THE MICROSCOPIC ANATOMY OF HUMAN ENDOCRINE ORGANS

A Thesis

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Master of Science

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by

Jack Edwin Newell

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sections stained by the Masson trichrome and simple stain techniques. Miss Toupin labelled many of the drawings, while Miss E. Green labelled some of the remainder. Miss Monk and the library staff have been most cooperative.

The writer wishes to acknowledge with appreciation the written permissions obtained from the following publishers: The Wistar Institute of Anatomy and Biology for permission to reproduce Figure 1, of "The Meningeal relations of the hypophysis cerebri" by H. G. Schwartz, appearing in the Anatomical Record, Volume 67, pp. 35-44; The publishers of The Bulletin of the Johns Hopkins Hospital for permission to reproduce Figure 1, of "On the glandular elements in the posterior lobe of the human hypophysis" by Dean Lewis and F. C. Lee, appearing in the Bulletin of the Johns Hopkins Hospital, Volume 41, pp. 241-277; The W. B. Saunders Company, Phila., publishers of A Textbook of Histology, by A. A. Maximow and Wm. Bloom, Fifth edition, 1948, pp. 700, for permission to reproduce Figure 503, page 572 (from Hertig and Rock, Carnegie Contrib. to Embryol., Vol. 29, 1941, Fig. 13).

The pen and ink drawings were done by the author and any shortcomings or errors in them are due to him.

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INTRODUCTION

One difficulty encountered in the histological study of any human organ is the continued reference in textbooks to animal material. To the student of human histology and pathology, this practice is most discouraging. The histology of one species is often similar to that of another species, but the finer details seldom coincide. The details of animal histology are important to the investigator in pure research, providing the histology of cats is used when he is working with cats, rats when working with rats, humans when working with humans and so on. In the early phases of this study, it soom became apparent that the authors of some textbooks were in many instances describing the histology of lower animals, the details of which were not strictly applicable to the human except in a general way only. Further, statements made and remade came to assume reality and finality but, in the light of more recent investigations, did not hold. For some reason, many papers dealing with human material have been neglected and are not included in the usual textbooks. An attempt has been made to list some of these, but the lists admittedly fall short. The earlier experiments and studies of pioneer investigators using animal material have contributed to the later study of human material by others and in this particular study only human material was utilized.

Human material suitable for study is extremely difficult to obtain. Most of the material used and studied was obtained at autopsy within half an hour of death. To collect the material for the few organs described, over one and one-half years were required. Ultrafresh material is seldom

seen by the pathologist. A parallel series of organs which have undergone the usual postmortem degenerations should be included but this work is confined to a more or less academic study until time and other factors permit further work.

The variations of most organs at different age groups are well recognized, but there are actually few comprehensive reports available. Such organs as the ovary and the uterus are well studied, but only recently has a report on the variations of the testes been published.

Not all the organs known to produce hormones have been included, but rather those organs whose major or secondary function is hormone production. Thus, the thyroid, parathyroid, pancreas, adrenal, testis, ovary, placenta and hypophysis cerebri are described. Each chapter contains a brief introduction, the nerve supply, blood supply, lymphatic drainage, gross description and embryological note followed by a more or less detailed description of the histology. It is impossible to attempt any summary of the endocrinology literature. The monographs of Professor A. T. Cameron and Professor Hans Selye are excellent source books and have been utilized for this aspect of the work.

Many problems are still unsolved. Many of these problems have more than academic importance. The problems have all been appreciated by former investigators; indeed, some form the basis of strong controversy in the literature. Many of the problems do not lend themselves to present methods of investigation. Others have apparently given way to the most recent means of investigation such as ultramicroscopes, enzyme staining, etc. The nerve supply of the testis, and especially the distribution of

the nerves within the organ, await confirmation. The distribution and the origin of the lymphatics within the testis is still to be determined satisfactorily. The lymphatic drainage and the distribution of the islets of the pancreas await further work and exploration. The blood vascular pattern of the human ovary and the blood supply within the human adrenal apparently require re-investigation. Spermatogenesis of the human has not been worked out in final detail. Ovulation in the human female has never been observed. A method of quantitative estimation of the islets in the pancreas and the interstitial cells of the testes is still wanting. Further confirmation of Spanner's work is being offered only in part. The lymphatic drainage of the parathyroids is still unknown. Days 3 to 6 inclusive are still unknown in the life cycle of the human zygote. The microscopic structure of the thyroid gland is far from being settled and there are still some who include interfollicular epithelium in their discussion of the organ, despite the fact that Reinhoff and other able investigators have disproved its existence. Lastly, the dural relations of the pituitary body are still ill defined.

Despite all these hiatuses in our knowledge of human histology, the respect for early investigators grows steadily when one studies their papers.

C H A P T E R O N E

THE ADRENAL GLANDS

(Suprarenal Bodies)

CHAPTER I

THE ADRENAL GLANDS

Introduction. The paired adrenal glands, one at the upper pole of either kidney, are composed of two distinct types of tissue, each of which has a different origin, produces different hormones, and has different staining reactions. On cross section, the fat laden pale yellow cortex is seen surrounding the dark red and grey vascular medulla.

Each normal gland[1] weighs from 3 to 5 grams, and measures from 40 to 60 mm in length, 20 to 30 mm in width, and 2 to 8 mm in thickness. The size and weight vary in different individuals, in sex, in health and disease.

I. THE CORTEX

The capsule. The well defined capsule consists of dense connective tissue embedded in which are arteries, arterioles, nerves, and collections of sympathetic ganglionic cells. Fine reticular fibres arise at right angles from the capsule, traverse the cortex and are found in close association with the radial cortical capillaries. These reticular fibres are well seen in silver, periodic acid, Mallory aniline blue, and Masson trichrome preparations.

The blood vascular system. Three arteries usually supply the adrenal gland, the superior suprarenal artery, the middle direct from the aorta, and the inferior from the renal artery[2]. The smallest

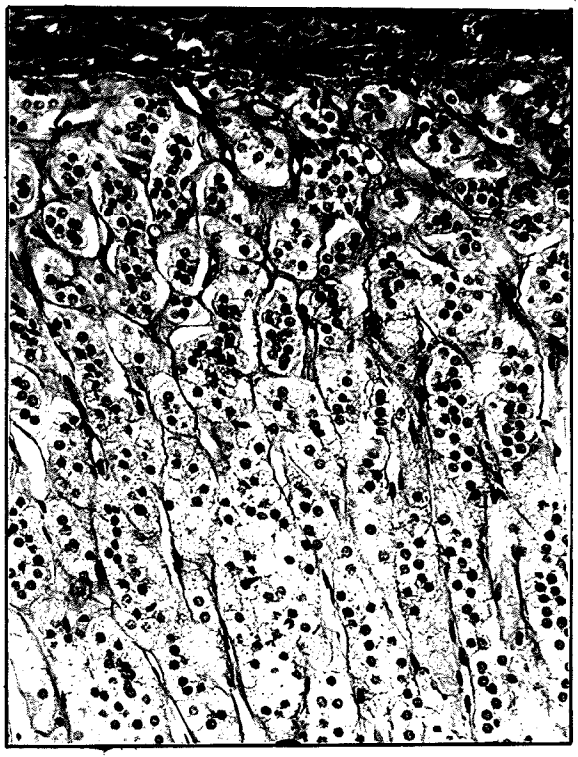


FIGURE 1

Reticulum of the capsule, zona glomerulosa and zona fasciculata. The fibres arise at right angles from the capsule supporting the endothelial cells and the parenchymatous cells. Formalin fixation. Hematoxylin and eosin, plus periodic acid. 200 x.

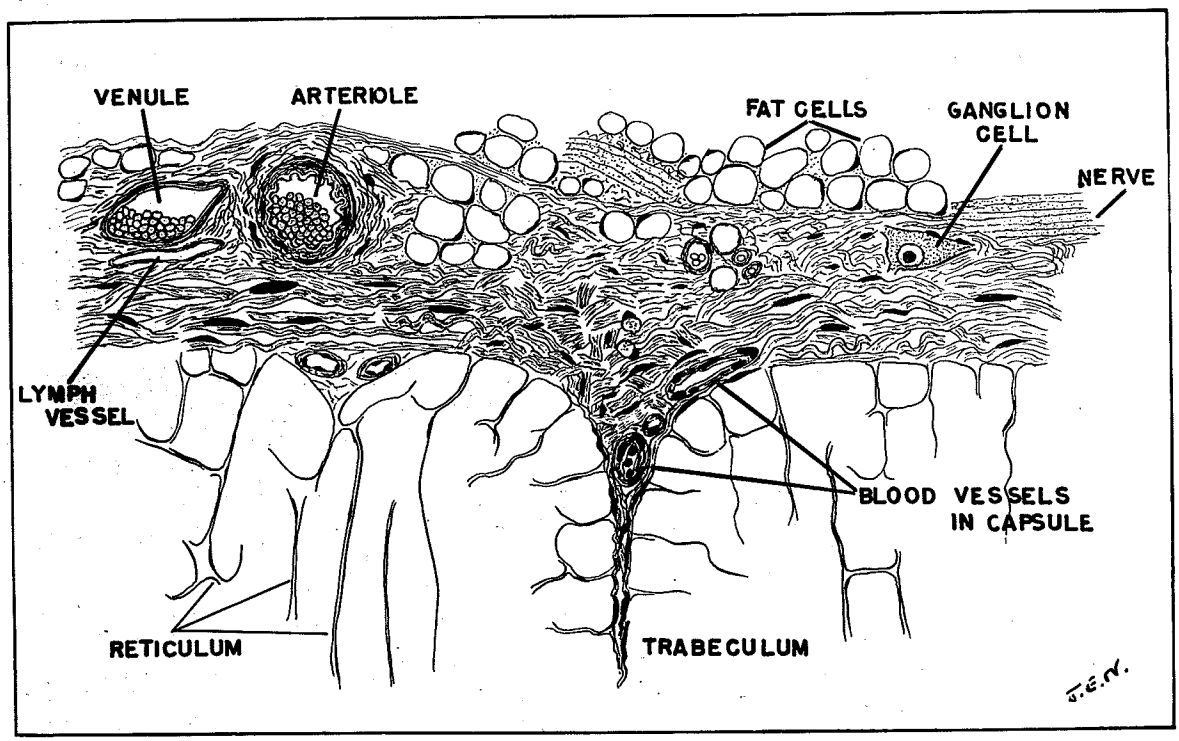


FIGURE 2

Composite drawing of the adrenal capsule. Different fields were used from various preparations. Approximately 350 x.

arterioles of the capsule empty directly into the capillaries of the cortex, while some of the larger arterioles pierce the cortex to empty into the medullary sinuses. The medullary sinuses, which originate from the continuations of the cortical capillaries and from the arterioles passing directly from the capsule, eventually drain into central veins which join to form a single central vein that leaves the gland at the hilus. The diagram of Flint[3] serves well to illustrate the vascular system of the adrenal gland (Cf. post). No lymphatics have been demonstrated in the substance of the gland except those around the larger veins of the hilus[4]. Endothelial cells and fixed macrophages line the capillaries and the sinusoids. The macrophages are part of the reticulo-endothelial system.

The cortex. Three vaguely defined layers of the cortex are recognized in the adult gland, an outer zona glomerulosa, a middle zona fasciculata, and an inner zona reticularis. The transition from one zone to another is gradual. Several names for the various layers have been proposed by different authors. The terms acceptable are listed on the right-hand side of Figure 5 (Cf. post), and are taken from Cowdry[5].

The narrow zona glomerulosa is situated just below the capsule, the small cells being arranged in clusters or in closely packed ovoid groups. There is close association with the capillaries which, being the first in Cowdry's vascular gradient[5], receive the arteriole supply from the capsular vessels. Most of the cells have an outer free border adjoining a capillary. The nuclei stain deeply, and in man irregular

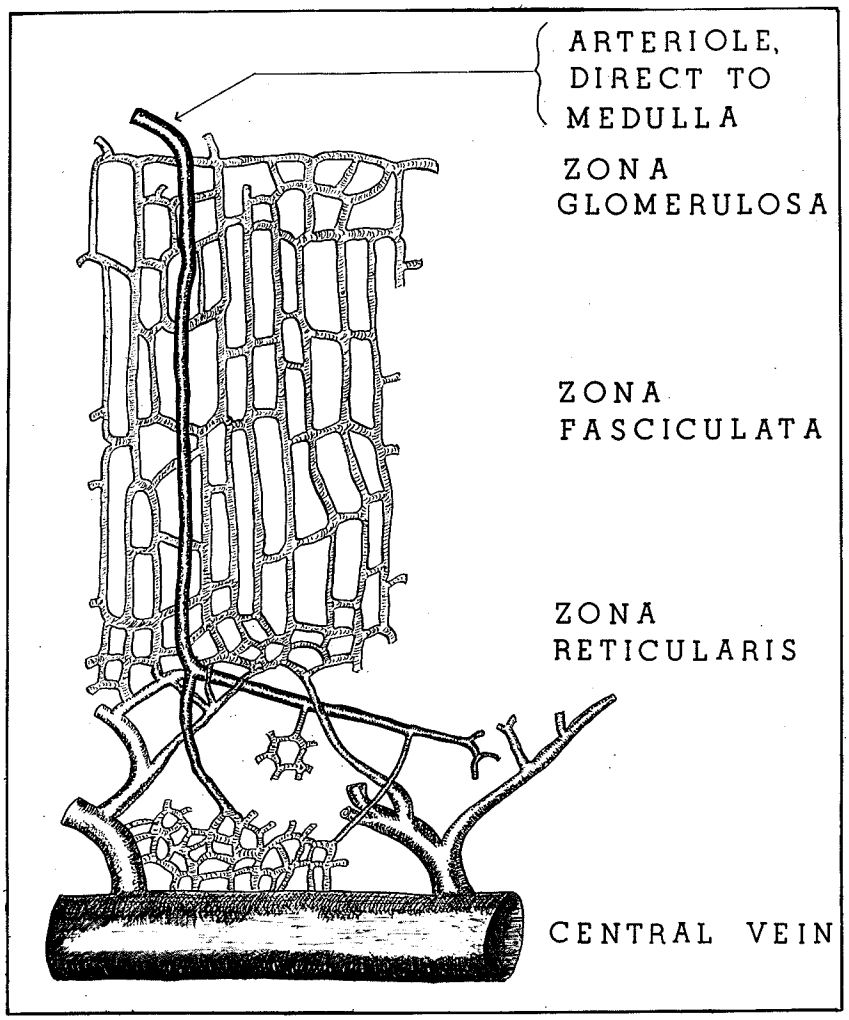


FIGURE 3

Diagram to show the arrangement of the intrinsic blood vessels in the cortex and the medulla of the dog's adrenal. (Redrawn and modified from Flint, J.M.) Contribution Sc. Med. .... Pupils W. N. Welch, Balt., 1900. 153-228.

FIGURE 4

Adrenal cortex and junction of medulla. Specimen from a 24 year old male who died of self inflicted gun wound. Death 8 hours after injury. Mercuric chloride formalin fixation. Masson's trichrome stain. 50 x. No. WGH A 7025.

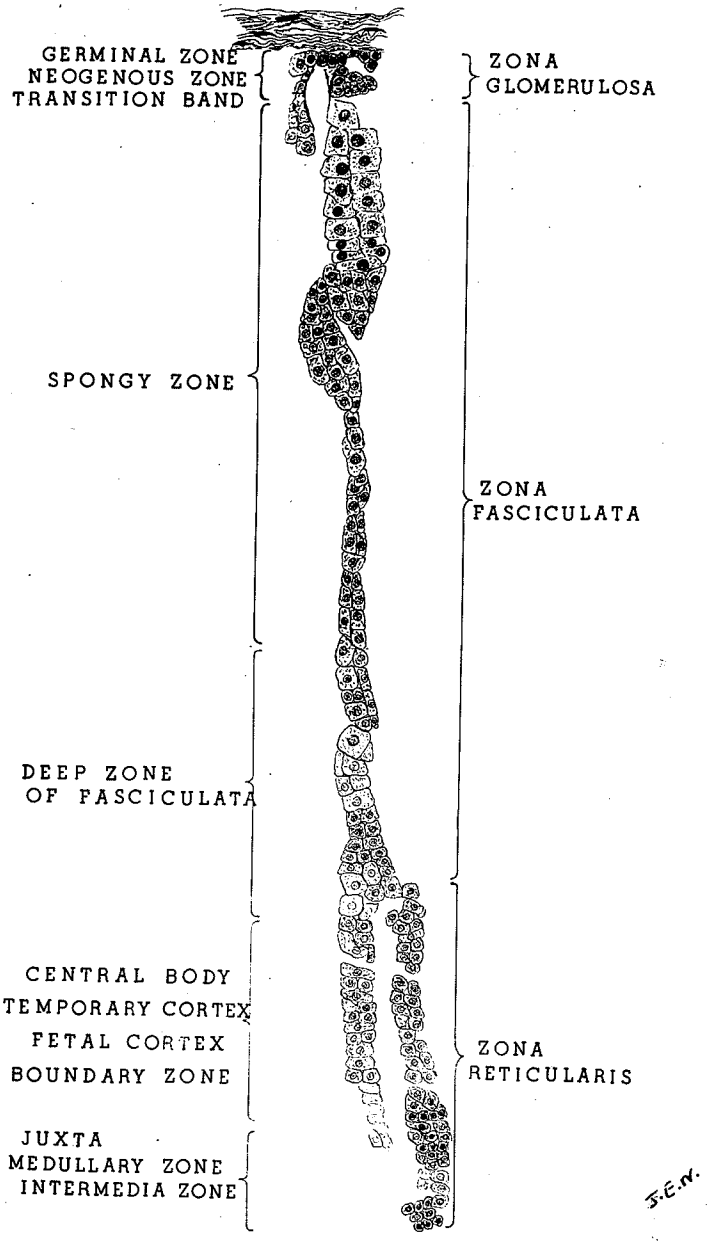


FIGURE 5

Drawing of a portion of the cortex. The names of the various zones given by different authors are listed on the left-hand side of the figure. Those accepted by Cowdry are listed on the right-hand side. From the same preparation as Fig. 4.

clumps of material in the scanty cytoplasm take on a chromatin stain. The cells of the layer contain little fat when stained with Sudan IV, Oil Red "O" or osmic acid. When present, the droplets are often seen on one side of the nucleus next to the capillary; however, this relationship is not constantly found. Many investigators find that the growth of the cortex occurs in this region[6, 7], the glomerulosa cells arising from indifferent cells resembling connective tissue (splanchnic mesoderm) and transform to the specific lipid rich spongiocytes.

In the wider zona fasciculata, the cells are polyhedral, larger, contain more fatty substance, and are arranged in columns which are more obvious. Capillaries and fine reticulum separate the columns and are well illustrated in material fixed in mercuric chloride formalin since the red blood cells are well preserved and take on a brilliant red stain. In paraffin sections, the lipids of the cells being dissolved out, the cells have a vacuolated appearance and are called "spongiocytes". The nuclei are centrally placed and occasionally there are two in the one cell. Groups of cells, having less lipid content, take on a deeper red stain in trichrome preparations, and are usually found in the deeper layers. Zwemer's work[6] explains the reason for this difference in lipid content of the cells. This work is discussed in connection with the life cycle of the cortical cells on page 17.

In the innermost zone of the cortex, the zona reticularis, the cells form a network, the columns projecting into the medulla for varying distances. A close relationship to the capillaries still exists. In the outer part of the zone, similarity of the cells to those of the

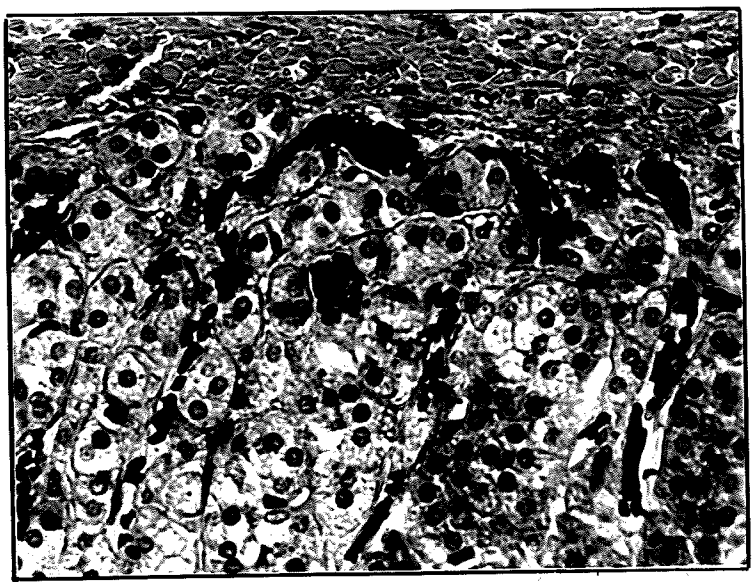


FIGURE 6

Zona glomerulosa and the capsule. The vascular spaces are congested, and appear as dark masses. Mercuric chloride formalin fixation. Masson's trichrome. 350 x. Same case as Fig. 4.

FIGURE 7

Zona fasciculata. Vertical columns of spongiocytes, vascular spaces and reticulum are seen. Same preparation as Fig. 4. 350 x.

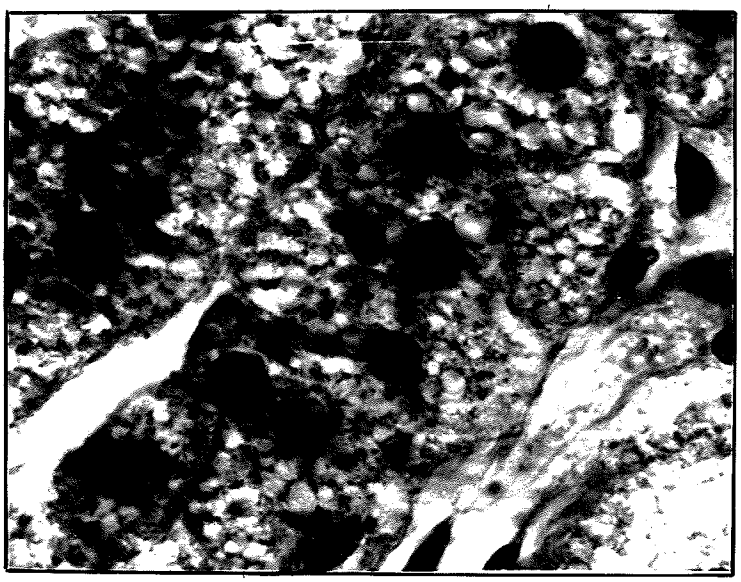


FIGURE 8

Appearance of the spongiocytes under oil immersion lens. 1200 x. Same tissue as Fig. 7.

fasciculata is noted, with one exception: there is less lipid material in the cells. Near the medulla, however, two distinct cell types are seen: a larger "light" cell, with granular cytoplasm and pale vesicular nucleus, and a smaller "dark" cell which has deeply staining cytoplasm and dark shrunken hyperchromatic nucleus. These dark cells are rich in lipid droplets and contain yellowish or brownish pigment granules (especially in older individuals).

The cortical lipids. The lipid droplets stained with conventional techniques (Sudan IV, Oil Red "O", and osmic acid) are small and numerous in some cells, fewer and larger in others. In any given cell the size is approximately the same.

The fat-like material is apparently a mixture of doubly refractive cholesterol esters and isotropic fatty inclusions of neutral and fatty acids[9].

Development of the cortex. The adrenal cortex develops from splanchnic mesoderm[11]. In embryos of 5 to 6 mm, the mesothelium at the level of the upper third of the mesonephros proliferates and sends buds into the mesenchyme at each side of the root of the dorsal mesentery[1]. Somewhat later in development, at the 17 mm stage, lateral migration of primitive cells of the medulla (sympathogonia) towards the cortical anlage begins. Migration is complete and the medulla formed at the 85 mm (to 100 mm) stage[11].

In fetal life the adrenal is almost all cortex, and at the third month is actually larger than the kidney. Von Gierke[32] gives the following

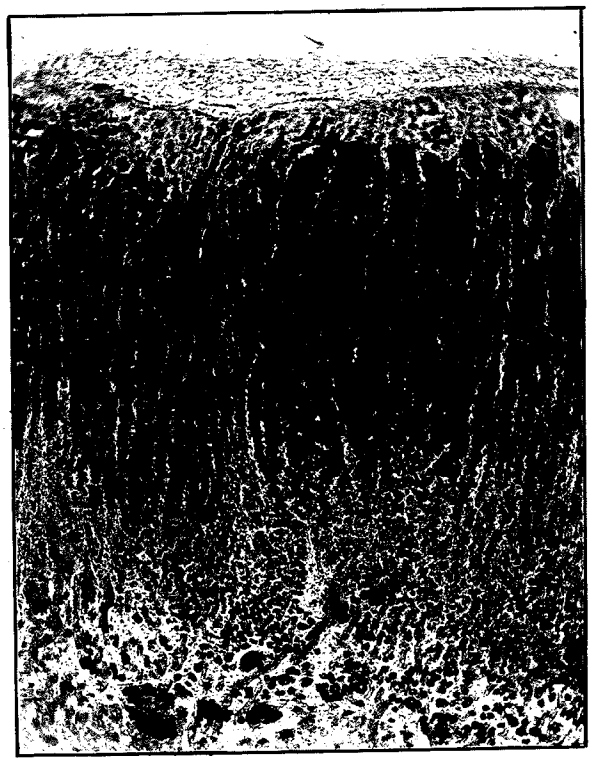


FIGURE 10

The distribution of lipids in the adrenal cortex of a 50 year old male who died suddenly of unsuspected brain abscess. Tissue obtained three quarters of an hour post-mortem. Formalin fixation. Oil Red 'O'. The stain appears black in the photograph. 50 x.

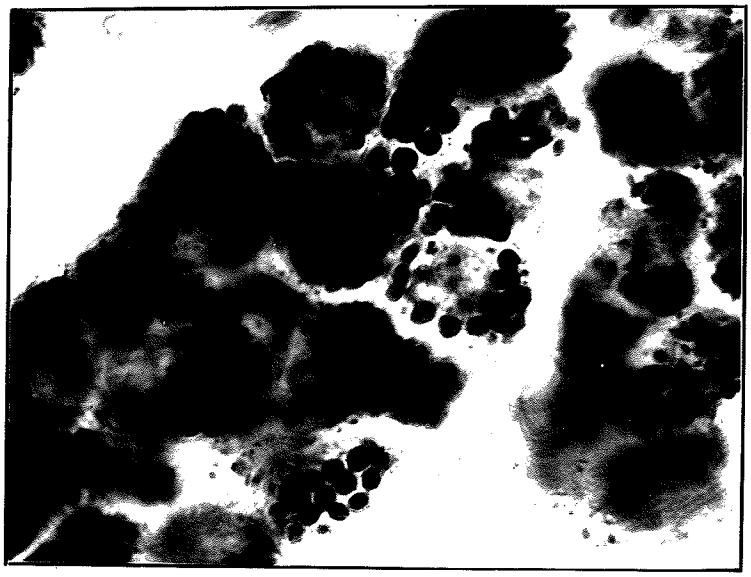


FIGURE 11

The same tissue as Fig. 10, seen with oil immersion lens. 1200 x.

size comparisons. During the first half of fetal life the adrenals are larger than the kidneys, at six months perhaps 1:2, at birth 1:3, and in adults the kidneys are larger than the adrenals in the ratio of 1:28. The cells of the fetal cortex contain no lipid material and are arranged in sheets instead of the columns described in the adult type of cortex. At birth there is a thin layer of cortex of the adult type around the medulla. The entire fetal cortex degenerates after birth, and is slowly replaced by cells of the adult type, the change being complete about the end of the first year of life[12].

Life history of the cortical cells. Zwemer[6] in agreement with other investigators finds that the cortex grows from without inward, the new cells being formed at the periphery and destroyed in the reticular zone. This investigator finds also that the glomerular cells arise from indifferent connective tissue-like cells in the capsule.

> These capsular cells lose their long processes, become short ovals, and take up lipoid droplets. A further increase in the amount of cytoplasm and marked increase in cell fats mark the transition to the spongiocytes. These retain and emulsify their fat content as they are gradually pushed inward by the formation of new cells. . . .
>
> As the cells secrete, the ratio of cytoplasm to nucleus is greatly diminished, so that the innermost regions of the cell cords consist of rows of nuclei with very small remnants of cytoplasm still surrounding them. In the end stage the cell is represented by a pyknotic nucleus which is finally phagocytosed. (cf Zwemer)

Ingle[8] has remarked that the three zones of the adult adrenal cortex represent different phases in the life history of the cortical cells. Thus, one may say that the reticularis is the graveyard of the cortex.

Variation in cortical morphology. As in many other of the specialized organs, the adrenal suffers early in post mortem changes. For this reason it is difficult to obtain strictly normal human material. Conditions existing in the organism immediately prior to death have profound influence upon the cytology and morphology of the cortex. Thus Zwemer[6] describes at least eight variations of the cortex, depending on the demand of the body for the corticosteroid hormones before death.

Variation in cortical thickness. There is marked variation in the thickness of the cortex and of the fat content of the cells. Thus Whitehead[10] demonstrated wide variations in the adrenal cortex of normal guinea pigs. There is extensive hypertrophy during fetal development, puberty, pregnancy, and in scurvy[5]. The effect of other hormones also influences the thickness of the cortex, being a factor in some of those conditions mentioned. Slight enlargement follows castration and the administration of adrenalotropic hormones[12]. The administration of oestrogens is followed by cortical hypertrophy, while androgens may cause regression in size[13]. In most, if not all, mammals, the adrenal cortex of the female is larger than that of the male[14].

Functions of the adrenal cortex. Many of the functions attributed to the adrenal cortex are based upon observations made on the adrenalectomized animal, the response of such animals to the injection of cortical extracts, and on patients suffering from Addison's disease.

The history of the physiology of the adrenals dates back to the original clinical and pathological observations reported by Addison[15]

in his 1855 paper. In this paper, he reported his observations upon eleven patients suffering from the disease which now bears his name. Five of these patients had tuberculosis of the adrenal glands, one had atrophy, and five had metastatic new growth involving the glands. Addison observed the pigmentation, the weakness, anorexia, nausea and vomiting, the constipation, and the emaciation that these patients suffered. Little has been added to the clinical picture since his time, and the weakness, pigmentation, gastro-intestinal symptoms, the wasting and the feeble pulse, together with the fatal outcome, still form the basis of the diagnosis at the present time[1].

