and correlated their histological structure with their clinical features. He concluded that basophile infiltration of the neurohypophysis does not bear any relation to high blood pressure, but appears to be mainly determined by age, but men in the prime of life show the change more often than women. Rasmussen (1936) agrees with the above conclusions.

Anselmino and Hoffmann (1931) again revived the concept that hypertensive toxemia is a pituitary dysfunction. They claimed to have demonstrated that during the toxemia there occurs an increased production of posterior pituitary hormones, namely, vasopressin, as evidenced

by its anti-diuretic and pressor effects. They pointed out that the most important clinical manifestations of toxemia are water retention and edema, an increase in blood pressure and capillary spasm, coma and convulsions. Identical effects can be produced by means of large doses of pitressin. The authors pointed out that substances having these pharmacological effects can be demonstrated in the *blood* of toxemic patients, and that as the clinical severity of the disease increases a greater concentration of these substances can be demonstrated. Furthermore, a dysfunction of one gland causes a dysorientation of the others, especially the thyroid.

The evidence of Anselmino and Hoffman has aroused considerable discussion. Their findings have not been substantiated (Hurwitz and Bullock, 1935). Levitt (1936) found no demonstrable effect on diurêsis from the injection of the cerebrospinal fluid of 25 non-hypertensive patients, or from ultrafiltrates of blood from 25 hypertensive or 15 pre-eclamptics. In 4 severe eclamptics *blood ultrafiltrates caused slight effects* on water excretion without effect on chlorides. At the same time there is much indirect evidence to support Anselmino and Hoffman. Hofbauer (1933) has pointed out that there exists a striking similarity in the blood chemistry of eclampsia and experimental posterior hyperpituitarism. The administration of posterior pituitary preparations results in hyperglycemia (Geiling and Eddy, 1928), increased formation of lactic acid, (Marenzi, 1934), considerable lowering of the CO_2-combining power of the blood (Draper and Hill, 1929) and an increase in inorganic phosphates (Urechia and Retezeanu, 1933). In eclampsia (and pre-eclamptic toxemia) there occurs an increase in lactic acid (Torre-Blancho, 1936) hyperglycemia (Siegel and Wylie, 1933), a compensated acidosis (Stander and Cadden, 1934) and an increase in inorganic phosphorus. According to recent experimental studies pituitary extracts interfere in some unknown manner with the oxygen utilization of the tissues. The cells thus become anaerobic in their activity and go into an oxygen debt, as evidenced by the fact that arterio-venous difference is markedly reduced. This lowered oxygen consumption by the tissues under experimental conditions is analogous to data found in actual eclampsia.

The antidiuretic effect of the posterior pituitary hormone, oxygen lack and decreased blood flow through the kidney (Richards), as result of angiospasm, account for the diminished urinary output and for the occurrence of degenerative changes, particularly endothelial damage, in the renal parenchyma of eclamptic patients (Hofbauer).

Hofbauer argues that in the light of the experimental evidence, pro-

longed spasm of the umbilical vessels can be readily elicited by histamine, hence the origin of placental infacts, particularly of the hemorrhagic type frequently encountered in placentas of pre-eclamptic and eclamptic patients, is readily traceable to such substances passing through the placental barrier. In other words, placental pathology in eclampsia has the same pathogenesis as the abnormalities found in other organs and should not be considered as having causal relations to the toxemia of pregnancy.

Fauvet (1933) points out that by administering pituitary extracts to animals he was able to produce all the symptoms, except eclamptic attacks. Even the pathologic-anatomic changes become manifest. Knepper (1934) believes eclampsia to be a combination of increased production of posterior pituitary hormones plus an allergic tissue reaction.

Smith and Smith (1934, 1935) approached the problem of toxemia of pregnancy and the endocrines from a different angle. They analyzed the urine and sera from 42 women in their last third of pregnancy, and determined the content of gonadotropic hormone (prolan) and estrone in these fluids. Of these women, 15 were normal, 22 had toxic symptoms without convulsions and 5 had eclampsia. One of the patients with toxic symptoms was definitely nephritic and showed normal levels of prolan and estrone. In no case diagnosed as toxaemia or eclampsia were both estrone and prolan within the limits of values found in normal women at the same period of gestation. In a number of women, however, there was unquestionably a nephritic element. All but one of the toxaemic and eclamptic patients, or 96 per cent, showed excessive amounts of prolan in the urine and in the serum, and 18 of them, or 69 per cent, had subnormal values for estrone. The urines and sera of one toxaemic patient were analyzed monthly from the second month to term. During the sixth month an abnormal increase in prolan and decrease in estrone appeared, which continued with fluctuations until delivery. This patient developed no toxic symptoms until the eighth month. From these results they concluded that a quantitative imbalance of these two hormones due to excessive amounts of prolan and, less consistently, to subnormal levels of estrone is typical of toxaemias of late pregnancy.

Smith and Smith noted further that in late pregnancy toxemia an excess of prolan has probably been present for some time before the appearance of toxic symptoms. The degree of excess of prolan does not always run parallel with the severity of symptoms. Quantitative

analyses have shown that the placentas of toxaemic and eclamptic patients contain excessive amounts of prolan and tend towards low levels of estrone as compared with placentas of normal pregnancy.

What does the increase in prolan imply? Is it the cause or the result of the toxemia? Is it a mechanism of attack or defense? What is the function of prolan during normal pregnancy? To these questions we have as yet no answers.

Langrock, Fishberg, Vorzeimer and Rappaport (1936) studied a group of patients having toxaemia of pregnancy. They noted that the large majority of this group presented a set of physical characteristics that occurred only infrequently in normal pregnancy. The significant characteristics were:

1. Adiposity of the so-called pituitary type.
2. Android pelvis.
3. Low basal metabolic rate.
4. Appearance of being older than the chronological age.
5. Decrease in serum proteins.
6. Overweight.
7. Hirsutism.

The writers point out that these signs are stigmata of endocrine dysfunction. That is, patients with such constitutional characteristics have a greater tendency to develop toxaemia of pregnancy than do patients without such characteristics. These observations have also been made by other clinicians (W. E. Caldwell, H. Aranow) though not with this systematic classification. The data presented by these observers are significant.

Fauvet (1936) discussed the possible relationship between the cortex of the adrenal and toxemia. He points out that there is a hypertrophy of the adrenal during pregnancy, and that this (hypertrophy) is absent during toxaemia, in fact, the adrenal is smaller than normal. Such hypertrophy may be produced by prolan or anterior pituitary extracts. He believes that severe toxaemia may be an expression of a hypofunction of the adrenal cortex. Fauvet states that he has found an increase in corticotropic hormone in cases of hypertension, but not in eclampsia. Cortical hormone lowers an increased blood lactic acid content. The blood of eclamptics contains a greater quantity of lactic acid than normal blood.

Anselmino (1936) calls attention to previous clinical observations that the symptoms of *pernicious vomiting* of pregnancy resemble those of adrenal insufficiency. Both present disturbances of carbohydrate metabolism. The hypertrophy of the adrenal cortex which is present in normal

pregnancy is absent in hyperemesis gravidarum. Anselmino sees in this form of toxemia a hyperfunction on the part of some hormones of the anterior hypophysis. He ascribes the increase in ketone bodies to an increase in the fat metabolism hormone. The inability to store glycogen he believes to be due to a defect in the contrainsular or carbohydrate-regulating hormone. Anselmino describes experiments tending to show that the blood and urine of patients with hyperemesis gravidarum contains greater quantities of these hormones. He confirms an observation previously made by Heim that in hyperemesis gravidarum there is an *increased* excretion of prolan which he believes to be of hypophyseal origin.

We have recently seen another form of toxaemia of pregnancy, namely, ptyalism. This patient spit up a minimum of 1,000 cc. of saliva per day. It is interesting to note that when pituitrin is injected directly into the ventricle marked salivation occurs (Asher, 1936). The saliva contained neither prolan nor estrone.

The association of toxaemia of pregnancy with the endocrine glands opens an entirely new field to speculation and subsequent experimentation. The following view of this relationship is in accord with some of the concepts proposed in this volume. Toxaemia of pregnancy may be considered as a response diametrically opposite to that obtained in the climacteric. In the latter we are dealing with a *loss of the ovary from an equilibrated organism.* As a result of this loss the organ systems undergo rearrangement, first, because of the *loss* of the ovarian hormones, and second, because of the loss of equilibrium between the other endocrine glands. Inasmuch as the hormonal pattern differs in every woman, the nature of the response to this loss of equilibrium will vary from patient to patient. On the contrary, in toxaemia of pregnancy we are dealing with the *addition of a gland of internal secretion—the placenta—to an equilibrated system.* Certain changes will occur in the pregnant woman, due, first, to the *addition* of the placental hormones (prolan, estrone, progesterone and possibly others) and second, to the loss of equilibrium of the remaining glands of internal secretion. The response of the remaining glands in the endocrine chain will in part depend upon their inherent stability. Unstable systems, or those previously compromised by hormonal disturbance, will be amenable to the greatest variation in response. And such response may be hyperfunction of the posterior pituitary gland, insufficiency of the adrenal cortex, thryotoxicosis, hypothyroidism, instability of the autonomic nervous system, and innumerable other dysfunctions. *Each patient must make her own inherent response.* It may be normal or abnormal

with an infinite number of intermediate variations. It is a most alluring problem and worthy of very much greater experimental effort.

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CHAPTER XXV

PRECOCIOUS SEXUAL MATURITY

The syndrome of precocious sexual maturity is extremely interesting, for a complete understanding of its mechanism would enlighten us on the mechanism and etiology of normal puberty. Precocious puberty may be associated with tumors of the gonads, the pineal, the hypothalamus and midbrain, the hypophysis as well as hyperplasia and tumors of the adrenal cortex, and inflammation of the midbrain. The cases (1, 2 and 4) described below present no such evidence, and seem to be wholly on a *functional* basis. The remaining case (3) was complicated by the presence of a tumor of the ovary.

Case 1. J. P., No. 399227, age 4 years. First seen by Dr. Walter Timme, to whom I am indebted for this case, January 3, 1934.

Chief Complaint. Overdevelopment of pubic hair. Overdevelopment of breasts. Overdevelopment of height. Overdevelopment of weight. Three years in duration.

Family History. Mother, 29, living and well; father 29, living and well. No exposure to tuberculosis. No familial history of carcinoma, gout, diabetes, asthma. No member of patient's family on either side has had disorder similar to patient's present illness.

Birth History. Only one pregnancy, born at term, labor not abnormal, instruments used. Child normal at birth (9 pounds) except for growth on forehead and nose (subsequently sebaceous cysts). No melena or jaundice. Sat up at 8 months, walked at 12 months, sphincteric control at 18 months, single words at 12 months, talked at 18 months.

Feeding. Formula from birth. Orange juice and cod liver oil started in first month and continued under medical doctor's care. Solid food at 12 months.

Behavior. Sunny, good natured child, plays with other children, is obedient, not nervous. Seems to have plenty of pep and life. Mother has noticed nothing abnormal in child's behavior. No mental precocity.

Infections. Whooping cough at 18 months, duration 2 or 3 months. No sequelae.

Operations. Growths removed from nose at 10 months and 18 months at St. Luke's Hospital.

Prophylaxis. Small pox and diphtheria injections at 10 months.

Present Illness. Patient was perfectly well during infancy and was seen by medical doctor about once a month. When *nine months old*, it was first noticed by mother and medical doctor that pubic hair was developing and that her breasts were beginning to enlarge. Weight and height at this time not known but ever since then she has grown rapidly, and breasts and pubic hair have maintained a

Blood: Hb.: 90 per cent. R.B.C.: 4,000,000. W.B.C.: 8150. Polymorphonuclears: 48. Lymphocytes: 48. Monocytes: 1. Eosinophiles: 1.

Glucose tolerance test: Control blood sugar: 101 mgm. Urine sugar: 0. Glucose: 48.3 grams. Blood sugar 1 hour, 117; urine sugar, 0. Blood sugar 2 hours, 119; urine sugar, 0.

X-ray (Dr. Abbott): X-ray of knees, hands and wrists show epiphyseal development to be about as usual for this age.

X-ray of pelvis (April 11, 1936. Dr. H. C. Moloy): The inlet is well formed but definitely elongated. The side walls are straight. The sacrum has an average curvature, five segments, and the outlet is narrow with male type of pubic rami.

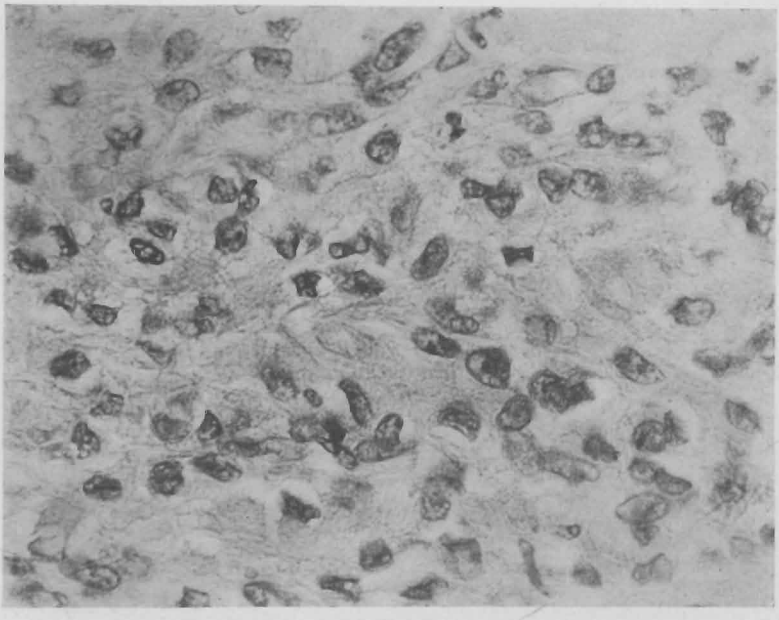


FIG. 151C. Case 3. Precocious sexual maturity. Section of ovarian tumor. No evidence of follicle formation. ×860. (Courtesy of Drs. H. B. Schoenberg and J. Felsen.)

Type of pelvis: anthropoid with gynecoid characteristics, straight side walls, masculine type of pubic rami and a long, narrow anthropoid type of sacrum.

Diagnosis. Precocious sexual maturity.

Progress Note (*November, 1936*). This patient has been observed at regular intervals for the past three years. The periods are regular, every 28 ± 5 days, and always associated with dysmenorrhea. The child is normal in all respects except for her sexual precocity.

Case 3. M. G., age 7 years. Referred by Dr. Henry Schumer. April 16, 1934. (Figs. 151A, 151B, and 151C.)

Chief Complaint. Menstruation: 2 months. Growth of breasts: past year.

Family History. The patient is the second of two children. The other sister is now 12 years old and has been menstruating for the past few months. She presents no precocity. The mother began to menstruate at 11 years.

Past History. Normal spontaneous delivery. No trauma. No previous illness except chicken pox. Growth, feeding and habits have been normal. Normal school course.

Present Illness. About a year ago it was noticed that the patient's breasts began to grow. This has been especially marked in the past 6 months, when pubic hair was also noticed. First menstrual period March 5–9, 1934, second period 5 weeks later, duration 3–4 days, moderate in amount and without pain.

Physical Examination. The patient is a young well nourished girl whose general body development corresponds to a youngster of her age, except that there is a definite feminine touch to her hips and waist. She coöperates well and answers questions intelligently.

Head: negative.

Eyes: pupils react to light and accommodation. No eye signs.

Mouth: deciduous teeth—10/10.

Neck: negative. Thyroid not palpable

Skin: texture normal, no pigmentation or excessive hair.

Lungs: negative.

Heart: no murmurs, no enlargement.

Blood pressure: 128/80.

Pulse: 88.

Breasts: well developed, full. Gland tissue palpable. Nipples and areoli of adult type.

Abdomen: soft, no masses. Right kidney palpable.

Mons veneris: covered by hair, of adult type.

Labia majora and minora: correspond to those of adolescent girl of 12 or 14 years.

Clitoris: small.

Vagina: patent.

Uterus (rectal): corresponds in size to that of seven year old girl, cervix forms greater part of organ, movable, firm, smooth.

Adnexa: behind the uterus is a firm mass about 7–8 cm. in diameter, movable, round, slightly sensitive, not adherent.

Tubes: not palpable.

Diagnosis. Granulosa cell tumor of ovary. Precocious sexual maturity.

Subsequent Course. Patient menstruated on May 1–7, 1934. Spotted slightly on May 13–15. Considering the phase of the cycle when the spotting occurred and the nature of this bleeding, a diagnosis of ovulation bleeding seems justifiable.

Follicular hormone (in the urine)—4 rat units per liter
Follicle stimulating hormone (in the urine)—negative. } April 22, 1934.

The patient was admitted to the Bronx Hospital for operation (Dr. Herman B. Schoenberg). Laparotomy May 21, 1934.

The right ovary was enlarged to about 8 cm. in diameter. It was solid, not adherent. Surface epithelium intact throughout. Left ovary small, without the slightest trace of follicles or corpora. The uterus is small and is composed of

two-thirds cervix and one-third fundus. No glands palpable. Parametria negative.

Pathological Report. (Dr. J. Felsen.) Specimen consists of a firm nodular encapsulated mass measuring 4 x 4 cm. On section mass is yellowish in color, soft.

This is a complex tumor composed of pale cells with central vesicular nucleus and granular cytoplasm, polymorphic, varying in shape from spindle to ovoid. The arrangement in places is suggestively perithelial and in others somewhat like glandular tissue. Many scattered areas of vacuolated and marginally spurred eosinophilic serous material are present. No suggestive follicles containing ova can be seen. The possibility of a luteoma or adrenal ovarian rest or perithelioma or teratoblastoma can be considered (fig. 151C).

Diagnosis. Teratoblastoma ovarii with lutein stroma.

Six months following operation the hormone findings were: Follicular hormone —negative. Follicle stimulating hormone—negative.

Following the operation the periods stopped and the breasts regressed a little. Early in 1936 the breasts began to swell again. Spotting on March 6 and 30, 1936. First regular menstrual period April 29, 1936. Since then the periods have occurred every 4 weeks, 3–4 days in duration, moderate in amount and without pain.

The breasts have continued their growth. Repeated rectal examinations show that the uterus corresponds in size to that of a girl of 10, but at the same time adult proportions are now becoming manifest, in that there is noticeable growth of the fundus. The remaining ovary cannot be palpated.

April 19, 1936: Follicular hormone—16 R.U. per liter. L.M.P. March 30, 1936. Follicle stimulating hormone—negative. April 29, 1936.

June 14, 1936: Follicle stimulating hormone—negative. L.M.P. May 26, 1936. Follicular hormone—8 R.U. per liter.

The general contour of the patient is distinctly feminine. She physically presents a woman in miniature. Her psychic reactions seem to be those of a girl of 10 years.

X-ray of pelvis (Dr. H. C. Moloy): The inlet conforms to the anthropoid type. The sacrosciatic notch is moderately wide, but has a male character to it. The side walls converge slightly. The subpubic arch is moderate in width, possessing straight pubic rami indicative of masculine characteristics. There is a fusion anomaly of the spinous process of the first sacral segment. The epiphyseal lines of the iliac crest are fairly well formed for the age of the patient. The pubic ischial rami are also fused which, I believe, is a little unusual for the age of the individual. While the pelvis possesses definite female characters, the outstanding findings other than this refer to the anthropoid characteristics with a few minor masculine characteristics.

X-ray of shoulders, wrists, ankles, elbows (June 18, 1936. Dr. Kasabach): Films of the various joints, including the hips, shoulders, pelvis, elbows, wrists and ankles show several interesting findings in the various centers of ossification of the bones. According to our anatomical chart, the epiphysis for the acromion processes and the apophyses of the os calci and the epiphyses of the upper ends of the femora usually appear and fuse at a later age than 12 or 15 years. In this child, the epiphyses for the acromion processes of the scapulae have already appeared. The apophyses of the os calci have also appeared and have almost

completely fused. These findings suggest that some of these osseous centers have undergone a developmental stage which is somewhat more rapid than one would expect in a child of 9 years of age. On the other hand, the centers of ossification and epiphyseal changes elsewhere in the body seem to be in accordance with the patient's age.

Recent hormone determinations have yielded the following results. The positive F.S.H. on October 26, 1936 denotes ovulation.