In 1856, Brown-Sequard extirpated the adrenals in experimental animals, and rapidly fatal outcome convinced him that the glands were indispensable to life[16].

In 1895, Oliver and Schafer[29] prepared an aqueous extract of adrenal gland, which when injected had profound pressor effects. Other workers, from 1867 to 1903, attempted organotherapy for treatment of Addison's disease in the form of whole desiccated gland or extracts, by mouth and injection. Some of the results were encouraging. For the next few years treatment using adrenine was attempted for Addison's disease, and as expected, with little success.

In the early twenties, interest was again aroused in obtaining a cortical extract, but it was not until the late twenties that Hartman and his associates[19] succeeded in obtaining extracts from the cortex which had profound effects upon the adrenalectomized animal. This was thought at first to be the hormone of the cortex and was named "cortin".

Later Hartman himself was able to show that there existed more than one substance. In 1934 crystalline compounds were separated which could maintain the life of adrenalectomized rats and dogs and by 1938 several crystalline compounds had been separated[21].

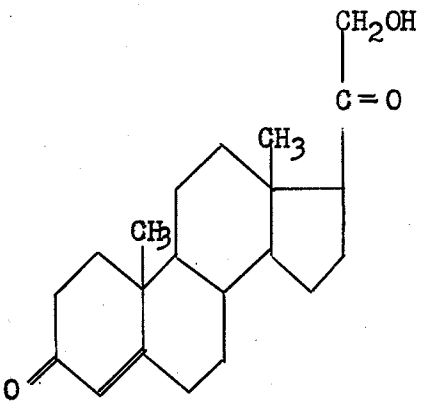
The cortical hormones. Repeated fractional extractions using benzene, water, isopropyl alcohol, and chloroform result in two types of compounds; the crystalline fraction which contains several steroids and the "amorphous fraction" which is very active material.

The following table is found in Soffer's monograph[1] and that which follows has been paraphrased from this work.

Whole adrenal cortical extract

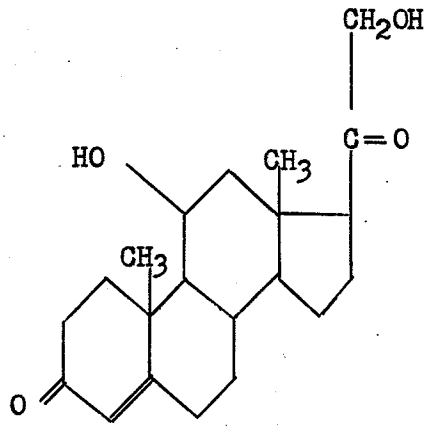
- Crystalline fractions
  - Desoxycorticosterone
  - Corticosterone
  - Dehydrocorticosterone
  - 17-hydroxycorticosterone
  - 17-hydroxy-11-dehydrocorticosterone ("E" of Kendall, "F" of Pfiffner & Wintersteiner)
  - 17-hydroxy-11-desoxycorticosterone ("S")
- Amorphous fraction

DESOXYCORTICOSTERONE

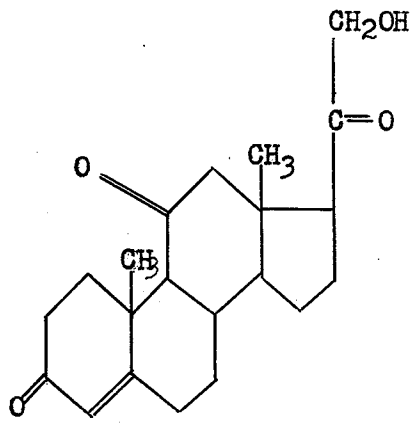


This hormone causes marked retention of sodium, chloride and water, and increases the urinary excretion of potassium and phosphorous. It has no effect upon carbohydrate metabolism or the pigmentation of Addison's disease. In adrenal insufficiency, it will restore the electrolyte pattern of the blood to closely normal picture, and will elevate the blood pressure. Its continued use may produce a temporary hypertension, edema and cardiac failure. When there is hyperfunction of the adrenal cortex, administration of the hormone produces an increased excretion of sodium and chloride in the urine.

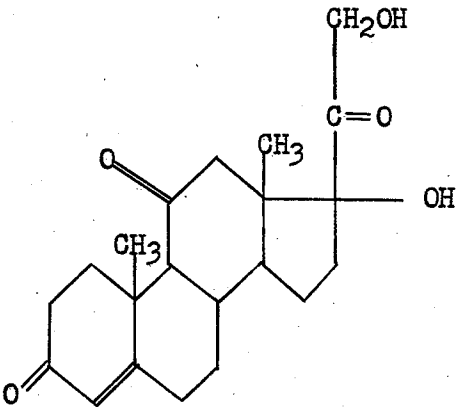
CORTICOSTERONE



DEHYDROCORTICOSTERONE

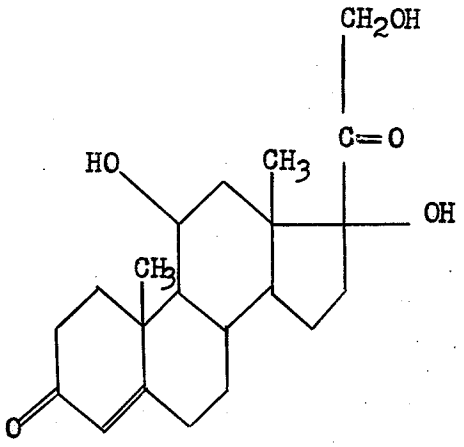


17-HYDROXY-11-DEHYDROCORTICOSTERONE ("E" of Kendall, "F" of Pfiffner, etc.)



These three compounds, (corticosterone, dehydrocorticosterone, 17-hydroxy-11-dehydrocorticosterone), exercise a marked effect on carbohydrate metabolism and correct defects in these substances in insufficiency. When these hormones are injected into the subject, glycogen is stored in the liver, the blood sugar levels are raised, and hypoglycemia is prevented. 17-hydroxy-11-dehydrocorticosterone has the most pronounced effect upon carbohydrate metabolism, being the only one of these three to form glucose from lactic and pyruvic acids; it produces a negative sodium balance with increased urinary output of this ion, while corticosterone and dehydrocorticosterone have little effect on electrolytes, causing minimal retention of blood sodium.

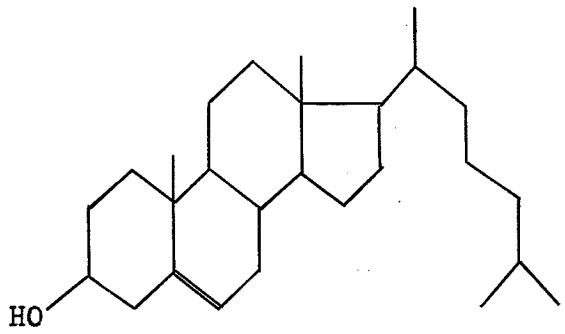
17-HYDROXYCORTICOSTERONE



This compound exercises a marked control over carbohydrate metabolism and also causes an increased urinary excretion of sodium, inducing a negative balance. (Cf. Soffer[1])

Amorphous fraction. This highly active residue is left after the removal of the crystalline fractions, and although it exerts no effect upon carbohydrate metabolism, it is exceedingly potent in its influence upon the distribution of electrolytes. According to Kendall[21] only one or two micrograms per kilo are required to maintain the electrolyte pattern in adrenalectomized dogs. Relatively large amounts of desoxycorticosterone are required to produce the same effects.

Cholesterol and ascorbic acid in the cortex. Cholesterol ($C_{27}H_{46}O$) is closely related structurally to the cholic acids of bile, Vitamin D_3, and to the hormones and other steroids of the adrenal cortex and gonads[22]. There is evidence that cholesterol is the parent substance of these compounds and its formula is included here.



Structural formula of cholesterol ($C_{27}H_{46}O$)
(according to Cameron[22])

Long[23] in a recent paper reviews some of the literature and reports new work when he writes of the conditions associated with the secretion of the adrenal cortex. The following is paraphrased from this paper:

> The chemical characteristics of the adrenal cortex are its high content of cholesterol and ascorbic acid. No other tissue of the body contains such a high quantity of these substances. The role of cholesterol with respect to cortical steroid hormones has been a matter of speculation for some time. On injecting adrenocorticotropic hormone of the anterior pituitary (ACTH), it was found that there is a decrease in the amount of cholesterol in the adrenal gland, while this decrease was not noted in other organs. Thus this response is regarded by Long to be a specific response to the trophic hormone. The ascorbic acid content also decreases in the adrenal gland following the injection of ACTH. Direct evidence, however, is lacking for the conversion of the adrenal cortical steroids from cholesterol, and the relationship of ascorbic acid is not known. However, the decreased amounts of cholesterol and ascorbic acid in the cortex following the injection of ACTH is

> associated with increased rate of secretion of the cortical steroid hormones, and it is probable that cholesterol is a direct precursor of the cortical steroids. Stimulation of the autonomic nervous system, with concomitant release of adrenine appears to be a major factor in stimulating the production of ACTH from the anterior lobe. The manner in which the adrenine produces this activation is not known. The author deals also with other factors such as the blood level of the hormone of the target gland. (Long[23])

The functions of the hormones. According to Cameron[22] the various compounds whose actions are associated with the known functions of the adrenals may be divided into two groups.

Group I compounds have a regulatory action of the blood electrolytes. Included here are desoxycorticosterone and a compound present in the amorphous fraction.

Group II compounds control carbohydrate metabolism and include those hormones which have an oxygen atom attached to

The exact number of hormones produced by the adrenal cortex is not known at the present time. Probably some hormones whose predominating activity is associated with sex function are intermediate products of metabolism. Cameron points out in his monograph that the two distinct types of activity of the compounds of Groups I and II suggest that at least two specific hormones are produced, and that it seems unlikely that four distinct Group II hormones should be produced. Cameron, in agreement with Hartman, believes that the four crystalline compounds so far isolated are the stable derivatives of a less stable compound, the true hormone.

Three groups of functions are listed by Long[24].

1. Control of carbohydrate metabolism, and protein metabolism.
2. Control of electrolytes and water metabolism.
3. Provision of a mechanism of resistance to various stresses such as those caused by bacterial toxins, histamine, shock, water, intoxication, low temperature, and low oxygen pressure.

The 17-keto steroids. The neutral 17-keto steroids are the urinary excretory products of androgenic metabolism and arise from the substances produced by the adrenal glands and the male gonads[1].

II. THE MEDULLA

The line of demarcation between the zona reticularis of the cortex and the medulla is not well defined as cords of the reticularis project into the medulla for varying distances.

Two types of cells are described in the adult medulla. These are arranged irregularly and seemingly without pattern. Ganglionic cells are found in groups or singly. They are not frequent in number. Axones from these ganglionic cells end in close association with the chromaffin cells which they innervate and stimulate to produce adrenine.

The chromaffin cells make up the mass of the medulla. They are irregular in shape, and have usually abundant cytoplasm. The tendency to shrink is marked even in well preserved tissue. Frequently a stellate shape is observed. These cells are characterized by the presence of fine brown granules in the cytoplasm when the tissue is fixed in

chromic acid or its salts. It is from this reaction, which is shared with other tissues of The Chromaffin System that the cells derive their name. The reaction is thought to be due to the polymerization or the oxidation of the adrenine (or its precursor). The cells are green when stained with either Schorml's or Weisel's method. Cramer[26] using osmic acid vapour was able to demonstrate adrenine granules in the chromaffin cells and actually found the granules in the neighbouring sinuses.

A third type of cell is occasionally seen in the medulla. These are arranged in small groups, are smaller in size, and take a deep stain. Many workers feel that these are immature sympathetic cells, while others believe that they are possibly lymphocytes[2].

The development of the medulla. The embryology has been presented by Rabin[11]. The medulla and the chromophile bodies develop from the primitive cells of the sympathetic ganglia or the sympathogonia, which in turn develop from the neural crest. In later stages of fetal development, the sympathogonia differentiate into two cell types, the ganglion cells and the pheochromocytes. The migration of the medullary tissues has been mentioned in connection with the development of the cortex on page 14. During this migration, according to Rabin, portions of the embryonic tissue may become split off and these develop into separate organs at varying distances from the aorta to form the organs of Zuckerkandl.

The blood supply. This has been discussed on page 4. The vascular spaces of the medulla are wider than those of the cortex, and

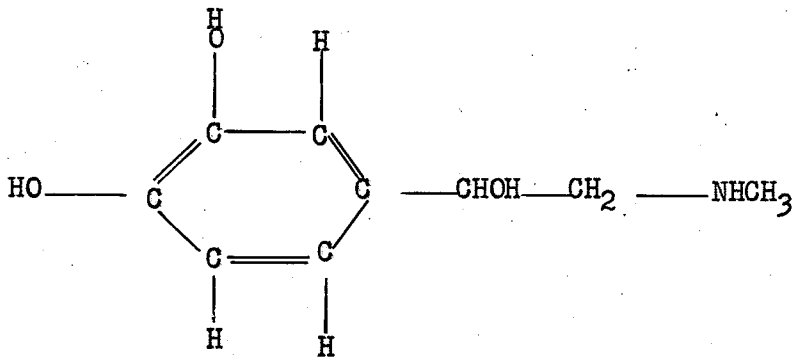
may properly be termed sinusoids. Sharpey-Schafer[25] has remarked that the medulla consists of "a solid cell-mass permeated by sinus-like blood vessels with the cells compactly arranged between and around them." The same intimate relation with the reticulum exists as in the cortex, although the reticulum is more irregular and not as heavy.

The nerve supply. The nerve supply of the adrenal is best discussed in connection with the medulla, for there is no known nervous control of the functional activity of the adrenal cortex[8].

The twenty to thirty nerves to each gland come mostly from the coeliac plexus (sympathetic), in part from the greater splanchnic nerves and possibly from the vagus also[2]. The innervation of the medulla is pre-ganglionic. The fibres pass without synaptic interruption to the cells they innervate. The greater splanchnic nerve conducts most of the fibres to the gland where they form a network in the capsule, penetrate the cortex without innervating it, and end in the medulla in close relationship with the cells there, each fibre being closely associated with a definite number of cells[27]. Other sympathetic fibres enter the gland through the hilus[2].

Stimulation of the splanchnic nerves leads to the liberation of an increased amount of adrenine from the medulla. Cramer's illustrations show the granules in the chromaffin cells and in the neighbouring sinusoids after the fresh gland was treated with osmic acid vapour[28]. The method of formation of adrenine is at present unknown, although theoretically, tyrosine is possibly the parent substance (Cameron[22]).

Adrenine. As early as 1894, Oliver and Schafer[29] demonstrated the remarkable rise in blood pressure following an injection of an extract of the adrenal medulla. In 1901, Aldrich and Takamine[30, 31], independently, isolated the compound adrenine $C_9H_{13}NO_3$. Abel[18] reported a method for producing a crystalline substance which he considered the active principle and which he called epinephrine. Subsequently it was shown that the hormone has the constitution:



CONSTITUTIONAL FORMULA OF ADRENINE

(Syn. - Epinephrine, Adrenaline)
(according to Cameron[22])

When injected, adrenine produces closely the effects obtained upon stimulating the sympathetic nerves, and for this reason has been called a sympathomimetic drug. The most striking effect is the constriction of arterioles of the skin, mucous membranes and cerebrum, resulting in an increased blood pressure which, however, lasts for a short time, the blood pressure rapidly returning to normal. Adrenine accelerates the enzymatic conversion of liver glycogen to glucose,

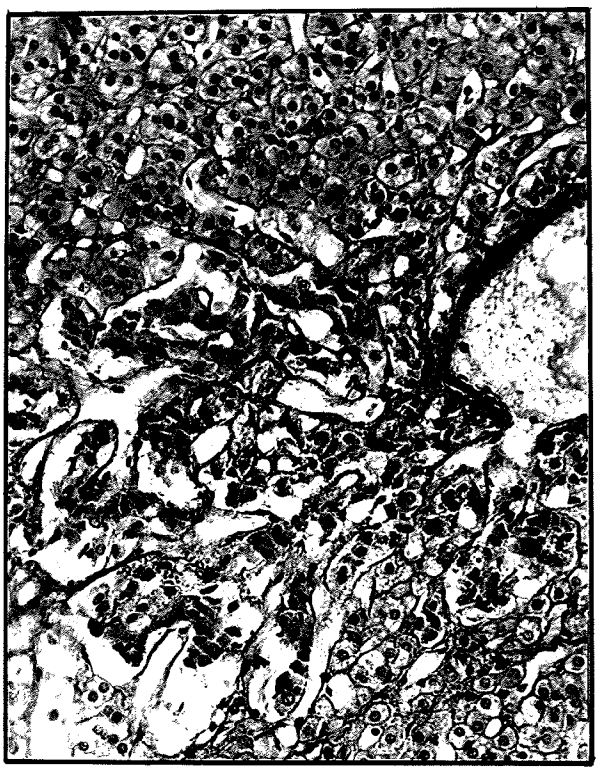


FIGURE 12

Reticulum of the medulla. The sinus-like spaces which permeate the tissue are wider than those of the cortex. A tributary central vein is partly shown. The junction of the zona reticularis and the medulla crosses the upper third of the figure. Same section as Fig. 1. 200 x.

and muscle glycogen to lactic acid, thus explaining the increased blood values of these two substances after the injection[22]. Although the effect of adrenine is usually the constriction of smooth muscle fibres, such is not always the case. The coronary arteries are dilated, heart rate is increased, with increased cardiac output; auriculoventricular conduction time is decreased; the arterioles of striated muscles are dilated while those muscles are contracted. The smooth muscles of the bronchi and bronchioles are relaxed. There is decreased motility of the muscle of the stomach and the remainder of the gastro-intestinal tract.

Experimentally, some animals survive the loss of the medullary tissue. Some writers feel that the hormone is unnecessary, and this is probably true in part, providing the experimental animals are kept in a normal environment. Man with diseased adrenals does not require replacement therapy. However, it is difficult to believe that such a potent physiological agent serves no useful function in body economy, simply because we are unable to attribute to it a function absolutely necessary for life. Long's recent paper, to which reference has been made, sheds some light on the problem.

As an emergency mechanism, secretion of increased amounts of adrenine has obvious advantages[22]. Such a statement is based on gross observations such as the rise of blood pressure, and those functions which are usually attributed to this hormone. Perhaps adrenine plays a more useful function in the maintenance of the race, and perhaps because of it, men are men, and mice are mice, a difference too often forgotten by men of science.

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C H A P T E R T W O

THE MALE REPRODUCTIVE SYSTEM

CHAPTER II

THE MALE REPRODUCTIVE SYSTEM

Introduction. The male reproductive system consists of the testes, with a complete system of excretory ducts (rete testes, epididymis and vasa deferentia); with auxillary glands (seminal vesicles and prostate), and the penis. In this discussion the testes only will be considered in detail, since these organs are the only part of the male reproductive system known to produce hormones.

Each testis is an oval body with flattened sides, varying considerably in size. The adult organ averages 38 mm in length, 25 mm in the antero-posterior diameter, and somewhat less from side to side[1].

Each organ is located in a serous sac which normally contains only a thin film of serous fluid to separate the parietal and visceral layers of the tunica vaginalis. The visceral layer of the tunica covers the anterior surface and the sides of the testis.

Two types of specialized tissue are found in the testis. Highly specialized stratified epithelium lines the seminiferous tubules. From this epithelium are produced the spermatozoa required for the propagation of the species. Production of spermatozoa is considered to be the basic function of the testis. The second tissue is found scattered irregularly throughout the testis as singly occurring or grouped Leydig cells, which are considered by most investigators to produce one or more of the male sex hormones, a secondary function of the testis.

The connective tissue framework. Immediately beneath the tunica vaginalis there is found the tunica albuginea, which is a thick, tough, white fibro-elastic capsule measuring from 0.4 to 0.6 mm in thickness. The deep surface of the tunica is very vascular, especially in the young, and has been called the tunica vasculosa. The tunica albuginea forms the basis for the entire fibrous framework of the organ. Posteriorly the capsule is thickened to produce a vertical ridge, the mediastinum testis. The mediastinum consists of dense fibrous tissue, a few strands of smooth muscle, and a few elastic tissue fibres. From the mediastinum radiate fibrous ribbons of the septulae testis. These pass forward and laterally to become attached to the inner surface of the tunica albuginea. In this manner the organ is sub-divided into 100 to 200 conical compartments, the lobules[2], each with the apex toward the mediastinum. The septulae are incomplete in places, especially toward the periphery, where the lobules communicate. Connective tissue extends from the septulae to support the contents of the lobules. Elastic tissue fibres increase with advancing age in the tunica albuginea.

The arterial supply of the testis. Hill[28], a student of Mall, gives an excellent account of the blood supply of the human testis. Okkels and Sand[32] in a recent paper, agree essentially with Hill.

The testicular artery arises from the anterior aspect of the abdominal aorta at a level slightly inferior to the origin of the renal arteries. Each slender artery passes obliquely downwards,

retroperitoneally on the psoas muscle, to reach the deep inguinal ring. From here the artery follows the spermatic cord to the testis. The testicular artery gives rise to a branch (external spermatic artery) high in the cord, shortly after that structure leaves the external abdominal ring. The external spermatic artery divides into smaller branches as it descends in the cord, and supplies the membranes of the testis.

The main trunk of the testicular artery ends in one or more terminal branches which become very tortuous just before reaching the mediastinum. The terminal arteries further divide near the mediastinum and send a great number of small arteries into the gland. From the mediastinum, vessels (ascending) follow the septulae between the lobules, radiating like 'spokes of a wheel.' The ascending arteries give off finer branches to the tubules.

Near the globus major, one large vessel from the terminal branches of the testicular artery goes to supply the tunica albuginea and encircles the testis, while on the inner or deep surface of the tunica, descending branches from this capsular vessel enter the gland substance and anastomose with the ascending arteries given off at the mediastinum.

A small branch from the terminal arteries descends to the globus minor and passes under the tunica albuginea to run under the capsule and anastomose with the capsular vessel arising at the level of the globus major. These capsular vessels send out many small arteries which are tortuous and which encircle the gland in the deep

surface of the tunica albuginea, termed the tunica vasculosa by Astley Cooper. Branches of these anastomose with vessels from the mediastinum. (Cf. Hill[28])

Arterial supply of the lobule. Each lobule receives an arterial supply from two or more arteries arising at the mediastinum (ascending) and an equal number from the capsular vessels (descending), and amongst all these there are rich anastomoses. Small arterioles arise from these vessels. These arterioles encircle the tubules and end as plexuses about them.

Hill[28] points out that the arteries and veins which supply each lobule are in the septulae and because of the presence of these vessels. the septulae are more conspicuous which give the testis a definite lobular appearance.

Venous drainage. The veins follow the general course of the arteries. Several large capsular vessels encircle the gland while on the deep or inner surface of the tunica albuginea. These empty into the pampiniform plexus. Capsular veins receive blood from the venules and veins on the inner surface of the tunica albuginea, from the tunica vaginalis and from anastomes with the ascending veins of the lobules. Blood is also returned to the pampiniform plexus by the descending veins which follow the course of the descending arteries. These empty into the venous plexus at the mediastinum. (Cf. Hill[28])

The convoluted pampiniform plexus eventually gives rise to a single spermatic vein on either side. The right spermatic vein enters

the inferior vena cava at an acute angle. On the left side the spermatic vein enters the renal vein at a right angle. According to Rivington[29], the testicular veins are supplied with valves.

Lymphatic drainage of the testis[30, 31]. Within the testis the peritesticular, peritubular and perilobular lymph capillaries form a rich network. According to Rouviere[31] lymphatic capillaries from the interstitial tissue pass into the septulae and then into the mediastinum; or more frequently to the tunica albuginea to follow the surface of the testis. All the tributaries eventually arrive at the postero-superior border of the organ. From here 4 to 8 collecting vessels ascend in the cord in the company of the veins[30].

Above the level of the inguinal ring, the ascent is retroperitoneal on the anterior surface of the psoas muscle still in the company of the spermatic vessels. At the level where the spermatic vessels cross the ureter, the lymph vessels leave the company of the veins and as well part from each other. In the upper part of the abdominal course the vessels branch, and also communicate with one another. Each testis has its own group of nodes which communicate with each other, and receive as well lymph from other organs.

On the right side, one to three nodes lie in the groove between the aorta and the vena cava, and one node is deeply placed between the two vascular trunks. In many cases nodes are found at a lower level arranged in a more or less irregular manner.

On the left side the nodes lie in the tissue to the left of the

aorta, generally grouped behind the origin of the inferior mesenteric artery. Occasionally a node is found as high as the left renal vein, and as low as the angle between the aorta and the left common iliac artery.

The secondary nodes are scattered irregularly amongst the para-aortic group, and receive lymph from the primary nodes, as well as from nodes above and below the level of the renal veins, and from still others in the chain of nodes along the outer side of the common iliac artery. (Cf. Jamieson and Dobson[30])

Nerve supply of the testis. The testicular plexus is derived from the renal plexus (formed from the coeliac plexus, the aortico-renal plexus and the aortic plexus, and also from the lowest splanchnics and branches from the vagus). The nerves accompany the testicular vessels and are derived from the tenth thoracic segment of the spinal cord through the renal and the aortic plexus. Fibres from the pelvic plexus are carried by the artery to the vas deferens to supply the testes and the epididymis (T11, 12 and L1)[34, 35]. The distribution of the nerves within the testes and the relationship of these nerves to the interstitial cells are discussed on page 66.

I. THE TUBULAR SYSTEM

General plan of the tubular system. Each lobule contains one to three, and sometimes more greatly convoluted seminiferous tubules (tubuli contorti) which are supported by delicate connective tissue. The seminiferous tubules are very long slender loops that are coiled or bent in sharp, short curves, each tubule forming several small compact convoluted masses[2]. The tubules measure from 30 to 70 centimetres in length and from 150 to 250 micra in diameter[3]. The combined length is estimated to be 250 metres by Maximow and Bloom[3], and by Johnson[16] as 800 feet for each testis.

According to Johnson[16], the commonest type of tubule found are the anastomosing loops connected in a complete series. Single anastomosing loops and single unbranching tubules are found, but are rare. Small diverticulae are found here and there along the tubules, and are more frequent near the mediastinal end of the tubules.

The tubules follow their tortuous course, converge on the apex of the lobule, pass abruptly into the straight tubules (tubuli recti) that enter the mediastinum and joint to form a racemose network of wider calibre, the rete testis. The rete testis consists of an irregular intercommunicating system of channels lined with cuboidal epithelium. The duct system emerges from the testis proper at this point as the ductuli efferentes. These pierce the tunica along the posterior border of the testis near the upper pole, forming the coni vasculosi, and connect the rete testis to the highly convoluted canal

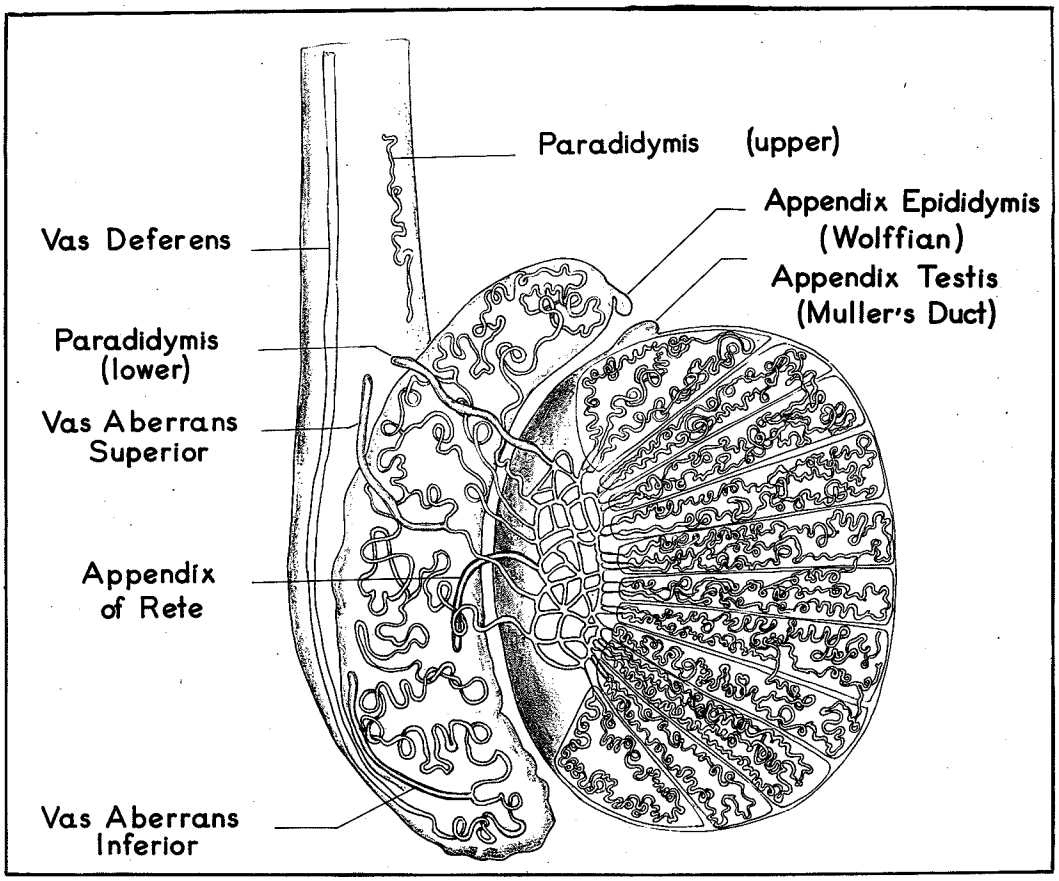

FIGURE 13

Schematic representation of the tubular system of the testicle. Redrawn and modified after Herman[37], and Johnson[16].

of the epididymis. (Cf. Piersol[4])

The epididymis has about 26 feet of tubules of inconstant volume and diameter[25]. Finally from the epididymis, there emerges the vas deferens to conduct the products to the exterior. (Cf. post)

The seminiferous tubules. A highly specialized stratified epithelium lines the seminiferous tubules. This epithelium rests on a well developed finely fibrillar basement membrane. A thin layer of condensed connective tissue is applied to the outer surface of the tubules.

Two kinds of cells are found in the complex epithelium; the sustentacular cells (of Sertoli) have a supporting and nutrient function, and secondly the germ or spermatogenic (sex) cells which after a series of proliferations and transformations give rise to mature spermatozoa. The histology of the seminiferous epithelium varies with age. In the testis showing active spermatogenesis, spermatogonia, primary and secondary spermatocytes, spermatids, immature and mature spermatozoa are present.

The cells of Sertoli. Numerous names have been applied to the Sertoli cells from time to time[19]; among these are the terms sustentacular cells, follicle cells, and the more descriptive 'sperm-nourishing cells.'

In the testis of the adult, during the period of active spermatogenesis, the Sertoli cells are slender, elongated, irregularly pyramidal cells, perpendicular to the basement membrane on which they

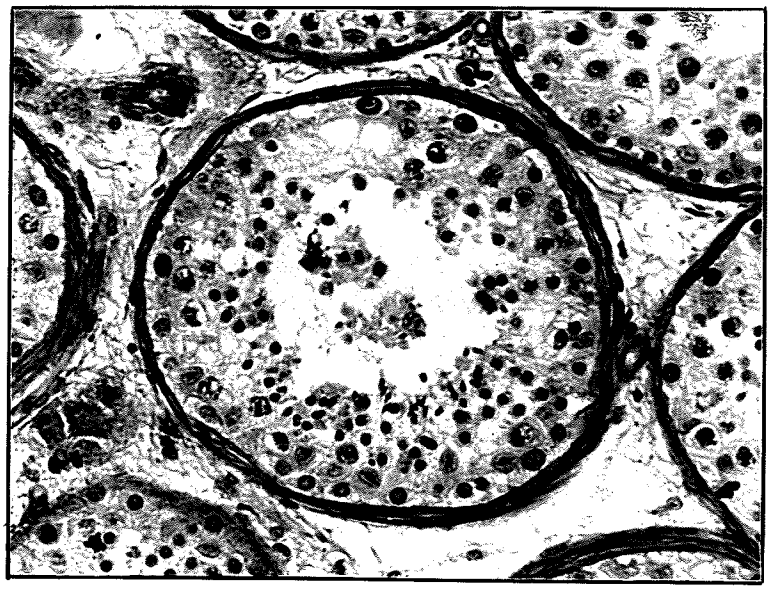

FIGURE 14

Seminiferous tubule showing the fibrillar structure of the wall, the seminiferous epithelium and the contents of the interstitial space. Stained with periodic acid, Schiff's reagent and hematoxylin. A 7088. 300 x.

rest. Wide divergence of opinion exists as to the nature of these cells, whether or not they form a syncytium[3]. Hanes[19] points out that Regaud in 1910 described the Sertoli cells as forming a syncytium, despite the fact that von Ebner in 1902 was able to tease the cells apart in physiological saline.

Although the cell outlines are indefinite, identification of the Sertoli cells is usually possible by recognition of the characteristic nucleus. The nucleus is found in the expanded basal portion of the cell, usually some distance from the basement membrane. The nucleus as a rule is oval in shape, with the long axis radially directed. The average size is 9 by 12 micra[3]. A compound nucleolus, consisting of an acidophil granule and one to three basophil granules, further characterizes the vesicular nucleus. Mitotic division is rare.

The Sertoli cells have several kinds of inclusions. Granules staining with iron hematoxylin, wavy fibrils, lipoid granules and a spindle shaped crystalloid (Charcot-Böttscher) may be found in the cytoplasm.

The Sertoli cells are presumed to have at least two functions. One function is to support the germinal cells. The second is nutrient. Spermatids and unripe spermatozoa are attached to the distal end of the cells. The spermatozoa lie with the heads attached to the cytoplasm of the cell as figured on page 49.

In the straight tubules, the Sertoli cells are more columnar in shape, in contrast to the pyramidal shape of those in the seminiferous tubules, where they extend out to the lumen, the distance

varying with the stage of the cell they are nursing[17].

The cells are highly resistant to noxious agents such as Roentgen rays, toxins of different sorts, and high temperatures, all of which are injurious to the more sensitive spermatogenic cells.

The spermatogenic cells. Spermatogenesis may be divided into two phases or stages. In the first phase, known as spermatocytogenesis, the spermatogonia undergo repeated divisions; primary spermatocytes are formed from spermatogonia; secondary spermatocytes are formed from primary spermatocytes; spermatids are formed from secondary spermatocytes. No further cell division occurs after the spermatids are formed. In the second phase, known as spermiogenesis, mature spermatozoa are formed from spermatids after a series of complex structural transformations. The structural details of the two phases are not completely elucidated for man[3, 17, 27].

Spermatocytogenesis. Spermatocytogenesis begins with the mitotic division of the spermatogonia, which are found situated close to the basement membrane. The primary generations of the spermatogonia are relatively large cells, each with a spherical nucleus which contains dust-like particles and a round body that stains similar to chromatin. The cytoplasm contains granular mitochondria and near the nucleus a pair of centrioles[3]. Occasionally there is a small rod-like crystalloid (of Lubarsch) which is smaller than those found in the Sertoli cells[17].

The spermatogonia of the first generations are pushed toward

the lumen by succeeding generations. Spermatogonia of the later generations are smaller, and tend to have a smaller nucleus, although the differences between earlier and later generations of the spermatogonia are not marked.

After an undetermined number of divisions, the spermatogonia develop into primary spermatocytes. These primary spermatocytes are moved towards the lumen of the tubule. After many generations, there is considerable variation in cell size and nuclear morphology. The fully developed cell is large, either spherical or oval, with the long axis of the cell perpendicular to the basement membrane. The nucleus is large and shows all the variations encountered in meiotic division. The secondary spermatocytes develop into spermatids.

Spermatids are relatively small cells having a small darkly staining spherical nucleus each about 5 to 6 micra in diameter. At the stage of spermatid formation, the phase of spermatocytogenesis is completed. (Cf. page)

Spermiogenesis. The phase of spermiogenesis consists of the production of mature spermatozoa from the spermatids, a process which requires a series of complicated changes in the latter. No cell division occurs in this phase.

While the details of spermiogenesis are not within the scope of this work, the paper of Gattenby and Beams[17] is mentioned as an excellent reference.

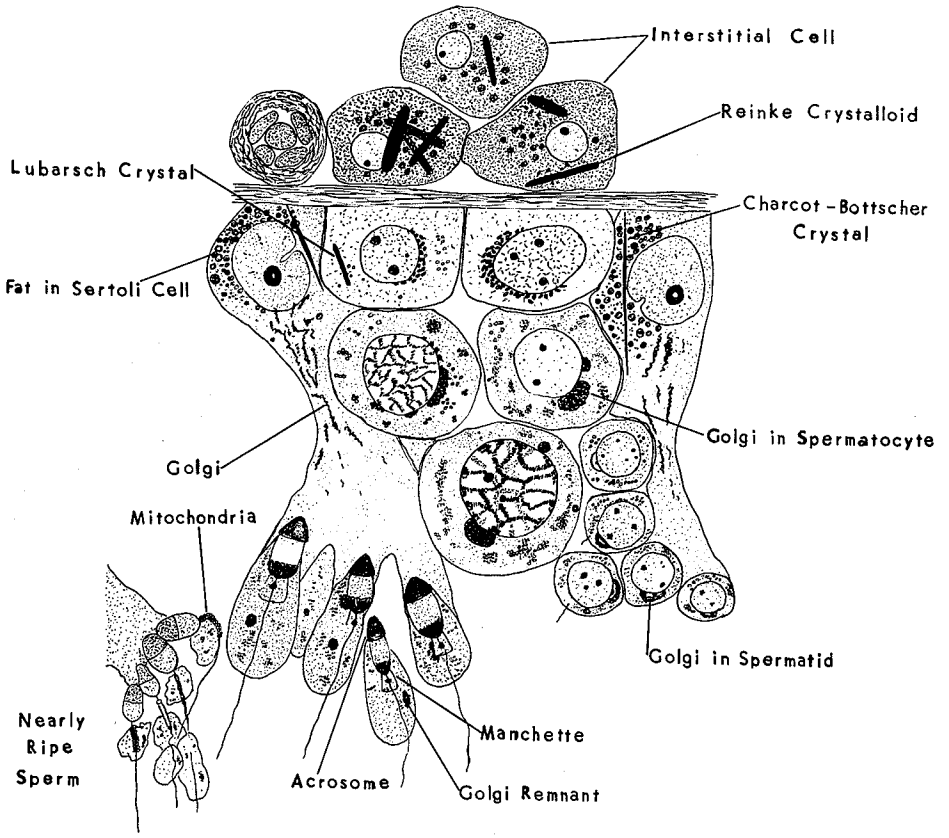

FIGURE 15

Diagram to show the development of the spermatozoa in the human testis. Redrawn and simplified after Gattenby and Beams.

Mature spermatozoa. Leeuwenhoek reported the discovery of spermatozoa to the Royal Society of London in 1677. The "living animalcules" were first seen by Ham who directed the attention of Leeuwenhoek to them in a specimen of human semen. Leeuwenhoek continued the study and found spermatozoa in enormous numbers in the semen of fishes, birds, insects and quadrapeds. (Cf. Bremer[2])

A mature spermatozoa consists of a head and a tail, the latter being divided into three parts.

The flattened head is ovoid and when seen on edge is pyriform in shape. The anterior margin is sharp and is supposed by some to be a cutting edge when the spermatozoon enters the ovum. The head has three subdivisions. The anterior two thirds is the galea capitis which stains less intensely with hematoxylin than the posterior one third. The galea is said to be covered with a thin, closely adherent structureless membrane[3] which originates as the acrosome from Golgi apparatus[17]. The head measures 4 to 5 micra in length and 2.5 to 3.5 micra in width; it is actually the condensed nuclear material of the spermatid and contains the chromosomes of that haploid cell. The chromatin does not appear to be arranged in threads or networks.

The neck is actually the point of attachment between the head and the tail. No marked constriction is found in human spermatozoa. Separation of the head from the tail occurs easily at this point.

The tail has three parts: the connecting piece (middle piece of some authors); the chief piece; and the end piece. The connecting piece measures 5 to 6 micra in length and is approximately 1 micra in

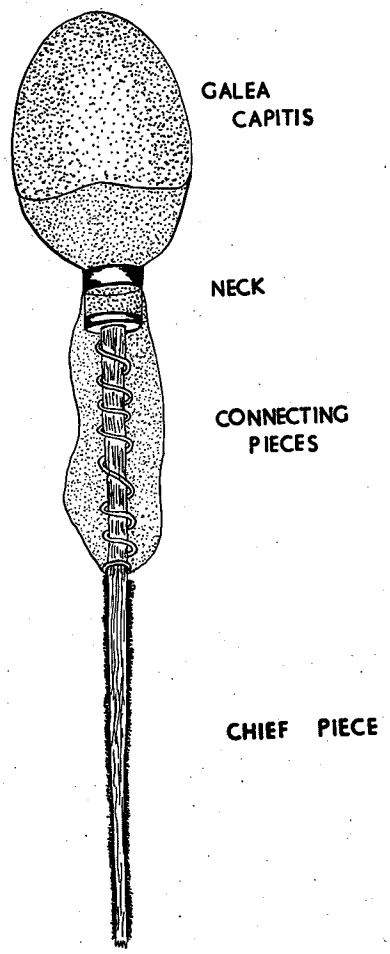

FIGURE 16

Meve's concept of the spermatozoa, based on the study of lower animals.

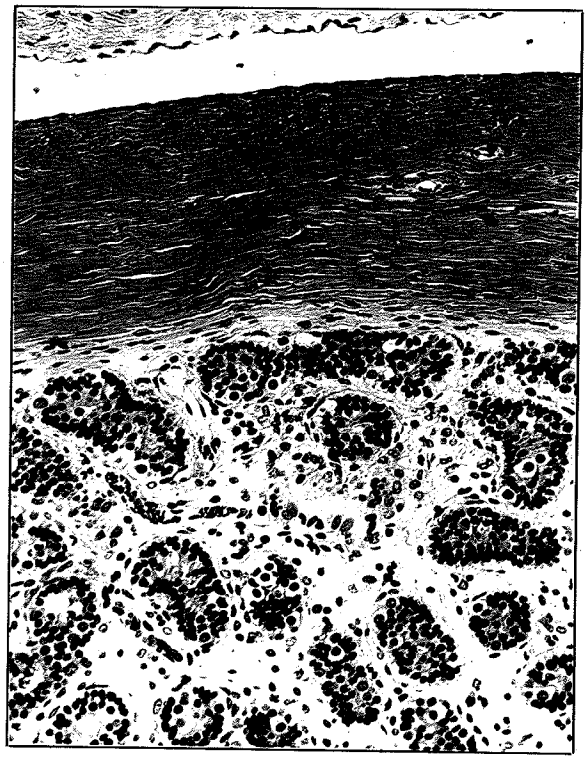

FIGURE 17

The capsule and tunica vaginalis of the testis of a twelve-year-old boy. Hematoxylin and eosin stain. 150 x. WGH A 7911.

diameter. It consists of cytoplasm, an "axial" element and two parts of the posterior acrosome. The spiral element, which is often figured in texts, does not exist in human material according to Gattenby and Beams[17] or Bowen[27]. The chief piece measures from 40 to 60 micra in length and gradually tapers as the tip is reached. An axial filament surrounded by a membrane makes up the chief piece. The end piece is simply the last 10 micra of the tail and is the naked prolongation of the axial filament. In ordinary preparations, the head and the tail are easily identified. Special methods of staining and special lenses are required to study many of the above details which are usually figured in text books of cytology.

Spermatozoa are produced in great numbers, the average count being 60,000 per cubic millimetre of semen. During the active reproductive period approximately 340 billion spermatozoa are produced by an individual[2].

Abnormal forms of spermatozoa. Abnormal forms of spermatozoa are very common. Moench[13] after the study of a small series concludes that normal semen may contain up to 20 per cent of abnormal sperm heads. When the sperm head abnormalities exceed 25 per cent, clinical sterility can be assumed. Among the fifty or more abnormal forms this author describes are microsperm, megalosperm, narrow irregularly staining aplastic head, coiled tail, abnormal attachment of the tail, no tail, multiple tails, thickened body. The most important variant from the normal, from the standpoint of fertility, is the frequent

finding of tapering narrow heads, or those that are sickle shaped. Motility of the sperm is not as important as their morphology from the standpoint of fertilizing power[14].

Spermatogenic wave. A marked difference in the stages of spermatogenesis is noted when examining one tubule after another in the same cross section. The process is not synchronous along the tubule. Spermatogenesis at one point is different in the stage reached at another point. The study of a longitudinal section of a tubule makes the difference more evident. Some laboratory animals have a definite wave pattern which is measureable and constant for the species. Such a condition does not exist for man. The reason for the spermatogenic wave is not known at the present.

Theory of determination of sex. Forty-eight chromosomes are found in the somatic cells of man[18]. These chromosomes consist of twenty-three pairs of varying shapes and sizes (autosomes) and one pair of sex chromosomes, which carry the genes controlling sex. In the female, the two sex chromosomes are similar and differ little from the autosomes. They are the X chromosomes. In the male, however, the sex chromosomes are unlike, one being the X chromosome of maternal origin and the other the Y chromosome of paternal origin. The latter is small and atypical[33].

During the division of the spermatogonia of man the chromosomes split in the usual way (mitotic division). Forty-eight chromosomes are found in each of the daughter cells. Primary spermatocytes

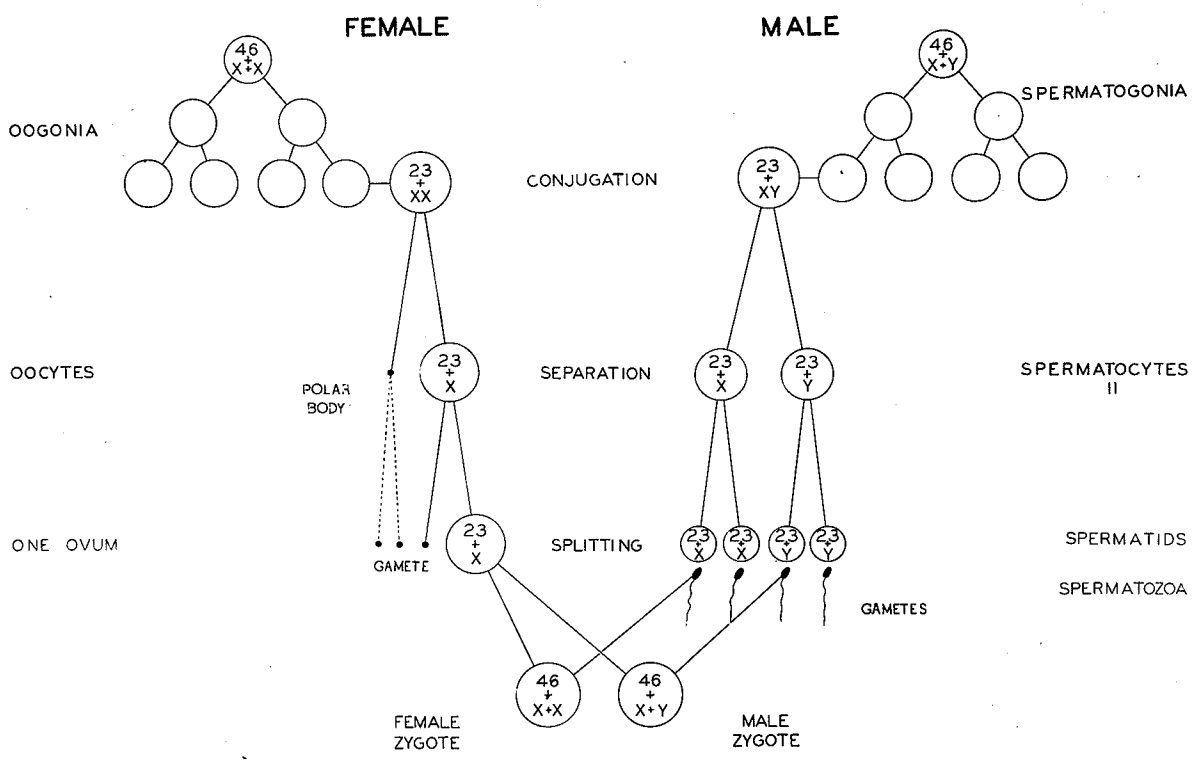

FIGURE 18

Diagram to illustrate the development of the mature sex cells of the male and female. Redrawn after Bremer, slightly modified.

develop from spermatogonia.

Through a process of re-arrangement of the chromosomes, primary spermatocytes develop from the spermatogonia. The chromosomes arrange themselves in pairs and fuse. In this conjugation, there is simply a reduction in the number of chromosomes and no reduction in the amount of the chromosome material. Each primary spermatocyte contains twenty-three paired autosomes and the sex chromosomes XY.

Secondary spermatocytes arise from the primary spermatocytes through a process of meiosis in which the amount of chromosome material is reduced. Each paired chromosome splits longitudinally, resulting in 23 autosomes and the X chromosome going to one secondary spermatocyte, and 23 autosomes and the Y chromosome going to the other. Thus in the human, the secondary spermatocyte and all cells subsequent to that stage have twenty-three ordinary chromosomes (autosomes of Painter[18]) and either an X or a Y chromosome to make up the twenty-fourth.

Secondary spermatocytes divide to form the spermatids. The chromosomes split longitudinally so that from each haploid secondary spermatocyte, two haploid spermatids are formed, each with twenty-three plus X chromosomes or twenty-three plus Y chromosomes, depending upon the chromosome makeup of the mother cell.

No division occurs in the transformation of the spermatids into spermatozoa. In theory there are two types of spermatozoa. One half of the spermatozoa contain 23 ordinary chromosomes plus an X chromosome, the other half containing 23 ordinary chromosomes plus a Y chromosome. Every ovum contains 23 chromosomes plus an X chromosome always[18]--never

a Y chromosome. Thus the sex of the organism depends entirely upon the type of the spermatozoa which unites with the ovum during fertilization. During fertilization, the total and original number of chromosomes numbering forty-eight is restored. If by union, there results an X-Y combination of the sex chromosomes, the new individual is a male. A female results if the combination is X-X.

The theory of determination of sex as outlined above is not universally accepted. It has not been proven in the case of humans. Two types of spermatozoa containing either an X or a Y chromosome have not been described morphologically for man thus far[3].

Semen. While only a small part of semen is actually a product of the testis, this discussion is included to give a short summary of Killian's paper[15].

Semen is the fluid medium in which the spermatozoa are transmitted during coitus. It is the product of the combination of many secretions from the auxillary sex glands such as the prostate and the seminal vesicles. It is thick, viscid, opalescent, slightly alkaline fluid, of 2 to 5 cc's volume. Few reports of the metabolism of the human spermatozoa are available. Killian[15] has reported some of his studies. This worker finds that the concentration of sugar in semen is from 4 to 6 times higher than that of the blood. When semen is incubated at 38° C, the sugar content falls off progressively for the first 6 to 9 hours, with a corresponding increase in lactic acid. The pH is slightly higher than of blood plasma, an average of 7.6. The urea content is about twice that of blood being about 60 milligrams

per 100 ml. Addition of solutions having a pH between pH 5 and pH 8 have no influence upon the motility of the spermatozoa. Solutions with a pH lower than pH 5 or higher than pH 8 depressed the motility, the maximum depression being at pH of 3 and 11 respectively. Also the lower the viscosity, the greater the motility. The addition of urea solutions increases the motility of the spermatozoa, and also decreases the viscosity of semen. (Cf. Killian[15])

The physiology of the tubular system. Not all spermatogonia become spermatozoa. Degenerating spermatocytes and spermatids may be seen in the lumen of normal tubules. Present also in the lumen is a colloid material, the source of which appears to be the autolysis of degenerating germ cells and apparently also from the Sertoli cells[25].

Physiologically speaking, the tubules form a closed system of tubules, open only at the straight tubules. The continuous formation of colloidal material, the degeneration of germ cells, and the proliferation of cells produces a head of pressure which forces itself along the tubules. This head of pressure can be easily demonstrated. After ligation of the vasa efferentia, the testis becomes tense and swollen, so much so that the tunica will on occasion rupture[25].

The movement of the secretions has been graphically described by Oslund[25]:

> This material moves slowly but steadily through the seminiferous tubules to the collecting tubules. Its movement, brushing along the sides of the tubules, causes the spermatic elements to slant much like grass or weeds in a slowly moving stream. (...)

The cellular elements including the spermatozoa are therefore carried passively along the tubules. This concept was put forward by Griffiths in his 1895 series of lectures[24]. Spermatozoa are inactive in the tubules[25], probably a fortunate circumstance considering the maze of tubular architecture. The column of fluid forces its way from the seminiferous tubules, through the straight tubules, the rete, the ducts of the epididymis and to the vas deferens.

There is no appreciable storage of spermatozoa in the genital tract[25]. Spermatozoa are not stored in the seminal vesicals[14, 25]. Indeed, rather than reservoirs, these appendages are considered as their graveyards[14]. Oslund points out that the epididymis is supplied with motor nerves. Moench believes that most storage of spermatozoa occurs in the epididymis, where in the tail they are kept inactive by lack of oxygen and "inhibiting secretions of the epithelium."

II. THE INTERSTITIAL TISSUE

Introduction. Filling the angular spaces between the tubules are thin collagenous fibres, blood and lymph vessels, nerves and connective tissue cells. Among these latter cells are fibroblasts, fixed and wandering macrophages, fat cells rarely, perivascular embryonic elements and the interstitial cells of Leydig. The intertubular connective tissue represents from 13.5 to 30 per cent (average 22 per cent) of the tissue of the testis[6]. About 66 per cent of the testis is tubular tissue. (Cf. Fig. , page)

In this discussion, the term "interstitial tissue" will be

used to include all those tissues filling the intertubular spaces. The term "interstitial cells" will be reserved for those cells described by Leydig.

The interstitial cells of Leydig. Leydig[5] in 1850 noted the masses of cells which contained either colourless or yellowish fatty globules and which are constantly in the interstitial tissue.

Several names have been assigned to these cells. Bouin and Ancel[7] in 1903 designated them as the "interstitial gland" of the testis. The undesirable term of "puberty gland" was introduced by Steinbach[8] in 1911.

Distribution and amount of interstitial cells. Between 8 and 28 per cent (average 12 per cent) of the testis is occupied by interstitial cells[6]. Such a high percentage is not apparent from the casual study of stained sections.

Generally the cells are found in rather dense masses, although single cells occur. They are found scattered throughout the testis, everywhere except within the tubules.

When the interstitial cells fill the intertubular spaces a close relationship to blood vessels naturally results. This arrangement is probably secondary, for if a decrease of the amount of interstitial tissue occurs, the close relationship to blood vessels does not appear to exist[9]. The cell nests are not especially vascular and are considerably less vascular than most of the other endocrines[10].

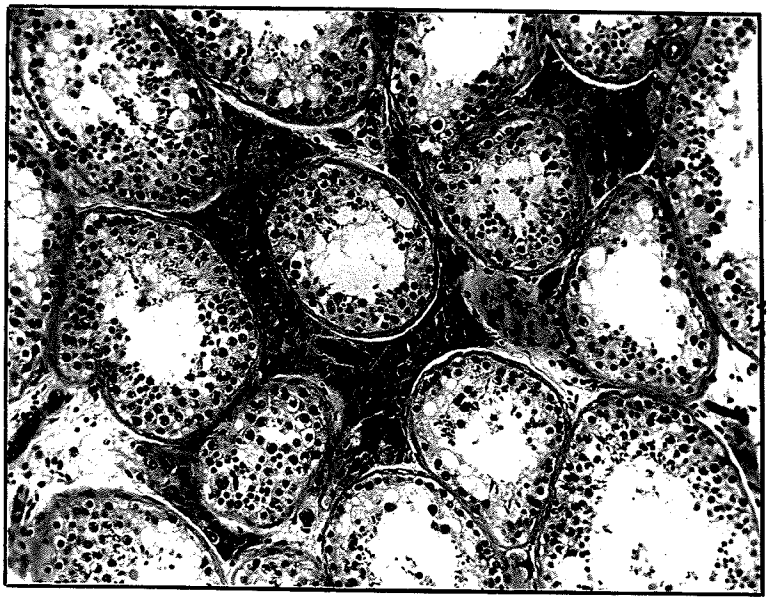

FIGURE 19

Interstitial cells almost completely surrounding the seminiferous tubule at the plane of section. Hematoxylin and eosin stains. A 7088. 125 x.

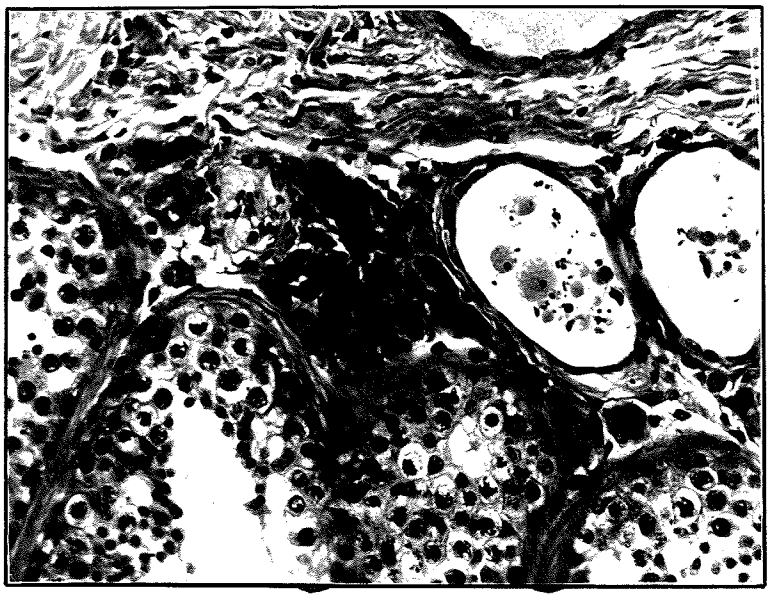

FIGURE 20

Interstitial cells situated just beneath the tunica vasculosa. Hematoxylin and eosin stain. A 7088. 260 x.

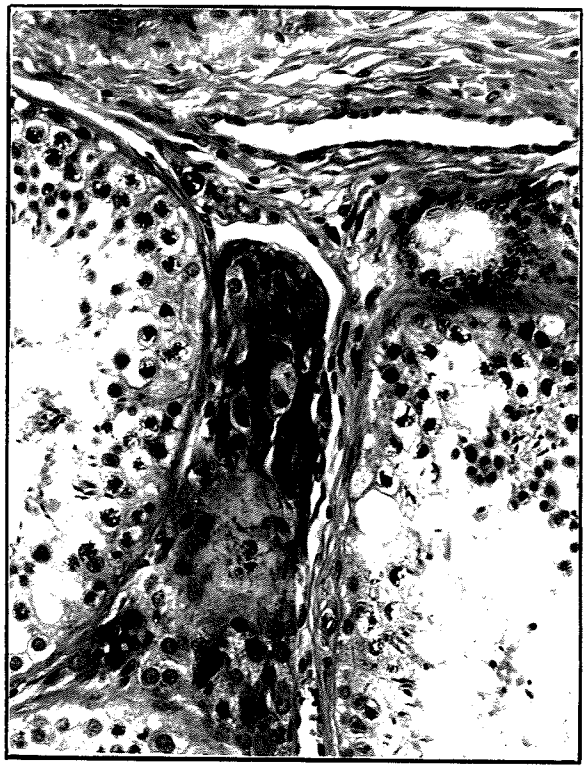

FIGURE 21

Interstitial cells situated just beneath the tunica vasculosa. Hematoxylin and eosin stain. A 7088. 260 x.