	P.M.P.
F.H.—(October 25, 1936)—none found	October 8–14, 1936.
F.S.H.—(October 26, 1936)—positive.	November 1–6, 1936.
F.H.—(November 1, 1936)—none found.	
F.S.H.—(November 2, 1936)—negative.	
F.H.—(November 5, 1936)—none found.	
F.S.H.—(November 6, 1936)—negative.	
F.H.—(November 15, 1936)—none found.	
F.S.H.—(November 16, 1936)—negative.	
F.H.—(November 22, 1936)—none found.	
F.S.H.—(November 23, 1936)—negative.	
F.H.—(November 29, 1936)—none found.	
F.S.H.—(November 30, 1936)—negative.	

Case 4. D. A. M., No. 464589, age 13 (figs. 152A and 152B). September 17, 1935.

Chief Complaint. Persistent vaginal bleeding for past 5 weeks.

Family History. Father and mother living and well. Two sisters, 19 and 16. Show no precocity.

Present Illness. Patient has menstruated regularly every month since the age of *6 months.* Secondary sex characteristics began to develop at six years, namely, breasts, pubic and axillary hair. These have progressively increased up to the the present time. Periods have always been regular, every four weeks, four to five days, moderate in amount and without pain. Patient had a regular period beginning July 20, lasting 3 days. One week later began to bleed again and has bled on and off for the past month. This has been associated with cramplike pains in the lower abdomen.

Physical Examination. Patient is a young girl with dark hair and blue eyes. She is very shy and not over-bright.

Head: negative.

Eyes: react to light and accommodation. No gross disturbance in visual fields.

Mouth: hypertrophied tonsils.

Teeth: some dental caries.

Neck: no thyroid enlargement.

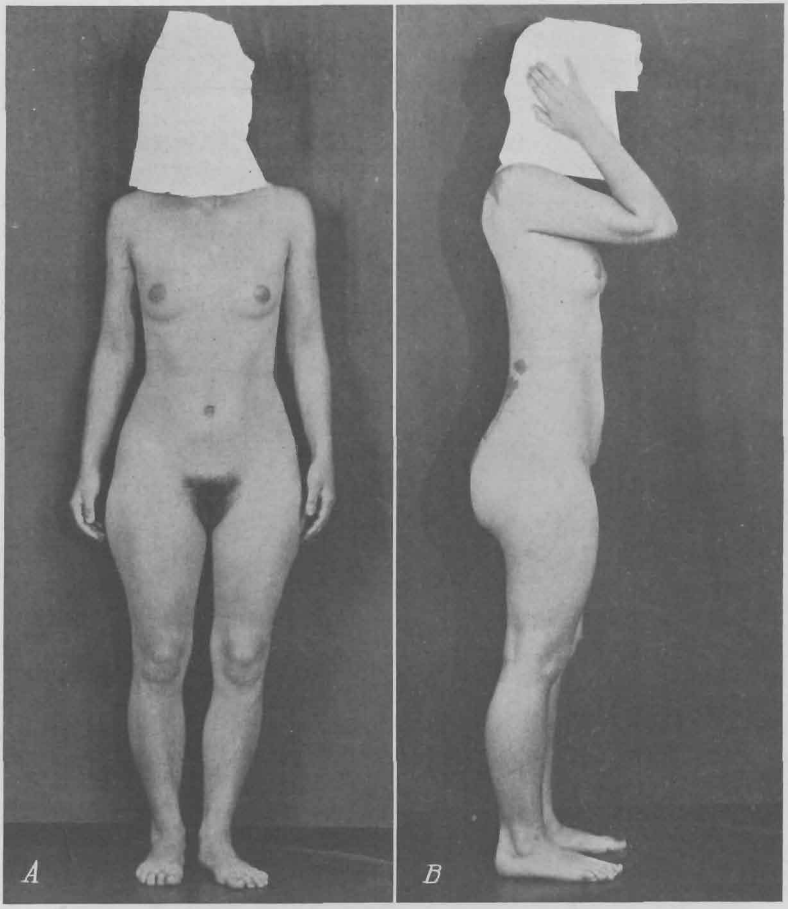
Hair: pubic and axillary hair well developed. Some excessive hair on legs, abdomen and upper lip.

Lungs: negative.

Heart: no enlargements, no murmurs.

Pulse: 84.

Breasts: well developed especially in the subareolar areas. The breasts closely resemble those seen after treatment with large doses of follicular hormone.



FIGS. 152A AND 152B. Case 4. Precocious sexual maturity. Menses began at 6 months. Present age 13 years.

Body: is distinctly feminine, except for the legs which are very solid and muscular, and show no convergence at the knees.

Skin: of normal texture, flat pigmented areas over back.

Abdomen: negative. Viscera not palpable.

External genitalia: well developed, resemble those of a postadolescent girl.

Clitoris: normal in size.

Vagina: patent.

Uterus (rectal): to the left of the midline, of adult proportions, and slightly larger than would be expected at this age.

Adnexa: to the right, in the region of the right ovary is a small ill defined cystic mass.

Impression. Precocious sexual maturity. Undetermined ovarian cyst?

Basal metabolic rate: 0 per cent.

X-ray of pelvis (Dr. H. C. Molloy): The inlet is round and wide having the general appearance of an adult pelvis although the bones are delicate. The suture lines are more advanced than the age of the patient. The sacrosciatic notch is wide. The side walls are straight. The subpubic angle is wide.

Type of pelvis: this is a round gynecoid type of female pelvis that demonstrates a maximum number of sex characteristics as far as the pelvic form is concerned.

X-ray of skull (Dr. Swenson): Films of the skull show no evidence of bony or intracranial pathology. There is a very interesting appearance of the petrous portion of the temporal bones. The internal auditory meatus on the left side appears larger than on the right. If an acoustic neuroma is by any chance suspected, this asymmetry might be due to such a process, or else we are dealing with an incidental asymmetry.

September 23, 1935: Follicular hormone—8 R.U. per liter. Follicle stimulating hormone—negative. L.M.P. September 30–October 3, 1935.

September 29, 1935: Follicular hormone—16 R.U. per liter. Follicle stimulating hormone—negative.

Progress Note. Early in December patient began to complain of occasional pain in the right lower quadrant. No nausea, vomiting or temperature. Reexamination on January 30 shows the right ovary to be distinctly above average size, freely movable (Professor B. P. Watson). The patient has not returned to the clinic.

It has been stressed in this volume that the establishment of sexual function requires, at least, the direct interplay of the adenohypophysis—the ovary—the genital tract and associated sex structures. This is the primary or direct mechanism. When any one of the three is absent normal sex function is impossible. This mechanism is then influenced by other glands of internal secretion, and probably by the vitamines, in an indirect manner. It is more than likely that this influence is exerted through the metabolism of the glands primarily concerned. This is the secondary, or indirect mechanism. We know that at puberty a more or less sudden exacerbation of the primary mechanism occurs. The point was previously made (Chapter XXIII) that this *primary mechanism functions at a very low rate in later intrauterine* life and during the preadolescent period. The pituitary contains gonadotropic hormone, the ovary in turn is activated to secrete estrone, and finally the genital tract shows very slow but progressive growth.

Etiology of the Onset of Puberty

Therefore, the onset of puberty is not brought about by any new, previously non-existent mechanism, but by a sudden increase in activity of a previously functioning system. This sudden increase function may occur through several mechanisms, namely:

The Sudden Activation of the Anterior Pituitary Gland. The activation of the adenohypophysis could theoretically come from within or without. Up to the present time no data are available to show a sudden increase in potency of the pituitary gland. Nor do we know of any other gland that is capable of suddenly stimulating the pituitary. Rowntree et al. (1936) made the interesting observation that estrus has been repeatedly demonstrated in the tenth generation of a thymus treated strain of rats at nine to twelve days, in animals weighing less than thirty grams. (Normally estrus occurs at about 60 days.) They also found mature follicles. Examination of the pituitaries of such precocious rats shows that the adult pattern (cell distribution) of the thymus injected animals is reached at an earlier period than in the control strain. Their findings suggest the theory that puberty is caused by the activation of the pituitary by thymus. Hence precocious puberty could be produced by premature activation. It must be pointed out that eleven generations of rats were required to produce this effect, and that a physiological response in rodents cannot be directly applied to the human.

The Progressive Maturing of the Ovary. We have previously pointed out (Chapter XXIII) that in the rodent the ovary will respond to stimulation only after it has matured to a certain extent. Apparently at about the age of 13 years the ovary has matured sufficiently to respond fully to the pituitary stimulus. Evidence to substantiate this point is lacking for the human. On the contrary, we have previously indicated (Chapter XXIII) that the human ovary is incapable of response after a certain age. Hence the theory is suggested that the ovary occupies the dominant position in the mechanism of puberty. When it has reached maturity, and it must be remembered that the hypophysis is probably an important force in this attainment, it responds to stimulation by accelerating its activity. Case 3 sheds some light on this point. The right ovary was replaced by a mass of granulosa-like cells. The left ovary was undeveloped. There were neither follicles nor corpora atretica. There was no evidence of functional activity, either past or present. Hence the etiology of the precocity could not have been primarily outside of the ovary, for the stimulus

would have involved both ovaries. Furthermore when the pathologic ovary was removed the precocity subsided for about 2 years. Then menstruation and growth of the breasts began anew. The remaining ovary has now ripened, truly at a premature age. But there seems to be some family predisposition to an early puberty, for both her mother and an older sister began to menstruate at about eleven. (We are constantly aware of the potential possibility of a metastasis from the original tumor. The patient is thoroughly examined every month, but to date, no such new growth has been found.)

As to the nature of the process by which the ovary attains maturity very little can be stated. It must reside within the fundamental metabolic processes of the cell. It is not primarily a matter of circulation, for the same arteries supply the ovary of the preadolescent as the mature individual. Nor is it a question of the number of primordial follicles, for opinion is still divided whether any such increase occurs after the ovary becomes a complete morphological entity.

The Release from the Antihormones. Collip's concept of antihormones has introduced another problem into the syndrome of precocious sexual maturity. We may assume for purposes of argument that for every hormone there is an antihormone. Under conditions of normal function only such quantities of hormone are released from the hormone-antihormone complex as are essential for normal function. Depending on the titer of the two components of the complex many shades of function are possible. Thus normal puberty would result from the liberation of the hormone (gonadotropic or ovarian) from its antihormone. Precocious puberty would result from premature liberation. This theory is very interesting from a speculative point of view, but evidence to support it is lacking. No antibody has been demonstrated for estrone or progesterone. Gegerson, Clarke and Kurzrok (1936 and unpublished results) have been unable to detect gonadotropic antibodies in patients with long standing amenorrhea or during lactation. It is true that gonadotropic antibodies can be produced both in the rabbit and the human by the parenteral introduction of large quantities of gonadotropic hormone in the form of pregnancy urine extract, anterior pituitary extract, or mare serum. But this implies the introduction of an antigen from without, a condition not found during normal function. Further development of the antihormone problem will be watched with great interest in this connection.

Evans has recently (1936) brought the topic of the gonadotropic antagonist to the fore (Chapter XXVII). This antagonist inhibits the ovary from being stimulated by the gonadotropic hormones. Pu-

berty could potentially be due to a removal of this antagonist, and precocious puberty to a premature removal.

Discussion

These cases of sexual precocity present many points of great interest. The preadolescent relationship between the fundus and the cervix remains in spite of regular periods. *The uterus does not exceed in size that of normal girls of the same age.* The endometrium has matured, the myometrium has not. This observation substantiates the point made in discussing the results of treatment of primary amenorrhea, that is, the uterus is the last (slowest) to respond to the effects of estrone. Case 1 substantiates this from another angle, for even though the secondary sex characteristics have been fully developed, especially the breasts, the myometrium and endometrium have retained their normal preadolescent state. Menstruation has not begun as yet.

Case 1 presented an additional precocity of growth. At four years of age her height was 57.5 inches (normal 41.5 inches). This implies excessive activity on the part of the growth hormone. The frequent association of sexual precocity and early ossification of the epiphyses is not present in this case. This is the more interesting in the light of case 3 in whom the osseous centers have undergone a developmental stage which is somewhat more rapid than one would expect in a child of nine.

The architecture of the pelvis presents another interesting problem in cases 2 and 3. In both the diagnosis is made of anthropoid type with masculine characteristics. We believe that the terms "anthropoid" and "masculine" used in this connection are merely descriptive. These patients present the effects of premature exposure to adult quantities of follicular hormone. No evidence has ever been presented that they are also producing male sex hormone, even though we have pointed out that whenever large quantities of estrone are produced, there is an accompanying production of androsterone, and v. v. It is difficult to believe that such small potential quantities of androsterone would be sufficient to modify the architecture of the pelvis in the presence of such large quantities of estrone. Case 4, on the contrary, presents at 13 years a fully developed gynecoid pelvis.

In the description of these cases we have pointed out that the breast development differed from the type produced in cases of primary amenorrhea (hypogonadal) by large doses of estradiol benzoate (progynon-B). These precocious girls presented fully developed breasts and in three gland tissue could be palpated. Since the fully developed gland in the human requires active corpus luteum function, the breast

development signifies a complete ovarian cycle, that is, follicle formation, ovulation and corpus luteum formation. Case 3 presents evidence of ovulation, as manifested by the sudden excretion of follicle stimulating hormone on a single day during the intermenstrual period (Kurzrok, Kirkman and Creelman, 1934).

The pigmentation of Case 4 may signify something or nothing. It is believed that pigmentation of this type may be a function of the adrenal cortex. As stated above, disturbances of the adrenal cortex may lead to precocity. This is all the more interesting in view of Reichstein's (1936) finding that certain fractions from the adrenal cortex (not cortin) possess estrogenic activity.

These cases of precocious sexual development are very much more than museum curiosities. A more complete understanding of the etiology behind this disturbance would go a long way towards the elucidation of some of the basic problems of sex-function.

CHAPTER XXVI

INTERSEXUALITY

Medicine has always exhibited an intense interest in the individual who presents the characteristics of both sexes in a varying degree. An enormous literature has grown up on the subject but only recently has a semblance of order arisen. Too much attention was paid to external morphological variations, and very little to inherent biological laws. The difficulties arose from the fact that in the hermaphrodite no correlation existed between the masculine or feminine characteristics. One could not determine the character of the gonads from a study of the habitus, the external sex characteristics or the psyche. Halban (1903) made an interesting observation that shed some light. He stated that there exist people *without gonads, but who present either a masculine or feminine habitus and internal genitalia.* He concluded from this that the differentiation into male or female during embryonal development is independent of the gonads. Furthermore, the sex of the fertilized ovum or zygote is therefore determined at the moment of fertilization. Further progress was made by Richard Goldschmidt (1931). His concept of intersexuality will be followed in this chapter.

Zygote Intersexuality

Goldschmidt chose for his experiments a butterfly, lymantria dispar. It was chosen because the sexes are easily discernible, and by crossing a given strain with others from a different locality he was able to produce various degrees of intersexuality. His work led to the conclusion that in the intersexes all organs develop according to one sex, but that at a given point of development a *deflection* (or turning, reversal, Drehpunkt) occurs and development continues according to the opposite sex. Whatever differentiation has been reached (determined) along the original pattern the intersex retains, but whatever has not developed at the onset of this second phase, now does so but according to the *opposite* sex. The earlier this deflection (turning point) occurs the fewer organs differentiate according to the original (genetic) sex, and the greater is the number that differentiate according to the opposite sex. Even the gonads may be involved in this reversal. Thus a gonad having been genetically determined as a testis may then develop

from the turning point on as an ovary. *It therefore becomes possible for a gonad to have the morphological make up of a testis and function as an ovary, that is, produce female sex hormone.*

Goldschmidt's experiments led to the conclusion that an intersex anlage results from the different valencies of the sex chromosome. That is, valencies greater or less than normal result in intersexes. Moszkow-

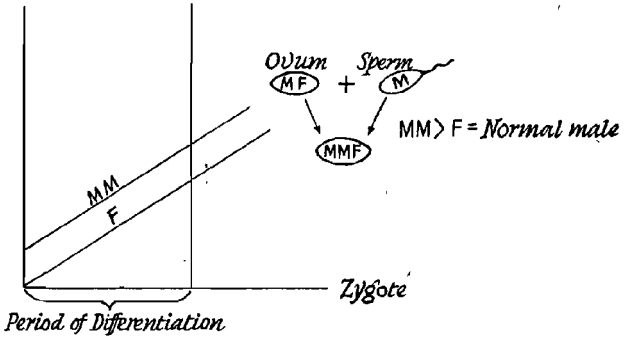


FIG. 153. Type I

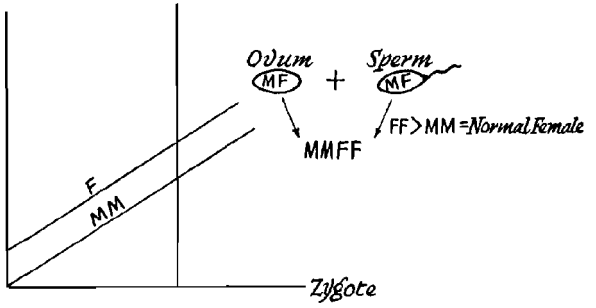


FIG. 154. Type II

ich (1936) illustrates this in the following manner: Every living cell is bisexual, that is, it contains within its nucleus masculinizing (M) and feminizing (F) factors. In the human they exist in the sex chromosomes. The only exception are the spermatozoa, of which there are two kinds. One half of the number has a sex chromosome, while the other half has not. So that the spermatozoa may be divided equally into M and MF types, hence of all body cells only one half of the sper-

matozoa contain no F component. On the contrary, *all ova* contain both an M and F factor, hence they are all MF. In the male M is dominant, in the female F. When fertilization occurs we have the possibilities shown in figure 153.

In this case the sperm has no F factor, hence in the zygote the M factor dominates. Curve MM signifies constant masculine dominance

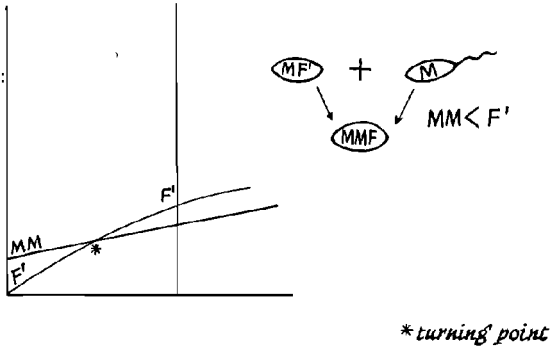


FIG. 155. Type III

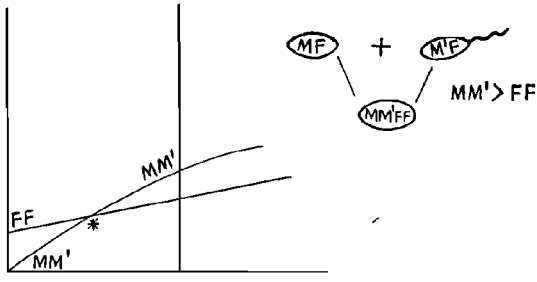


FIG. 156. Type IV

in the development of all organs. The individual is a normal male. (See fig. 154.)

In this case the sperm has an MF factor, hence in the zygote F factor predominates. Curve FF signifies constant feminine dominance in the development of all organs. The individual is a normal female. (See fig. 155.)

Let us suppose that the F′ factor in the ovum has a feminizing va-

lency twice as great as normally. Hence in the zygote F′ will predominate in spite of two MM factors. The organism will begin its development *apparently as male*, but because of the dominance of the F′ factor a reversal occurs and the organism *continues its development as a female.* (See fig. 156.)

Let us suppose that the M′ factor in the sperm has a masculinizing valency twice as great as normally. Hence in the zygote MM′ will dominate over FF. The organism will begin its development as a *female*, but because of the dominance of the MM′ factor a reversal occurs and the organism *continues its development as a male.*

Goldschmidt's experiments show that Halban was correct in his assumption that the organs can differentiate as male or female in the complete absence of gonads. Goldschmidt calls an individual who began embryonic development as a female and finally ended as a male, a feminine intersex. The reverse is known as a male intersex. Both types compose the zygotic intersexuality. The gonads do not influence the development of this type of intersex, but instead are influenced by it. Hormonal differentiation occurs in the human during the second embryonal month, hence zygotic differentiation occurs in the entire absence of sex hormones.