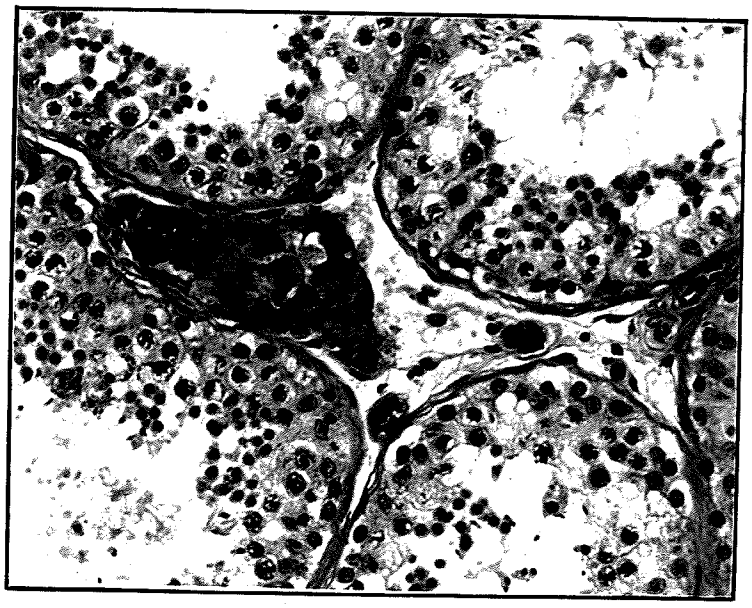

FIGURE 22

A group of interstitial cells in the intertubular space, showing relationship to blood vessels and connective tissue. Hematoxylin and eosin stain. 260 x.

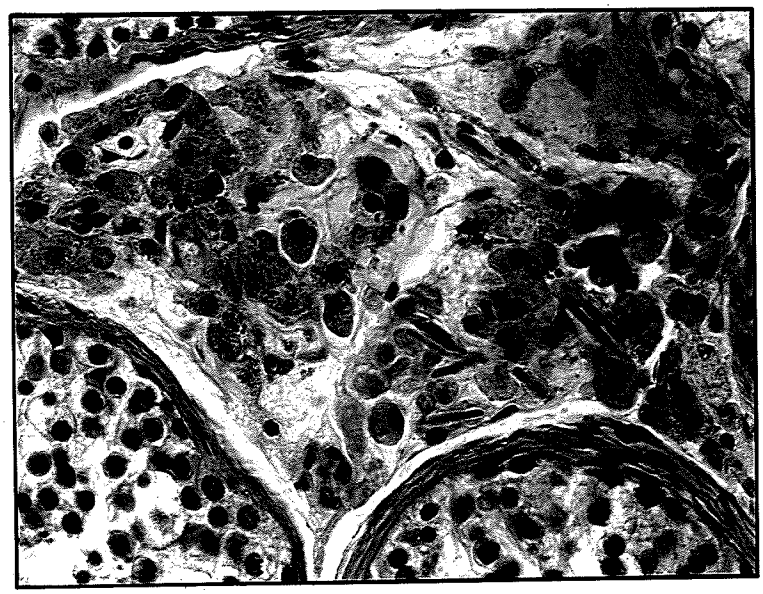

FIGURE 22 a

The interstitial tissue and cells of the testis of an elderly man, showing the pigment and crystalloids. H & E. 350 x. (WGH 2185-50).

The interstitial cells appear first in the 19.5 mm human embryo[38]. Most authors agree that they probably arise from mesenchymal cells. The cells are abundant at birth; however, they subsequently disappear. At the time of puberty they reappear. Spermatogenesis commences at the time of their reappearance.

Histological details. Typically the interstitial cells are large, irregular polygonal cells, measuring from 15 to 20 micra[4]. Numerous inclusions make the abundant cytoplasm granular.

The nucleus measures from 6 to 10 micra, mostly 7.6 micra[11]. Usually there is one nucleus, although some authors have reported the rare finding of binucleated cells. Mitosis is rare[10]. One, or occasionally more nucleoli show distinctly after most staining reactions. The spherical nucleus is vesicular and has a typical arrangement of chromatin and small granules.

A large idiosome is present in the endoplasm near the nucleus. Two rod-shaped centrioles are usually present. The mitochondria are in the form of filaments and granules. A typical Golgi net is found in the interstitial cells. Various round granules which are larger than the mitochondria have been described by some authors. No agreement has been reached as to the nature or significance of these granules. (Cf. Rasmussen[9])

Abundant fatty globules are found in the fully developed interstitial cell. There is considerable variation in the size of the globules. These fatty substances consist of cholesterol,

cholesterol esters, and smaller amounts of cerebrosides and phosphatides[12]. The role of the lipoid material is not known[9].

Brown pigment in the form of granules is usually not found in the human interstitial cells until the adult stage is reached[9], after which time the amount of the pigment increases. In old age, numerous cells may be packed with granules of pigment.

The first description of elongated crystalloids in human interstitial cells was given by Reinke in 1896. These inclusions are generally regarded as characteristic of humans, although they have been described in a few other species. The crystalloids do not appear until after puberty, after which time they persist[9].

Four characteristics of the interstitial cells in humans may be listed here. Compared with the interstitial cells of other species, those of the human have relatively large amounts of lipoid material. The crystalloid of Reinke is absent or rare in other species. No seasonal variation in the number or appearance of the Leydig cells is found in man. The cells form large groups or collections which are easily identified. In many of the lower animals, the Leydig cells are found singly placed, and are often difficult to identify. In other animals the interstitial cells are very numerous.

Relationship between nerves and the Leydig cells. Details of the relationship of the nerves supplying the testis and the Leydig cells are due mostly to the work of Okkels and Sand[32], whose paper is summarized in the paragraphs to follow.

According to Okkels and Sand[32], the nerves of the human testis are more numerous than has been previously assumed. The nerves on reaching the organ in company with the blood vessels, continue to follow the course of the latter into the interior of the gland, some passing directly through to the mediastinum, but the majority and the larger nerves following the arteries which run in the deep layers of the tunica albuginea. From the deeper layers of the albuginea, they pass into the interlobular septae.

Three types of connections are described by Okkels and Sand: (a) perineural Leydig cells are found in the region of the hilum or at a point just after the nerves have entered the tunica vasculosa. The contact is essentially superficial; (b) while the nerves are still in the albuginea, and are about to enter the septulae, occasionally are found intraneural Leydig cells scattered throughout the nerve 'like raisins in a bun'; (c) when the large masses of Leydig cells are reached, the nerve splits up and blends with the cells, where the neurofibrils stop at the surface of the cell, but do not penetrate into the cytoplasm. These investigators have not been able to demonstrate an intimate relationship between the nerves and the Leydig cells before puberty.

The larger nerves follow a periarterial course until the parenchyma of the gland is reached. During this course, it is probable that vasomotor nerves are given off. The nerves apparently have a functional significance and Okkels and Sand suggest that this function is secretory. (Cf. Okkels and Sand[32])

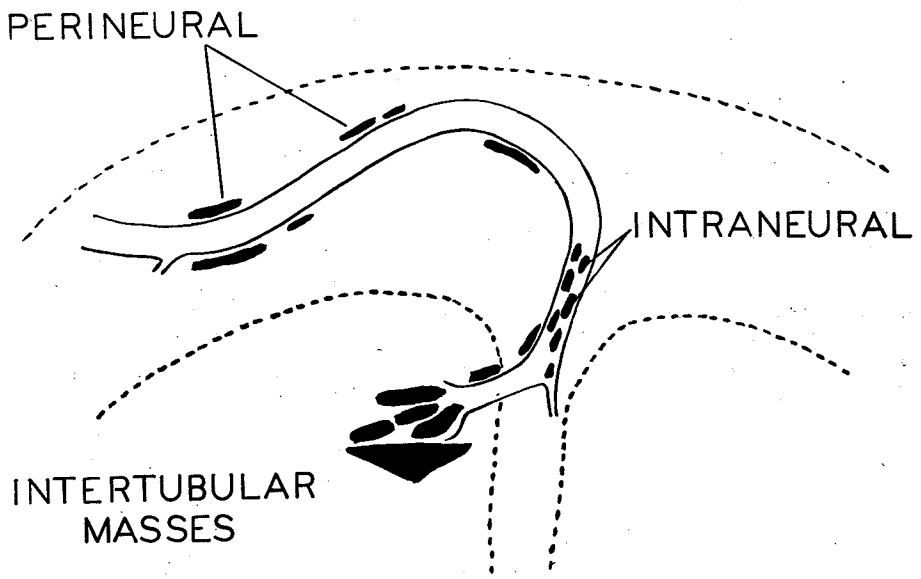

FIGURE 23

Diagram illustrating the varying relationship of interstitial cells to a nerve as it courses from the capsule of the testis into the septum. Redrawn after Okkels and Sand.

Variation of the testes with age. Full size or function of the testis is not attained until puberty or afterwards. In a boy of ten years of age, the organ is almost round, and is firm and compact. The organ measures 10 by 10 by 15 mm in size. The seminiferous tubules are almost indistinguishable from one another. The tubules are not greatly convoluted and are closely packed together. There is little interstitial tissue, most of which is fibrocellular connective tissue. Each tubule consists of a solid column of cells. All the cells are in a quiescent state.

In the adult, the organ is softer, larger and more plump. When the organ is cut in the fresh state, the yellow tissue bulges. From the surface the tubules may be picked up with fine forceps and are seen to resemble fine contorted threads. The tubules are larger than those of the young male. The tunica propria is thin.

The process of aging leaves its effect upon the male genital system but the changes are not so uniform when compared to similar changes in the female genital system. Nor are the changes found in old age so well known. There is a lack of published data. Spermatogenesis ceases at a period varying greatly with the individual. Reports found in the literature vary greatly with respect to the upper limit of this period. In many individuals there occurs with failing spermatogenesis a thickening of the basement membrane of the tubule. The connective tissue of the tunica propria increases in thickness, usually associated with a decrease in the size of the tubule. As well there is an increase in pigment found in the interstitial cells

FIGURE 24

The testis of an infant male of one month. The tubules have no lumen. The intertubular tissue is very cellular, but shows no interstitial cells. CH 48-2067. Hematoxylin and eosin. Low power.

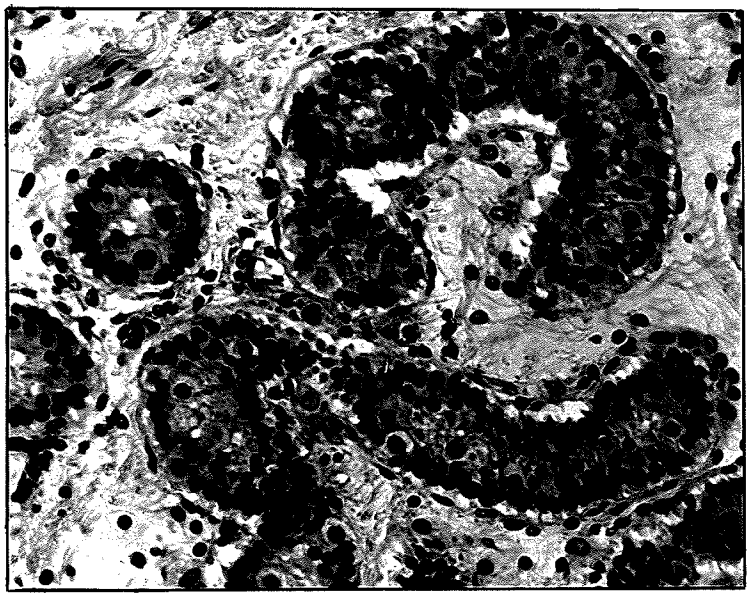

FIGURE 25

The tubules and interstitial tissue of the testis of a normal twelve-year-old boy who died of accidental electrocution. Autopsy twelve hours post-mortem. Hematoxylin and eosin stain. 275 x. WGH A 7911.

of many individuals. (Cf. Engle[36])

Note on development of the testis. The development of the testes and the ovaries is closely associated with the development of the urinary system. The development of the genital system is complex owing to the fact that its different portions arise from diverse primordia. The contributions from these primordia vary qualitatively and quantitatively in the two sexes.

While the sex of the future embryo is determined at the moment of fertilization by the presence or absence of the X-chromosome, the identification of the sex of the embryo is not possible until the 17 mm stage.

The primordia of the sex glands appear in embryos of 4 to 5 mm (CR) length. The genital ridges appear as thickenings of the coelomic epithelium on the medial aspect of the mesonephros. The basement membrane separating the coelomic epithelium from the underlying mesenchyme disappears. Cords of cells proliferate from the epithelium and project into the mesenchyme. Until the 17 mm stage, these developmental changes in these ridges are the same in either sex.

At about the 13 mm stage, the gonadal blastema becomes divided into cords by the development of fibrous tissue bundles. The sex cords are at first joined to the germinal epithelium. At about the 25 mm stage, the dense tunica albuginea forms and separates the cords from the epithelium. Later, the cords extend into the region of the mesorchium where they form a network, the rete testes. Some of the

cords become canalized to form the seminiferous tubules, the cells of which are either the sustantacular cells or the primordial germ cells. Some of the shorter cords do not become canalized, and persist as small groups to become the interstitial cells.

The ovaries and the testes in late fetal life occupy a different position from that in which they are found in the embryo. The change in position is most marked in the case of the testes, since these organs pass from the abdominal cavity through the abdominal into the scrotal sac.

By the third month of fetal life (70 mm), the testis lies retroperitoneally in the false pelvis. It remains at the abdominal end of the inguinal canal until the seventh month. During the seventh month it passes through the inguinal canal. Normally the testis is within the scrotum by the end of the eiththh fetal month. (Cf. Hamilton, Boyd and Mossman[33])

The hormones of the testes. The male sex hormones are discussed in Chapter V, together with the other hormones of reproduction.

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C H A P T E R T H R E E

THE FEMALE REPRODUCTIVE SYSTEM

THE OVARY

CHAPTER III

THE FEMALE REPRODUCTIVE SYSTEM

Introduction. The female reproductive system consists of the ovaries, oviducts, uterus, vagina, mammary glands, and the external genitalia. Of these, the ovaries produce hormones and discussion will be restricted to them. Discussion of the placenta is found in Chapter Four.

Embryology, anatomy, and function of the ovaries and testes have many similarities and as may be expected, many differences. A comparison of the ovaries and testes is made on page

General description of the ovaries. Textbook descriptions of the normal ovary are often too restricted and stereotyped to allow for the great variations which may fall within the normal range of the organ. Measurements generally give range from 2.5 cm to 5 cm in length, 1.5 cm to 3 cm in width, and 0.6 cm to 1.5 cm in thickness. It is fairly common for the normal ovary to attain larger proportions. All too often these "large" ovaries are regarded as pathological and are sent to the surgical laboratory for examination.

There are many quite normal variations of the surface appearance. A pale smooth or slightly pitted surface is considered by many as the normal appearance, while a vigorous and healthy organ with its bulging follicles, deep scars, hemorrhagic and non-hemorrhagic corpora lutea is often incorrectly considered abnormal.

Cross sections of adult organs show variations from specimen to specimen. The thin, pale, germinal epithelium on the surface cannot be distinguished by the naked eye. The tunica albuginea immediately beneath the epithelium is much thinner than in the testes and is not prominent. An ill-defined rather firm cortex surrounds the softer more vascular medulla. Near the hilus a few gaping slits, the rete ovarii, are present. Follicles which are actively growing may reach the size of a few millimetres and are thus visible to the naked eye. Corpora lutea are usually easy to identify, especially later in their development when they assume a fairly characteristic shape and yellow colour. Corpora albicantes and the smaller corpora fibrosa are white in colour because of the large amount of fibrous tissue present in these structures. Atretic follicles may attain great size, frequently reaching the diameter of a centimetre or more. These follicles are filled with clear fluid which is often under considerable tension. When the follicle is cut across, the fluid escapes and the wall of the cystic structure above the surface of the ovary collapses, while the wall beneath the surface is held in shape by the stroma.

The blood supply of the ovary. The ovarian arteries arise from the aorta in a manner similar to the testicular arteries in the male. The arteries arise from the antero-lateral aspect of the abdominal aorta, near the origin of the renal arteries. From here they descend retroperitoneally, cross the external iliac arteries approximately an inch below the level of their origin, and then descend into the

infundibulo-pelvic ligament. After reaching the mesosalpinx, each ovarian artery anastomoses with the corresponding uterine artery, and after giving off some branches to the oviduct, traverses the mesovarium to enter the hilum of the ovary[7].

The veins emerge from the hilum to form a pampiniform plexus which communicates with the plexus of the uterine vessels. Two veins issue from this plexus and ascend retroperitoneally in a manner similar to the testicular veins[7].

Lymphatic vessels from the ovaries are associated with the lymphatics of the body and fundus of the uterus. They drain into the lateral and pre-aortic groups[7].

Vessels within the organ. Recent studies of the vascular patterns of the human ovary show that standard descriptions found in text books are no longer tenable. Delson, Lubin, and Reynolds[8] report their studies and the rediscovery of Farre's description of 1858.

The main artery as it courses in the hilum of the ovary is undulant, tortuous and is somewhat flattened. From the main artery arise the primary arteries which are mostly tortuous and undulant. Secondary arteries arise from the primary vessels. As these become smaller they show a tendency to spiralling. The smallest vessels that these investigators were able to inject with plastic were the tertiary vessels. These showed regularly the greatest spiralling. The spirals were in the form of a counter-clockwise helix of gradually diminishing diameter.

The veins show no spiralling and are larger and irregularly flattened. Anastomoses are frequent. The veins drain into the pampiniform plexus in the hilar region. (Cf. Delson, Lubin and Reynolds[8])

The nerve supply. Fibres from the coeliac, renal and superior mesenteric plexuses supply the ovary. The origin of the ovarian plexus is often difficult to determine by dissection. The fibres are very fine and surround the vessels to the ovaries in the form of a network. Following the vessels, they reach the hilus where twigs are given off to supply the oviduct and others continue with the vessels in the ovary and branch with them. (Cf. Müller[9])

After reaching the ovarian stroma, only fine non-medullated nerve fibres are found. Müller[9] could find no ganglionic cells. Fine non-medullated nerves reach the follicles. The "egg layer" (cortex) is devoid of nerves. (Cf. Müller[9])

Note on embryology of ovaries. The early development of the gonads in either sex is similar until the 17 mm stage (seven weeks) is reached. The essential features of the early stages were given briefly in connection with the development of the male gonads at the end of Chapter II.

About the time the 13 mm stage (six weeks) is reached, the gonadal blastema becomes divided into cords by the formation of fibrous tissue bundles. The cords are at first attached to the germinal epithelium, but soon become broken into isolated cell masses.

The small groups of cells resulting from the fragmentation become grouped to form the primordial follicles. (Cf. Hamilton, Boyd and Mossman[20])

A summary of the fate of the primary follicles, i. e., development of the Graafian follicles, Corpora lutea, corpora albicantes, and atretic follicles. Probably no other organ has so many variable components. The follicles which have been designated as: (a) primordial, (b) primary, and (c) vesicular (maturing and Graafian), are not static but are changing from day to day. The corpora lutea as well undergo changes rapidly. A definite pattern is followed in the case of each structure, but before considering the detailed microscopic picture of each and the various phases of its existence, a brief summary of the development of an ovum and its subsequent possible fates is given.

From the germ cells there develop the primary follicles, each of which is essentially an ovum surrounded by a single layer of flattened follicular cells. As the follicle grows, the follicular cells become more numerous, and eventually are separated from the ovum by the development of a clear, glassy membrane known as the zona pellucida. Fluid collects in the interspaces of the follicular cells. As this follicular fluid collects, it enlarges the interspaces until a continuous follicular cavity is formed. Eventually the ovum with its zona pellucida and surrounding follicular cells is situated as a rounded mass of cells (cumulus oöphorus) on the inner surface of the lining of the vesicle. The follicle (now mature and called the Graafian follicle in this discussion) contains fluid which is under

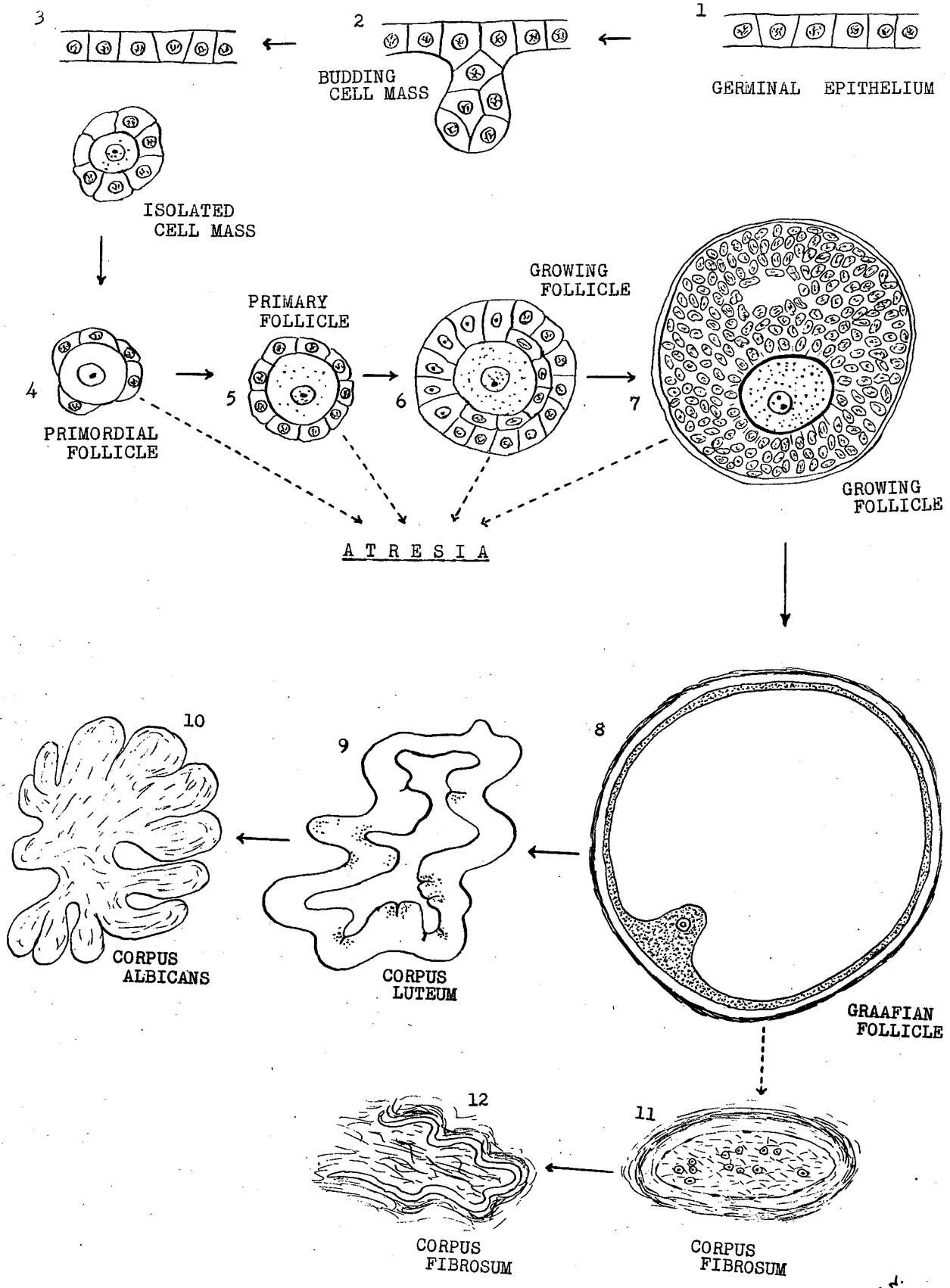

FIGURE 26

The development of the Graafian follicle and its subsequent fate. Based on several authors.

considerable tension. The follicle ruptures to release the ovum and some of the liquor folliculi. Following the rupture, the wall collapses and changes soon occur in the lining follicular cells to become the cells of the corpus luteum. The subsequent history of the corpus luteum depends upon the presence or absence of conception. If conception has occurred, the corpus luteum continues to grow in size and increases in function until the period between 50 and 60 days of the gestation, after which it undergoes regression. In the absence of conception, regression of the corpus luteum occurs forthwith, without continuation of growth. Finally the corpus luteum is replaced by collagenous connective tissue of the corpus albicans.

Not all primordia develop along the same plan as given above. Countless numbers become vesicular follicles, but as a rule only one, infrequently more than one, reaches the stage of maturity to discharge an ovum. When ovulation occurs, for some unknown reason, the other follicles "racing to maturity" undergo an arrest of development and become atretic follicles.

The germinal epithelium. Except at the hilum, the ovary is covered with a single layer of cuboidal or columnar "germinal epithelium." This layer is described as modified peritoneum or modified mesothelium. As age increases, the cells tend to become flattened. No basement membrane is found beneath the layer. A slight condensation of collagenous fibres, termed the tunica albuginea, is directly beneath the germinal epithelium. Compared to the structure of

FIGURE 27

Germinal epithelium of the ovary of a sixty-three year old woman. The epithelium is cuboidal. Masson's trichrome stain. 400 x.

FIGURE 28

Germinal epithelium. Higher magnification of the same section as Fig. 85. The basement membrane is seen where the epithelium is missing (artefact produced in handling of the tissue). Masson's trichrome stain. 800 x.

the same name in the testis, this tunica is thin and delicate.

There is general agreement among various workers that the germinal epithelium arises from coelomic epithelium in embryonic development[4].

Throughout the functional period of the ovary, the germinal epithelium shows marked activity of growth. Groups of cells from the epithelium migrate into the cortex and finally reach the deeper cortex and lose their attachment. Whether these cells develop into ova or degenerate is not definitely known. (Cf. Schwarz et al.[5])

Cortex and medulla. Division into cortex and medulla is poorly defined in the ovary. Follicles and their derivatives are found in a characteristic stroma of compact spindle-celled connective elements of the cortex. The cortex is wide during the reproductive period making approximately one half to three quarters of the cross-section. The medulla has a loose connective tissue stroma and numerous large contorted blood vessels. At the hilus, the medulla is not covered by the cortex. Smooth muscle fibres extend in from the mesovarium at the hilus, elsewhere a few may be found, chiefly around the blood vessels.

A few slit-like spaces are found at the hilus. These are the rete ovaree and are lined with a simple low epithelium.

In the senile ovary, the cortex is a relatively thin zone of connective tissue. The vessels of the medulla show endarteritis obliterans and in the walls of many of them there is often extensive calcification. Corpora lutea are not present. Primary follicles and

atretic vesicular follicles are found, although they are rare.

Origin of ova. While consideration of the different phases of maturation of the developing ovum is not within the scope of this discussion, brief reference is made to the work of Swezy and Evans[1]. According to these authors, the human embryonic ova arise by proliferation from the germinal epithelium. During the period between seven weeks to three months of intrauterine development only growth and multiplication take place. Maturation phases of the ova are found during the period from three months to five months. From five months to seven months maturation phases which are similar to those found in the male germ cells are found.

In the ovary of the adult, embryonic ova have disappeared. Definitive germ cells arise then in one of two methods: first by proliferation from the germinal epithelium of the surface of the organ, and secondly by division of germ cells already existing in the organ. (Cf. Swezy and Evans[1])

Schwarz et al.[5] support the view that new ova are formed after birth. The origin is mainly from the germinal epithelium according to these workers. Following is a quotation from their paper:

> (...) The concept that in the mammals the ova are all formed before birth and remain quiescent until cycle maturity has no foundation in fact. On the contrary, all of the ova in adult life are new formations and are being constantly produced and are constantly being destroyed. (...)

Primary follicles. Simkins[6] defines the primordial follicles as an ovum which is inconstantly and incompletely surrounded by flat and ellipsoidal cells. The average diameter of the primordial follicles is 30 micra. Primary follicles are completely surrounded by spherical cells and have an average diameter of 50 micra. In the ovary of the newborn, the primordial follicles greatly outnumber the primary follicles, while at 14 years, their numbers are about equal[6].

The single layer of spherical to cuboidal epithelium rests on a basement membrane and surrounds the ovum or germ cell. The nucleus of the ovum is large and vesicular with a loose network of linin threads, small chromatin granules and a large chromatin nucleolus.

Surrounding the follicle, the stromal cells are lightly stained and give the impression of a capsule. This is not a true capsule. The appearance is mentioned here because of the subsequent fate of these cells. They become the thecal cells to be discussed later.

Counts of the follicles vary considerably, probably due to different techniques[5] and are subject to such wide variations that estimates have no significance[6]. Maximow and Bloom[2] mention the figure of 400,000 ova in the ovaries of a newborn. v.Hannsemann[3] gives the number of ova in a single ovary between 40,000 and 80,000 in the newborn. v.Hannsemann found only 20,000 ova at the tenth year of life and by the beginning of puberty 16,000 in each ovary. While the numbers are very variable, the tendency for a progressive decrease in the total number with age is established[2].

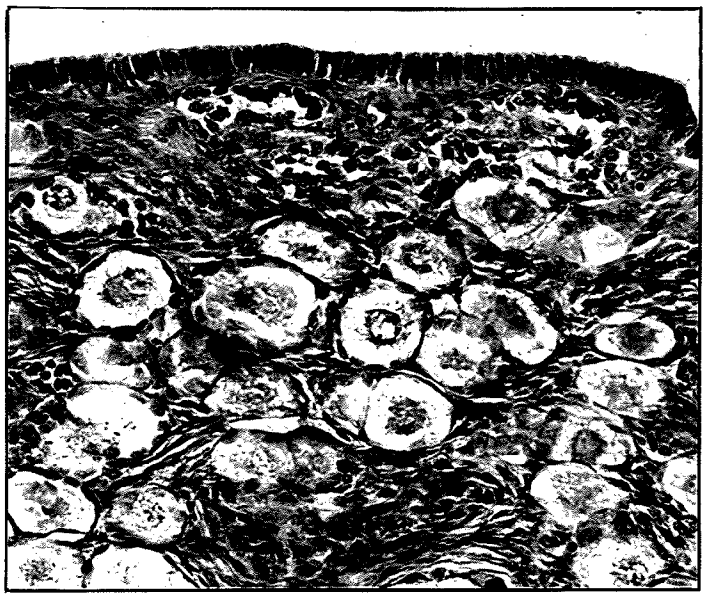

FIGURE 29

The germinal epithelium and cortex of the ovary from a female infant, aged 3 months, showing columnar germinal epithelium and many primary follicles. Hematoxylin and eosin stain. CH 48-4191.