Hormonal Intersexuality

This form of intersexuality occurs in individuals whose sex has already been determined (zygotic). Hormonal intersexuality depends upon the action of a sex hormone upon an individual of the opposite sex, that is, female sex hormone on a male, or male sex hormone on a female.[1] The action is reversible. Hormonal sexuality occurs as the result of tumors or hyperplasia of the adrenal cortex, of masculinizing tumors of the ovaries, called by Robert Meyer arrhenoblastomas, and certain tumors of the anterior hypophysis (Cushing). Their effect depends upon the age of the individual, being in general more profound the less developed the individual is. There are characteristic changes in the distribution of hair and fat, in the growth of the clitoris and larynx, and in the breasts. Associated changes in the function of the genital tract occur. It is of importance to remember that such tumors (adrenal, ovarian and hypophyseal) may occur without producing inter-

[1] It must not be forgotten that even in zygotic intersexuality we are dealing with chemical complexes within the gene. The M and F factors are chemical molecules that direct development in a certain direction, but whether they are hormones or catalysts we do not know.

sexuality. Furthermore, certain tumors of the adrenal produce feminization instead of the usual masculinization. This depends upon the character of the metabolism of the tumor or hyperplasia. Reichstein (1936) has shown the presence of both androgenic and estrogenic activity in the cortical fractions of the adrenal. What factors govern the production of either of the sex hormones by these tumors? The answer may be the character of the tumor metabolism, the nature of the stimulus that produces the growth and, finally, the genetic pattern of the host. The last concept brings us back to the zygotic intersexuality of Goldschmidt. May the M and F components of some individuals be so delicately balanced that an addition of one or the other sex hormones furnishes the impetus to precocious maturity (when young), or towards heterosexual characteristics. May not other individuals be so preponderantly M or F, that their systems appear "buffered" against the action of the opposite sex hormone. It will be of great interest to determine whether tumors of the adrenal cortex (or arrhenoblastomas) which do not produce the characteristic heterosexual changes, produce excessive sex hormone, but that the hormone fails to "make an impression" upon the individual.

Moszkowich proposes to differentiate the zygotic intersexes by the presence of prostatic tissue. The male intersex can be differentiated from the female intersex by the position of the prostate in relation to the primary urethra, that is, that portion of the urethra between the bladder and the junction with the vagina to form the urogenital sinus. When the prostate lies cephalad to this junction the individual is a male intersex, and when caudal to it a female intersex.

Case. F. P., age 36, No. 458373. Autopsy No. 11949.

This case was first seen by Dr. Arthur H. Blakemore and will be published by him in extenso. I am greatly indebted to him for permission to publish the following description.

History 419950. Chief Complaint. Pain in lower abdomen and constipation for 6 weeks.

Family History. There is no familial history of hermaphroditism, carcinoma, or syphilis.

Past History. No acute illness. He was born a hermaphrodite, always dressed as a male, took a male part. Has had no social difficulties. Developed normal sexual attraction to female at 12. Married 7 years ago and has had normal frequent intercourse with reasonable pleasure. Had nothing to suggest menstrual periods. His wife's single pregnancy resulted in a normal boy, now 6 years old.

Present Illness. About 6 weeks ago while working as a painter, patient developed sharp pain in the right loin which after about one hour shifted and settled in the suprapubic region, and there was dull ache in the right lumbosacral region. The pain has occurred off and on for the past 6 weeks and has been aggravated by

heavy meals. Constipation has been present since the onset of pain and has increased to the point at which daily enemata have been necessary. No nausea, vomiting, or blood in the stools. The pain has not been colicky in type. He has lost 14 pounds in the past six weeks. During the present illness there has been slight burning on urination for which he has been taking urotropin.

Physical Examination. Temperature 100°; pulse 100; respiration 20; blood pressure 160/105. A well developed and well nourished young adult appearing decidedly male in facial features. Female type of breast and figure. Head: eyes, ears, nose and mouth, negative. No cardiac enlargement, rough apical systolic murmur. Rhythm regular. Abdomen: prominent, particularly in the lower half. On the right side arising out of the pelvis and extending almost to the umbilicus a large firm non-tender mass is felt. On rectal examination this mass is felt occupying the pelvis.

Genito-urinary. Feminine distribution of hair. Very small penis with extreme hypospadias. About 1 cm. behind urethral meatus is a very small opening lying between two ridges similar to the labia and containing no testes. A catheter can be passed in this opening extending upward and backward for a distance of 7 cm. Fluid of a very mucoid consistency is exuded on the catheter. Reflexes normal. Extremities negative.

Laboratory Findings. Blood count: W.B.C. 10,360; polymorphonuclears 77 per cent; urine negative save for a moderate number of W.B.C., singly and in clumps in the sediment. X-ray No. 135843; July 9, 1935; abdomen shows no evidence of calcification in the abdominal mass. Its outline is not discernible. The left kidney outline is obscured. Stereoes and lateral films of the pelvis were taken following the injection of lipiodol into the perineal opening just before the glans. This outlined a smooth pocket about 5 cm. long. It is wider below and narrower above as might well be seen with a pseudovagina. This points almost directly posteriorly, and there is no lipiodol entering the lumen of possible uterus or cervix.

The pelvis is of the android type. Another x-ray of the pelvis was made with the comment that it is basically a female pelvis with masculine characteristics, and can be duplicated many times in individuals of female sex. Cystoscopy July 12: third degree hypospadias. There appears to be a normal bladder except for compression from the mass outside of the bladder which forces the vault down. Veromontanum rudimentary. Aschheim-Zondek Test negative. F.S.H. determination 3 out of 4 negative, with a slight stimulation on the fourth.

Course. A diagnosis of hermaphroditism with teratoma of ovary or abdominal testis was made and operation performed one week after admission. A right round ligament, ovary, Fallopian tube and uterus were found. There was apparently no cervix uteri, nor were there any discernible structures on the left side. The ovary on the right was partially replaced by a huge degenerating tumor, the pedicle of which had twisted through 180°. It was densely adherent to the rectum and sigmoid and filled the cul-de-sac and nearly the entire pelvis. There was considerable hemorrhage beneath the capsule. There was no evidence of extension or metastasis. Tumor, uterus, right ovary and tube were removed. Diagnosis of the surgical specimen No. 58089 was malignant teratoma of the ovary. No testicular tissue was found. The teratoma was thought to be of a solid variety which is exceptionally rare and appeared to be very malignant. The patient made a good recovery from the operation and returned home on the 15th day postoperative. He came to the hospital again two months later complaining of

abdominal pain which was constant and dull, situated chiefly in the right lower quadrant and in the right costo-vertebral angle. During the past five days he has been having tenesmus and many small watery stools. He has lost strength and weight. Physical examination disclosed a tense, full, tender abdomen with many masses palpable in the epigastrium, in the suprapubic region and in both lower quadrants. Slight shifting dullness. X-ray of the chest showed the lung fields to be clear. Patient remained in the hospital for 2½ weeks, extremely ill, and running a low grade fever. Two days before his death his urine was positive for F.S.H. and showed four units of ovarian hormone per liter.

Diagnosis. Pseudo-hermaphroditism. Teratoma of right ovary.

Autopsy by Dr. Pearce, October 24, 1935: (Only part of the autopsy protocol is presented.) The body is that of a young adult individual whose skeletal and muscular development is excellent. The general configuration is masculine. The calvarium is symmetrical, presents no bony abnormalities, and is covered with an abundant growth of graying black hair. The pupils are in mid-dilatation. There are no petechiae of the conjunctivae nor is there any icterus of the sclerae. There is no discharge from the nose. The front teeth appear in good condition, but post-mortem rigor prevents examination of the inside of the mouth. The mucous membranes of the mouth are bright cherry red in color. There is fairly well marked post-mortem lividity and moderate rigor. The trachea is in the midline. The thyroid is not palpable. The thorax is symmetrical. The breasts are moderately full and have a soft fatty consistency. The nipples and areolae are female in type. A few small shotty glands are present in the neck, but there is no general adenopathy. The abdomen is distended. No masses, however, can be felt through the abdominal wall. There is a healed surgical incision measuring 18 cm. in length reaching from a point just above the lateral to the umbilicus to the symphysis. The pubic hair has the female distribution. The penis measures 5 cm. in length (fig. 157A). The prepuce is reflected off the glans and descends laterally and posteriorly for a distance of about 4 cm. and its lower portions have the appearance of labia minora. There is a third degree hypospadias and what appears to be an urethral opening is present at the most posterior point to which the prepucial folds reach. A small amount of somewhat watery mucoid material is present in the meatus. There is no vaginal opening. The rectum appears normal and running from it to the urethral opening there is a raphe resembling that seen on the scrotum. No testes can be felt in the rather loose pigmented skin on each side. There is no clubbing of the fingers or toes. The nail beds are bright pink. Needle puncture wounds are present on the anterior and medial aspects of both thighs and in the left antecubital space. The abdominal fat is bright yellow in color. The abdominal musculature is red and well developed.

Adrenals. Are normal in size and shape. Their cortices are well filled with lipoid, and are demarcated from the narrow pearly gray medulla by reddish brown intermediate zones. The right adrenal together with the kidney to which it is attached has been pushed upward by the tumor in the right lumbar gutter and right side of the abdomen and has come to lie in a loose mass of tumor tissue between the liver and the diaphragm on the superio-lateral aspect of the former.

Pelvic Organs. The bladder is lined by wrinkled shiny white mucosa which is slightly injected at the fundus and in the region of the trigone. In the latter situation it is also somewhat roughened. Both urethral orifices are freely patent.

The *urethra* is large, measuring 2 cm. in diameter. At a point 3 cm. from its opening in the bladder it is joined by the *vagina* through a lumen 0.5 cm. in circumference. The two structures then continue as a common canal to the external meatus. This portion which is lined by mucous membrane resembling that of the urethra is 4 cm. in length. It is surrounded by erectile tissue resembling a corpus spongiosum. The vagina begins at the described opening in the urethra and extends upward posterior to the urethra for a distance of 7 cm. At its uppermost portion there is a small structure about 2 cm. in length and 1 cm. in width resembling cervix. This contains a narrow lumen filled with brownish-red mucoid material. Its upper pole is buried in a mass of tumor tissue and in this region there are several black silk sutures. The mucosa lining the vagina is not remarkable. The wall is thick and is made up of tough fibrous tissue. The cervix and upper part of the vagina lie behind the bladder. There is a mass of firm dense tissue 0.5 cm. in width between the lower portion of the vagina and the uterus. It appears to be composed of thick fibrous tissue in which no definite structures can be made out. Between the vagina and rectum is a small amount of loose areolar tissue in which lies an extensive venous plexus. No structures resembling seminal vesicles can be found nor is there around the vagina or urethra any semblance of prostate. The rectum itself is normal. No testes or ovaries can be found on either side.

Uterus (S.P. No. 58089) (fig. 157B). The section consists of large, intertwining masses and bands of smooth muscle between which lie areas resembling endometrium. In these regions there are gland-like spaces lined by columnar epithelium. They vary greatly in size, some being extremely small, while others occupy several low power fields. They are for the most part empty, but some of the larger ones contain red blood corpuscles and lymphocytes, and an occasional polymorphonuclear. Beneath the epithelium there is a loose cellular tissue closely resembling that found in the normal endometrium. Here, too, occasional small hemorrhages have occurred. The combination of these glandular elements with the rather dense irregularly arranged bands of smooth muscle suggest an adenomyoma. Masses of dark, yellowish-brown pigment often occurring in monoculear cells are found in places between the smooth muscle bundles. This resembles hemosiderin.

Right Uterine Tube (fig. 157C). The section consists of rather loose areolar tissue among which are clumps and bands of smooth muscle, varying in size. At one side there is a cross section of a Fallopian tube which does not differ in any essential from the normal. At another point and occupying one low power field

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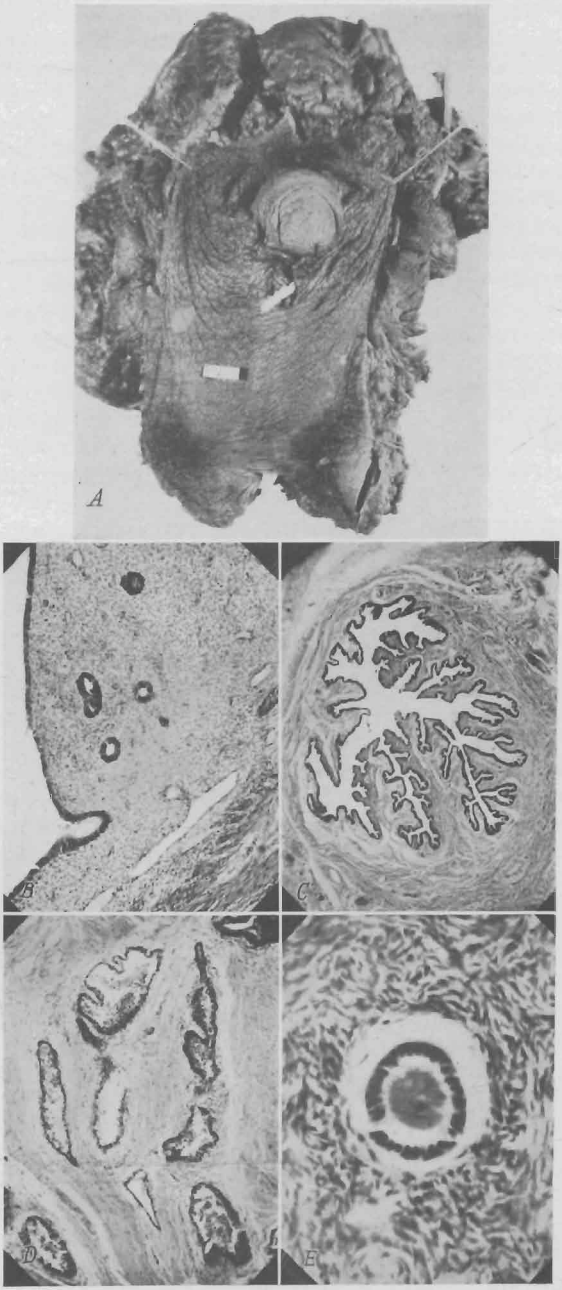
FIG. 157A. Case 1. Intersexuality. Penis and perineal region. Note the catheter in the common urethra and vagina. (Courtesy of Department of Pathology.)

FIG. 157B. Case 1. Intersexuality. Section through endometrium. (Courtesy of Department of Pathology.)

FIG. 157C. Case 1. Intersexuality. Section through tube. (Courtesy of Department of Pathology.)

FIG. 157D. Case 1. Intersexuality. Adenoid structure at junction of urethra and vagina. Resembles prostatic glands. (Courtesy of Department of Pathology.)

FIG. 157E. Case 1. Intersexuality. Ovary showing follicle. (Courtesy of Department of Pathology.)



FIGS. 157A–E

are four glandlike structures, the lumina of which are filled with acidophilic coagulum. These are lined by closely packed columnar epithelium, and each is surrounded by a distinct broad muscular wall. At a distance from these and entirely separated from them there is a cystic structure about 1½ low power fields in size. It is lined in part by columnar epithelium and in part by heaped up layers of polygonal epithelial cells whose cytoplasm is intensely basophilic. The lumen is empty. Many dilated lymphatics are scattered through the section.

B. This is a section taken from the left side of the uterus in the region of the site of the Fallopian tube. This consists of bundles of smooth muscle and connective tissue among which lie several tubular structures lined by columnar epithelium and surrounded by a muscular wall varying in thickness. One of these is much larger than the others, and appears dilated. There is likewise much less muscle in its wall. It is impossible to determine whether these structures represent a rudimentary Fallopian tube or whether they are merely parts of a paraovarium.

Right Ovary (*fig. 157D*). Much of the section consists of normal ovarian stroma, in which there is one perfectly formed primary follicle containing an ovum. In addition there are several normal appearing corpora albicantia. Beneath the typical ovarian stroma is a broad zone of loose fibrous tissue containing several small hemorrhages.

Right Ovary and Tumor. This is a section of the ovary including a portion of the tumor arising from it. Again there is typical ovarian stroma and several corpora albicantia. No primary follicles are present, however. In the loose fibrous tissue seen in the other section there are here numerous anastomosing gland-like spaces lined by columnar epithelium which extend into the surrounding tissue infiltrating between the connective tissue fibers in solid cords and in adenomatous projections. There are no basement membranes around the gland-like spaces. The epithelial cells are deeply basophilic, varying considerably in size. Their nuclei are for the most part round or oval. An embryonic type of fibrous tissue surrounds the epithelial cells and forms the stroma for them. This varies greatly in density, sometimes there are closely packed masses of nuclei with dense fibers between them, while in other areas there are few nuclei and few delicate fibers. The nuclei likewise vary greatly in size, some being extremely large and vesicular, while others are small. None of them contain much chromatin. A few mitotic figures can be found both in the stroma and the epithelium. Much hemorrhage and a considerable amount of necrosis is present.

Tumor. Parts of this section resemble the neoplastic elements described in the preceding one. There are, however, several additional features. In many places the stroma of embryonic fibrous tissue is much denser in appearance, resembling that of a fibrosarcoma. There are numerous large and small nodules of only slightly atypical cartilage. Sometimes the transition from the embryonic stroma to the cartilage is almost indistinguishable. A few of the cartilaginous islands are surrounded by acidophilic hyaline material closely resembling osteoid. Also present are several groups of intercommunicating large blood-filled spaces, apparently lined by epithelium and resembling a cavernous hemangioma. As in the preceding section hemorrhage and necrosis are prominent.

Pancreas. The acinar cells are normal save for postmortem change. The islands of Langerhans are numerous. They vary quite markedly in size, some being very small, while others are large, and sometimes elongated. There seems

to be a slight increase in the amount of connective tissue around the islands and within them. Pancreatic ducts are not remarkable.

Adrenal. The cortical cells contain a moderate amount of lipoid. In the inner zone of the cortex at the junction of cortex and medulla the cells are rather markedly autolyzed The medulla itself is normal save for an advanced degree of shrinkage of the cells. Chromaffinity, however, cannot be seen.

Breast. The section consists of normal resting mammary gland. There are large dense masses of collagen in which lie groups and single gland acini. The epithelium is not remarkable, although the majority of the glands are small. Some of the glands are dilated and contain granular pink precipitate

Thyroid. The gland is damaged by autolysis, and much of the colloid has escaped into the interacinar tissues. The acini vary slightly in size, but are for the most part lined by low cuboidal epithelium and are well filled with colloid.

Pituitary. The gland is wide and flattened. The anterior lobe is composed for the most part of acidophilic cells. A moderate amount of colloid is present at the junction of anterior and intermediate lobes. The posterior lobe is normal.

Cervix. One end of the section is lined by squamous epithelium. In the remainder there is no mucosa, although there is a small portion somewhat suggesting endometrium. Beneath this are bundles of smooth muscle similar to those in the uterus.

Junction of Vagina and Urethra. One side of the section is covered by squamous epithelium of the vagina, and the other by pseudostratified columnar. At the tip these two meet in a series of finger-like projections covered by the latter type of epithelium, much of which has been torn off. Between the two surfaces are duct-like spaces lined with heaped-up epithelium suggesting that seen in the prostatic ducts (fig. 157E).

Pineal Gland. The gland is quite cellular but comparison with others at this and greater age shows that this cellularity is within the limits of normal variation.

There is no doubt that this case presents an intersex, and probably of the male type (accepting Moszkowich's dictum of the position of the prostate). This individual began embryonic life as a male but shortly, at an early turning point (Goldschmidt's "Drehpunkt") *continued differentiation as a female.* The case would belong to Type 3 of Moszkowich's classification, where MM < F'. The development as a female continued far enough, so that follicles and corpora lutea (as evidenced by the corpora albicanta) formed. Unfortunately an insufficient number of hormone analyses were made. An estrone determination done one day before exitus showed 4 R.U. per liter. Considering the histological make-up of the ovary, we would expect estrone production.