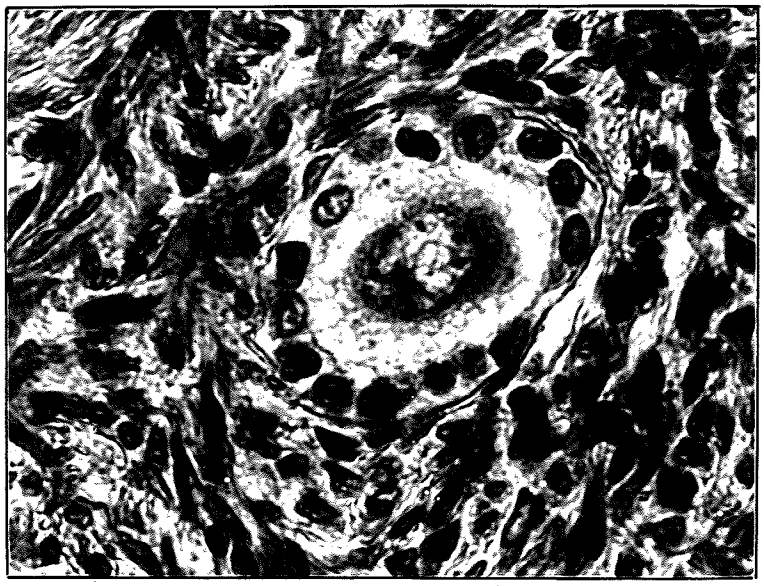

FIGURE 30

Primary follicle of an adult ovary. The nucleus shows some degeneration. Masson's trichrome stain. 800 x.

Growing follicles. As development progresses in the follicles, the nucleus of the ovum increases in size, and the cytoplasm increases in amount. Yolk granules of two types appear in the cytoplasm at a later date. Simultaneously, changes also occur in the follicular cells and the adjacent connective tissue. It is believed that ten to fourteen days are required for a mature follicle to develop from a primary follicle[2].

By the time the ovum is 60 to 80 micra in diameter, a highly refractile membrane, the zona pellucida, surrounds it[2]. The chemical nature of this thin layer of clear substance is not known, although from its staining reaction it must contain mostly protein[10]. It stains deeply, is highly refractile, and increases in thickness as the ovum and follicle mature. Striations and layers have been described by older writers. These striations are never seen in fresh specimens[10]. (Cf. Corner)

Enlargement of the follicle in the early stages is due mostly to the rapid proliferation of the follicular cells. While still a single layer, the follicular cells increase in size and number, their form changing from flattened to columnar cells[11]. Proliferation continues until the ovum is surrounded by a solid mantle of darkly staining cells many layers thick. Growth of the follicular cells is eccentric being more rapid at the pole containing the ovum with the result that the follicle is oval in shape[2]. No blood vessels are found among the follicular cells[12], which are now also called the granulosa cells.

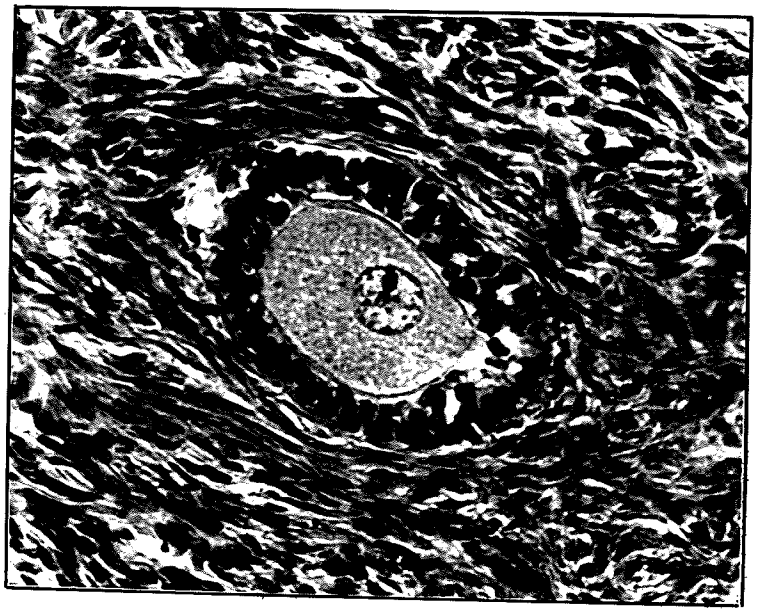

FIGURE 31

A growing follicle. Masson's trichrome stain. 400 x.

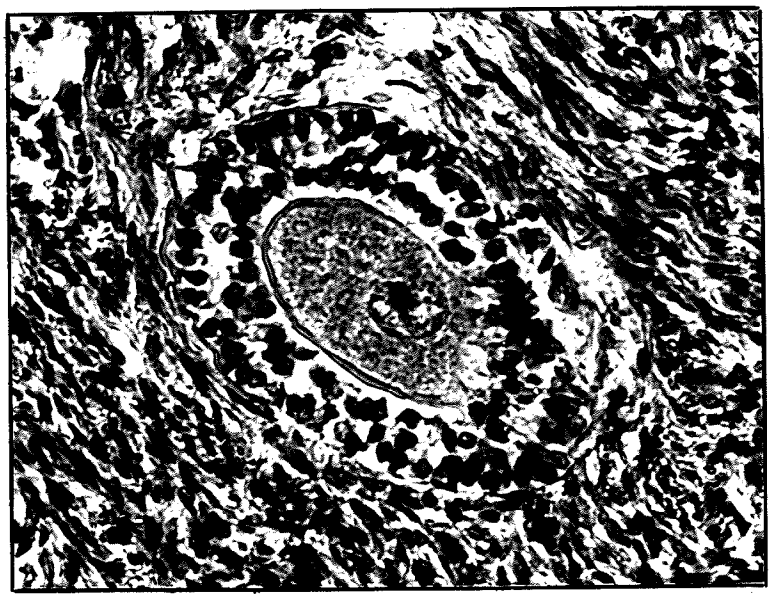

FIGURE 32

A growing follicle which has proceeded in development slightly further than the follicle shown in Fig. 31. Masson's trichrome stain. 400 x.

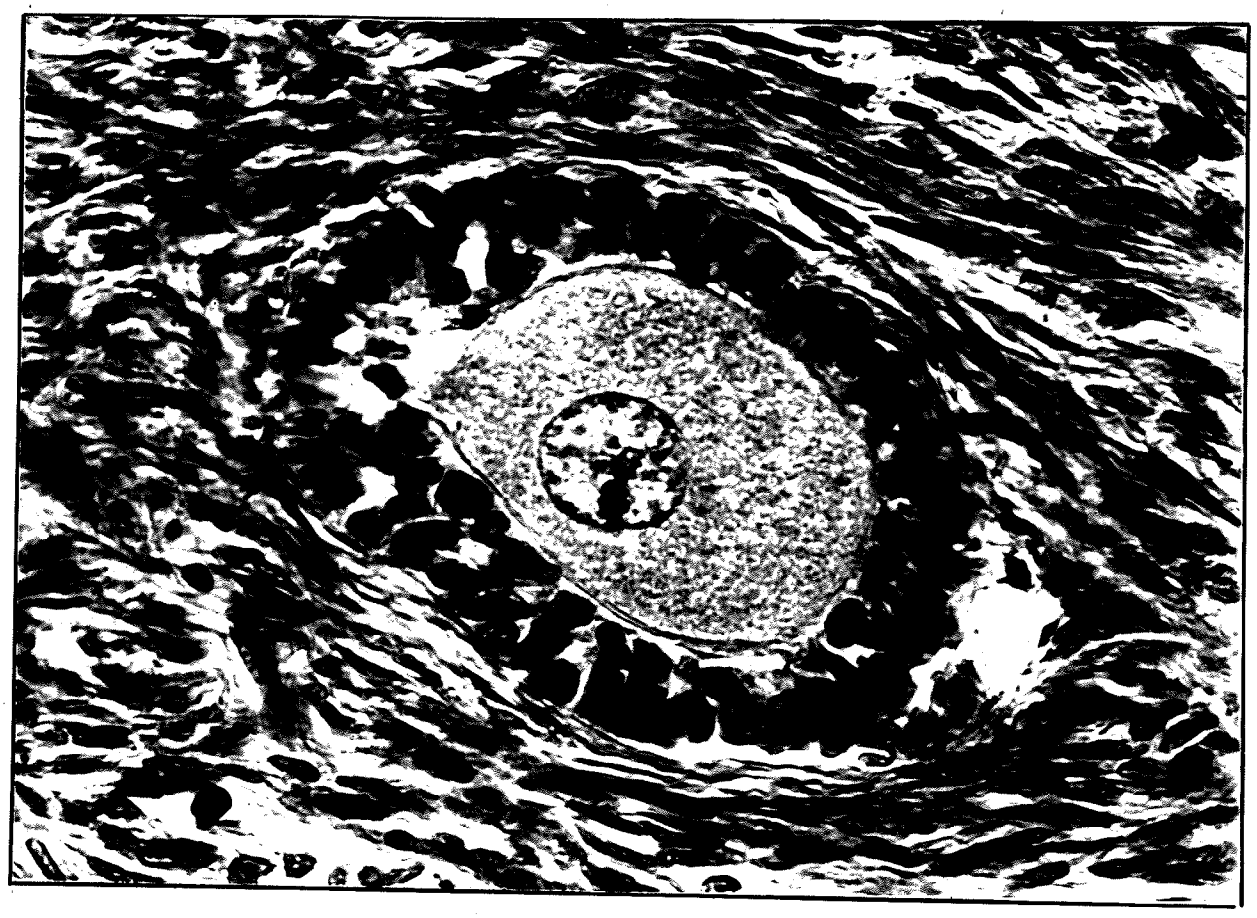

FIGURE 33

The growing follicle. The follicular cells are three and four layers thick. Masson's trichrome stain. 800 x.

When the follicle has reached a diameter of 0.2 mm, irregular spaces filled with liquor folliculi appear between the granulosa cells. As more fluid accumulates, the collections of fluid coalesce, and as a result, the follicle increases in size and is now termed a Graafian follicle[2].

The Graafian follicle is a vesicle filled with fluid. The granulosa cells have darkly staining nuclei and cytoplasm. The cells are arranged in sheets, densely packed together, and assume a polyhedral shape. At one side of the vesicle, there is a mass of cells which form the cumulus oophorus (discus proligerus), deriving its name from the fact that the ovum is embedded in this mound of cells.

The granulosa cells adjacent to the ovum form the corona radiata, where the cells are arranged radially to the zona pellucida. Next to the bodies of Call-Exner, and next to the theca interna, the granulosa cells tend to be radially arranged and to be columnar in shape. (Cf. Corner[10])

Call-Exner bodies may be present in the growing or mature follicles. These spherical bodies measure from a few micra to as large as 100 micra in diameter. They are not found in all follicles. About them the granulosa cells are arranged in a radial fashion, and the cells tend to be columnar. The Call-Exner bodies stain faintly with eosin and sometimes have basophil granules in their centres. (Cf. Corner[10]) Very little is known of their origin, composition, or functional significance.

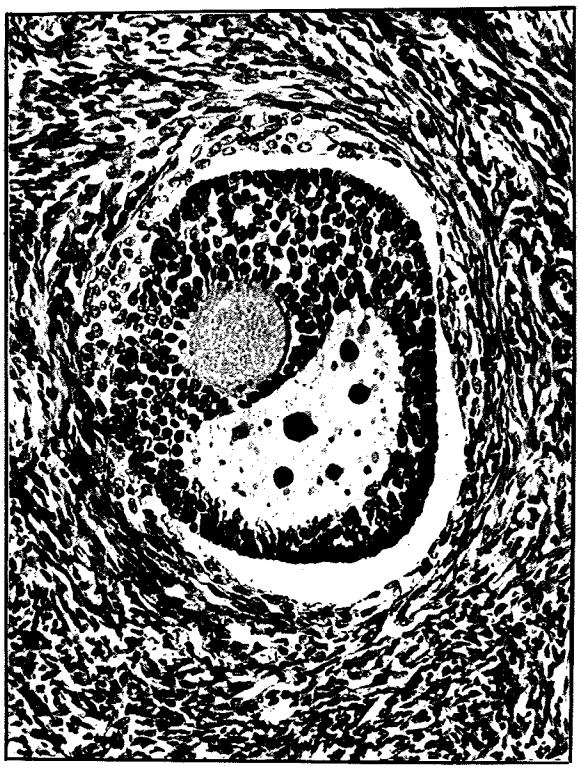

FIGURE 34

The developing follicle with early formation of the vesicle. A Call-Exner body with typical radial arrangement of the cells around it is situated just above the ovum. See also Fig. 35, Page 98. Masson's trichrome. 225 x. A 6566.

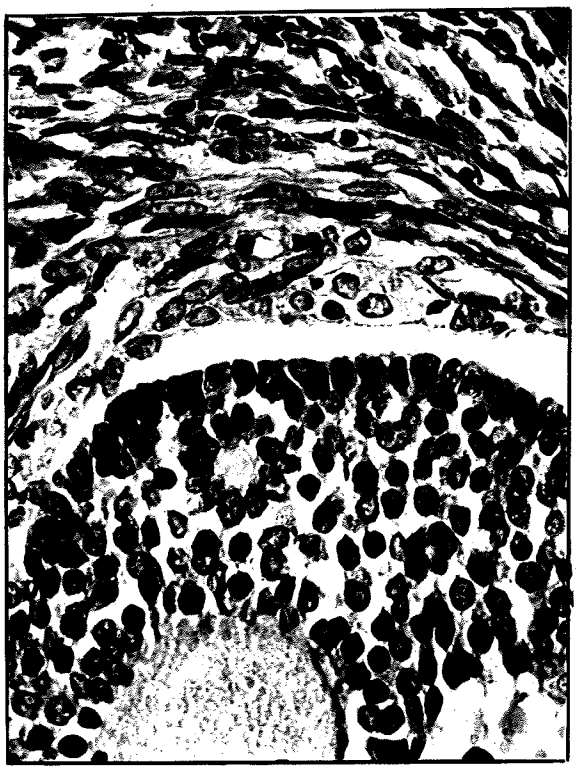

FIGURE 35

The Call-Exner body shown in Fig. 34.
Masson's trichrome stain. 500 x.

Mature follicles. Mature follicles (Graafian) are cystic structures which may reach the size of 8 mm in diameter. Most of the features of a mature follicle have been discussed in connection with the growing follicle. It is difficult to determine whether a follicle is still growing, has reached maturity, or is beginning to involute[2].

The wall of the mature follicle has three layers: (1) the membrana granulosa, (2) the theca interna, (3) the theca externa.

The innermost layer of cells is the membrana granulosa (the stratum granulosum or granulosa-lutein layer resulting from a transformation of the follicular cells of the growing follicle). This layer completely surrounds the central cavity or antrum which contains the liquor folliculi. The protein content of the liquor folliculi accounts for the granular appearance in fixed, stained sections. Shrinkage in sections is common and difficult to overcome. Considerable tension of the follicle results as more and more of the fluid collects. Accumulation of the fluid accounts for the rapid increase in size of the vesicle as it approaches maturity.

The membrana granulosa is supported by a well defined basement membrane, which also serves as a landmark to demarcate the membrana granulosa and theca interna in microscopic sections.

The cumulus oophorus has been described in the section dealing with the growing follicle.

The two layers of theca reach their greatest development at this time in the life history of the follicle. As growth begins in the Graafian follicle, a change is noted in the stroma of the ovarian

cortex in the immediate vicinity of the follicle. In the earliest stages of development of the follicle the connective tissue stroma surrounding the follicle is similar to the stroma found elsewhere. As development proceeds, two layers emerge. When the follicle reaches the 2 to 3 mm stage, the innermost layer becomes very vascular ("the perigranulosa vascular wreath"). The cells of the innermost theca layer are loosely arranged and form the theca interna. During the development of the follicle these cells are very sensitive to hormonal stimulation and take the appearance of "epitheloid" cells which are polyhedral in shape and which have oval nuclei and fine lipoid droplets in the cytoplasm[12].

The theca externa, however, retains its appearance of condensed, laminated, concentrically arranged connective tissue cells which are fusiform in shape[2]. The layer has many large blood vessels. As far as is known, the rather condensed ovarian stroma of the theca externa plays no important part in the life history of the follicle[12].

The ovum. The ovum in the mature follicle reaches a diameter of 120 micra or more[2]. A thick structureless membrane, termed the zona pellucida or oolema, surrounds the ovum and is closely applied to the surface. The large eccentric nucleus measures up to 25 micra in diameter, has a thick nuclear membrane, slightly granular linin network, and a large nucleolus (the macula germinativa)[2]. Yolk granules of different sizes and of different colour are found scattered throughout the cytoplasm when the ovum is seen in the fresh state[2].

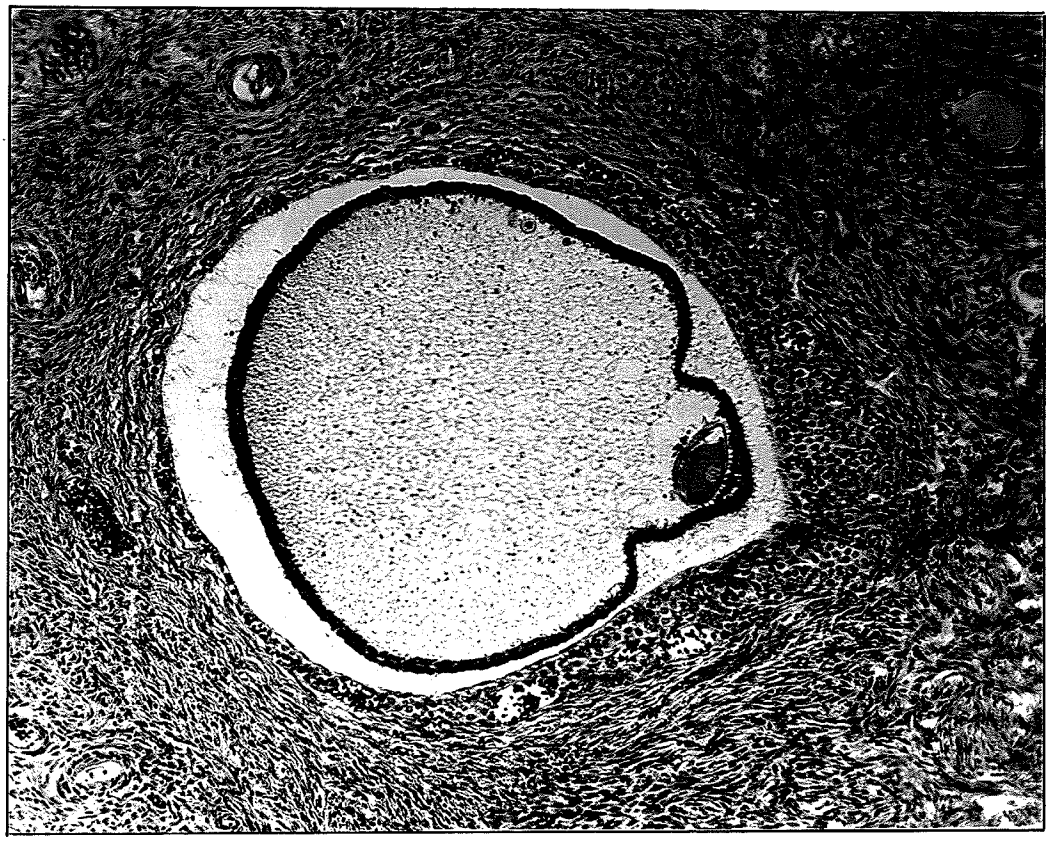

FIGURE 36

Graafian follicle showing signs of atresia. The space between the granulosa lutein cells and the theca lutein cells is an artefact. The growing follicle in the upper right hand corner is shown in Fig. 32, page 94. Masson's trichrome stain. 125 x.

Oögenesis. The development of the mature ova from the primordial sex cells is similar in many respects to the development of spermatozoa in the testes. Spermatogenesis is discussed in Chapter II.

The primitive female sex cells are the oögonia, which correspond to spermatogonia in the male gonad. An oögonium divides an indefinite number of times. Each daughter cell resulting from these mitotic divisions has 48 chromosomes, consisting of 46 ordinary chromosomes and a pair of X chromosomes.

Oögonia give rise to primary oöcytes by the process of conjugation, in which the chromosomes arrange themselves in pairs and fuse. In the process, the number of chromosomes is reduced to one half, but here there is no reduction in the amount of chromatin material. When reduced to one half, the number of chromosomes is said to be haploid, the full number being diploid. The chromosomes of the primary oöcytes divide by meiosis to produce secondary oöcytes. From the division of each primary oöcyte, there results two cells of unequal size. These cells have haploid nuclei. By far the greater mass of the cytoplasm goes to the secondary oöcyte. The other cell is very much smaller, has the same amount of chromosome material as the oöcyte and much less cytoplasm, and is known as the first polar body or polar cell. The polar body may divide, giving rise to two polar bodies or it may degenerate without the division taking place. All haploid female sex cells contain 23 ordinary chromosomes and one X chromosome.

A secondary oöcyte divides by meiosis to produce ova and smaller polar bodies. During the divisions from primary oöcyte to secondary

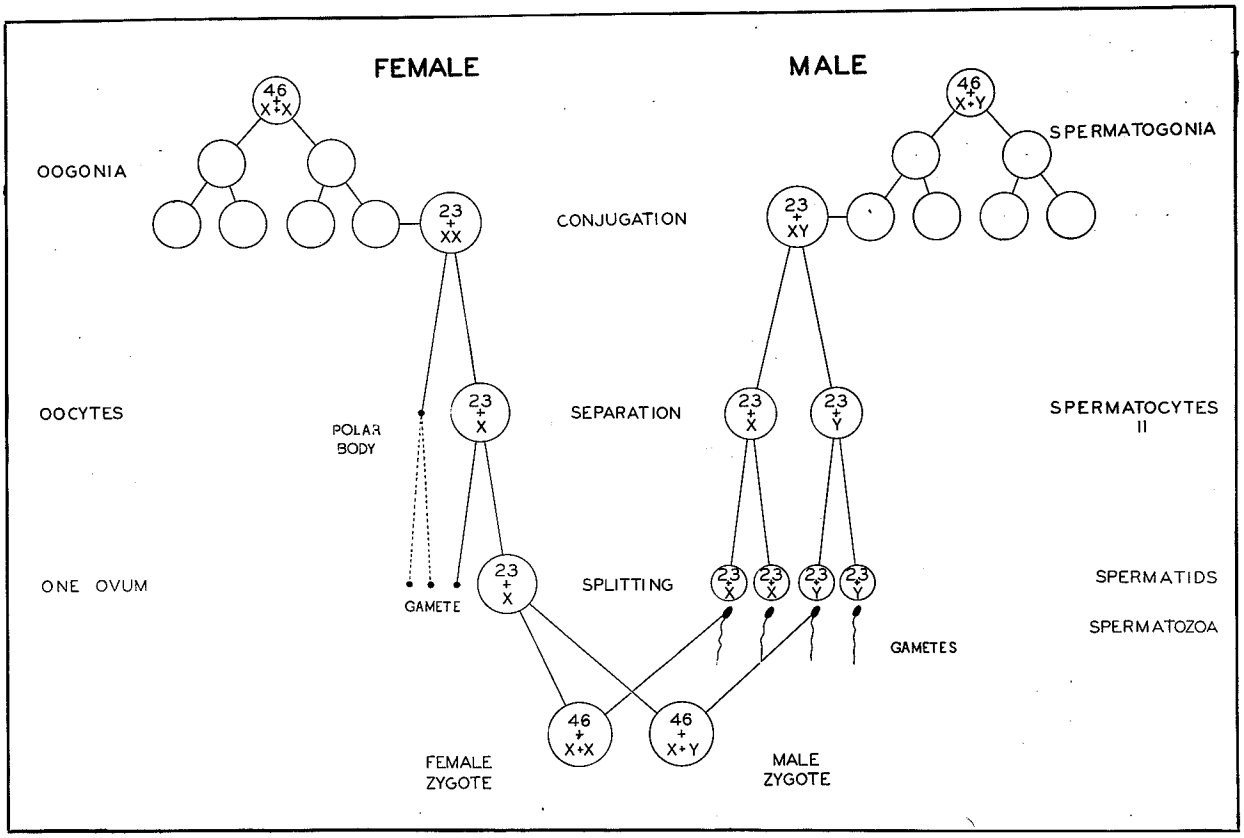

FIGURE 37

Diagram to illustrate the development of the mature sex cells of the male and female. Redrawn after Bremer, slightly modified.

oöcyte to ovum, there results only one ovum from one primary oöcyte, and not four sex cells as in the case of spermatogenesis.

As in the case of the male sex cells, much of the process above outlined is still theoretical and has not been observed in man. Many observations of similar nature have been made in lower animals, and it is assumed that the same cell divisions are present in human oögenesis. (Cf. Bremer[19])

Further development of the Graafian follicles. Following the stage of maturity, the further development of the Graafian follicles follows either of two courses. First, the follicle may mature, discharge its ovum and then become a corpus luteum. The corpus luteum is further discussed on page . Secondly, atresia of the follicle may occur, the details of which are given in the section below.

Atresia of the follicles. At the time of ovulation from a follicle, countless numbers of other Graafian follicles are approaching maturity simultaneously but only rarely does ovulation occur from more than one follicle. Why one follicle should be so selected is not known. The remainder undergo involution and regression, commonly termed atresia folliculi.

King (quoted by Gardner[11]) lists various forms of regression: (a) absorption, (b) atresia with cystic formation, (c) atresia with proliferation of the stratum granulosum and cystic formation, and (d) atresia with proliferation of the stratum granulosum and no cystic formation.

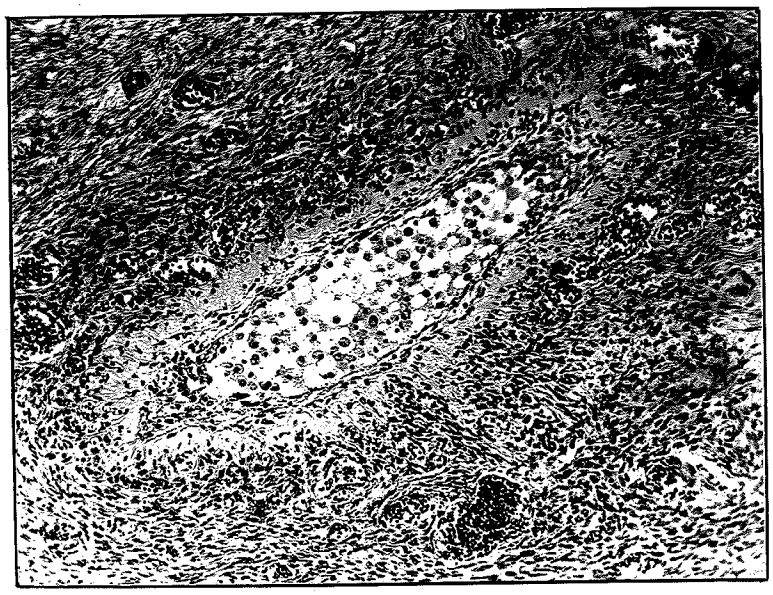

FIGURE 38

Forming corpus fibrosum.
Masson's trichrome stain.
100 x.

FIGURE 39

Corpus fibrosum. Masson's trichrome stain. 100 x.

The cells lining the atretic follicles are granulosa cells. They are considerably smaller than the lutein cells and are somewhat smaller than the granulosa cells of the follicle from which they originated. Occasionally if a section in the correct plane happens to be obtained, the cumulus oophorus and the degenerating ovum is seen. Lining the rest of the follicle the cells are found in layers which vary in thickness from four to eight cells thick. In others, the cells are thinned out to layers of one to two cells in thickness. The shape of the cumulus oophorus in older cysts varies greatly and is often distorted in shape. (Cf. Gardner[11])

Atretic follicles are found in the ovaries of all normal women during their active reproductive period of life. Such follicles are found also in the ovaries of young girls and of women after menopause. In these as well as in active ovaries, they must be considered as arrested developing follicles. These follicles are usually less than one centimetre in diameter, but occasionally and probably quite normally may reach larger dimensions. In time they disappear, leaving as their mark a small mass of fibrous tissue which differs in pattern outline from the corpus albicans. To this mass of fibrous tissue is given the name corpus fibrosum[12] or corpus candicans[13].

I. THE CORPUS LUTEUM

Introduction. The transitory nature of the corpus luteum adds to the difficulty of the study of this structure. It is not static, and any description must take into account its continuous and relatively rapid transformation from one stage to another[13]. The whole picture is pieced together from the study of many ovaries together with a correlation of clinical and operative findings.

Robert Meyer (quoted by Novak[14]) was the first to call to attention that the corpus luteum passes through certain stages corresponding to phases in the endometrium. The corpus luteum undergoes changes from day to day[14].

The corpus luteum arises from the granulosa cells of the Graafian follicle after the ovum is discharged from the follicle. The changes begin immediately after ovulation. Immediately after the rupture of the Graafian follicle of course there is no demonstrable change of the granulosa cells. Soon however, the walls collapse and the early corpus luteum is a thin walled collapsed structure. The thin walls are moderately undulating and have a grayish yellow colour instead of the brilliant yellow of the later stages. At this stage the structure is easily overlooked; it is inconspicuous and its discovery is largely accidental[14]. The common description of the bright yellow thick layer with a wavy outline is the structure of the corpus luteum seen only at certain of the later stages.

Reference to the influence of conception upon the development

and history of the corpus luteum has been made on page . If conception has not occurred, the corpus luteum begins forthwith to show degenerative and regressive stages prior to the onset of the menstrual flow. If conception has occurred, then the corpus luteum continues to grow and function until the pregnancy is definitely established. After 50 to 60 days it then begins to show signs of regression. In either case, conception or no conception, the ultimate outcome is fibrosis to form the corpus albicans which is a functionless mass of fibrous tissue. In time, the corpus albicans itself disappears.

That the corpus luteum is indispensable to menses was pointed out by Novak[14] in 1916. During the menstrual life of a normal woman, lutein tissue in some stage or another is always to be found in the ovaries. On the other hand, in non-menstruating women, corpora lutea are absent. Nor are corpora lutea found in the ovaries of the fetus, new born, or girl before puberty. For a number of months after the last menstrual flow at menopause, regressive corpora lutea are found in different stages. The amennorrhoea of lactation is explained by other hormonal factors suppressing the action of the corpora lutea. (Cf. Novak[14])

Four stages in the history of the corpus luteum of menstruation have been described. The terms are mostly due to Meyer (Arch. f. Gynäk., 1911, 93:354--quoted by Novak[14]):

1. Stage of proliferation or hyperemia
2. Stage of vascularization

3. Stage of maturity (blossom)

4. Stage of regression.

II. THE CORPUS LUTEUM OF MENSTRUATION

Hyperemic stage. Within a few hours after ovulation, structural changes occur in the wall of the follicle. The follicle collapses. The large ovoid or polyhedral theca interna cells which contain lipoid droplets resemble the lutein cells at this stage more than they do the granulosa cells. The theca layer is characteristically hyperemic while the granulosa layer is avascular. The picture then differs little from that of the wall of the mature follicle.