Case 2. "P". This case is one of three sisters whose history and physical findings is to be presented in extenso by Mishell (1937). They had the following features in common. Feminine external genitalia, normal appearing breasts, inguinal hernias containing testes including epididymus and rete. Internal genitalia were absent. Figures 158A and 158B present one of the gonads found

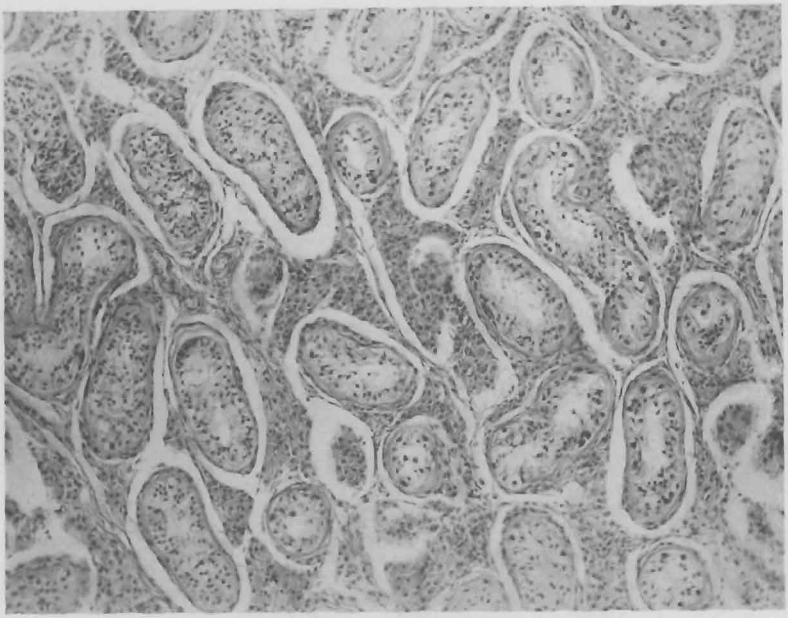


FIG. 158A. Case P. Testes of intersex type. Tubules resemble those of cryptorchid testes. Note marked overdevelopment of interstitial cells. ×160. (*Courtesy of Dr. D. R. Mishell.*)

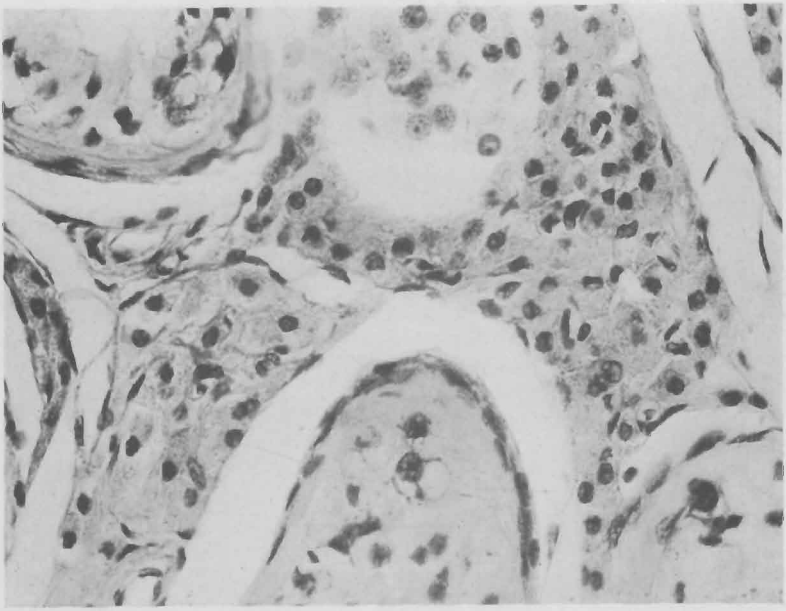


FIG. 158B. Case P. Testes of intersex type. Marked overdevelopment of interstitial tissue. ×1000. (Courtesy of Dr. D. R. Mishell.)

in case 2. There is an absence of spermatogenesis, though cells resembling spermatogonia are present. There is a marked overdevelopment of the interstitial tissue. The following hormonal titers were obtained:

	Estrone	*Follicle Stimulating Hormone*
Before operation	72 R.U. per liter	Not done
After operation:		
July 15, 1936	None	Not done
August 10, 1936	None	Positive—greater than 70 M.U. per liter
August 20, 1936	None	Positive
September 17, 1936	None	Positive
September 18, 1936	None	Positive
September 19, 1936	None	Positive

The finding of estrogenic hormone before the removal of the testes and its absence after their removal indicates that the estrogenic substance was produced by the testes. It is believed that all testicular hormone is produced by the interstitial tissue, hence we believe that it was also the source of the estrogenic hormone. But whether this substance was actually estrone or a modified testosterone molecule we do not know. This case leads to the following concept. According to Halban it is impossible to determine the character of the gonads from the nature of the external habitus. To this we would add that it is impossible to prognosticate *in special situations* (as in this case) the character of the gonadal hormone from the histologic picture of the gonads.

Hormonal Intersexes—Arrhenoblastoma

In a previous Chapter we have discussed hormonal intersexes due to disturbances of the adrenal cortex and also the anterior hypophysis. A third type is caused by masculinizing tumors of the ovary. Considerable work tending to clarify the microscopic characteristics of these tumors has been done by Robert Meyer (1931) and Novak (1936). To date no comprehensive hormone studies are available, though in a case of arrhenoblastoma Szathmary (1934) reports an absence of estrone before operation and 130 M.U. per liter of urine after the removal of the arrhenoblastoma. One would expect the production of male sex hormones by these tumors.

Here the histologic picture ranges between highly differentiated testicular adenomas, through a group showing varying numbers of tubules and acini, to a highly undifferentiated group which resembles sarcoma and may show an occasional epithelial tubule. The more undifferentiated the tumor type the greater are the masculinizing fea-

tures. About one-third of the adenomas show any degree of masculinization. The symptoms are usually amenorrhea, hirsutism, deepening of the voice, hypertrophy of the clitoris, a loss of subcutaneous fat making the figure more angular, and a decrease in the size of the breasts. Some symptoms may be more extensively developed than others. Upon the removal of the tumor the feminine characteristics return.

Phelan (1934) reported an interesting case and through his kindness I am able to reproduce a microphotograph of the tumor (fig. 159). The

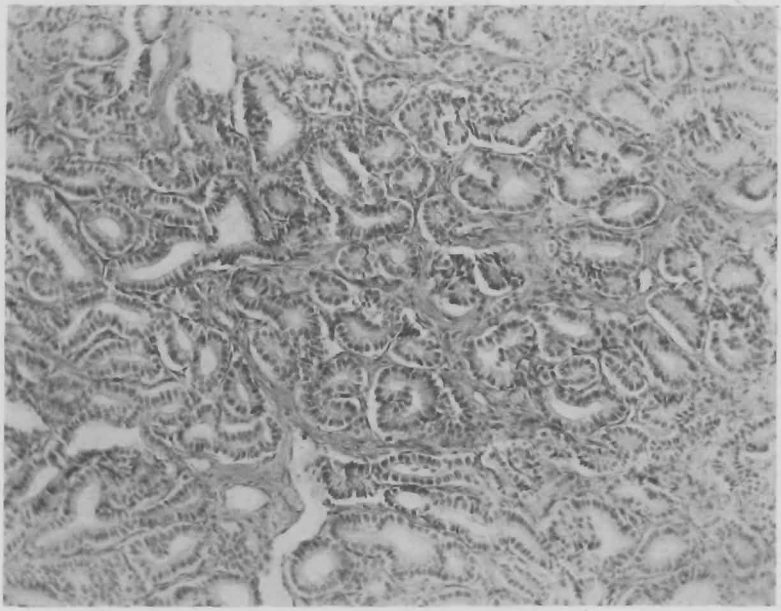


FIG. 159A. Arrhenoblastoma. The tubular structure is very distinct. ×160. (Courtesy of Dr. G. W. Phelan.)

patient was twenty years of age, had never menstruated and had a masculine distribution of hair. Voice coarse and distinctly masculine. Breasts flat, general figure angular. Vaginal examination revealed a well-formed introitus, save that the labia were smaller than normal. Clitoris hypertrophied (2.5 cm. long and 0.5 cm. thick). Vagina readily admitted examining finger. Cervix felt as a small dimple and could only be identified through the speculum. Uterus could not be felt, but sound passed the cervix for 2.5 cm. The left fornix presented a small (size of tangerine) solid, slightly movable tumor. This was

removed at operation. The uterus was found small and atrophic. Two months later she had a short period, followed by a full flow one month later. Four months after operation she became pregnant and she subsequently miscarried. Another pregnancy followed and she gave birth to a living child. There has been a gradual diminution of the hirsutism, the voice became softer. She was able to nurse after delivery. Libido and orgasm now normal. No change noted in clitoris.

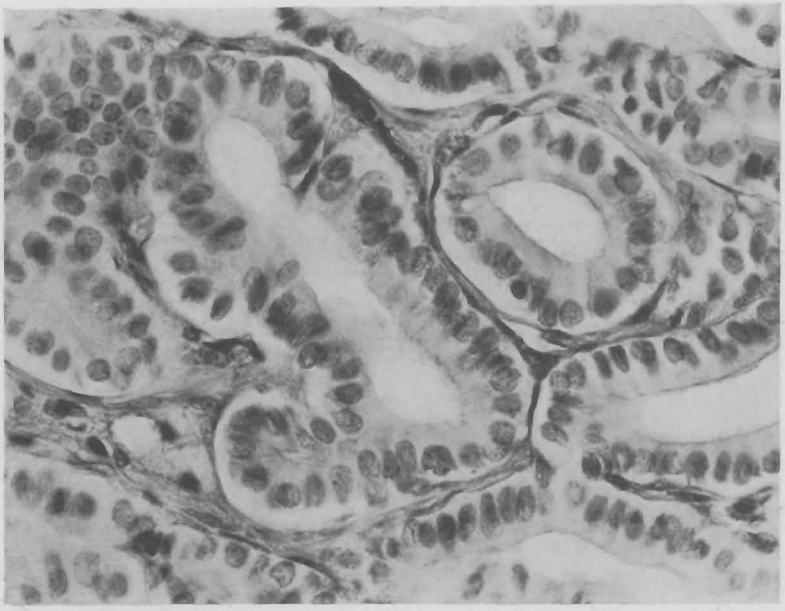


FIG. 159B. Arrhenoblastoma. Stroma appears active. ×800. (Courtesy of Dr. G. W. Phelan.)

The tumor was 2.5 x 1.5 inches, firm, with yellowish depressions. The tumor is surrounded by a connective tissue capsule from which trabeculae extend into the mass proper. There are numerous gland-like spaces lined by a single layer of tall columnar epithelium.

This patient was genetically a female and then continued development along feminine lines probably for a number of years. At some time before puberty the deflection (Drehpunkt) occurred and the patient continued development as a male. The source of the male sex hormone was the arrhenoblastoma. The damage that the male hormone could do was the stimulation of those secondary sex characteristics in the patient that male hormone normally develops in the male. We make

the point, *that the system will utilize that hormone which is present in excess*, in this case, male sex hormone. When the source of the masculinizing hormone was removed, her own female sex hormones came to the fore and she renewed her development as a female.

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CHAPTER XXVII

HORMONAL STERILIZATION

Any contraceptive method, when successful, acts by producing temporary sterility. This *temporary sterility* lasts only a few hours and at best, with safest "safe period," a number of days. Unfortunately the variations in the time of spontaneous ovulation, the possibility of induced ovulation, and the variations in the length of the cycle, render the date of ovulation relatively unpredictable. When ovulation is so variable the "safe period" must fluctuate along with it. The "safe period" will only be reliable when it will be possible to prognosticate exactly the date of the next ovulation. Hence, at the present time, there is no completely satisfactory method of rendering the woman temporarily sterile for a longer period than a few hours, or at most, a few days. Hormonal sterilization deals with methods that render the woman temporarily sterile for the duration of at least one menstrual cycle.

No satisfactory method of hormonal sterilization has as yet been devised. But several methods of approach are potentially possible.

It is self-evident that when an ovum is not available for fertilization pregnancy cannot occur. The *anovulatory cycle* furnishes one method by which the organism limits its fertility. The character of this infertile cycle has been fully discussed in Chapter XV. It is essential to repeat that when ovulation is absent, a corpus luteum fails to form, the endometrium retains its postmenstrual characteristics, and bleeding occurs cyclically about every four weeks. Anovulatory cycles occur more frequently than was previously suspected. They form the usual type of cycle during lactation. Kurzrok, Lass and Smelser (1937) have noted more than six successive sterile cycles. Fifty-five to seventy per cent of the cycles during lactation are sterile. Kurzrok and Wilson (1937) have under observation a number of (non-lactating) patients exhibiting anovulatory cycles at irregular intervals, and several patients in whom numerous successive cycles are sterile. The patients exhibit no other abnormality. They cannot differentiate the normal from the sterile cycle. If such cycles could be produced at will we would have available a safe contraceptive method.

The association of the anovulatory cycle with lactation suggests a

relationship to prolactin. It was first observed by Dresel (1935) and confirmed by Lahr and Riddle (1936) that prolactin is capable of temporarily suppressing ovarian cyclic activity in both mice and rats. But even with continuous injection of prolactin cycles are again resumed. After stopping the injections there was almost immediate resumption of normal estrus in all cases. The ovaries of these anestrous rodents disclosed the retention of large corpora with large cells apparently not in stages of degeneration. Whether the anestrus was brought about by progesterone secreted by the corpora lutea is undecided. Daily dosages of 0.3 mgm. of crystalline progesterone do not inhibit estrus, but huge doses, 4.0 mgm. daily, do (Selye, Browne and Collip (1936)). It is of interest to note that Kurzrok (et al.) noted the spontaneous resumption of ovulatory cycles after several anovulatory ones, during the lactating period. It is essential to study the effect of prolactin on normal non-lactating women, for it may produce anovulatory cycles, and in this way present a method of hormonal sterilization.

The exact method by which the anovulatory cycle is brought about is not known. Evans et al. (1936) have published very important observations bearing on this problem. It has previously been observed (Evans and Long, 1931) that intraperitoneal injections of anterior pituitary extracts produced gigantism in rats and partial or complete inhibition of estrus. Smith (1927) observed the repair of the gonadal, thyroid and adrenal subnormalities of hypophysectomized rats by means of daily intramuscular implants of anterior lobe tissue. He found that simultaneous intraperitoneal injection of saline extracts of bovine anterior pituitary tissue, while not interfering with thyroid and adrenal repair, prevented gonadal repair. Evans reports the separation of a *gonadotropic antagonist* which prevents or decreases the effect of gonadotropic hormones. The antagonist is most effective when given intraperitoneally. It prevents the action of the follicle stimulating hormone, but does not interfere with luteinization. When administered in the last half of pregnancy, the same excessive luteinization is observed, pregnancy is prolonged, and normal parturition does not take place. When administered to normal adult rats, the antagonist permits extensive luteinization of ovarian follicles. Evans calls attention to the parallel effects of the administration of the antagonist and of estrone. Estrone, in addition to stimulating the production of luteinizing hormone (Hohlweg, 1934; Magath and Rosenfeld, 1933; Hisaw et al., 1934), has a remarkable depressant effect on the ovarian response to follicle stimulating hormone (Fevold, Hisaw, and Greep, 1936). Evans et al. suggest

that estrone stimulates the secretion of the antagonist and thereby inhibits follicular development.

The gonadotropic antagonist may be responsible for the anovulatory cycle. But we doubt whether this is the whole story, because if the human follicles were to become luteinized, as do the rat ovaries, one would expect to find a premenstrual endometrium on the basis of a functioning corpus luteum. It is conceivable that luteal tissue formed within a ripening unruptured follicle does not possess lutein activity. The human ovary during an anovulatory cycle has not as yet been adequately studied. The effect of injections of the gonadotropic antagonist on ovulation in the human may have an important bearing on the problem of hormonal sterilization.

During the administration of large doses of estradiol benzoate (20,000 R.U. of progynon-B) for the treatment of dysmenorrhea, it was noted that the normal menstrual rhythm was frequently disturbed. As a rule the intervals between periods were lengthened, but occasionally shortened. We are unable to prognosticate in any given patient the effect of this high dosage of estrogenic hormone when given during the first two weeks of the cycle. Wilson has examined repeated biopsies during such delayed cycles and has noted that when the cycle is prolonged by four weeks, the first six weeks of this new cycle represent the proliferative phase and the last two weeks the premenstrual phase. In other words, ovulation is delayed about four weeks. We are now studying the effect of *repeated* injections of large doses of estradiol benzoate, for the purpose of postponing ovulation still further. The flow at the termination of such postponed cycles is not abnormal in either quantity or duration. It is conceivable that the delay in ovulation is due to estradiol stimulating the secretion of the gonadotropic antagonist. Hence estrone, by inhibiting ovulation, offers a third possibility for hormonal sterilization.

Another method of inhibiting ovulation has been observed by Parkes and Rowlands (1936). The rabbit has the peculiar property of ovulating only as a result of copulation and, on the average, some ten hours after it. Parkes and Rowlands observed that if they injected gonadotropic anti-serum into the rabbit immediately after copulation, ovulation would not take place. The observation was confirmed by Gegerson, Clarke and Kurzrok, (1936). Ten cubic centimeters of a potent gonadotropic antiserum was sufficient to inhibit ovulation. The nature of such antisera is a disputed question. It is conceivable that such antiserum (shown by Gegerson et al. (1936) to inhibit the gonadotropic

action of pregnancy urine extract, anterior pituitary extract and pregnant mare serum) may be related to or identical with the gonadotropic antagonist. This work has not as yet been transferred to the human, although the sera of two patients injected with about 6,000 to 8,000 R.U. of Follutein over a period of seven months showed the presence of gonadotropic antibodies. The injection of gonadotropic antisera for the prevention of ovulation in the human is a distinct possibility.

If serial sections are made of mouse or rat ovaries which show a positive Aschheim-Zondek Test, one frequently finds ova surrounded ("locked") by corpora lutea. Apparently luteinization has been so rapid and extensive that the follicle became luteinized before rupture. Hamblen and Ross (1936) studied the effect of pregnancy urine extract (800 to 8,200 R.U.) on the human ovary. They noted the formation of follicular cysts, with active granulosa and fairly well preserved ova, and surrounded by a proliferated theca interna with prominent theca luteum cells (theca lutein cysts). Whether doses of 20,000 R.U. of pregnancy urine extract or more would produce changes similar to those found in the rodent is not as yet known.

Burdick and Pincus (1935) and Whitney and Burdick (1936) noted that daily injections of estrone begun on the day of mating ordinarily resulted in the retention of ova in the Fallopian tube in both mice (5 R.U. per day) and rabbits (100 to 150 R.U. per day). All ova, whether they descended into the uterus or remained in the tubes, showed definite signs of degeneration by the fourth day after copulation. The prevention of pregnancy resulting from estrone injections during the early preimplantation period may be due to (*a*) the degeneration of the ova long before they are capable of implantation and (*b*) secondarily to the prevention of uterine entry of the blastocysts. These experiments raise the question whether a normally fertilized human ovum can be tube locked by an excess of estrone and secondly, whether the degeneration of the fertilized ovum can be made extensive enough to *completely inhibit* further development.

The potentialities of hormonal sterilization are tremendous. The problem is important enough to warrant extensive work on the human.

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CHAPTER XXVIII

FUNCTIONAL STERILITY

The discussion will be limited here to those factors in the problem of infertility and sterility that are either solely, or in part, under control of the hormones. Hence such topics as the pelvis infections and tumors will not be discussed.

We can best understand the problem of infertility if we stop to consider what happens under normal circumstances when fertilization does occur. Superficially the problem does not appear complicated, but on close inspection we find such an involved process that one often wonders how pregnancy does manage to take place.

Let us consider the phenomena that occur immediately after coitus. The slightly alkaline semen is injected into a highly acid vaginal canal. The cervix with its alkaline wick of cervical mucus dips into this seminal pool. This cervical plug extends from surrounding margins of the external os and passes upwards beyond the internal os to fuse imperceptibly with the mucus of the uterine cavity. The acidity of the vagina and the alkalinity of the cervix are both expressions of normal ovarian function. We believe that the former is definitely controlled by estrone and the latter probably so. It is interesting to note that when the sudden discharge of a large quantity of thin glairy cervical mucus occurs during a single day in the intermenstruum, forming thereby an external sign of ovulation, that day corresponds to the peak in estrone excretion. Miller and Kurzrok (1932) believe that the variations in pH between the vaginal walls, the semen, and the cervical plug serve a definite purpose. This variation in hydrogen ion concentration plus the fact that—vaginal wall—semen—cervical mucus—form immiscible phase boundaries, serve to form an electrical system that orients the spermatozoa into the cervical plug. The spermatozoa must leave the seminal pool in a short time for the constant influx of lactic acid from the vaginal walls would soon overcome the buffer action of the semen and render the sperm immobile. Hence, the migration of the spermatozoa into the cervix cannot be left to chance. They must be *oriented or directed* into the cervical canal. Miller and Kurzrok believe that the current set up (and it is measurable!) across these phase boundaries guides the *negatively charged sperm* into the cervical plug, for under the

Like bait on a fishing-hook.

conditions normally present *the plug forms the positive pole of the system.* The same explanation may be offered in answer to the questions:—why do not the spermatozoa leave the seminal pool and pass outward through the vagina towards the vulva? There is less obstruction than in the thick, glairy, sticky, cervical mucus. Under the system present the *vaginal walls carry a negative* charge, hence *repel* the negatively charged sperm (fig. 160).