Stage of vascularization. Blood vessels in the hyperemic theca interna proliferate and grow into the granulosa layer. Small capillaries growing into the granulosa cell layer have been demonstrated by Brewer[15] approximately 24 hours after ovulation. Capillaries extend completely through the granulosa cell layer and project into the central cavity by three days after ovulation. Growth of the vessels is rapid as evidenced by mitotic figures found in the endothelium. The majority of the capillaries are distended with blood and extravascular blood is marked throughout the entire granulosa cell layer. The entire picture during the first three days of the life of the corpus luteum is one of extensive and rapid vascularization of the granulosa cell layer. (Cf. Brewer[15]) Changes are noted in the granulosa cells. The cells increase in size, stain less intensely,

have a relatively small nucleus and often a vacuolated appearance.

Five days after ovulation, the capillaries are tortuous and arranged so that most of the granulosa cells are in contact with a vessel. There is an ingrowth of connective tissue along with the capillaries which project into the central cavity. Hemorrhage into the cavity occurs from these vessels. During the next few days there forms a sort of lining separating the granulosa cells from the central cavity.

While the lutein cells are developing from the granulosa cells, the cells of the theca interna are regressing. With a loss of lipoids and a decrease in size, they take the appearance of ordinary connective tissue cells. Trabeculae, which are wedge shaped, grow into the granulosa-lutein layer to produce the familiar undulating appearance of the corpus luteum. The corpus luteum now has a bright yellow colour, wavy outline and hemorrhagic centre.

According to Brewer[15], during the period of eight to ten days after ovulation, there is gradual diminution of the hyperemia in the granulosa-lutein layer, the capillaries becoming straighter and narrower, with little or no extravasated blood. In the connective tissue core, however, the vessels are dilated and filled with blood. According to the same author, after ten days, there is little or no blood in the granulosa-lutein layer. The vessels are mostly collapsed and the connective tissue around them increases. (Cf. Brewer[15])

The granulosa cells become larger and acquire a more functional and active appearance as the vascularity of the granulosa-lutein layer

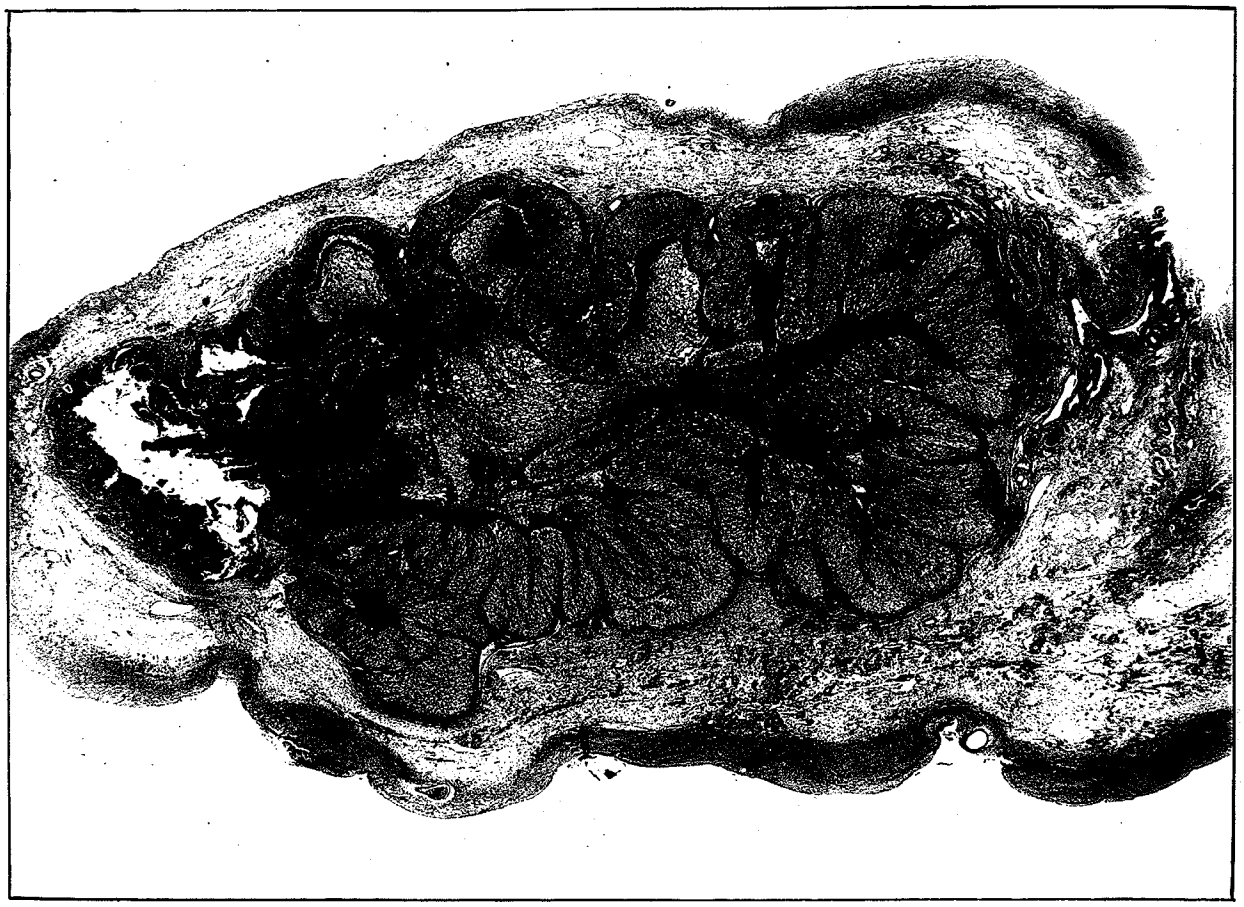

FIGURE 40

Corpus luteum of menses in the ovary of a young woman. The tissue has been torn in the left-hand side. Hematoxylin and eosin stain. G 4566. 10 x.

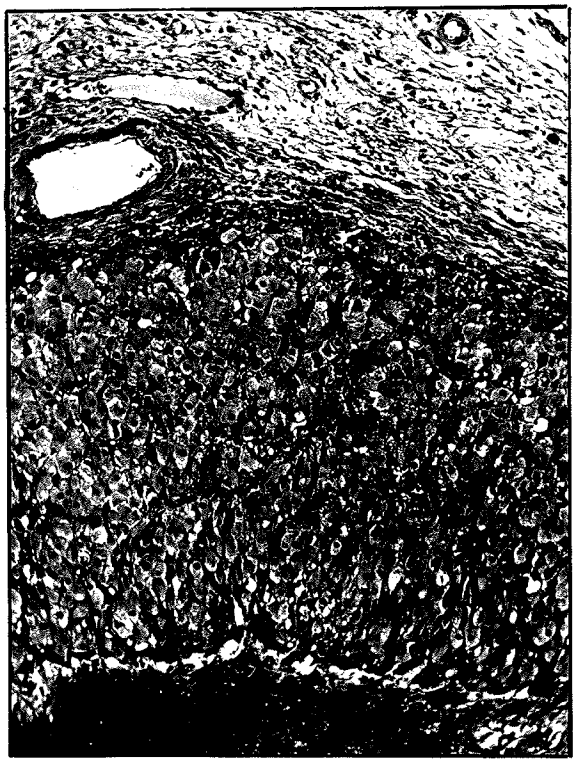

FIGURE 41

Higher power field of the same corpus luteum as seen in Fig. 40. 100 x.

increases. All cells do not develop or regress simultaneously, so that the processes are patchy. At seven to eight days after ovulation, some cells contain small lipoid droplets. The greatest increase in lipoid content is observed in the cells along the border of the cavity. (Cf. Brewer[15])

At the end of the vascularization stage at eight to nine days after ovulation, some of the granulosa-lutein cells begin to degenerate. The cells showing degenerative changes have a large amount of clear cytoplasm and pyknotic nuclei. At the end of eleven days the lipoid droplets are larger, and there are extensive zones of necrosis.

Stage of maturity. Mature corpora lutea may be large structures measuring up to 1.5 cm in diameter and larger. The central core is delimited by the surrounding lutein tissue. Fluid which is either clear or bloody may be found in the central space, which is also very variable in size. The cavity may be small due to the presence of a great deal of connective tissue. The folded bright yellow lutein tissue varies greatly in thickness and may measure up to 5 mm in thickness. The corpus luteum is an enormous body when its size is compared to that of the primary follicle.

Offshoots from the theca interna invade the band of granulosa-lutein cells and carry with them many blood vessels. The theca cells in the septa often become epitheloid in appearance and often show an alveolar arrangement. These are the paralutein cells of Pinto (1905). Their endocrine function, if any, is not known.

As maturity proceeds, the blood in the central cavity is gradually resorbed. Complete resorption seldom occurs; some of the pigments remain in the tissues and in the macrophages. Along the inner edge of the lutein zone a layer of fibroblastic tissue is developed.

Stage of regression. The stage of maturity is apparently restricted to a few days. Meyers in discussing Brewer's paper[15], states that he has found evidence of degeneration of the corpus luteum between days 22 and 24 of the menstrual cycle. According to Brewer[15], regression of the corpus luteum begins at the end of the stage of vascularization (four to six days before onset of menses) in that he has demonstrated at this time a marked decrease in the amount of blood in the blood vessels of the granulosa-lutein layer, fatty degeneration and atrophy of the granulosa-lutein cells, as well as an increase in the amount of visible fats in the cells. There is as well an increase in the amount of cholesterol in the cells of the corpus luteum. Other workers quoted by Brewer have shown a decreased amount of pregnadiol excreted in the urine after this time. (Cf. Brewer[15])

Corner, on the other hand, (quoted by Novak[12]) places the beginning of regression about two to three days before the onset of menses. His conclusion is based on observations upon the rhesus monkey. Actually all these times are very close if one considers them from the same reference point in the cycle. Meyers used as his point of reference the beginning of menses, Brewer the moment of ovulation,

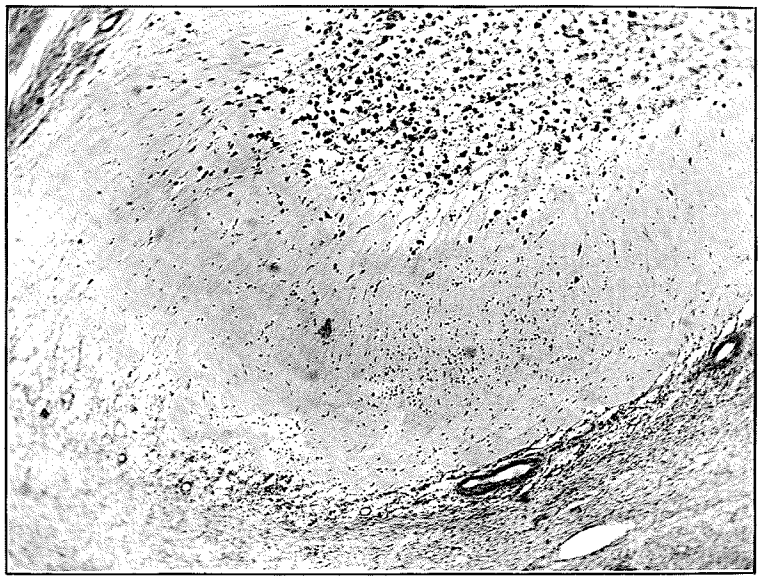

FIGURE 43

Degenerating corpus luteum. The granulosa lutein layer shows some hyalinization. Masson's trichrome. 50 x.

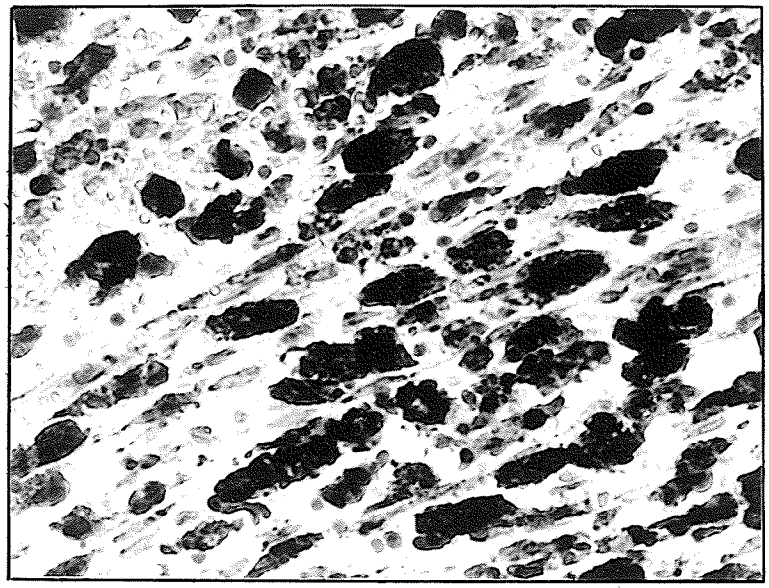

FIGURE 44

Macrophages filled with lipoid material in a degenerating corpus luteum. Stained with Sudan III. The dye appears black in the photograph. 400 x.

and Corner also the beginning of menses. If all the stated times are expressed in the same terms, they fall within two to three days of each other.

The degeneration of the granulosa-lutein layer is a slow and gradual process[17]. The transformation need not progress uniformly at all points of the wall[18]. In the early phases of regression, the process is reversible, should pregnancy occur. Regression is not sudden in onset and begins at the onset of the menstrual flow. Typically not all cells are involved. Immediately prior to the onset of menses, many histological variations are encountered in the granulosa-lutein layer. Uniform degeneration may occasionally involve all the cells. Degeneration may occur in localized groups of cells, or degeneration may be minimal and scarcely evident. Variations within the same corpus luteum may occur. (Cf. Brewer and Jones[17])

In regression, there is an increase in the amount of lipoids to be found in the cells. This increase of lipoids is the basis of comparison with the adrenal cortex. The comparison is applicable only to the older corpora lutea and is not applicable to the early corpora. Only old corpora lutea undergoing regression have a decided yellow colour[18]. Not all species have a yellow corpus luteum. The yellow colour has been traced to carotin found in the diet[10].

Fibrosis increases and continues until the whole structure is converted into a mass of connective tissue. The lutein layer shows also some hyalinization so that the contour of the layer is easily recognized. The yellow colour may persist for some time but after many

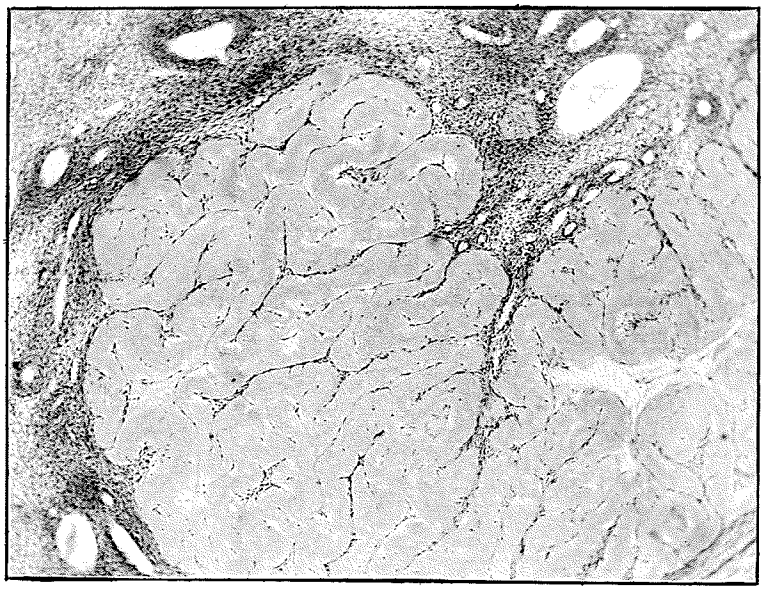

FIGURE 45

Corpus albicans. Only part of the structure is included. Masson's trichrome stain. 50 x.

weeks, the familiar white corpus ablicans is well established. In time, the corpus albicans disappears to leave no trace[11].

III. THE CORPUS LUTEUM OF PREGNANCY

Material for the study of the corpus luteum in all phases of pregnancy is difficult to obtain. Few reports are available. The account which follows is mostly from the paper of Gillman and Stein[16].

Although there are wide individual variations, a growth pattern is followed. After conception, the corpus luteum increases slowly in size. The early increase in size is due mostly to the formation of a relatively large cavity containing fluid within the centre of the structure. According to Gillman and Stein[16], the cavity increases slowly in size until the 50th day of gestation, after which it enlarges rapidly until the 60th day, and from then on it decreases rapidly in size. The decrease in size is due to a decrease in the size of the cavity which continues to decrease until about the fifth month when obliteration is complete. The amount of lutein tissue remains fairly constant throughout the pregnancy.

Septa from the theca continue to produce a folded appearance of the granulosa-lutein layer. Between the septa are reticular fibres which support the glandular cells and the fine capillaries. The thecal layer, so prominent in the early months of pregnancy, gradually diminishes to completely disappear by the fifth month.

Dilated blood vessels and lymphatics are prominent in the capsule of the early corpus luteum. Fibrous tissue lines the central

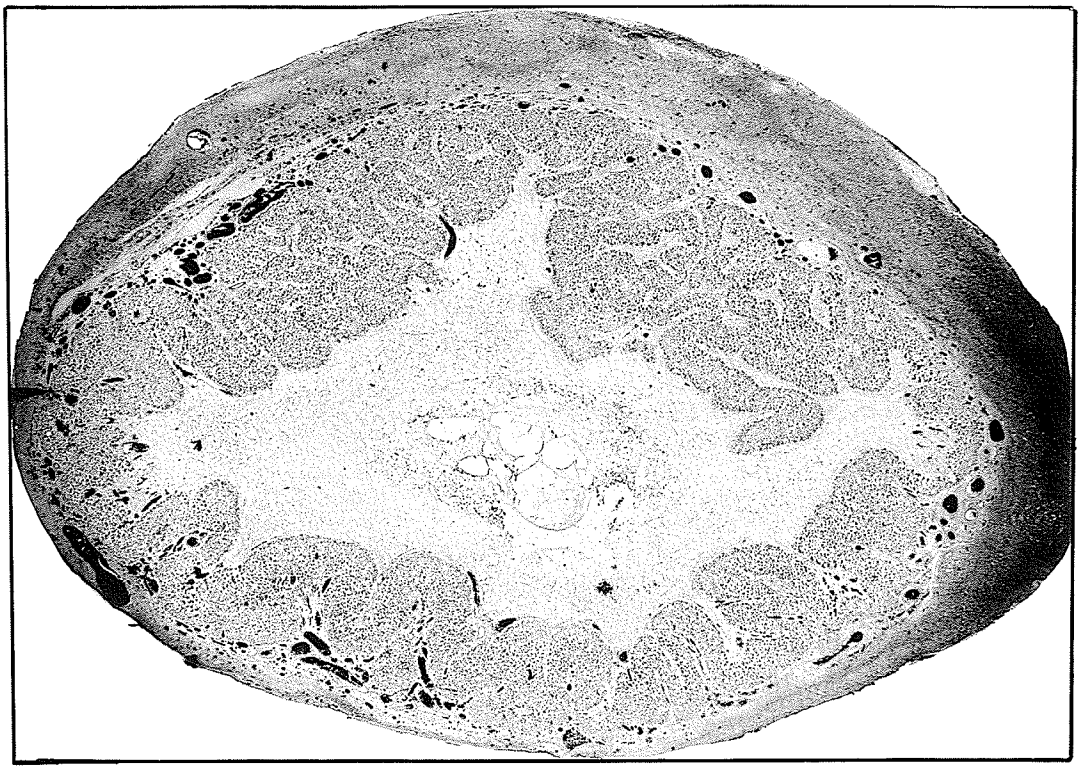

FIGURE 46

The corpus luteum of pregnancy at three months. From the ovary of a twenty-nine-year-old woman who died following air embolism, resulting from a vaginal douche with soapy solution.

cavity and consists of widely separated young fibroblasts in a faintly staining matrix.

The cells of both the granulosa-lutein and theca-lutein layers have inclusions, some of which are common to both types of cells, while other inclusions are found in only the granulosa-lutein cells.

Of these inclusions, the lipoids have received much attention. The lipoids were first described by Meyer in 1911. Gillman and Stein[16] find that the number of fat containing cells is greater at the beginning of pregnancy than at term, although fat is found in some cells throughout the whole of the gestation. Generally the lipoid is found in the form of small globules. Some may fuse to form larger masses. Cholesterol is found in relatively small amounts and is characteristically scarce in the corpus luteum of pregnancy.

Secretory granules which stain red after Mallory's stain are found in approximately 50% of cells in the early months. The granules are not specific for pregnancy and are also found in the corpus luteum just before the onset of menses.

Colloid droplets staining with Mallory's stain are cell inclusions which are labile in nature and disappear quickly after death. They are seldom seen in autopsy material unless they have undergone degeneration and have become calcified. These bodies are found only in the corpus luteum of pregnancy. Some of the colloid bodies have vacuoles.

The granulosa-lutein layer. Cells of the granulosa-lutein layer form the bulk of the corpus luteum in early months and at term all of it. Reticular fibres are irregularly arranged throughout the layer.

Compared with the theca-lutein cells, these cells are larger. Staining is less intense. The larger nucleus is vesicular and contains one or two and occasionally more prominent nucleoli which are oval or rod shaped. The nuclear membrane is delicate and the chromatin within the nucleus is arranged in a delicate linin network.

Sixty or more fine, dark red granules are found in each cell when stained with Mallory's connective tissue stain. They are contrasted against the dark blue of the cytoplasm. The granules are scattered throughout the cell or they may be arranged in a peripheral manner. About 30 per cent of the cells contain these granules in early pregnancy while at term only 2 per cent contain them.

The lipoid occurs as fine droplets which occasionally fill the whole cell. They are characteristic of early pregnancy.

In the first two months of gestation, less than 15 per cent of the granulosa-lutein cells contain one to two colloid granules measuring from 3 to 10 micra in diameter. At term, 50 per cent may contain colloid. The droplets increase in size and in numbers as the gestation progresses. The cell may disintegrate and leave only the colloid. Staining is variable. With Mallory's stain, the colloid droplets may stain red, blue or yellow. With eosin and hematoxylin, the granules take a pale pink stain. The colloid bodies are labile

and are seldom found in autopsy material. No colloid bodies are found in the theca cells.

Vacuoles in the form of round colourless spaces in the granulosa-lutein cells are most numerous in early stages, especially in the cells bordering the central cavity. The vacuoles gradually enlarge to fill the whole cell. Specimens from the latter half of pregnancy have fewer vacuoles. The contents of the vacuoles become progressively more stainable and form the colloid droplets. Vacuoles are not found in the theca-lutein cells.

Chromidial substance in the form of filaments, triangular or club shaped bodies in the cytoplasm are found in the cells of both the granulosa-lutein and theca-lutein layers. It is most abundant in the early months of gestation. A progressive decrease in the amount of chromidial substance is seen throughout pregnancy. Much of this work of Gillman and Stein awaits confirmation. (Cf. Gillman and Stein[16].)

The theca-lutein layer. The reticular fibres of the theca-lutein form a regular fine network and support regular groups of cells. The cells are closely associated with blood vessels. The cells are small, polyhedral and measure 15 micra in diameter. The round nucleus averages 7 micra in diameter. Within the nucleus, a single large round nucleolus is found. Compared with the nucleus of the granulosa-lutein cells, the nucleus is darker. The granules found in the theca-lutein layer are slightly larger than those of the granulosa-lutein layer and average 2 micra in diameter. Only a few are found in each cell. The

lipoid granules are relatively large and few are found in each cell. The colloid bodies are absent.

IV. THE TESTES AND OVARIES COMPARED

Until the 17 mm stage (seven weeks) of development is reached, the ovaries and testes have similar embryological histories. In each case the gonads arise as thickenings of coelomic epithelium. Later in development, the germ cells arise as part of the sex cords which are developed from the coelomic epithelium. Both the male and female gonads descend during development, the descent being most marked in the case of the male. The male gonads must be in an environment slightly cooler than body temperature in order that spermatozoa may be produced. During development a complete excretory system is developed to convey the countless numbers of spermatozoa to the exterior. On the other hand, the female has what seems to be a rather precarious and incomplete duct system.

Development of the germ cells in the testes and ovaries is essentially the same. Both ova and spermatozoa are haploid cells as a result of mitotic and later the meiotic cell division. One half of all spermatozoa have 23 ordinary chromosomes plus an X chromosome; the other half have 23 ordinary chromosomes plus a Y chromosome. All ova have 23 ordinary chromosomes plus an X chromosome (never a Y chromosome). From one primary spermatocyte, four spermatozoa develop. From one primary oöcyte, only one ovum develops (the one half of the chromosome material going to the polar bodies). On the one hand,

spermatozoa are produced by the thousands and with no apparent economy, while on the other hand ova are produced one at a time. Several ova may be developing, of course, at the same time, but only one is extruded as a rule.

Structurally the ovary is more complex than the testis. In the ovary, there are more variable components which undergo cyclic changes that may occur from day to day. The testis has interstitial cells which are not found in the ovary. Senile changes occur in both male and female gonads, the changes being more pronounced in the case of the ovaries and occurring at an earlier age as a rule.

The testes produce more androgens than oestrogens, while the ovaries produce more oestrogens than androgens. The difference is quantitative rather than qualitative. It is interesting to note that the ovaries, testes and adrenals all begin their development in approximately the same part of the body and all produce in varying amounts the steroid compounds which influence the sex pattern of the individual. The ovaries have one function for which the testes have no counterpart in the production of progesterone. Both organs are influenced by the hormones of the anterior lobe of pituitary as well as by hormones of other endocrine organs.

V. THE HORMONES OF THE OVARIES

The hormones of the female and male gonads are discussed in Chapter V, in which the hormones of reproduction are considered.

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C H A P T E R F O U R

THE PLACENTA

CHAPTER IV

THE PLACENTA

While nutrition of the fetus and removal of its metabolites are functions of the placenta which have been recognized for a long time, the production of hormones has been proved only within recent years. The presence of oestrogenic substances in the placenta was demonstrated early[18]; however, their presence was attributed to storage of the hormones by the placenta and not to the production of them in the organ. In the early 1930's, Collip produced crude active extracts which he believed contained hormones produced by the placenta. Subsequently other investigators produced crystalline compounds and within recent years hormones have been produced by placental tissue growing in tissue cultures[21, 22].

Material for the study of implantation and nidation of the embryo is difficult to obtain. Early studies of these processes were made on laboratory animals. Grosser[8] reviews some of the early history and points out that fowl and rabbits were the first animals to be studied. These animals have features which are similar to each other and which are not found in other species. The deductions based on the studies of these and other animals were used to bridge the wide gaps in our knowledge of early human and primate development. Gradually the gaps have been narrowed by further studies of human material.

The early development of the placenta is intimately associated with the development of the ovum, although ovulation, fertilization and

migration of the ovum occur before implantation which is the initial stage of placentation. The entire life history of the placenta may be divided as follows:

(a) Implantation (nidation).

(b) Placentation.

(c) Growth and maturity of the placenta.

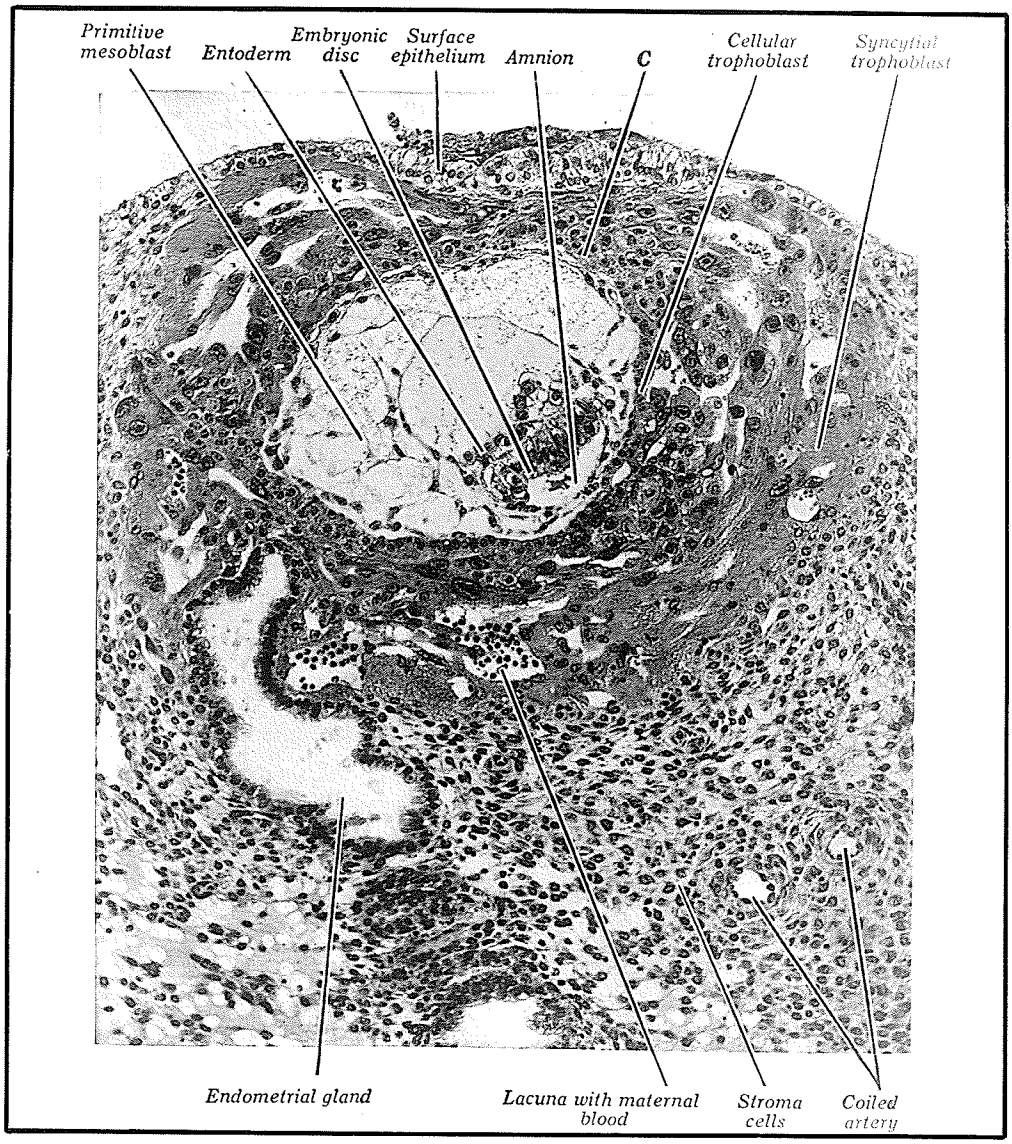
(d) Expulsion of the Placenta.

Rock and Hertig[1] have traced human development during the first two weeks. The first part of the discussion in this Chapter is found mostly in their report.

A zygote results from the union of an ovum and a spermatozoon. The term zygote has been used very little in recent literature and the term ovum is used to describe the newly formed organism up to the fourteenth day. The term embryo denotes the organism up to the end of the second month, and from this time on, the term fetus is used. In this discussion, the term ovum is used instead of zygote.

Development before implantation. The mature ovum is fertilized, traverses the oviduct, and undergoes segmentation into at least two blastomeres during the first twenty-four hours after ovulation, according to the deductions of Rock and Hertig[1]. By the third day, the ovum is in the uterine cavity. The eight cell stage is reached by the fourth day.

Development between days 3 and 7 has not been observed in the human and deductions are based on observations on the monkey[1]. In this



Photomicrograph of a section through the implantation site of the eleven-day Hertig-Rock ovum, magnified 140 diameters. The bulk of the ovum consists of irregular masses of trophoblast (syncytium) which is invading the endometrium. Within the syncytial trophoblast is the cellular trophoblast, the cells of which are arranged as simple epithelium except at C. The cellular trophoblast immediately surrounds the primitive chorionic mesoblast in which the embryo is suspended. (After Hertig and Rock, Carnegie Contrib. to Embryol., V. 29, 1941, Fig. 14) Reproduced from "A Textbook of Histology," by A. A. Maximow and Wm. Bloom, Fifth edition, p. 572, with written permission from the publishers, The W. B. Saunders Company, Phila.

animal, during the period between days 3 and 6, a multicellular morula develops and it acquires a central segmentation cavity and so becomes a blastocyst. At one pole, a small mass of cells remains grouped, forming the inner cell mass which gives rise to the embryo proper. The other cells spread out to form the cyst wall and give rise to the trophoblast and allied structures.

Implantation. The cavity of the blastocyst is probably formed during the fourth or fifth day, following which there is differentiation of three kinds of cells; the trophoblast on the wall of the blastocyst, the ectoderm and endoderm of the embryo. Contact with the endometrium occurs about the sixth day. The segmentation cavity collapses as it does in the monkey. "The newly differentiated syncytial trophoblast at the embryonic pole engrosses some of the superficial epithelium and Streeter puts it "forages" its way into this tissue." (Rock and Hertig[1])

The ovum becomes embedded in the endometrial stroma and this process is known as interstitial implantation. In the monkey, at day 9, the blastocyst attaches itself to the endometrium and begins to implant. The development in the monkey is not so rapid as in the human. There is intense edema and congestion of the endometrium in the neighbourhood of the ovum[2].

At 11 days, the ovum is a slightly raised pale area, a little less than 1 mm in diameter. It is translucent and is surrounded by congested endometrium[2]. By this time the ovum is buried in the endometrium[1].

Placentation. Implantation represents the initial stage of the process of placentation[2].

In the nine-day-old specimen described by Rock and Hertig[1], it is illustrated as being almost entirely surrounded by endometrium. The segmentation cavity is redistended and the ovum, consisting of ectoderm and endoderm, is found within it. The enlarging amniotic cavity is bounded dorsally by the early amnion which is a product of the adjacent cytotrophoblast. The mass of syncytium has increased six times in amount. Lacunae have formed in the synctial mass, most of which, if not all, communicate to form a network of canals. In their specimen, one lacuna contains maternal blood, forming the first stage of the placental circulation. (Cf. Rock and Hertig[1])

By twelve days, the ovum is 1 mm in diameter and is entirely embedded in the endometrium although the site of entry is not quite covered. The embryo itself measures 0.1 mm in diameter. It consists of a pyriform plate of pseudostratified ectodermal cells and a thin layer of endodermal cells. The mesoderm has not yet appeared. The amniotic sac is a thin-walled cyst on the dorsal surface bounded by amnion and ectoderm. The embryo lies in a cavity which is five times its size and which is filled with fluid. A lacework of mesoblasts subdivides the extraembryonic cavity into numerous spaces, one large and many small.

Trophoblast surrounds the whole central space that contains the embryo. The trophoblast exhibits two layers, an inner cytotrophoblastic layer and an outer syncytiotrophoblastic layer. The

cytotrophoblast surrounds and gives rise to the primitive mesoblastic tissue. The syncytiotrophoblast has made contact with the endometrium and has "engrossed and digested the maternal stroma in which it grows." A portion of a thin-walled sinus has been dissolved. Maternal blood has filled the fused lacunae within the syncytium. At this stage, the ratio of syncytiotrophoblast to cytotrophoblast is about three to one, a relationship which is soon lost. (Cf. Rock and Hertig[1])

By the fourteenth day, the cytotrophoblast has grown rapidly and by this time is arranged in masses of discrete cells that project into the syncytium[1]. These are the primary villi[2] which have no mesodermal core. Except at the zone of contact with the endometrial stroma, the syncytium forms now only a thin layer lining the lacunae. The greater amount of trophoblast is made up of cytotrophoblast and a correspondingly less amount of foraging syncytiotrophoblast. Extensions from the mesoblast project into the primary villi forming the supporting structure of the villi which are known as secondary in type. Maternal blood is found in the lacunar spaces. The whole ovum now measures from 2 to 3 mm in diameter in the fixed state. (Cf. Rock and Hertig[1])

Growth and maturity of the placenta. By the fifteenth or sixteenth day, secondary villi have appeared and these contain a core of extraembryonic mesoderm in which a plexus of embryonic vessels soon develops. The core of mesoderm is immediately surrounded by a layer of cytotrophoblast which in turn is covered with a layer of

syncytiotrophoblast. The tips of the secondary villi continue to extend as columns of cytotrophoblast. Mesoderm gradually extends into these tips. The ends of the villi fuse at the periphery of the growing ovum to form a trophoblastic shell which is composed mainly of cellular trophoblast. (Cf. Wislocki[2])

Changes in the endometrium. The endometrium adjacent to the growing ovum undergoes a number of changes. In the zone where the trophoblastic shell and endometrium meet and intermingle, the maternal tissue undergoes degeneration. This area has been called the "junctional," "composite," or the "penetration" zone. The endometrium is divided into three layers. The superficial layer is the stratum compactum, in which the stromal cells are transformed into a zone of large, characteristic polygonal decidual cells. The middle zone is the stratum spongiosum which is characterized by very much dilated actively secreting glands. The thin stratum basilis is adjacent to the myometrium.

The decidua is divided into three regions: basalis, capsularis, and parietalis. Each term indicates the relationship of the decidua to the ovum. The decidua basalis (serotina) is immediately beneath the ovum and forms the maternal part of the placenta. The decidua capsularis (reflexa) is over the ovum and separates it from the uterine cavity. The decidua parietalis (vera) lines the remainder of the uterine cavity. All the decidua is cast off with the fetal membranes during the third stage of labour. After parturition, the endometrium

is renewed by subsequent growth of those residual portions of the stratum basalis which remain intact.

At an early stage, the chorionic villi are present over the entire chorionic vesicle. The villi become long, branched, and grow more profusely where the trophoblast of the ovum is in contact with the deeper and more vascular endometrium. These constitute the chorion frondosum, and eventually develop into the placenta as we know it at term. Over the remainder of the ovum, the villi are much shorter and their development is minimal. By the third month of gestation, the villi, together with the decidua capsularis, dwindle and leave a relatively smooth membrane termed the chorion laeve. During later development, with enlargement of the fetus and the amnionic sac, the chorion laeve eventually fuses with the decidua parietalis of the opposite wall, thereby obliterating the uterine cavity until the end of gestation. (Cf. Wislocki[2])

Angiogenesis. Towards the end of the first month of gestation blood begins to circulate in the capillaries of the chorionic villi. The red cells are nucleated. The secondary villi continue to grow and branch. They project into the intervillous spaces. A certain number of villi attach to the decidua and become anchoring villi. (Cf. Wislocki[2])

According to Hertig[9], there is morphological evidence that angioblasts and primary mesoderm originate simultaneously by delamination and differentiation from the chorionic trophoblast.

Further origin of angioblasts and primary mesoderm is usually associated with the metamorphosis of trophoblastic columns into primary villi and continues only as long as the primary villi are being formed. The isolated vascular primordia thus formed soon possess the power of independent growth and lumen formation, resulting not only in the vascularization of the chorion and primary villi but also of the secondary villi as well. (Cf. Hertig[9]) It is important to note that some other authors do not support Hertig's theory.

Villi at 12 weeks. The villi at the twelfth week of gestation are large and have both cytotrophoblast and syncytiotrophoblast. The outer syncytiotrophoblast layer stains bluish gray with hematoxylin and the cytoplasm is very finely granular. The nuclei stain darkly and are somewhat irregular in shape. The cytotrophoblast (Langhans' layer) is made up of large, clear cells, which are discrete and have large pale staining nuclei. The stroma of the villi consists of loosely knit, embryonic connective tissue, with scattered nuclei. Blood vessels are few, small in calibre, and contain nucleated red blood cells. The endothelial cells are large and embryonic. Hofbauer cells are present. These contain lipoids, and appear vacuolated[3].

Villi at twenty weeks. The villi become progressively smaller as the gestation advances. Such a change is evident at this time if the villi are compared with those of an earlier gestation. The cytotrophoblast has begun to disappear by this time. There are more blood vessels present and the stroma has become slightly more dense. Nuclear

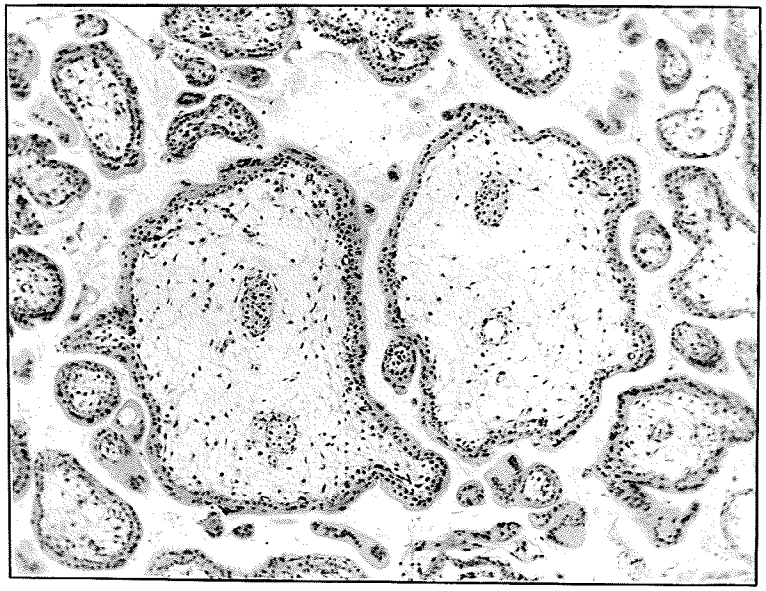


FIGURE 47

Placental villi of a six weeks gestation. Hematoxylin and eosin stain. WGH 1208-48. 75 x.

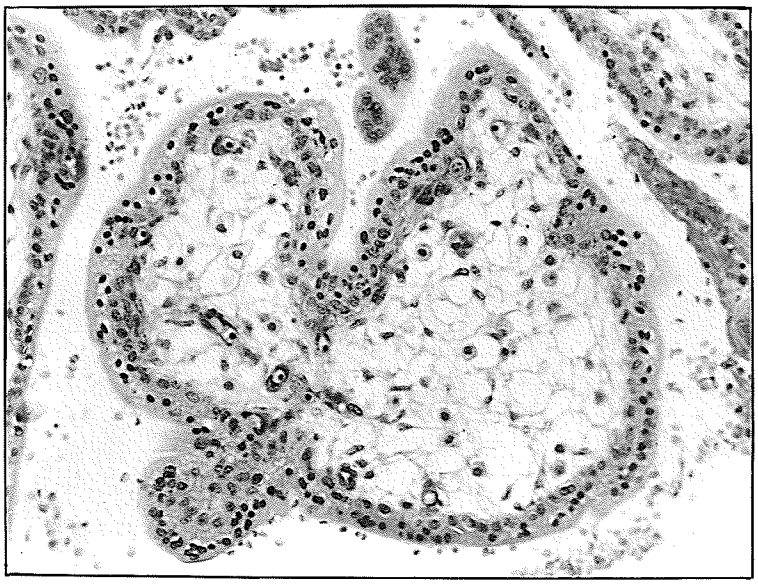


FIGURE 48

Villus of six weeks gestation, showing cytotrophoblast, synctiotrophoblast, loose stromo and few blood vessels. WGH 1208-48. Hematoxylin and eosin stain. 175 x.

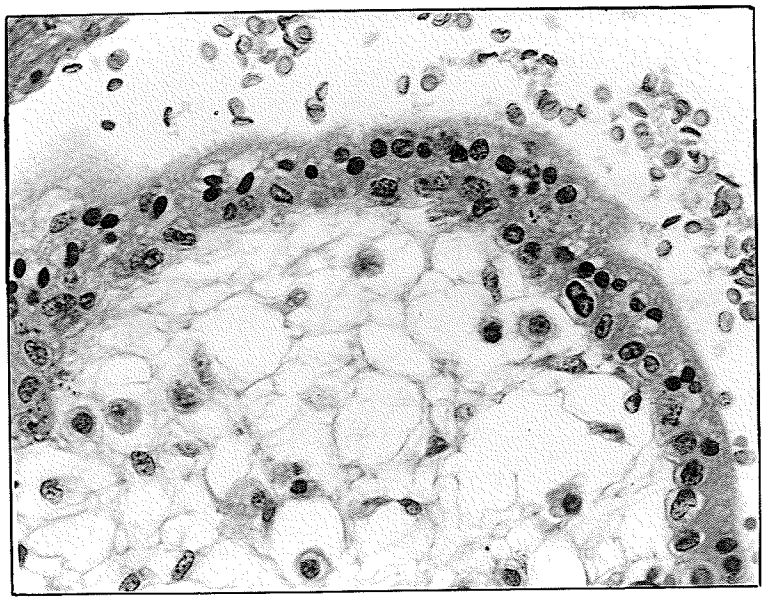


FIGURE 49

Villus of a six weeks gestation. The Langhans layer and syncytium are well differentiated. The loose stroma has few blood vessels. Hofbauer cells are present. Hematoxylin and eosin stain. 400 x.

knots are present, although they are not as numerous as at a later date. (Cf. infra)

Villi at thirty weeks. The villi are still smaller. Here and there on the syncytial layer the nuclei are grouped together, leaving adjacent clear areas in the syncytium devoid of nuclei. The groups of nuclei have been termed nuclear knots, and these are regularly present in the placental villi after nineteen weeks. By thirty weeks the cytotrophoblastic cells are rare. The stroma is still slightly more compact and has still more capillaries. The red corpuscles in the capillaries are without nuclei. The endothelial cells lining the capillaries are mature in that the cells are more compact and the nuclei are flattened.

Villi at forty weeks (term). At term the villi are smallest and are more numerous in a unit area than at any other time of the gestation. The syncytium at term is thin and acidophilic with many nuclear knots attached to the side of the villus. Cytotrophoblast is characteristically absent. The capillaries make up two thirds of the connective tissue core. Hofbauer cells are not present[3].

Expulsion of the placenta. Expulsion of the placenta occurs during the third stage of labour. Normally, separation occurs along the spongy layer of the decidua basalis.

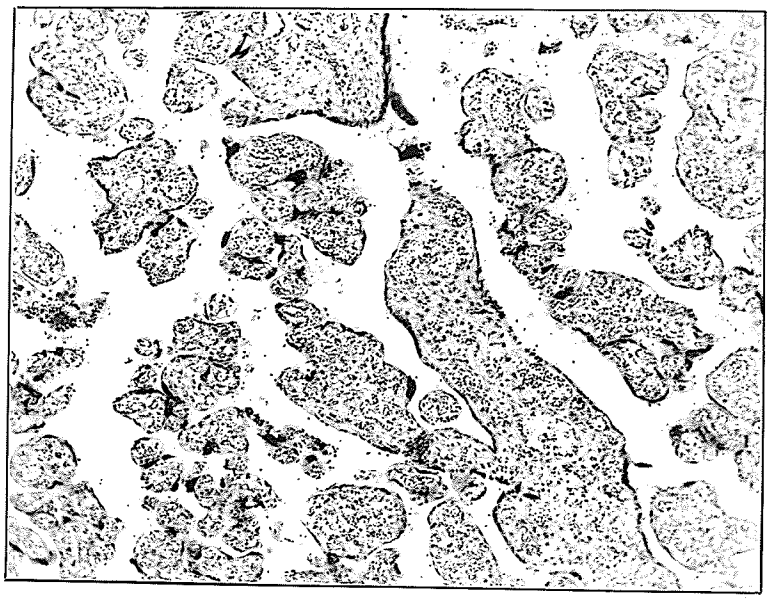


FIGURE 50

Low power field of the villi of a twenty-nine weeks gestation. WGH 4062-48. Masson's trichrome stain. 75 x.

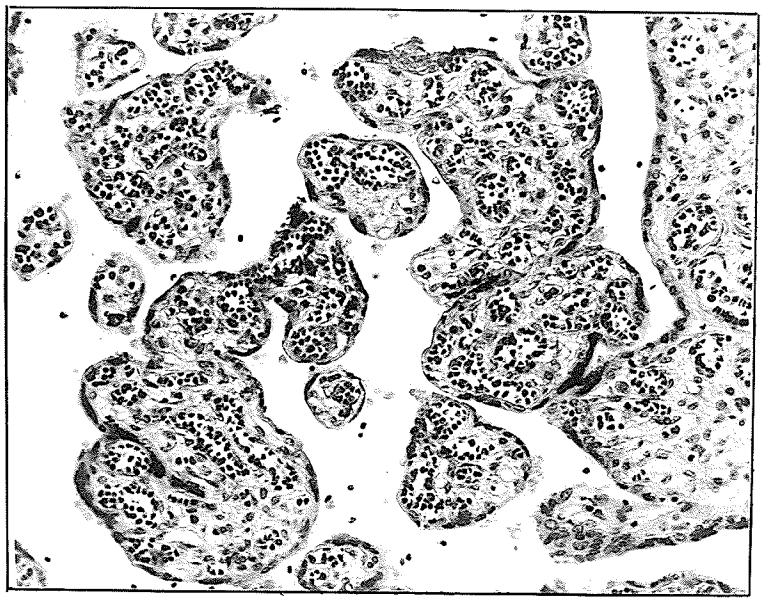


FIGURE 51

Villi at twenty-nine weeks. Stroma is denser, and there are more blood vessels. Masson's trichrome stain. 175 x.

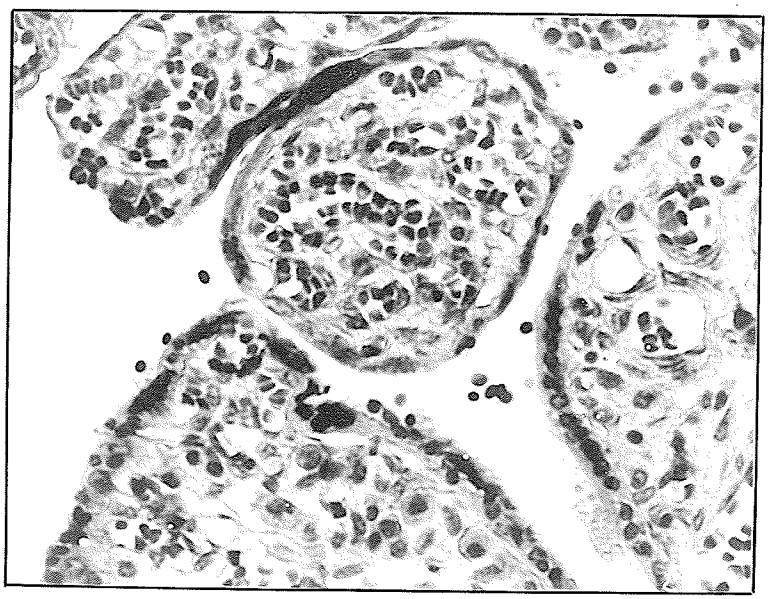


FIGURE 52

Villi at twenty-nine weeks. Langhans layer is beginning to disappear. The red cells in the blood vessels appear nucleated due to the intensity of the staining. Masson's trichrome stain. 400 x.

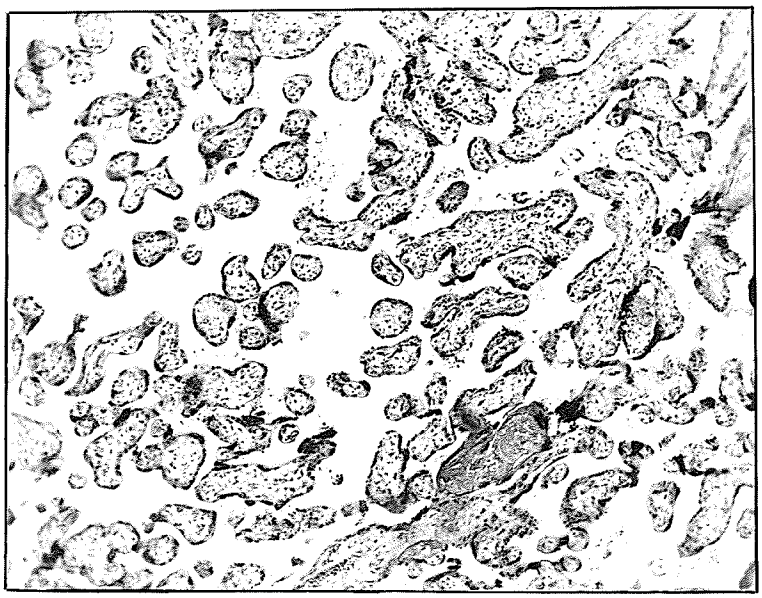


FIGURE 53

Villi at full term. Hematoxylin and eosin. 75 x.

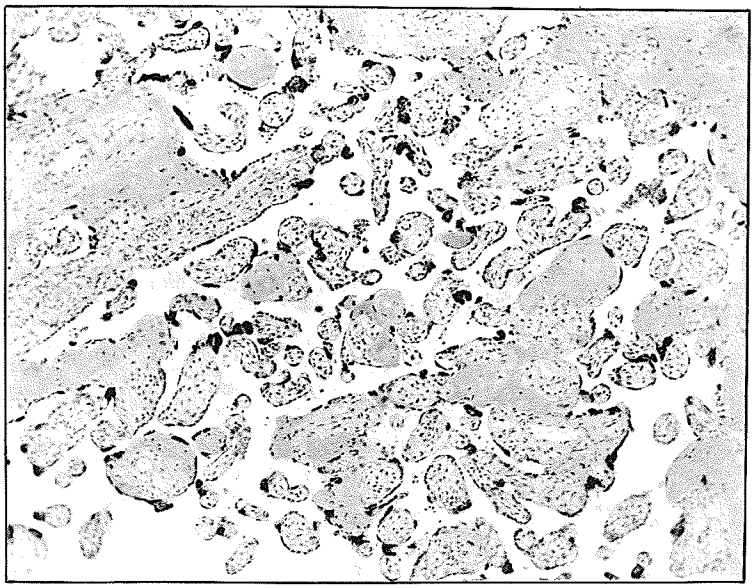


FIGURE 54

Villi at full term, showing nuclear knots and extensive hyalinization. Hematoxylin and eosin stain. 75 x.

I. GROWTH CHANGES IN PLACENTA DURING GESTATION

Changes in the villi. Morphological changes occur in the villi from the time of their earliest formation until parturition. The altered structure is not a wearing out process but in all probability a change with a definite purpose benefiting the fetus[3]. While the more obvious details of morphology are well known, the finer details of cytology and physiology are still to be determined[3]. The following features should be noted when studying a series of placentae at different ages:

(a) The number and size of the villi.

(b) Change in the nature of the mesoblastic core.

(c) Increase in the size and number of capillaries.

(d) Change in the endothelial lining of the capillaries.

(e) Appearance and disappearance of the Hofbauer cells.

(f) The presence of nucleated red cells in the capillaries in the early months and the gradual change until mostly non-nucleated at term.

(g) The disappearance of the cytotrophoblast (Langhans' layer).

(h) The appearance of nuclear knots.

(i) The persistence and thinning of the syncytial layer.

(j) Degenerative changes, the deposition of fibrin, and calcification.

Many of the features listed above have been discussed and of those, a brief summary will suffice. The Hofbauer cells, the cytotrophoblast,

the syncytium, and the degenerative changes will be discussed more fully.

The number of villi in the early stages is naturally small. These early villi are relatively large. As the gestation proceeds, the number of villi seen in a unit area of section increases, and at the same time the average size progressively decreases. The early mesoblastic core of the villi is embryonic in nature. With aging, the stroma becomes somewhat more dense. Blood vessels do not occur until the end of the first month. They become progressively larger and more abundant. At term, the capillaries are often referred to as sinusoidal-like vessels. The only tissues to separate the fetal and maternal bloods are the endothelium, syncytium and the argyrophil fibres. The endothelial lining of the capillaries becomes more adult in appearance as the gestation proceeds in that it is thinner, the cells are smaller, and the nuclei are smaller and stain more darkly.

Hofbauer cells. Hofbauer[7] in 1905 described in detail the large cells found scattered throughout the stroma of the villi at a certain period during pregnancy. These cells were recognized long before Hofbauer gave his description[5,6]. (Cf. Meyer)

The cells are large with rather granular cytoplasm which contains lipoid granules or vacuoles. The nuclei vary considerably in size, position, and staining reaction. In sections, the cells are detected by their large size and nucleus, contrasted with the nuclei of the mesenchymal cells. Their position within the villus is not constant[6].

Hofbauer cells are phagocytic in nature, they exhibit amoeboid movements in fresh specimens, and take up neutral red dyes. Their number present is erratic. Lewis[6] when studying the material of the Carnegie collection, was unable to find these cells in the 2, the 3, or the 5 mm stage of embryo. They were present in the 5.2 and the 5.5 mm stages. From six to twenty weeks the cells are abundant and easily found. At term they are again relatively few. (Cf. Lewis[6])

Hofbauer cells are found to be increased in some pathological conditions. They are associated with degenerative processes of the mesenchyme (Cf. Meyer). The origin of the Hofbauer cells is not known at the present[6].

The syncytium. Wislocki and Bennett[4] have recently given a full description of the trophoblastic layers. According to these authors, there is more or less general agreement that the syncytial trophoblast is derived from the cytotrophoblast layer. Cells in transitional stages have been found. Mitotic figures are frequently seen in the cytotrophoblast, while they have never been described in the syncytium. The syncytium has more darkly staining nuclei which are small and irregular in outline as compared with those of the cytotrophoblast. This latter observation is used as an inference that the nuclei of the syncytium are older than those of the cytotrophoblast. Furthermore, the cytotrophoblast disappears as the gestation progresses. (Cf. Wislocki and Bennett[4])

The outer surface of the syncytium is variable. Fixation plays

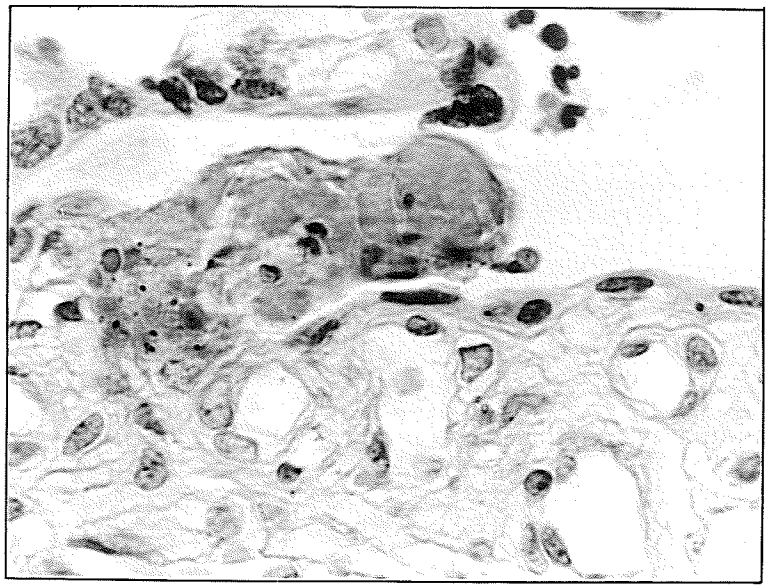


FIGURE 55

Nuclear knot--full term villus. Hematoxylin and eosin stain. 700 x.

a part in this appearance. Whether there is a true brush-border has long been a matter of contention. Wislocki and Bennett were able to find areas on the surface where the cell was covered with regularly distributed protoplasmic processes of equal size and length. Their distribution is not constant. In other areas the cytoplasmic processes are more irregular in size and length, and more indefinite in pattern. These are called cytoplasmic streamers. As term approaches the brush border and cytoplasmic streamers are less conspicuous and more difficult to demonstrate. At term, the outer border of the syncytium is composed of fine, closely but unevenly set hair-like irregular processes. The surface of the syncytium adjacent to the cytotrophoblast is relatively smooth. As the cytotrophoblast disappears during the course of the pregnancy, contact between stroma and synctyium increases greatly. (Cf. Wislocki and Bennett[4])

Vacuolization is characteristic of the syncytium. Vacuoles appear in the syncytium early in development during the period of implantation. With the coalescing of these vacuoles the beginning of the lacunar system is laid down. A very marked vacuolization appears at the time of the formation of the syncytial trophoblast and continues in lesser degree until the primary villi appear and the chorionic circulation begins. Vacuolization of the syncytium of the secondary villi, after the circulation is established, is much less evident. (Cf. Wislocki and Bennett[4])

Fat droplets are present in the syncytium throughout the entire gestation. They diminish in size and number as time goes on, but fat

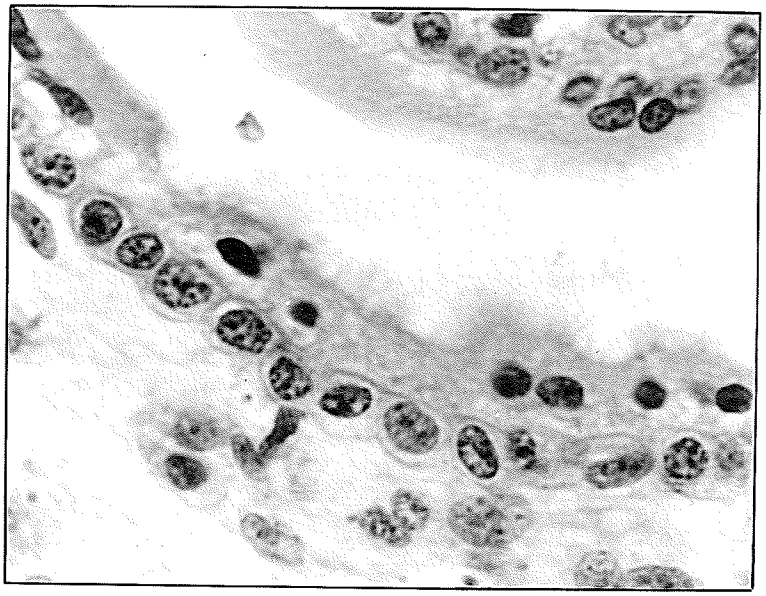


FIGURE 56

Cytotrophoblast and syncytiotrophoblast of a six weeks gestation. The "brush-border" is also seen. Hematoxylin and eosin stain. 800 x.