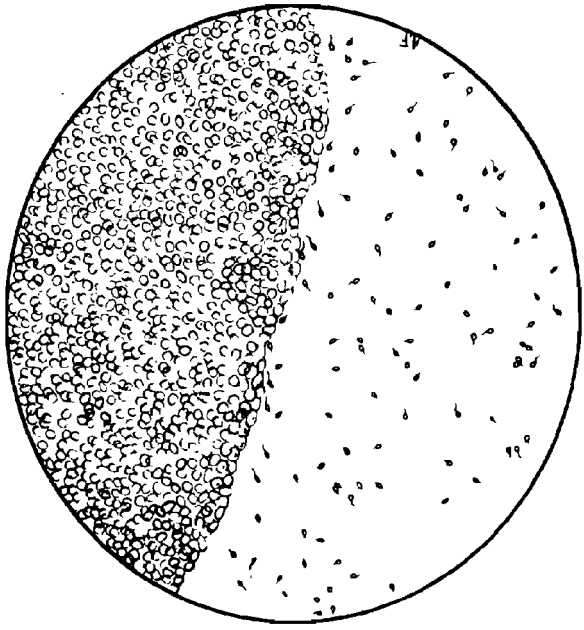

FIG. 160. Contact between semen and infected mucus. Note the failure of penetration and orientation.

Such a delicately balanced system of variations in hydrogen ion concentration acting across phase boundaries can be subject to considerable damage. Supposing the semen and the cervical mucus were badly infected; their pH would shift towards the acid side and thereby destroy or reverse the currents across these phase boundaries. The acid cervical plug would form the negative pole and thereby repel the negatively charged spermatozoa. This is one of the reasons why a

badly infected cervix is such a bar to fertilization. It might be asked at this point: What effect have variations in testicular function (the male sex hormones) on the pH of the semen? We do not know.

This brings up the therapeutic value of alkaline douches in the treatment of infertility. The reason usually given for their use is that they overcome the acidity of the vagina. But the very acidity of the vagina is most essential for the further progress of the spermatozoa into the cervical canal. Claims for excessive acidity in the vagina are frequently made. We know of no functional conditions that produce such excessive acidity, nor of any pH measurements made during such conditions. The only time a *dilute* alkaline douche is of value is when there is a very moderate endocervicitis. The dilute alkali dissolves the excessive mucus at the cervix and allows newly formed mucus to reach the cervical canal. Excessive alkalinity would temporarily lessen or overcome the acidity of the vaginal walls and thereby destroy the orienting mechanism. And even the slightly alkaline douche should be taken several hours before coitus.

Having been oriented towards the cervical plug, the spermatozoa must now pass *through* this plug. It is important to keep in mind the viscosity and the density of the mucous plug. The writer feels that the uterus takes no part in the passage of sperm through the canal. Its rôle is entirely passive, and the spermatozoa pass through it by means of their own powers of locomotion. We believe that sperm can pass through this gelatinous medium because they possess a lytic substance, probably an enzyme, which enables them to dissolve cervical mucus (Kurzrok and Miller, 1928). The lytic substance can also be found in completely aspermic semen. The clinical demonstration of the migration of spermatozoa through (normal) cervical mucus can be demonstrated by the following test. (Miller and Kurzrok.)

1. Place a small drop of semen, about 3 to 5 mm. in diameter, on the center of a clean, dry glass slide. It is essential that a goodly number of motile spermatozoa be present.

2. Remove a small fragment of cervical mucus, 3 to 5 mm. in diameter, from the cervical *canal* and place it on the slide about 3 mm. away from the drop of semen. This is best carried out by wiping the cervix clean, thereby removing the mucus that may have been in contact with the acid vagina for a considerable time. The cervix is then gently squeezed with a sponge forceps. The drop of mucus expressed in this manner is then used for the test.

3. The space between the two drops (semen and mucus) must be

completely dry and not contaminated by either semen or mucus. Drop a cover glass squarely on the drops. The weight of the cover glass will usually be sufficient to bring the margins of the drop, into contact. If only a *slight* (1 mm.) space intervenes between them, then *very gentle* pressure on the slide will bring the surfaces together. If this is not sufficient then repeat the test bringing the drops closer together.

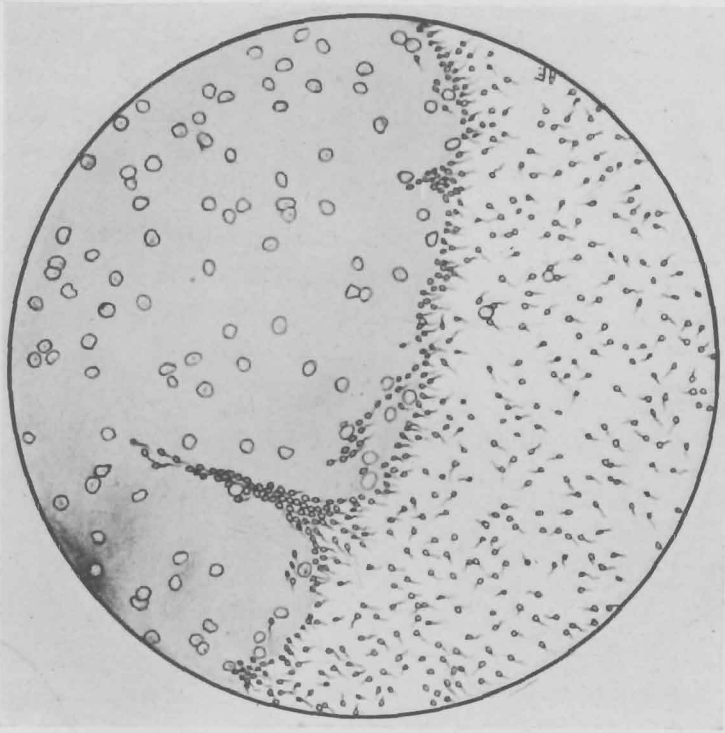

FIG. 161. Contact between semen and normal mucus. Orientation and beginning penetration.

4. An immiscible phase boundary is thus set up between the semen and the mucus.

5. Examine the contact zone first with the low power and then with the high dry power.

6. When the test is positive, that is, when the spermatozoa penetrate the mucous plug the following phenomena may be observed. The sperm swimming *close* to the mucus occasionally seem to act as if they were "pulled into" it. Their heads are directed against the mucus and

by a rapid oscillation of the tail penetration of the plug begins. In a short while a whole phalanx of sperm is driving forward. Those at the tip of the phalanx frequently float back into the semen drop, and in doing so they usually swim backwards, that is, tail first. Often the phalanx divides and several channels are then begun. This phenomenon may be observed in various stages throughout numerous points in the contact zone. If one focuses carefully at the tip of the phalanx a

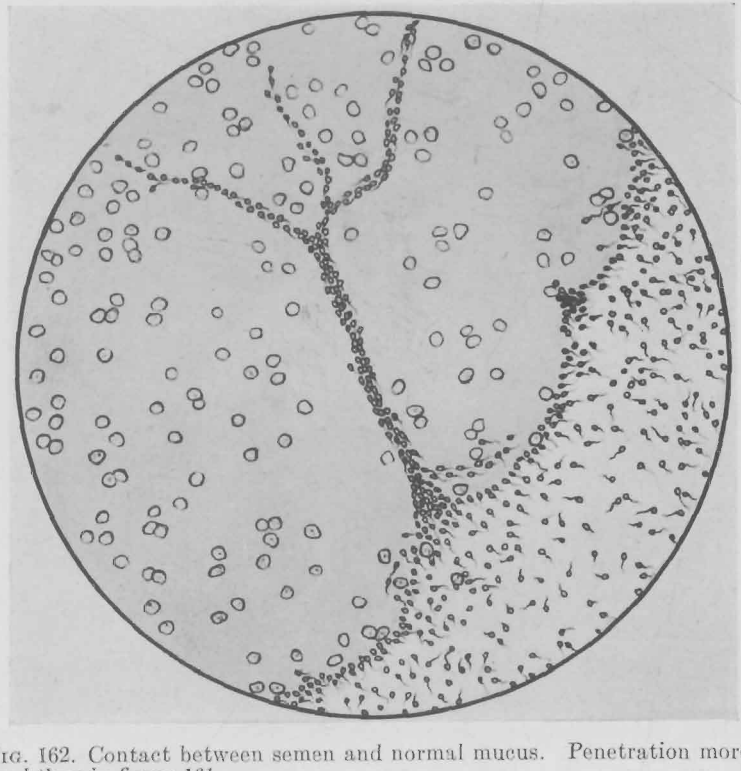

FIG. 162. Contact between semen and normal mucus. Penetration more advanced than in figure 161.

halo of dissolved cervical mucus may be seen (figs. 161 and 162). At the end of two or three hours the entire process stops, probably because of the drying and cooling of the preparation.

7. When the test is negative, that is, in the absence of penetration, the sperm swim past the plug without making the slightest effort to enter it. This is usually the case when the mucus is infected. Occasionally, even in the absence of any visible change in the mucus the

spermatozoa will make no effort to penetrate it, but when the same semen specimen is tested against a plug from another patient penetration occurs (fig. 163).

8. It is our impression that the penetration occurs, in general, best at about the time of ovulation. Of all the functional states that we have had occasion to examine, the mucus from a distinctly hypoplastic uterus appears to be definitely resistant to sperm penetration. This implies that a morphologically inadequate uterus is also inadequate as to its secretions.

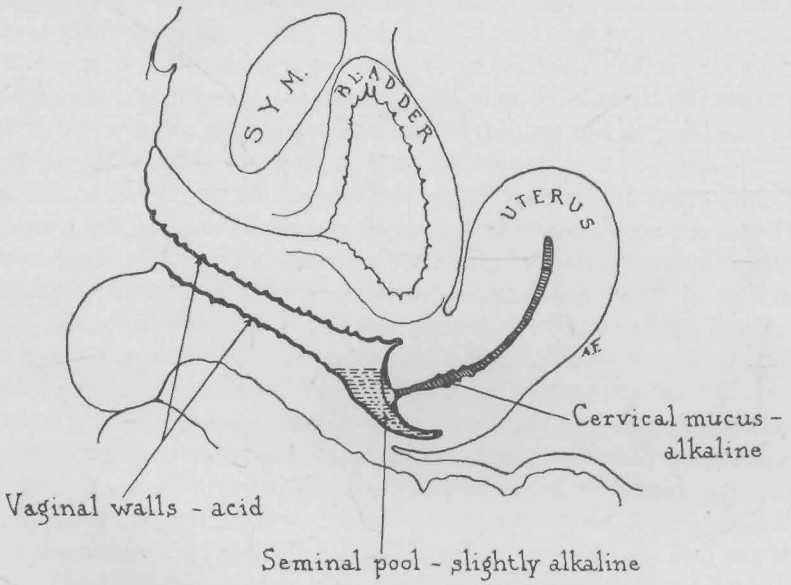

FIG. 163. Diagram showing contacts between vaginal wall, semen, and mucus

We believe that this test is of distinct clinical importance, and carry it out when the semen specimen shows numerous motile sperm. It was our impression that in two cases the penetration of the mucous drop occurred with greater facility after the male had been treated with gonadotropic pregnancy urine extract, all other factors remaining the same. Much further study is needed to completely substantiate this point.

Our *therapeutic* maneuvers as applied to the cervix offer an aid to this penetration mechanism. Pregnancy occasionally follows gentle dilatation of the cervix, especially when carried out late in the *postmenstrual* phase. The secretion of cervical mucus is constant, and adequate

drainage is essential. It is quite conceivable that if the drainage were inadequate the mucus plug could undergo physical and chemical change, due to such factors as dehydration, change in hydrogen ion concentration, and proteolytic digestion. Such change in the plug may be sufficient to render it partially or completely impermeable to spermatozoa. The gentle dilatation and cleansing of the cervical canal removes such mucus and allows fresh mucus to come into the cervix and thereby form a new plug.

Another observation in this connection has been made by my former associate, Dr. Ch. Birnberg. He noted that in many cases when cervical dilatation was carried out during any day in the second week of the menstrual cycle, follicle stimulating hormone appeared in the urine on the day following the dilatation. This means that stimuli from the cervix passed to the anterior pituitary gland and resulted in increased activity of the gland. Such increased activity could serve as an aid to ovulation and, secondly, by stimulating the ovaries change the character of the cervico-uterine secretions. It is of interest to note in this connection that in our studies of human ovulation as evidenced by the sudden excretion of follicle stimulating hormone, coitus, *when accompanied by orgasm on the part of the woman*, resulted in the sudden excretion of F.S.H. in her urine on the day following. In the absence of orgasm F.S.H. did not appear. This may have an important bearing on the problem of induced ovulation.

A definitely decreased number of spermatozoa begin to enter the uterine cavity about four hours post coitum. It is doubtful whether more than 10 to 20 per cent of the sperm that were ejaculated into the vagina ever get into the uterine cavity. The loss is due to the necessity of surmounting the barriers of the highly acid vagina and the highly viscous cervical mucus. In addition one is impressed, when examining a semen specimen, by the fact that not all spermatozoa seem to be endowed with the same amount of energy. Some are very sluggish from the outset. It is essential for the sperm to traverse (for them) the large expanse of uterine surface. How do they locate the tubo-uterine opening? Is it a "hit or miss" proposition, or are they oriented towards the tubo-uterine opening by some such mechanism as a potential gradient extending from the mucus at the external os to the tube? Do the spermatozoa enter both tubes in relatively equal numbers, or does the ovary containing the Graafian follicle exert an orienting and selective influence? What rôle does the ciliated current play in this upward migration of sperm? The direction of the ciliated current is *outwards* towards the

cervix and *against* the upward invasion of the sperm. Professor E. G. Miller and the writer had calculated that the rate of flow of the ciliated stream outward was greater than rate of progress of the sperm upwards, and yet the sperm do get into the tubes. To make this possible it is essential to assume a mechanism that either accelerates the speed of the spermatozoa, or inhibits or reverses the ciliated current. We are everywhere faced with a total lack of knowledge concerning these problems, especially in their application to the human. Is it a wonder then that we have so many unexplained cases of functional sterility?

Further problems present themselves. What is the reaction of the uterus to the semen? What controls the uterine contractions while the sperm are swimming upwards towards the tubes? What opens the tubo-uterine sphincters and allows the passage of spermatozoa into the tubes? We have at least partial answers to these questions. Kurzrok and Lieb (1930) have shown that normal semen causes a profound relaxation of the uterine muscle. This relaxation lasts for about one hour or more, and is independent of the phase of the menstrual cycle. The relaxation involves all muscle coats of the uterus and during this period of relaxation the uterus is almost completely refractive to pituitrin or adrenalin. We consider this to be the normal reaction of the uterus to semen, and in all likelihood due to a specific concentration of acetyl choline (or its derivatives) in the semen. The concentration of acetyl choline is wholly independent of the presence of spermatozoa (Cockrill, Miller and Kurzrok, 1935; Kurzrok, Miller and Cockrill, 1936; Miller, Cockrill and Kurzrok, 1937). In our opinion this relaxation is responsible for the opening of the tubo-uterine sphincters, and allows the passage of spermatozoa into the tubes. (See Chapter XVI.)

On the contrary a given semen specimen may produce marked spasm of the uterus, the spasm lasting about an hour or more. A semen specimen may produce relaxation in one uterus and contraction in another. Or a given uterine strip may react to one semen specimen by a relaxation and to another specimen by a contraction. These phenomena are in part due to variations in concentration of acetyl choline (or its derivatives) in the semen. The spasm involves all uterine muscle coats and sphincters. We believe that these factors may greatly influence the progress of the spermatozoa at the internal os, through the uterus and through the tubo-uterine sphincters. Fertilization is a function of time, and if the motion of the spermatozoa is prolonged enough their fertilizing power may be seriously impaired. This is more readily understandable when we consider the source of energy available to the spermatozoa. If the sole sources of energy are the molecules

within the sperm head, then protracting the journey towards the ovum would greatly diminish the available stores. Furthermore, if contractions of the uterus were maintained throughout the ascent of the sperm within its cavity, motion of the spermatozoa would be

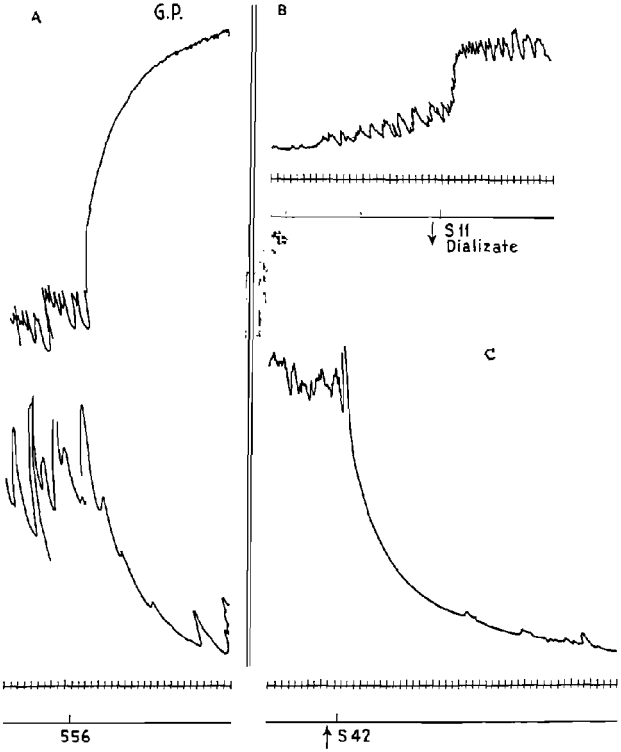

FIG. 164. Reaction of human uterus to human semen. *C* shows the profound relaxation of a uterine strip produced by normal semen. *B* shows an abnormal or spastic reaction. In *A* a human and guinea pig uterine strip have been suspended in the *same* chamber, hence under identical conditions. The addition of human semen (0.25 cc. in 100 cc. Ringer solution) causes marked spasm in the guinea pig uterus, and relaxation in the human.

hindered. The evidence available at the present time favors a uterine "pace maker" somewhere in the region of the tubo-uterine sphincters, the contraction wave passing from that point towards the cervix. But what external factors control these properties of semen? Are they

functions of the male sex hormones? If so, which hormone? How may these factors be modified therapeutically? A great deal of work is imperative for the solution of these problems, for how can we understand and *treat* the abnormal if we do not understand the normal physiological processes?

The importance of uterine hypoplasia in the problem of functional sterility should not be overlooked. The problem has been discussed in part in Chapter XX. It is reasonable to expect that morphological growth of a hypoplastic uterus as the result of the therapeutic exhibition of estrone is accompanied by an improvement in the functions and secretions. Where pregnancy is the desired effect in the treatment of genital hypoplasia we must be guided by certain principles, namely:

1. The estrogenic hormone may be administered in smaller doses throughout the cycle, or in several larger doses during the first half of the cycle. The total parenteral dosage during any cycle should not exceed 75,000 to 100,000 international units. If mouth medication is used the total unitage should be multiplied about five times.

2. When large doses are given during the postmenstrual phase the last dose should not be given later than the twelfth day of the cycle.

3. Large doses of estrogenic hormone should not be given during the secretory phase of the cycle for they may interfere with the development of the secretory phase of the endometrium. The result would be a "mixed endometrium" (Wilson and Kurzrok, 1937), which could be unsuitable for nidation of the fertilized ovum.

4. *Very large doses* of estrogenic hormone, 200,000 I.U. or more, frequently prolong the cycle and postpone ovulation.

The writer does at least two endometrial biopsies just preceding the flow, that is, one biopsy each month for two or more successive months.

The presence of a secretory endometrium is evidence that ovulation had taken place during the cycle. A careful study of the endometrial fragment may give some inkling as to the extent and character of the endometrial development and transformation.

With regard to the effect of the tubal secretions on the spermatozoa, and the effect of sperm on the tubes, very little exact knowledge is available. Possibly our ideas concerning the duration of life and fertilizing power of the spermatozoa would undergo considerable change if we had some inkling of tubal physiology under various conditions. To speak of therapy in this connection, at this time, is sheer nonsense.

Many observers are of the opinion that some infertile patients actually do become pregnant but the patients miscarry a few days after the expected onset of the period. Several such cases have given a positive

Aschheim-Zondek Test on the first or second day of the expected period. By the time the result of the test was known bleeding had already set in. This bleeding simulated a normal menstrual period in every respect. We believe that a common cause of such early miscarriages is the inadequate secretory preparation on the part of the endometrium. As a result of these observations we have adopted the following policy towards these patients. When a sterile patient with regular cycles skips her period by two days, the patient is put to bed. One half of an international unit of progesterone is injected intramuscularly every day, or one international unit every other day. When this dosage is exceeded the patient frequently complains of a fulness in the pelvis and a sense of approaching menstruation. Very frequently bleeding does begin. It is our belief that the progesterone improves the endometrial bed of the ovum, by increasing secretory activity. It is quite true that most of such skipped periods have turned out to be false alarms (as evidenced by a microscopic examination of the passed fragments), but here and there one does succeed. Considering the fact that therapeutic results do not exceed 10 to 15 per cent, when a *large* series of sterile cases is considered, that is, patients infertile for two or more years, we feel justified in using all methods of therapy that are not detrimental to the patient.

The reason for our lack of success with our best therapy is relatively simple. We do not as yet understand the physiology of normal human reproduction.

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CHAPTER XXIX

THE MALE SEX HORMONES

In view of the fact that many patients coming to the attention of gynecologists and obstetricians show definite masculinizing characteristics, it is considered advisable to include a chapter on the male sex hormones. No attempt will be made to cover the field; for such information the reader is referred to the articles by Moore (1932) and Steinach (1936). We will limit ourselves to a brief discussion of the chemistry and certain physiological interrelationships.

There are two standard methods in use for determining the presence of the male sex hormones.

The Bird or Capon or Comb-growth Unit

This test is based on observations made by Pezard (1911) by means of testes transplants. He noted a direct relationship between the size of the comb and the amount of testes tissue implanted. This method was extended further by Gallagher and Koch (1929), who measured the sum of the width and length, in the single-combed Leghorn capon. Laqueur et al. (1930) substituted a planimetric measurement of the comb surface. Schoeller defined a capon unit as that amount of male sex hormone which injected on two successive days produces an increase of 20 per cent in comb area on the third day, in two out of three capons.

The Seminal Vesicle Regeneration Test

Steinach (1936) had observed in his early experiments with infantile castrates a growth of the secondary sex characteristics as the result of testes implants. The seminal vesicles showed a macroscopic increase in width and length. Voss and Loewe (1931) called attention to microscopic changes and defined their unit as the minimum amount which, when injected in three doses in thirty-six hours into a four week castrated mouse, brings about in 100 hours certain changes in:

a. The normal height of the epithelial cells.

b. The presence of secretion granules and vacuoles.

c. Cell division with numerous mitoses.

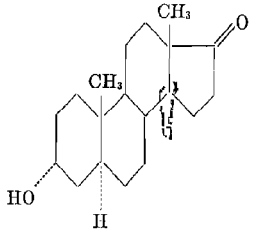
d. The presence of acidophile secretion in the lumen of the seminal vesicle.

The two tests are not equally sensitive to the same hormone.

The isolation of the male sex hormones was a matter of the utmost difficulty. The hormones are present in minute amounts in male urine or in testes. One hundred liters of male urine contains 100 mgm. of hormone (androsterone) while one ton of bull testis tissue contains approximately 150 to 200 mgm. of the pure testes hormone (testosterone) (Koch, 1936).

Chemistry

In their work on the male sex hormone Gallagher and Koch (1934) noted that the crude hormone isolated from urine was not identical with the lipoid fraction from testes. The urinary male hormone concentrates were resistant to alcoholic potassium hydroxide whereas the testis tissue concentrates were very easily destroyed thereby. Subse-



Androsterone

FIG. 165

quent work has demonstrated three male sex hormones: androsterone and dehydroandrosterone from male urine, and testosterone from the testis-tissue.

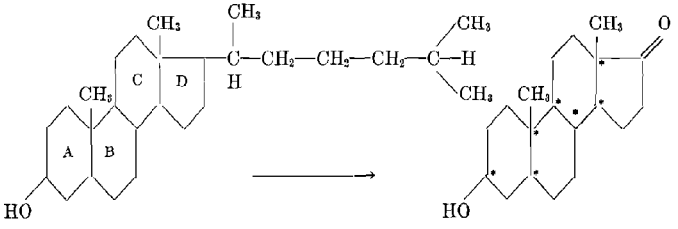
Androsterone. This substance was first isolated in crystalline form by Butenandt and Tscherning (1931) and to the former belongs the credit for determining its structural formula. Ruzicka (1934) was the first to synthesize the hormone from cholesterol. It is remarkable that Butenandt established the structural formula of androsterone when he had only 25 mg. of material available.

The chemistry involved in the production and synthesis of the male sex hormones is very complicated. Only a few pertinent facts can be given in this chapter. For a complete discussion of the subject the reader is referred to the very comprehensive monograph by Fieser (1936).

Androsterone ($C_{19}H_{30}O_2$) is represented by the structural formula in figure 165.

Androsterone is a saturated compound, containing a secondary alcohol and a ketone group. It melts at 182° to 183° (corr.) and is optically active. The hormone is very stable chemically.

Ruzicka noticed the similarity in the formula between androsterone and the sterols, and assumed that the hormone might be a degradation product of these substances. The most readily available starting material for such a synthesis was dihydrocholesterol. He oxidized dihy-



Dihydrocholesterol

3-hydroxyaetioallocholanone-17

(* points to the asymmetric C atoms)

FIG. 166

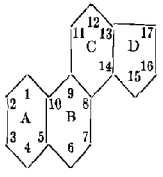
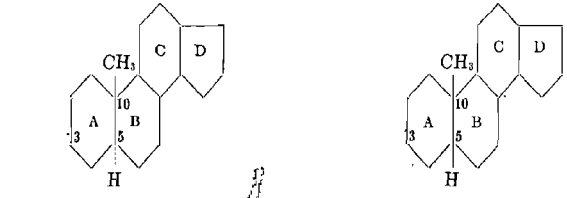


FIG. 167

drocholesteryl acetate with chromic anhydride in boiling glacial acetic acid. The greatest fraction obtained was acidic, but a small neutral fraction yielded a crystalline semicarbazone. On hydrolysis a substance was obtained which had definite physiological comb-growth activity and had the expected composition of androsterone. The natural hormone was the more active, hence Ruzicka felt that he was dealing with an isomer. There is ample opportunity for such isomerism in the hydroxyaetiocholanone structure. Corresponding to the seven asymmetric carbon atoms present, no less than 128 stereoisometric forms are possible (Fieser). (See fig. 166.) The position of the various car-

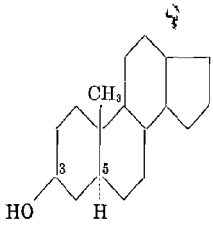
bon atoms in the nucleus is shown in figure 167. The problem is simplified by the fact rings C and D have identical configuration in all the natural sterols and bile acids. The junction C/D is known to be trans and a similar linkage probably exists between rings B and C. The linkages that interest us here are between rings A and B. The isomerism is dependent specifically upon the configuration at the asymmetric center C_5, the hydrogen atom at this point bearing either the cis or the trans relationship to the methyl group at C_{10}. (See fig. 168.)



(Cholestane series)
Trans relationship or allo series

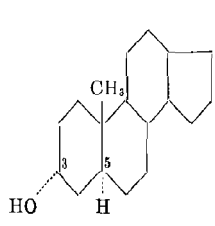
(Coprostane series)
Cis or normal series

FIG. 168



Dihydrocholesterol
(trans)

FIG. 169



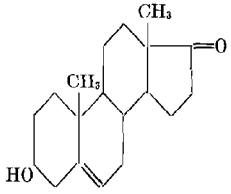
Epidihydrocholesterol
(cis)

FIG. 170

Another important type of stereoisomerism has to do with the configuration of the carbon atom C_3, which in all of the known sterols of natural occurrence is the point of attachment of the lone hydroxyl group. By convention the configuration of cholesterol or dihydrocholesterol is represented as in figure 169 and that of the epimeric form as in figure 170. In figure 169 the hydroxyl group bears the trans relationship to the hydrogen atom at C_5 while in figure 170 the substituents are cis (Fieser).

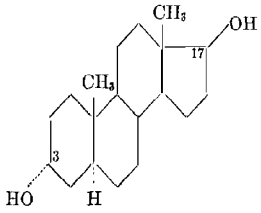
Ruzicka prepared the four possible isomers having the structural

formula of androsterone, but varying only in the positions of the OH and H groups on carbon atoms C_3 and C_5 respectively. He found that the ketone obtained as an oxidation product of epidihydrocholesterol was identical in every respect with androsterone. It is interesting to

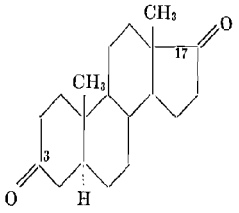


Dehydroandrosterone

FIG. 171

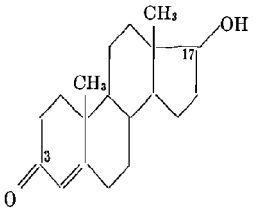


Androstanediol 3,17



Androstanedione 3,17

FIG. 172



Testosterone

△ 4-5 Androstene-ol (-17) on (-3)

FIG. 173

note that the male hormone belongs to the cholestane (allo) series, while pregnanediol corresponds in configuration to coprostane. Hence in the conversion of cholesterol into androsterone it is necessary for the OH group on C_3 to shift from the trans to the cis position. So

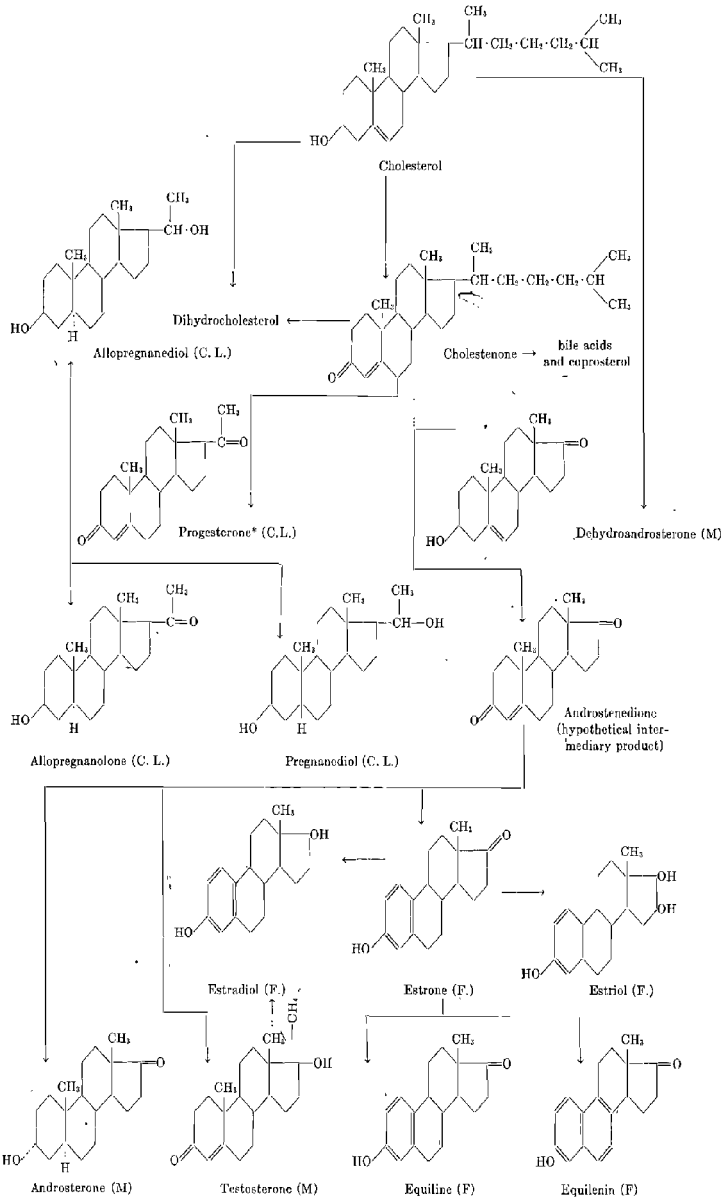


FIG. 174. The sterols, the sex hormones and their interrelationship

M denotes members of the male hormone group. F denotes members of the female hormone (theelin) group. C.L. denotes members of the corpus luteum group. * denotes the only active member of the corpus luteum group.

that if cholesterol is the precursor of androsterone in the organism, a biological mechanism must be present to make this rearrangement.

Dehydroanderosterone. Dehydroandrosterone is a second male sex hormone found in the urine (Butenandt and Dannenbaum, 1934). It was also isolated from cholesterol by Ruzicka and Wettstein (1935). It possesses about one-third of the comb-growth activity of androsterone. (See fig. 171.)

It is interesting to note that dehydroandrosterone is intermediate in degree of unsaturation between androsterone and estrone, and may be a natural precursor of substances of both groups.

A number of derivatives of androsterone have been prepared by converting the keto and hydroxyl groups into two keto or two hydroxyl groups. Figure 172 gives the compounds obtained.

Their order of biological activity is as follows:

	Capon Unit Equivalent
Androstanediol—3,17	45–50 gamma
Androsterone	150–200 gamma
Androsterone acetate	150–170 gamma
Androstanedione—3,17	300 gamma

As expected, the presence of an additional hydroxyl group (by reduction of the ketone group) enhances the potency three times. The same effect was noted between estrone and estradiol. On the contrary the presence of two keto groups greatly reduces the activity of the compound. The acetate does not alter the effective dose but exhibits a marked protracted action. This may be due to the time required for hydrolysis, or to a slower process of absorption within the organism.

Testosterone. The question arose whether the hormone in the testis was identical with the one in the urine. Or were we dealing with a condition similar to that found in the female, that is, that estrone is an excretory product, and that estradiol is the active hormone of the follicular fluid. The previously cited work of Gallagher and Koch showed that testes extracts were inactivated by boiling with alkali while the male sex hormone fractions from urine were not. Further observations were made by Laqueur et al. (1935) who compared the effects of administering to castrated rats equal amounts, in terms of capon units, or urinary and testicular male hormone extracts. The animals receiving testicular extracts showed extraordinary growth of the seminal vesicles. The average weights in the case of the controls, the animals receiving urinary extracts and those receiving testicular extracts were in the ratios: 1:14:67. This divergence of activity pointed clearly to the presence in the testes of a substance other than androsterone.

David, Dingemanse, Freud and Laqueur (1935) isolated a crystalline substance from testis tissue. Their yield was 10 mg. of hormone from 100 kg. of testis tissue. They called this substance testosterone. It is about 10 times as powerful as androsterone in promoting comb growth, 10 gamma being the equivalent of one capon unit. The synthetic production of this hormone was then accomplished by Butenandt (1935) and shortly afterwards by Ruzicka (1935). Their starting point was dihydroandrosterone. Testosterone has the chemical structure shown in figure 173. The double bond is between carbon atoms 4 and 5, the alcoholic group (=ol) on C_{17} and the keto group (-on) on C_3. Testosterone is optically active and melts at 154° to 154.5 corr.

Figure 174, modified from Tscherning (1936) and Ruzicka (1936) shows the chemical relationship between the sterols and the sex hormones, and the interrelationship between the latter.

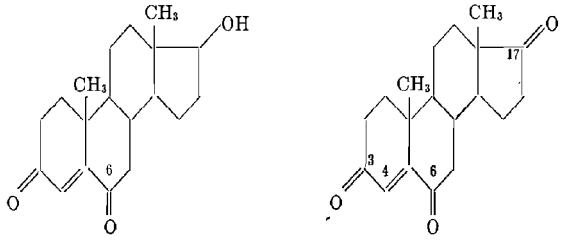


FIG. 175

It is of great physiological and clinical interest to isolate compounds that are intermediate between the male and female sex hormones. Possibly such substances may be produced in the various intersexes. Butenandt and Riegel (1936) have studied such a series. They increased the oxidation of androsterone by either increasing the number of double bonds (removing hydrogen) or by adding oxygen groups. They produced two such compounds, namely, 6-oxo-testosterone and Δ^4-androstenetrione-(3–6–17) (fig. 175). Two milligrams of either substance will produce estrus in a mouse, while 4 mgm. will not produce comb growth, nor will 16 mgm. produce a change in the seminal vesicles. It is rather startling to note that two substances of such high potency as testosterone (capon unit = 10 gamma) and androstenedione (capon unit = 100 gamma) will lose their properties as male sex hormones and gain those of estrone, through the mere addition of an oxygen. The

relation of Butenandt's oxidation products to Reichstein's adrenosterone (Chapter XX) will be awaited with great interest.

Further Physiological Action of Male Sex Hormones

The effect of a sex hormone on the organism of the opposite sex is of great clinical interest. Recent experimental data (Korenchevsky, Dennison and Simpson, 1935) have yielded some interesting facts. These workers studied the effect of prolonged treatment of male and female rats with androsterone and its derivatives, alone or together with estrone.

Effect on Castrated Male Rats. Androstanediol and androsterone stimulated the secondary sex organs. The former was the more potent and the atrophied organs were restored to normal or more than normal weight.

A coöperative effect on the seminal vesicles and prostate was seen between androsterone and estrone, though a quicker and greater effect could be obtained by increasing the dose of male hormone preparation. An antagonistic action on the adrenals appeared to occur between the male hormones and estrone.

The male sex hormones had a stimulating effect on the heart, liver, kidneys and adrenals. There are distinct clinical potentialities in these observations.

Effect on Ovariectomised Female Rats. The injection of large doses of diol caused a partial recovery of the atrophied uterus and vagina. The effect was slight even with large doses of androsterone.

The injection of androsterone and of diol increased the rate of involution of the thymus, decreased the weight of the adrenals and (with large doses) of the hypophysis and slightly increased the weights of the kidneys and liver, thus in general producing results similar to those obtained in castrated male rats.

Simultaneous injections of estrone and large doses of androsterone or diol showed a "coöperative recovery effect" on the weights of the uterus and vagina and to a smaller degree on the thymus, while there seemed to be a slightly antagonistic effect between the actions of estrone and the male hormones on the adrenals.

Selye, McCuen and Collip (1936) reported that treatment with testosterone benzoate stimulates the development and secretion of the mammary gland in normal and castrate female and male rats. Furthermore, testosterone is not estrogenic in doses sufficient to stimulate the mammary gland. Hence the effect of the male hormone on the

mammary tissue cannot be attributed to its transformation in the organism into the female sex hormone. Jung and Shafton (1935) found that proliferation of mammary tissue is normally demonstrable in boys between 12 and 17 years of age.

The effect of the male sex hormones on the female organism can be profound. The potentialities are enormous when one considers the number of variations the hormone molecules may undergo in the body chemistry, both under normal and abnormal conditions.

I am indebted to Dr. Erwin Schwenk for his advice concerning this chapter.

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CHAPTER XXX

METHODS OF HORMONE ASSAY

The assay of the sex hormones in blood and urine has given valuable aid in the treatment and diagnosis of various obstetrical and gynecological dysfunctions. Only those methods will be given that can be carried out in a fairly well equipped hospital laboratory.

Extraction and Assay of Estrone

I. Urine. *A. Method of Kurzrok and Ratner (1932).* The twenty-four hour urine specimen is measured and 900 cc. placed in a one liter Erlenmeyer flask. This is tested and made slightly acid with dilute acetic acid. The hormone is more easily extracted from an acid solution. The urine is then saturated with sodium chloride (approximately 50 grams) which decreases the solubility of ethyl acetate in the urine. The treated urine is then covered with ethyl acetate to the top of the flask, and the flask is connected as in figure 176. The second flask (*B*) of 300 cc. capacity is filled with 200 cc. ethyl acetate. The hot plate is turned to medium heat and the ethyl acetate distills over through the upper side arm, is condensed and drops to the bottom of flask (*A*) from which it returns through the lower side arm to flask (*B*). The drops of ethyl acetate in passing upward through the urine extract the hormone. This provides continuous extraction for 48 hours. It is automatic and needs no watching. The extracted urine is then discarded, and the ethyl acetate extract, which contains the hormone is concentrated by vacuum distillation (fig. 177). The distilling flask (*A*) is filled one-third full with ethyl acetate extract and connected with the condenser (*B*), a receiving flask (C), a second receiving flask (*D*), a trap (*E*) (provided with stopcock) and finally onto an aspirator type of suction pump. *After* the distilling flask has been heated in a steam bath, the suction is turned on. The remaining extract is allowed to drop into the flask (*A*) through a thistle tube which is provided with a stopcock. When all the ethyl acetate has been added it is concentrated to as small a volume as possible and 6 cc. of propylene glycol is added through the thistle tube. The distillation is continued until all of the ethyl acetate has been removed. This last step should be done slowly to prevent bumping and loss of the hormone. The oil now contains the hormone originally present in the 900 cc. of urine.

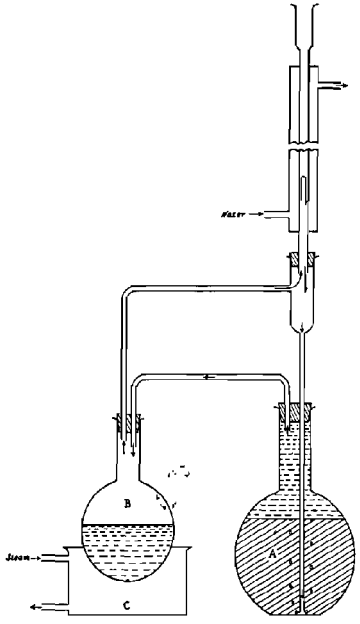

FIG. 176. Estrone extraction apparatus. A hot plate can be used instead of the steam bath. The condenser should be long, for ethyl acetate is fairly volatile.

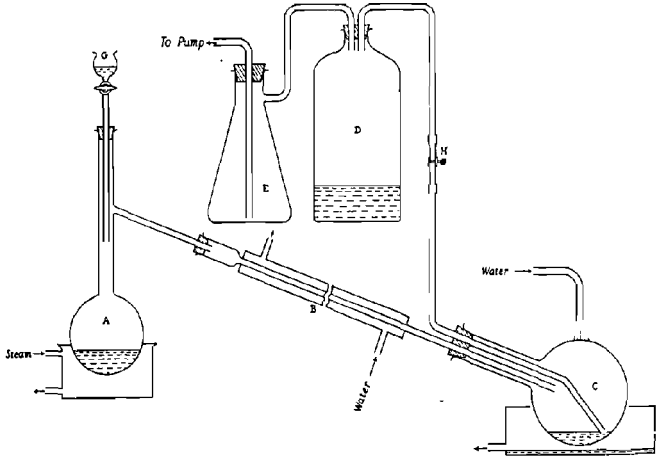

FIG. 177. The concentration apparatus. The receiving flask (*D*) should be 3- to 5-liter capacity. It acts as a reservoir and is only emptied of ethyl acetate when full. Aside from removing and replacing the distilling flask, the apparatus need not be disconnected.

This 6 cc. of oil, prepared as described above, is used for the biologic assay. One group of three ovariectomized rats is used for the assay of each specimen. They are injected with the oil subcutaneously in the back, at 9 a.m. and 5 p.m. of the first day and at 9 a.m. of the second day. One rat receives 0.5 cc. oil at each injection and the other two rats receive 0.25 cc. each at each injection. Vaginal smears are taken 24 and 32 hours after the last injection. A No. 13 dental spatula is very convenient for obtaining the vaginal secretion which is then spread in a drop of saline and examined under the microscope. *A positive smear must show the cornified cells characteristic of estrus.* Mucus, leucocytes and epithelial cells must be absent to establish a positive result. The hormone content of a liter of urine, expressed in terms of rat units may be calculated from the following formula.

$\frac{6}{y} \times \frac{1000}{900}$ equals rat units per liter of urine when y equals total amount of oil injected into a single rat producing a positive result.

Notes: The distance between the two horizontal arms in figure 176 should be three to four inches.

The condenser *should not* reach the level of the upper horizontal arm.

Animals that have repeatedly shown negative smears should be injected with potent estrogenic hormone (10 R.U.) once a month. This prevents excessive atrophy of the genital tract.

B. Bergen and Smelser Modification[1] *of Estrone Extraction.* The twenty-four-hour specimen of urine is measured and 750 cc. of it is poured into a 2-liter flask. The urine is made slightly acid with dilute acetic acid. Fifty grams of sodium chloride is added to the acidified urine and to this is added 750 cc. of ethyl acetate. This is shaken *vigorously* for three minutes and then poured into a separatory funnel. The urine fraction is drawn off. The ethyl acetate fraction is allowed to stand a few hours, then drawn off and distilled according to the method described above. The biologic assay is the same as above.

If an emulsion results during the shaking allow the ethyl acetate fraction to stand over night; then transfer to a clean flask. Centrifuge the emulsion if it is very heavy.

II. Blood. *A. Method of Fluhmann (1934).* The test depends on the injection of small amounts of untreated serum into spayed mice. A positive result is indicated by the production of a "mucification"

[1] Harold Bergen and Jane Smelser of the College of Physicians & Surgeons, Columbia University. Method not previously published.

of the vaginal mucosa. The method may be applied to quantitative studies, provided that a sufficient number of test animals is employed.

The blood is obtained by venipuncture and immediately placed in a test-tube for transmission to the laboratory. From 25 to 40 cc. are taken each time, according to the amount required. The clear serum is secured by centrifugalization, and the cells are discarded. If it is kept sterile, the serum may be left in the ice-box for days and even weeks without losing its potency.

The animals used are adult female mice, which have been spayed 6 to 7 days prior to the day the test is begun. This is important, for if the period of castration is shorter the vaginal mucosa may show unusual changes difficult of interpretation at the end of the test, while if it is longer the mice are not as sensitive to the action of estrin. It may be possible to "sensitize" old castrates by the administration of a cornifying dose of estrin some days before they are used, but this method has not been resorted to. The usual procedure has been to do a bilateral oöphorectomy on the mice on a Tuesday and to begin all tests on the same day of the following week.

A total of 4.5 cc. of blood serum is given to each mouse, and the test is completed in 72 hours.[2] The serum is administered subcutaneously in the back, and the site of each injection is varied as much as possible to facilitate absorption. Three injections of 0.5 cc. are given daily, at 8:00 a.m., noon, and 5:00 p.m., for three consecutive days. On the fourth morning the animal is sacrificed and the vagina is carefully dissected free. This is a simple procedure and is accomplished by making a long incision in the abdominal wall and separating the symphysis pubis. As many as 20 to 30 autopsies may be done in a half hour. The vagina is fixed in formalin, mounted in paraffin, and transverse sections are made at different levels and stained with hematoxylin-eosin.

In the preliminary report of this work no differentiation was made of the intensity of the changes elicited in the mouse vagina, but this has been very unsatisfactory. It has now been found possible to recognize six reactions, as follows:

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[2] An attempt is now being made to shorten this period to 48 hours, and it has already been shown that mucification may be readily induced in that time. On the basis of some unpublished observations it seems that the intensity of estrin effects does not depend altogether on the total amount of hormone injected but also on the concentration maintained in the test animal. It thus may be possible to intensify the reactions elicited by giving the test animal 6 injections of 0.75 c.c. in two days.

Reaction 0. Atrophy of the vagina. Mucosa shows two days of low cuboidal epithelium. Occasional leucocyte.

Reaction 1. Vaginal mucosa shows two layers, a basal of low cuboidal epithelium and a superficial of tall columnar cells. A few leucocytes.

Reaction 2. The superficial cells are high, begin to show stratification, and secrete mucus. There is a well-marked increase in leucocytes which may also be found in the lumen of the vagina.

Reaction 3. The epithelium of the vaginal mucosa is composed of several layers, and the cells at the surface are of the mucified variety. A characteristic feature often observed is a folding-in of the mucosa, a type of festooning, which is evidence of the rapid growth. The mucosa is invaded by large numbers of leucocytes which occur also in the lumen along with epithelial debris.

Reaction 4. The mucosa is made up of from 6 to 10 or 12 rows of cells, the lower resembling the basal cells of squamous epithelium while those at the surface are still of the tall mucified variety. In marked cases early cornification may be observed between the stratified epithelium and the mucified cells. Leucocytes have disappeared or are present in small numbers.

Reaction 5. The vagina is now lined by fully developed squamous epithelium with cornified cells at the surface. There are no leucocytes.

As in all biological procedures, individual mice vary greatly in their responses to the same dosage. The accuracy of the test thus depends on using as many animals as possible for each specimen. In this work two or three mice has been the minimum employed for each test. After the response for each mouse has been estimated, the numbers are added and then divided by the number of mice used in order to give the reaction. For instance, if three mice are used for a test and reactions 3, 2 and 2 are observed, the reaction elicited by that specimen would be given as 2.3.

B. Modified Method of Frank and Goldberger (1926). 1. Fifty cubic centimeters of blood is required. It is dehydrated by anhydrous sodium sulphate.

2. The powder is twice extracted with 200 cc. of 95 per cent alcohol.

3. The alcohol fractions are combined and evaporated to dryness on a water bath.

4. The residuum is taken up in 5 cc. of olive oil and injected into spayed mice.

5. The bioassay is according to the Allen-Doisy Method.

C. Neustaedter Modification of Frank and Goldberger Method (1936). Transfer 40 cc. of blood from a syringe to a petri dish containing 30 to 40

grams of anhydrous Na_2SO_4. Mix thoroughly with a glass rod. Fan until the mass has the consistency of fudge. Transfer to a mortar and grind to a very fine powder as the mixture dries. Return the powder to the petri dish for storage if the procedure must be interrupted at this point. Should the mixture not harden, add more Na_2SO_4. Transfer the red powder to a 250-cc. Erlenmeyer flask and add 100 cc. of ether. Stopper tightly with rubber and agitate for 20 minutes in a Holtz shaker. Rest the flask on a specially constructed board at an angle of 45° to separate the ether from the sludge by sedimentation. Decant the supernatant fluid and centrifuge. Repeat the process twice (that is, use 300 cc. of ether in all). Evaporate the centrifugalized ether to dryness in a casserole before an electric fan. Dissolve the lipoid residue in 6 cc. of benzene to which 0.6 cc. of olive oil is added. Fan off the benzene. If the air is full of dust, it is preferable to cover the tube with filter paper and set aside for spontaneous evaporation since injection of dirt leads to infection in the animals. Sterilize the olive oil which now contains the lipoid extract by autoclaving at 15 pounds for 15 minutes. This extract may be kept for several days if rubber stoppered and stored in the dark.

The extract is injected into mature, castrated female mice in divided doses using a sterile tuberculin syringe. On the first day 3 injections are given at 4-hour intervals. Two injections at the same interval are made on the following day. The extract is introduced into the mouse dorsally and subcutaneously. Beginning on the third morning, vaginal spreads are prepared twice daily for 4 days. The smears are made with a small bent glass tube drawn to a fine tip. By means of a rubber bulb, a drop of water is repeatedly injected and reaspirated from the vaginal canal so that a uniform and typical sample of vaginal secretion is secured. The specimen is transferred to a clean slide, dried, fixed and immersed in aqueous thionin (1 per cent) for 1 minute; excess stain is removed with water. Examine the vaginal smear for an estrus reaction (denoting 1 mouse unit of estrin and considered the norm).

Method of Extraction and Assay of Follicle Stimulating Hormone

I. Urine. *A. Modification of Zondek's Method.* Extraction: 1. Pour 47 cc. morning urine into a 250 cc. centrifuge tube. Test and make slightly acid with dilute acetic acid.

2. Add 200 cc. 95 per cent ethyl alcohol and allow to stand for 2 to 24 hours.

3. Centrifuge and discard the supernatant fluid.

4. Add 20 cc. ethyl ether and shake well, breaking up the precipitate. This removes the estrogenic substances.

5. Centrifuge and discard the supernatant fluid.

6. Dry the precipitate until all ether is evaporated.

7. Add 7 cc. distilled water and shake constantly for 10 minutes.

8. Centrifuge and save the supernatant fluid, which contains the follicle stimulating hormone. The preparation is then ready for animal assay.

Animal Assay. 1. Three immature female mice weighing from 6 to 8 grams are injected twice daily for three days. Each mouse receives 0.25 cc. at each injection, subcutaneously in the back. The injection is made with a 1 cc. tuberculin syringe and No. 24 hypodermic needle. The mice are not injected on the fourth day and are autopsied on the fifth day or 100 hours after the first injection.

2. A macroscopic examination of the ovaries and uterus is made. A positive test shows enlarged, reddened ovaries showing large follicles, and an enlarged and distended uterus and an open vagina. A negative test shows small white ovaries and threadlike uterus. This extract should be kept in the refrigerator. Each mouse receives the equivalent of 10.5 cc. of urine. If the test is positive it means that there are at least 100 M.U. of Follicle Stimulating Hormone per liter. By diluting the remaining hormone solution it is possible to determine the total amount of hormone present.

B. Method of Levin and Tyndale (1936). The chloroform-preserved urine is chilled, siphoned from any sediment and acidified to a pH 5 with acetic acid. For each liter of urine, 20 cc. of a freshly prepared aqueous 10 per cent tannic acid solution are added. An immediate precipitate forms. After thorough mixing, the precipitate is allowed to settle in the cold room and is then collected at the centrifuge. The bulky precipitate, which contains considerable water, is extracted once with at least 5 parts of 95 per cent ethyl alcohol followed by 3 to 4 extractions with 80 per cent alcohol. Alcohol is removed by several washings with acetone and the residue is freed of acetone by reduced pressure. In addition to removing considerable pigment and other inert material, the alcohol-acetone treatment removes any estrogenic substances which may be present.

The resultant powder (100 to 200 mgm. per liter of urine) contains 75 to 100 per cent of the original gonadotropic activity of the urine[3] as

[3] Application of this precipitation to other types of urine (pregnancy, normal women, normal men) has yielded excellent results. Hellbaum et al., 1935 (Hell-

indicated by parallel assays of the raw urine (or alcohol precipitates thereof). The activity of the dry tannate is quite stable, no loss being noted even after storage for more than a year at room temperature.

Assays are conducted by giving 20- to 22-day-old mice (8 to 12 grams body weight) subcutaneous injections once daily for 3 days, the animals being sacrificed 72 hours after the first injection. Because with minimal doses ovarian weight alone is an unreliable criterion, the mouse unit has been tentatively defined as that amount which, when administered in the above manner, will cause vaginal canalization, at least a 200 per cent increase in the weight of the uterus drained of fluid (controls 4 to 7 mgm.), and a slight increase in ovarian weight as compared to uninjected controls. Such a unit is very sensitive and in the absence of estrogenic substances is quite accurate. We have never been able to demonstrate the presence of any estrogenic activity in the alcohol washed tannates.

II. Blood. *Method of Fluhmann (1931).* The technic of the test used in this study has been described in two previous communications, and is based on the "pregnancy test" of Aschheim and Zondek. It consists in obtaining, under sterile precautions, from 15 to 20 cc. of venous blood from the patient to be examined. This is then allowed to stand or is centrifuged and from 3.0 to 5.0 cc. of the clear serum are injected subcutaneously, twice daily in from 0.5 to 1.0 cc. doses, into an immature female white mouse. (In view of the study by Engle and Rosaco the immaturity is determined by the age and not by the weight of the mice, and they are used only if between seventeen and twenty-two days old.) In the case of a positive result, the vaginal introitus of the mouse is established by the fourth or fifth day and the animal is then killed and its ovaries fixed in Zenker's solution. They are embedded in paraffin and serial sections are made and studied. The conditions which indicate the presence of anterior pituitary hormone are grouped into three categories by Aschheim and Zondek, as follows:

Anterior Pituitary Reaction One (APR I)—(The "ovulation" reaction): The ovaries show the presence of normal ripening follicles, while if autopsy is delayed normal corpora lutea are seen and ova may be found in the Fallopian tubes.

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baum, Fevold and Hisaw, Proc. Soc. Exp. Biol. and Med. **32**, 1566) have reported similar experience with pregnancy urine. Others (Thomsen and Pederson-Bjergaard, Comp. rend. Soc. d. Biol. **120**, 1143, 1935; Pederson-Bjergaard, Zbl. f. Gyn. **60**, 372, 1936; and Friedman and Weinstein, Proc. Am. Physiol. Soc., p. 54, 1936) have applied our method to normal urines with confirmatory results.

APR II—(The "hemorrhagic cyst" reaction): The ovaries grossly show the presence of fine reddish dots, which correspond to hemorrhages into normal and abnormal follicles, and are apparently due to the intense congestion set up by the hormone. In the present study the finding of this reaction is not charted, since it is invariably an accompaniment of reaction III and does not seem to yield any important additional information. In the diagnosis of pregnancy, however, it is of considerable value as it is readily perceptible grossly.

APR III—(The "luteinization" reaction): In this case the ovaries present a process of luteinization of the cells of follicles and the formation of abnormal structures resembling corpora lutea in which ovulation has not occurred and the ovum remains imprisoned. It is also worthy of note that these structures occur much sooner than normal corpora lutea would be formed following ovulation and in one instance they were noted forty-eight hours following the first injection of serum from a patient with an early pregnancy.

The changes in the vaginal mucosa which accompany the various processes seen in the mouse ovaries are also of interest and have been studied in a smaller series in view of the recent observations of Wiesner. In the case of APR I, the vaginal mucosa shows the cornification characteristic of the stage of estrus, and is identical to the change elicited by the ovarian follicular hormone in spayed mice (Allen-Doisy test). On the other hand, when APR III is obtained alone the vaginal mucosa is altogether different, as it is composed of tall cylindrical cells at the surface ("pseudopregnancy" of Evans and Long; "mucification" of Wiesner and Patel), and is similar in appearance to the mucosa of a mouse during gestation.

Method of Assay of Luteinizing Hormone

1. Method of Zondek (1935). a. The material is taken up in water.

b. It is then injected into infantile mice (6 to 8 grams) in six divided doses over a period of 36-hours. All injections are subcutaneous.

c. The animals are destroyed 100 hours after the first injection.

d. A positive test is presence of fully developed corpora lutea.

Zondek (1935) states that when young rats are used instead of mice they should be destroyed 120 hours after the last injection. Corpora lutea develop more slowly in the rat.

2. Method of Loeser (1931). Infantile rats are used. A positive test is the reaction obtained in 5 out of 10 infantile rats. At least one well-formed corpus luteum must be present in each ovary.

Method of Assay of Corpus Luteum Hormone

1. Method of Corner and Allen (1929); Allen (1930). An adult doe, preferably one which had been isolated for a month or more, is mated to one or two bucks and insemination proven by discovery of spermatozoa in vaginal smears made immediately after mating. The animal is thereupon again isolated until conclusion of the experiment. About 18-hours after mating the animal is anesthetized with ether and the abdomen is opened under aseptic precautions. If ruptured follicles are found in the ovaries as expected, the ovaries are removed. It is our practice to clamp the ovarian pedicle tightly with a small artery clamp, to transfix and ligate the pedicle with black silk on the proximal side of the clamp, and to cut away the ovary along the distal side of the clamp. Bleeding points are carefully controlled by ligature with silk, avoiding ligation or kinking of the Fallopian tube. Complete removal of the ovaries (to insure total ablation of the corpora lutea) is of course essential to accuracy of the test. A piece of the uterus about one or two centimeters long at the middle of the left cornu is removed between ligatures after the mesometrial vessels supplying the excised portion are tied and finally the cut ends of the uterus are approximated by tying together the ends of the two uterine ligatures. The portion of the uterus thus removed is preserved in Bouin's fluid for use as a histological control. The incision is closed in two layers with silk. The extract is administered subcutaneously in the dorsal region immediately following the operation and once daily thereafter until five doses have been given. The animal is killed 5 days after the operation, one day after the final dose, and the genital tract and other tissues are removed for examination. The sites of the ovaries are carefully searched for surviving ovarian tissue and any suspicious tissues are examined under the dissecting microscope and if necessary preserved for sectioning. The right uterine cornu, the left cornu above the ligature, and the two Fallopian tubes are successively cut from the reproductive tract and washed out into watch-glasses with normal salt solution by means of a hypodermic needle and syringe, in order to obtain the embryos. Finally the parts of the genital tract are fixed in Bouin's fluid for sectioning. A fairly accurate provisional estimate of the result can be obtained without waiting for paraffin sections, by examining with a dissecting microscope in a strong light the surfaces of razor-cuts made through the specimen after a few hours' hardening in the fixing-fluid. The result of the test is measured by the degree of progestational proliferation induced in the uterus, according to a standard described below.

The test is considered fully positive if at the time of necropsy the endometrium has attained throughout both cornua a condition similar to that of the uterus at the 8th day of normal pregnancy (as seen in sections taken from the undilated portions of the uterus between the implantation sites).

A "rabbit unit" is the minimum dose of an extract which suffices, when divided into 5 daily doses, to alter the uterus of a doe weighing 3 to 4 kgm., under the specified experimental test, to a state equal to that described in the preceding paragraph.

2. Method of Clauberg (1930). a. An immature female rabbit weighing about 800 grams is used.

b. The animal is injected with 10 M.U. of estrone daily 8 days before the corpus luteum injections are begun.

c. Corpus luteum extract is then injected for 5 days and the animal killed on the next day.

d. The uterus is submitted to microscopic study and the rabbit unit determined by the same method used by Allen and Corner.

Extraction and Assay of Male Sex Hormone From Urine

1. Method of Callow (1936). In order to obtain a result which is correct within ±20 per cent it is necessary to take a three-liter sample of an average urine for extraction so as to allow for administration of a total dose of extract equivalent to a half liter of urine per bird in a group of five capons. It is important that the urine should be kept fresh; it is preferably stored at 0°C. after collection, without preservative. The proportion of concentrated hydrochloric acid required to bring the urine to pH 1 is determined with a 100-ml. portion; with a fresh urine about 20 ml. per liter is necessary. Methylviolet is used as indicator, the color produced by the urine mixed with an equal volume of the indicator solution being compared with that produced by decinormal hydrochloric acid (the Capillator technique is convenient). The whole sample is then rendered strongly acid (pH below 1) by the addition of sufficient hydrochloric acid to bring it to pH 1, plus 20 ml. per liter in excess.

The mixture is placed in a flask fitted with a reflux condenser and a tube from the top of the condenser leading to an arrangement for absorbing fumes in alkali, and is then brought to the boil within ½ to 1 hour and boiled for 1 hour. It is then transferred to a continuous extraction apparatus and extracted with benzene for 20 to 24 hours. The benzene solution (500 ml.) is freed from acid by extracting twice with 50 ml. portions of a 2 N sodium hydroxide, and washed with water.

The benzene solution is evaporated to dryness and the residue is extracted four or five times with 10-ml. portions of redistilled ether. The ethereal extracts are filtered through a coarse sintered-glass filter, and the filtrate is evaporated bit by bit in a small, weighted, flat-bottomed tube. Sufficient arachis oil is added from a syringe to the residue (100 to 300 mgm.) to make the volume up to 3 ml. and the mixture is heated and stirred until all the oil-soluble material has dissolved.

This solution, containing the equivalent of 1 liter of urine in 1 ml. provides for daily injections of 0.1 ml. per bird in a group of five capons over a period of five days in the usual method of assay, giving, with a normal urine, an easily measurable response.

The yield of male hormone obtained in this way from pooled samples of normal female and male urine has amounted, respectively, to 29 and 26 international units of male hormone activity *per* liter (equivalent to 2.9 and 2.6 mgm. of androsterone per liter).

2. Female Bitterling as a Biologic Test Animal for Male Hormone (Kleiner et al., 1936). *Experimental.* The female bitterling develops an ovipositor which depends from the ventral margin of the body slightly anterior to the origin of the anal fin. In the quiescent state the ovipositor is seldom visible, but in those individuals in which it is visible out of season it is very small and rarely reaches 5 mm. in length. During breeding activity the organ is prolonged until it may reach 5 cm. in length and at spawning it is inserted into the inhalant siphon of a mussel, usually Unio or Anodonta, and the ova extruded into the gill-folds. Fertilization is effected by sperm which is liberated near and drawn into the inhalant siphon of the mussel and passed over the embedded ova. Hatching occurs within the gill-folds and the fry liberate themselves, in a post-larval state, two or three weeks after oviposition.

Description of Test. To a small aquarium is added 4 liters of water, 2 liters from a stock tank and 2 liters from the tap. Two female bitterlings are placed in the tank and kept there 24 hours before introducing the material to be tested. At the end of the 24-hour period readings are made of the size of the ovipositor. The scale used is as follows: If the ovipositor is not visible the reading is 0; if the length of the ovipositor equals the length of the first ray of the anal fin the reading is 10; an ovipositor which reaches halfway down the first ray is 5; etc. (That is, the length of the ovipositor is compared to that of the first ray of the anal fin in equal units running from 0 to 10). No fish with ovipositors exceeding 3 on the scale, before the addition of any test material, are used.

Ovipositor readings are taken at 24-, 48-, and 72-hour intervals and maximum growths under these conditions are usually observed at the second reading.

We tentatively define a bitterling unit as the amount of material which, when added to a tank containing 4 liters of water and 2 female bitterlings, produces an increase in the length of the ovipositor of one or both fish of 7 or more on the scale within 48 hours. For assay it is suggested that a series of dilutions may be set up and the lowest dilution giving a positive reading may be considered to contain at least one unit, providing a positive reaction is also given in the next higher dilution.

Technique for Assay of Urine. In attempting to determine the amount of male hormone in normal male urine, the following procedure was employed, using normal male medical students as subjects: For each assay, 4 aquaria were set up, containing 4 liters of water and 2 female bitterlings in the usual manner. To each tank was then added 10-, 25-, 50-, and 100-cc. portions of urine respectively. Twenty-four-hour samples of urine were used. The smallest amount causing a positive reaction was considered to contain one unit. The number of units in a 24-hour sample was then calculated. It was noted that in many instances the urine was toxic and even fatal in amounts from 50 to 100 cc. Various methods have been tried in the attempt to detoxicate the urines and finally it was found that dialysing is all that is necessary. By this means dialysed urines representing as much as 200 cc. of the original may be used without any ill effects whatever. Ordinarily the procedure is the following: Measured amounts of urine, usually 200 cc., are dialysed in membranes of cellophane ("plain transparent" not "moisture-proof") against running tap water for 18 to 24 hours. The volumes are then measured and amounts equivalent to 10, 25, 50, and 100 cc., respectively, of the original urine are added to 4 aquaria, each of which contains 2 female bitterlings. The ovipositors of the fish must have been read on the preceding day and also just before addition of the dialysed urine. Subsequent readings are made in 24, 48, and 72 hours, and the number of units determined in the manner suggested above. The average excretion of male hormone is about 35 bitterling units per day with a range of approximately 15 to 75 b.u. (see table 11). Further work to check and enlarge this series is in progress.

Preliminary tests have indicated the unreliability of using casual specimens for even rough quantitative work. We have noted that successive samples obtained during the day from the same individuals

have been exceedingly variable in the amounts of hormone excreted and some samples are even entirely devoid of the hormone. Apparently 24-hour samples of urine are needed in order to determine the output of this hormone with any degree of accuracy.

3. Method of Darby,[4] (unpublished). *Chemical Procedure.* Funk et al., (1929) and Adler (1934) have shown that a substance which promotes comb growth in capons can be extracted from acidified urine by lipoid solvents. Extracts from urine which has not been acidified are inactive. However, as Adler has shown, the active substance can be demonstrated in such extracts after treatment with strong acid. It seems that the male hormone is ordinarily excreted in a physiologically inert form.

The first step, then, in the assay of this substance, is the acidification of the urine sample (preferably a 24-hour specimen) by the addition of 1 ml. of concentrated HCl for each 100 ml. of urine. The final pH

TABLE 11

Daily Excretion of Male Hormone by Normal Adult Males

NUMBER OF 24-HOUR URINE SPECIMENS	NUMBER OF CUBIC CENTIMETERS OF URINE WHICH, WHEN ADDED TO 4 LITERS OF WATER, PRODUCE A POSITIVE REACTION	BITTERLING UNITS EXCRETED DAILY
5	10– 15	75–120
15	25– 35	24– 45
10	75–100	9– 22

should be between 1.0 and 2.0. Thymol blue is a convenient indicator to use for this purpose. The acidified urine is then allowed to stand for several days at room temperature, to allow the hormone to be hydrolyzed to the free state. If there is need for immediate assay hydrolysis can be speeded up by gentle heating (50°C. for four hours); but it should be remembered that heating tends to destroy some of the hormone, so should be avoided whenever possible.

The urine is then placed in a large separatory funnel, and shaken thoroughly with three-quarters of its volume of ethyl ether. The ether layer is separated and placed in another container, and the urine extracted twice more with fresh portions of ether (about one part ether to two parts urine). The urine is then discarded. The ether extracts are pooled together in the separatory funnel, and washed with two 25-

[4] Hugh Darby, Ph.D., Fellow, Dept. of Biochemistry, College of Physicians and Surgeons, Columbia University.

ml. portions of N/5 NaOH. This alkaline washing serves to remove all of the acid, practically all of the pigments, and any estrogenic substances which may be present. It is followed by several washings with 100-ml. portions of distilled water, until the washings when tested with brom-thymol-blue have a pH of about 7.2 to 7.4. If enough time is allowed between washings for the water to settle out of the ether, three washings will suffice; but if the changes of water are added in rapid succession, more than three may be needed to reduce the pH to the desired level.

The ether now contains the hormone free from estrogenic material, but there remains a certain amount of water dissolved in it. In order to get rid of this water, as it will interfere with the ultimate dissolution of the active residue in oil, some anhydrous sodium sulphate (about 2 grams per 100 ml. of ether) is placed in the funnel with the extract. This absorbs practically all the water present, and clings to the walls of the separatory funnel so that the ether can be decanted quantitatively. If there is enough water to dissolve any of the salt (that is, if any droplets appear amongst the salt crystals), more sodium sulphate is added.

The dried ether extract is next placed in a large distilling flask and the ether distilled off over a water or steam bath. The distillate can be saved and used repeatedly. When the residue has been reduced to about 10-ml. volume, the distillation is stopped, and the concentrate is transferred with a capillary pipette to a 50-ml. Erlenmeyer flask. The distilling flask is rinsed with a little ether, which is added to the concentrate. The last of the ether is then evaporated from the concentrate over a water bath (60°C.), leaving a gummy or syrupy residue. This is taken up in 10 ml. of sesame oil, with thorough mixing. It is now ready for injection.

The chemical procedure can be summarized as follows:

(1) Acidification of urine specimen.

(2) Hydrolysis over several days of storage.

(3) Ether extraction of urine (three times).

(4) Washing of ether extract with alkali (twice) then with water, to neutrality.

(5) Removal of dissolved water from extract.

(6) Concentration of extract by distillation; and finally, after transfer to small container, by evaporation.

(7) Dissolution of the residue in sesame oil.

Biological Procedure. At the second Conference on the Standardiza-

tion of Sex Hormones, held under the auspices of the League of Nations in 1935, the following standard was adopted for the assay of the male sex hormone: namely, the amount of growth produced in the comb of a capon by the daily injection for six days of 0.1 mgm. of androsterone. The amount of growth is expressed as the percentage increase in the area of the comb. The unit dose of androsterone (0.1 mgm. = 100 gamma) is physiologically equivalent, in the capon, to 15 gamma of testosterone. Hence testosterone is about seven times as active as androsterone. More will be said of the relative activities of these compounds later. Suffice it to say here that the capon test is the recognized method of assaying male sex hormones.

Capons to be used for this test should be between one and two years old. Leghorns are quite commonly employed.[1] If possible, they should come from the same brood. Many agricultural schools will be pleased to supply capons or assist in caponizing the birds. They should be caponized very young, as the operative mortality increases with age. When caponization is complete, the comb shrinks to a small white mass on the head, and the wattles below the beak become so small as to be hardly noticeable. Sometimes caponization is incomplete. In such cases, although the bird at first shows a shrinkage of the comb, it later grows a small but bright red comb. These incomplete capons, or slips, as they are called by poultrymen, are useless for experimentation.

The preparation under test is injected intramuscularly into the breast of the capon, care being taken not to inject into a feather follicle. A tuberculin syringe fitted with a No. 27 needle, three-quarters of an inch long, has proved satisfactory. The dose is administered daily on alternate sides of the breast for six consecutive days.

The oil used in the preparation should always be sesame oil, as the work of Miesscher et al. (1936) has shown that the solvent can markedly affect the amount of response obtained. The writer, when injecting known quantities of a pure preparation of testosterone into fishes, also obtained entirely different results depending on whether the material was dissolved in propylene glycol or olive oil. As sesame oil is distinctly superior to other solvents which have been tried experimentally, it should be used as the standard carrier.

Measurement of Response. The reaction of the capon to the administration of the hormone is the commencement of growth within the first 24 hours. The first observable sign of response is the replacement of the white color in the comb and wattles by a bright pink. This reaction does not lend itself to quantitative measurement, hence

changes in the size of the comb are used as an index of response. The procedure generally recommended for measuring the comb is to make a shadow picture whose area may then be determined with the aid of a planimeter. This measurement is made at the start of the test, prior to the first injection, and is repeated daily until the comb has passed through its maximum size, and has begun to regress. In the presence of the hormone, the comb grows steadily over the injection period, and for a few days thereafter. It then starts to regress. The speed with which the comb reaches its maximum size with a given hormone dosage has been found to be somewhat variable; and in the case of testosterone, at least, depends to a large extent on the nature of the acids with which

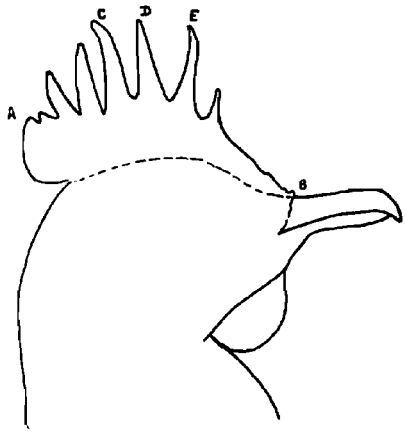

FIG. 178. Points of reference for comb measurements

it is esterified. Hence it is important to continue the measurements until the maximum growth has been reached and passed, to make sure of observing the complete response.

The writer has simplified the technique of estimating comb area as follows: The comb is measured along its length (distance *A–B* in fig. 178) with a pair of dividers, and the length recorded in centimeters. The three longest prongs of the comb (*C*, *D* and *E* in diagram) are measured from their tips to the base, where the comb joins the head. These heights are also expressed in centimeters, and are added together and divided by three to give an average prong height. This value is multiplied by the length *A–B* to give an arbitrary over-all area. The

measurements are repeated daily throughout the assay period. The original area (length × average height) is subtracted from the new area as obtained each day, and the percentage of growth calculated by dividing the (100 × the difference) by the original area. The daily percentage increases are then plotted against the time in days. A typical set of figures is given in table 12.

Estimation of Hormone Present. The dosage of test material used depends on what kind of information the assay is expected to give. If the only answer wanted is whether there is male sex hormone present or not, then the simplest procedure is to give a daily injection of 0.5 ml. of the preparation to each of three birds for six consecutive days. If the hormone is present in large quantities, the combs will show an increase well above 60 to 70 per cent by the eighth day. This method

TABLE 12

DAY	LENGTH OF COMB (A–B)	HEIGHT OF PRONG				AREA	PERCENTAGE INCREASE
		C	D	E	Average		
0	4.9	2.9	2.7	2.4	2.67	13.1	
1	5.1	3.0	2.8	2.5	2 77	14.1	8
2	5 45	3.15	3.1	2 6	2 95	16.1	23
3	5.6	3.2	3.1	2.7	3.00	16 8	28
4	5.7	3.2	3.1	2.8	3.03	17.3	32
5	5.8	3.2	3.15	3.0	3 12	18 1	38
7	5 8	3 25	3 20	3.0	3.15	18.3	40
8	5.6	3.25	3.15	2.9	3 10	17 4	33
10	5.6	3 25	3.15	2 85	3 08	17.2	32

will not enable the experimenter to tell how much male sex hormone is in his preparation, but will furnish a rough idea of its activity. The reason for the inadequacy of this procedure for quantitative purposes will be apparent from the next paragraph.

If a careful assay is needed, the basis of the international standard must be understood. The international unit of activity is the amount of growth produced by the daily injection of 100 gamma (0.1 mgm.) of androsterone for six consecutive days: i.e., a maximum increase in the comb area of 30 per cent. This maximum is generally reached by the eighth day. In measuring the activity of an unknown preparation, the dosage level must be adjusted to the point where six equal daily injections produce the standard 30 per cent increase in comb area, *and no more.* That is to say, a 60 per cent increase does not mean exactly twice the hormone dosage which produces a 30 per cent increase; much

less does an increase of 120 per cent indicate four times the unit dosage level. The figure of 30 per cent has been selected as the dosage level at which the bird reacts with the utmost sensitivity. Hence, to return to the practical problem of assaying our 10 ml. of oily material of unknown activity, the following procedure is recommended.

First inject 0.5 ml. of the preparation into one capon daily for six days. Calculate the percentage increases, and plot the figures obtained. If the maximum goes above 60 per cent, then the remaining material (7 ml.) must be diluted with sesame oil. The physiological response indicated by 10 to 50 per cent increases in comb area is about proportional to the amount of hormone administered; but above 60 per cent the response is no longer proportional to the dose, hence there can be no blanket rule for dilution. The writer uses the following scheme. If the maximum response is 60 per cent, dilute by adding one part sesame oil to one part of preparation. If the response is 80 per cent, add three volumes of sesame oil to one volume of preparation. Experience with this material and familiarity with the graphs obtained following excessive doses soon give an idea of what dilutions ought to be made. If enough birds are available, it is advisable to inject some standard preparation in sesame oil at several different dosage levels. The graphical records of the responses can then be kept for reference. The dilution of the unknown finally selected should be tested on three or four birds to average out biological variations. If instead of a 30 per cent increase, a slightly higher percentage is obtained, let us say 45 per cent, it is permissible to estimate this as a response to 1.5 capon units of male sex hormone.

Having arrived at a dosage level corresponding to approximately one unit of activity, the next problem is to calculate the total number of capon units in the preparation. For example, in the case already tabulated on page 181, the growth obtained (40 per cent) corresponds to 1.33 units. This was produced by a daily dose of 0.5 ml. of undiluted preparation. Hence the total unitage of the whole 10 ml. of preparation is $1.33 \times 2 \times 10 = 26.6$ units. (In cases where dilutions have to be used to bring the activity within range of measurement, the dilution factor has to be included in the above calculation.) In this particular case, two 24-hour specimens, of a total volume of 3 liters, had been extracted; hence the unitage was 13.3 units per day, or 8.9 units per liter. The foregoing procedure does not define the chemical nature of the active material in the urine, but it does furnish a measure of its physiological activity.

Other Methods of Assay. It must be remembered that the capon method of assay is the only one which has been standardized, and is therefore of great value in enabling various workers to compare their results. It is therefore the method of choice whenever possible. However, other animals have been employed, chiefly the castrate rat.

In the rat method of assay, the hormone is estimated from the amount of growth induced in the seminal vesicles of castrated animals. Young growing castrates are injected with the hormone for ten days, and killed on the eleventh day. The seminal vesicles are dissected out and weighed, and compared with the weights of the seminal vesicles of uninjected castrate controls. The difficulty of this method lies in the establishment of the control value. A number of animals, castrated at the same time as the experimental group, must be carried parallel with them, and sacrificed at the same time. All the rats must come from the same stock, and be as nearly homozygous as possible, in order to keep variability at a minimum. At the start of the experiment, about 20 days after castration, the rats should weigh between 60 and 70 grams. There is reason for believing that the rat, in contrast to the capon, shows qualitatively different responses to different forms of male sex hormone; and it has been suggested that both ought to be used for testing (Korenchevsky et al., 1935; Miesscher et al., 1936). However, no standard procedure has yet been agreed upon.

Another animal which has been suggested in this connection is the female bitterling, *Rhodeus amarus*. Kleiner et al. (1936) reported that the ovipositor of this fish lengthens in response to the presence of male sex hormone in the water. The possibility of utilizing the reaction for assay purposes is limited by its lack of specificity. It was originally offered as a test for female sex hormone. In our experience, the length of the ovipositor is highly variable in untreated normal bitterlings and may be increased by a number of stimuli, such as high temperature or the addition of any foreign material to the aquarium that causes fouling of the water. According to Kleiner et al., pure preparations of androsterone and testosterone are effective within narrowly limited ranges of concentration. The writer has attempted to repeat these observations with crystalline compounds, but has been unable to do so. The female bitterling does not seem to offer any possibilities whatever for assaying unknown amounts of hormone in urine.

Since the whole subject of the sex hormones is in a state of rapid development, it is essential for anyone interested in clinical applications to keep in close touch with the current literature.

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AUTHOR INDEX

C

N

S

T

SUBJECT INDEX