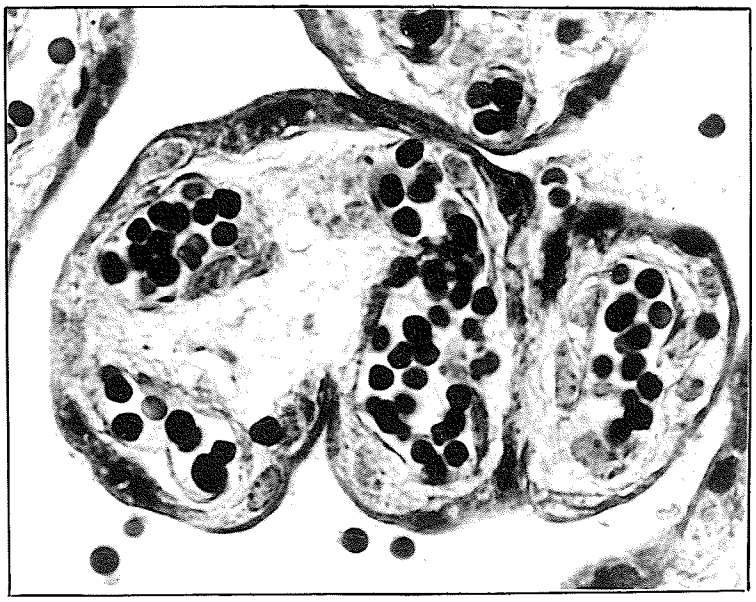


FIGURE 57

Villus at full term. Trophoblast is now almost all syncytium. Stroma is comparatively dense and has many large blood vessels. Hematoxylin and eosin stain. 700 x.

is present at term in the form of very fine droplets. (Cf. Wislocki and Bennett[4])

The development of nuclear knots in the syncytium is characteristic and becomes more marked as the pregnancy advances (Cf. post). With the development of nuclear knots, the syncytium is irregularly thinned over the surface of the villi.

The cytotrophoblast (Langhans' layer). The villi of the very early blastocyst are made up of cytotrophoblast alone. After the appearance of the mesenchyme and during the first few weeks, the mesenchyme is everywhere separated from the syncytium by cytotrophoblast. Gradually the cytotrophoblast disappears, apparently transforming into syncytium. There is no agreement when the cytotrophoblast completely disappears. The fifth and sixth months are mentioned most frequently. Wislocki and Bennett[4] believe that they find very occasionally cells at term which probably represent cytotrophoblast.

The cells of the cytotrophoblast are spherical, oval, or somewhat polyhedral. Their size varies considerably, probably because they are dividing. Mitoses are rather frequent. The faintly granular cytoplasm is clearer, and takes less stain than that of the syncytium. In many cells there are faint vacuoles.

No fat is found normally in the cytotrophoblast. The Golgi apparatus is of the usual net type, while that of the syncytium is of the dispersed type. (Cf. Wislocki and Bennett[4])

The reticulum. Reticular and argyrophil fibres demonstrated by silver impregnation methods (Bielschowsky) are fine and hair-like during the early weeks of gestation. During the later months and at term these fibres are heavier, longer, and more numerous. This point is demonstrated in Figures 58 and 59

Degenerative changes. Degenerative changes occur in the placenta, the most frequent of which are fibrin deposition and calcification, but not all placentae show these changes. The degree varies from specimen to specimen.

The earliest and most frequent change noted is the deposition of fibrin. Subchorionic deposition of fibrin is seen in nearly every placenta after the sixth month[3]. This fibrin is in the form of flat white plaques of 2 to 5 mm in diameter, and occasionally may be larger in size. Fibrin is deposited also on the villi and under the syncytium in nearly all placentae after the sixth month[3]. The deposition is associated with the degenerative changes in the syncytium. During the last half or third of the gestation when nuclear knots are forming, the thinned syncytium frequently becomes the site of fibrin deposition. If sufficient fibrin is deposited to encase the villus, ischemic necrosis of that structure may result. Intervillus deposition of fibrin may also occur.

No placenta is entirely free of calcification at term. Its presence is of no significance, other than perhaps a sign of senility of the organ. Calcium deposits are most frequently found along the stems of the main villi or in the placental septae[3]. (Cf. Hellman)

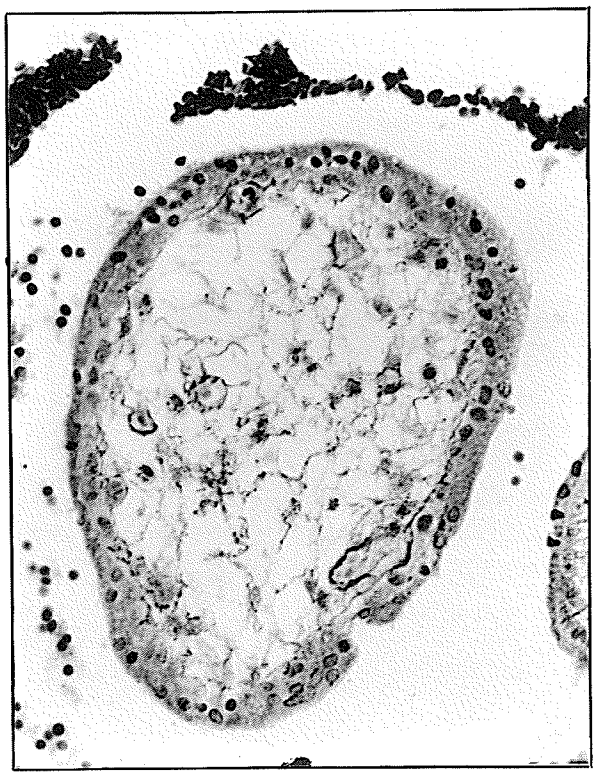


FIGURE 58

Reticulum of the placental villi at six weeks. Compare with Fig. 59, page 159. Bielschowsky silver impregnation. 300 x.

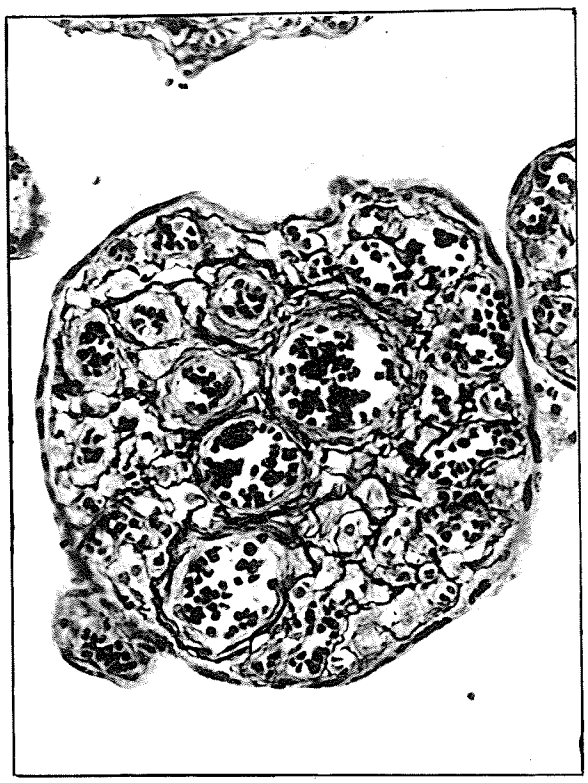


FIGURE 59

Reticulum of placental villus at term.
Bielschowsky silver impregnation.
300 x. WGH 4062-48.

The placenta at term. The placenta at term is a disc-like organ measuring 15 to 20 cm in diameter and up to 3 cm in thickness. It weighs approximately 500 grams or one seventh of the weight of the fetus. The fetal surface is smooth and is covered by vascular chorion containing fetal vessels. The amniotic sac is closely applied to the chorion on its fetal side. At the placental margin the membranes are commonly fused. (Cf. Earn[16])

The maternal surface is dark in appearance and is divided into cotyledons which appear as mounds when the expelled placenta is examined. Separating the cotyledons are septae, which are decidual and trophoblastic in origin. These septae do not reach the chorionic plate[17].

The cord which carries one vein and two arteries is usually attached eccentrically to the fetal surface, although central attachment is found in many specimens. Its outer surface is covered with amniotic epithelium and the vessels are supported in a mucous connective tissue known as Wharton's jelly.

Further descriptions of the placenta at term are found in the recent review by Earn[16], and in the papers of Dodds (Anat. Rec. 1922, 24:287) and Dees-Mattingly (Amer. J. Anat., 1936, 59:485).

Placental circulation. Two systems of circulation exist in the placenta, the fetal circulation in the chorion and villi, and the maternal circulation in the intervillous spaces and the subchorionic space. The systems are separate and normally do not mix.

There are two main theories regarding the circulation of blood

through the placenta. The earliest is that of Bumm, which has been reproduced in texts and has held universal sway since the end of the last century. In 1935, Rudolf Spanner announced his concept of the anatomy and circulation of the placenta. While there is not universal acceptance of Spanner's theory, it appears to be the more correct of the two. The essential features of the two theories are shown in Figure and Figure

Bumm's theory. Maternal blood enters the placenta by small arteries of the decidua, which are continuous with the spiral arteries of the uterine walls. After the blood circulates among the villi and bathes them, it leaves the placenta by veins which drain into those of the uterine muscle.

The villi, in whose stroma are the vessels carrying circulating fetal blood, hang in the intervillous spaces, "like branches of a tree." Interchange of metabolic substances occurs across the placental barrier which here consists of trophoblastic cells, stroma, and endothelial cells of the vessels in the villi. (Cf. Johnstone[17])

Spanner's theory. Falkiner[13,15] has summarized the work of Spanner. The account which follows is mostly from Falkiner's papers.

The structural unit of the placenta is the cotyledon, of which there are a varying number in each placenta, ranging from fourteen to thirty. Each cotyledon is separated from others by septa, which are complete at the base of the placenta but do not reach the chorionic plate. Maternal blood is fed into the base of the cotyledon by the

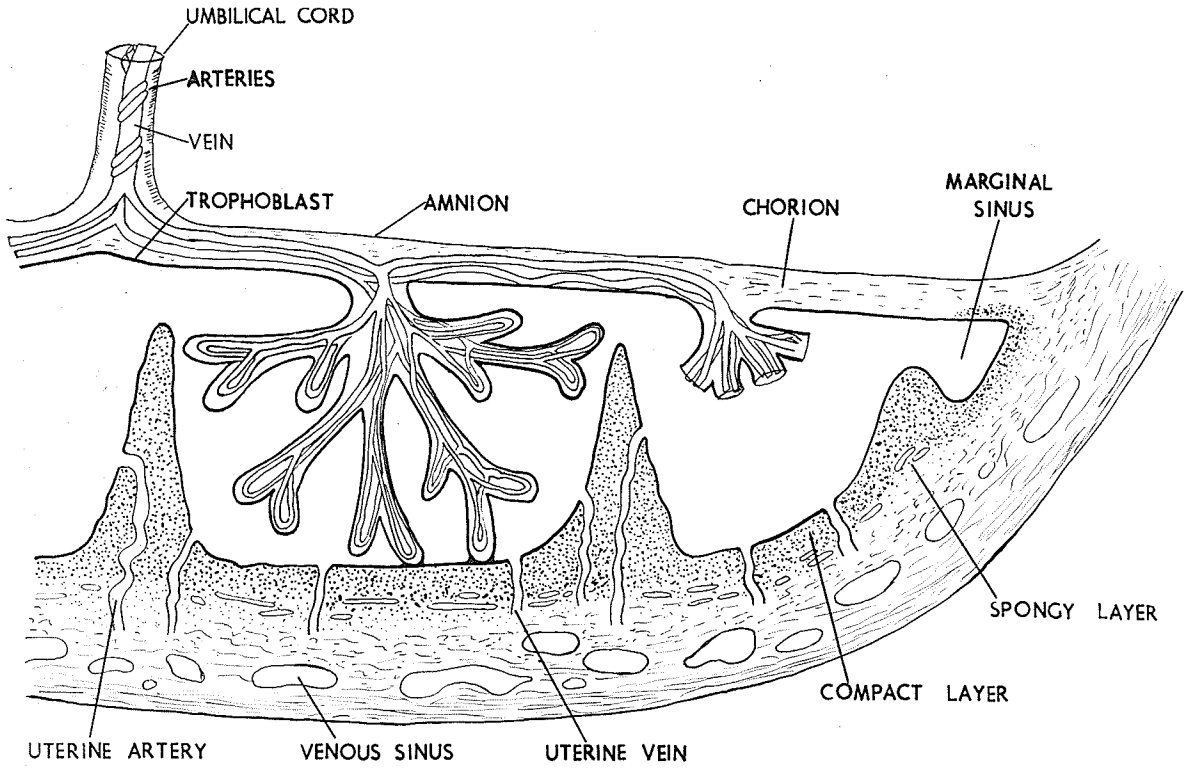


FIGURE 60

Bumm's concept of placental circulation.
Redrawn from Arey[19].

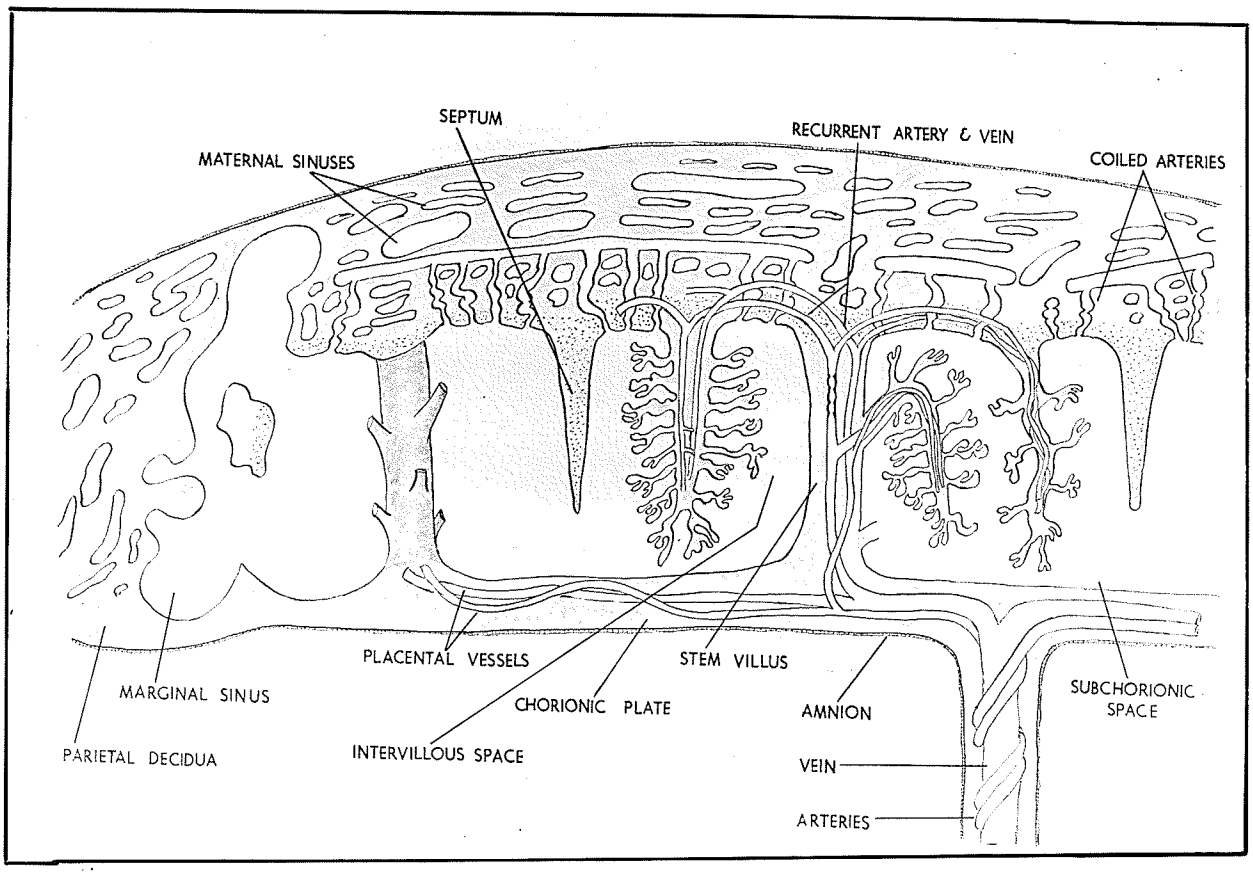


FIGURE 61

Spanner's concept of placental circulation. Redrawn after Bremer[12].

spiral arteries. The blood wells up over the septa to form a lake under the chorion (the subchorionic space) and from here enters the marginal sinus which drains directly into the uterine veins.

Each cotyledon has at least one main villous trunk. These large villi are the only structures to cross the subchorionic space[13] which measures from 0.5 cm to more in depth. The space is not seen in the separated and collapsed placenta because of the loss of blood but is well demonstrated in the specimen which has been injected in situ[16].

During early development, while the implantation cavity is enlarging, the decidua is split and many vascular units consisting of arterioles and venules are opened up[15]. Gradually hundreds of the spiral arteries described by Bartelmez open into the implantation cavity[15]. The coiled arterioles of the endometrium gradually become the coiled utero-placental arteries. Spanner[14] counted as many as 488 openings in one specimen. The distribution of the arteries in the base of the cotyledon is irregular, and they do not pass across the septa. (Cf. Falkiner[13])

Maternal blood flows from the subchorionic space into the marginal sinus. The marginal sinus is circular, following the outer edge of the organ, and measures from 2 to 3 cm in diameter in places, while at some points the sinus may be as narrow as 0.5 cm in width. Incomplete septa often divide the sinus. (Cf. Falkiner[13])

Spanner holds that the return of venous blood of the placental circulation is entirely limited to the periphery of the organ via the circular sinus[15] and not by veins from the intervillous spaces. Blood

from the circular sinus drains into a fine network of venous channels situated just under the decidua. This system has no direct connection with the intervillous space in the central portion of the placenta, and is drained into a more superficial network which in turn is drained by the tributaries of the ovarian and uterine veins. (Cf. Falkiner[13,15])

Fetal circulation. The fetal vessels running from the chorion into the villous trunk pass right across the chorio-decidual space and enter superficially into the substance of the decidua[17]. The vessels then turn back towards the chorionic plate and re-enter the chorio-decidual space, still within the villi. Thus, instead of appearing like the branches of an ordinary tree, such as the spruce, they resemble more the appearance of the "weeping willow" or "an old fashioned chandelier." (Cf. Johnstone[17], Falkiner[13,15])

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C H A P T E R F I V E

THE HORMONES OF REPRODUCTION

CHAPTER V

THE HORMONES OF REPRODUCTION

Introduction. The difference between maleness and femaleness is quantitative rather than qualitative. Thus the male has female potentialities and similarly the female has male potentialities. In early development, unless one set of characters clearly predominates, the individual becomes an intersex or hermaphrodite (Cf. Moore[16], Cameron[2]). Hormones acting upon a specific genetic constitution result in those characteristics associated with maleness or femaleness. Cameron[2] states:

> More than one endocrine compound is essentially (but not necessarily exclusively) associated with "maleness," and more than one endocrine compound is essentially (but not necessarily exclusively) associated with "femaleness" ...

The testes are the principal source of androgens, while the ovaries are the principal source of oestrogens. The adrenal cortex produces weakly androgenic and oestrogenic substances, while the placenta produces strongly oestrogenic substances[2].

Androgenic substances are generally assumed to be produced by the Leydig cells of the testes[36]. The androgens are required for the development, maintenance and control of the male reproductive system. The secondary sex organs (penis, prostate, seminal vesicles) as well as such factors as body contour, pitch of voice, hair distribution, and in lower animals at least, the sex drive are influenced by the androgens.

Oestrogenic substances control and maintain the secondary sex organs (uterus, vagina, clitoris, mammary glands) and as well those characteristics usually associated with femaleness such as body contour, hair distribution, and pitch of voice.

Neither the testes[16] nor the ovaries are automatic but in turn are controlled by other influences in the body. The chief of these influences are those hormones of the anterior lobe of the pituitary known as the gonadotrophins, of which there are at least two. Nor is the pituitary the only source of the gonadotrophins. The placenta produces a gonadotrophin which is active only in the presence of an intact pituitary[2,14].

Close association and interplay between the hormones of the anterior lobe of the pituitary and those of the gonads exist. Hormones of the adrenal cortex and the thyroid gland also exert an influence. With these factors, together with the fact that the male produces oestrogens as well as androgens, and the female produces androgens as well as oestrogens, the picture becomes one of great complexity. The effect of all these factors on the individual organ and in turn upon the whole body is thus dependent upon the summation of many factors.

Selye[1] in his monograph on endocrinology, notes that approximately 5000 papers a year are published in the field of endocrinology. The task of tabulating and evaluating these publications can be immediately appreciated. The discussion in this chapter is based mostly on standard monographs by authorities in the field of

endocrinology, such as Selye[1], Cameron[2], Allen, Engle, Moore[16], Papaniculeau et al[9], as well as Fluhmann[13] and Novak[3]. Very little reference has been made to original contributions.

The hormones. The hormones associated with sex and reproduction form two groups of compounds. The gonadotrophins and prolactin, which are proteins, make up the first group, while the androgens, oestrogens, and progesterone, which are steroids, form the second group. The compounds are listed on page 172 in tabular form.

Follicle stimulating hormone (FSH). This hormone can be obtained from pituitary glands and from the urine of normal, menopausal and castrated humans. It is protein in nature. The hormone causes the growth of follicles in the ovary and the development of the seminiferous tubules in the testis. Its action alone does not stimulate the production of sex hormones. For this a small proportion of luteinizing hormone (LH) is needed.

THE HORMONES OF REPRODUCTION

CHEMICAL NATURE	GROUP	NAMES	SOURCE
PROTEIN	GONADOTROPHINS	Follicular stimulating hormone (FSH)	Anterior lobe of pituitary
		Interstitial cell stimulating hormone (ISCH) OR Luteinizing hormone (LH)	Anterior lobe of pituitary
		Anterior pituitary-like (APL)	Placenta
	LUTEOTROPHIN	(Synonyms - prolactin, lactogenic hormone, mammotrophin, luteotrophin (LTH)	Anterior lobe of pituitary
STEROID	ANDROGENS	Testosterone Androsterone Dehydroandrosterone	Mostly from the interstitial cells of testes. Also from the adrenal cortex.
	OESTROGENS	-oestradiol estrone estriol	Mostly from the follicular cells of the ovary, also from the Corpus luteum and the adrenal cortex.
	PROGESTERONE	progesterone	Corpus luteum

Luteinizing hormone (LH).@ LH is a glycoprotein which augments the action of FSH, the combined action leading to the production of the sex hormones of the ovary and ovulation. Luteal tissue development and development of corpora lutea are stimulated. Under the influence of LH the corpus luteum is formed from the pre-formed graafian follicle. LH is presumably the ovulation producing hormone, but apparently does not regulate the corpus luteum once it has formed (Cf. Goldzhier[14]). In the testes, LH stimulates the interstitial cells to produce androgens[2,14].

The molecular weight of LH (ICSH) shows a species variation. In sheep, the molecular weight is estimated to be 40,000 and in hogs 100,000, while in the human the molecular weight is not known.

Chorionic gonadotrophin (APL). The placental gonadotrophin is chemically and biologically distinct from the pituitary hormones. It is a glycoprotein of a molecular weight between 60,000 and 80,000. Galactose is part of the molecule[14]. Experimentally, APL is without effect in the hypophysectomized animal, but is effective in the

@ According to Selye[1], the luteinizing hormone (LH) is identical with the interstitial cell stimulating hormone (ICSH). Since the human ovary does not have interstitial cells, clinicians object to the term ICSH. Cameron[2] on the other hand, uses the term, preferring LH as a synonym. In this discussion, the term luteinizing hormone is used with the understanding that ICSH and LH are identical.

presence of an intact pituitary[2].

Human trophoblastic cells grown in continuous culture produce chorionic gonadotrophin. The type of cell responsible is the Langhans cell[17,19].

Prolactin. Prolactin augments the action of LH (ICSH) being required before the corpus luteum produces progesterone, and is essential for the actual secretion of milk in the mammary gland. Evidence suggests that the hormone is responsible in part for maternal behaviour[2]. Further evidence suggests that luteotrophin (LTH) and prolactin are identical. (Cf. Goldzhier[14] quoting Evans et al.) (Also Cf. Selye[1])

Androgens. Androgens control the development and maintenance of the secondary sex characters associated with maleness. Androsterone, dehydroandrosterone and testosterone are the three major androgens. Those isolated from the adrenals are relatively weak in their androgenic properties. Of the androgens, testosterone is the most potent, and has been isolated from the bull testes only. It is assumed to be the chief androgenic substance of other species. (Cf. Cameron[2])

The androgens are steroid in nature and are closely associated in their chemical constitution to the hormones of the adrenal cortex and to cholesterol[2]. Contemporary opinion is greatly in favour of considering the Leydig cells as the producers of the androgens of testicular origin[1].

Oestrogens. Oestrogens control the development and maintenance of the secondary characteristics usually associated with femaleness. Estrone, estriol and estradiol are the most important of this group. -oestradiol is the most potent natural occurring oestrogen and has been isolated from the ovaries of sows, human placenta and stallion testes[2]. Oestrogens exert their greatest effect on tissues of Müllerian origin such as the oviducts, uterus, and upper portion of the vagina[3].

Progesterone. Progesterone produces changes in the uterus and vagina following oestrogenic stimulation. The secretory changes of the endometrium, deposition of glycogen in the epithelium, and increase of vascularity are due to progesterone. Inhibition of ovulation is said to be another effect. The hormone is required for the maintenance of pregnancy, should pregnancy occur. (Cf. Cameron[2])

Methods of study. Castration of man and domesticated animals has been practised for centuries, affording ample opportunity to show the effects of loss of gonadal tissue. Experimental investigation has played an important role. Early experimentation consisted of the removal of the gonads. Removal of the testis, and grafting was next performed. Extracts of gonads were found to correct some of the defects produced by castration.

At the present time, many concepts are based on experimental evidence. Conclusions drawn from experimentation of one species do not necessarily apply to another species.

Clinical observations of functioning tumors in which an excess of a hormone is produced has given another approach to the function of some of the hormones.

Castration of pre-pubertal females is rare. Examples of hypogonadism and congenitally absent ovaries have been observed. The post-pubertal female castrate is common and the syndrome produced by loss of ovarian tissue in the female adult is well known.

The human male castrate. Castration effects vary greatly depending upon the period at which the testicular tissue is lost. Pre-pubertal castration (early eunuchism) has a more profound effect upon the body than post-pubertal castration (late eunuchism). In this discussion, the most severe form of hypogonadism is considered, since this state gives us an indication of the functions of the male hormones. The graphic description of Selye[1] is excellent.

Early eunuchism. Aplasia of the gonads, surgical removal for disease, or destruction of the gonads for any reason before puberty leads to marked deviations from the state of maleness. The testicular deficiency occurs before the morphogenesis of organs and structures has proceeded far enough to give the individual definite male characteristics.

By the time the third decade is reached, early eunuchs present usually a fairly typical picture. Excessive growth of the long bones of the extremities gives the patient an awkward appearance. The limbs are disproportionately long compared to the body. The gait is

affected. Poor muscular development together with lack of muscular strength, lack of initiative and drive add to the plight of the intraverted, shy and frequently sullen early eunuch. The boyish voice is in keeping with the infantile face which lacks beard and which lacks the bone differentiation of the adult, but which is in contrast with the senile tiredness and fine wrinkles. Sebaceous secretions are diminished, the hair and the skin are dry. The patients do not become bald. Distribution of hair over the rest of the body is female in type. The pubic, axillary and lanugo hair is sparse. The tall patients are thin. The short patients tend to be fat, the adipose tissue being found in the breasts, the hips and the abdomen. The general body configuration is female in type, the broad hips accentuating the picture. The testes are absent. The penis and other accessory sex organs are small and infantile. Libido and potentia are markedly diminished or absent.

Excretion of androgens, 17-KS and oestrogens is decreased since the main source of such compounds is removed. The continued excretions of small amounts of these substances is probably attributable to the adrenal cortex. Gonadotrophin excretion is increased since the inhibiting influence of the testicular tissue is lacking. (Cf. Selye[1])

Late eunuchism. Post-pubertal loss of testicular tissue does not affect the organism to the extreme degree seen in pre-pubertal castration. The appearance of the late eunuch differs but little

from the normal male adult. There may be atrophy of the male accessory organs, but these organs tend to remain fairly normal in size. Growth of bone and growth itself is not disturbed. The beard and pubic hair are normal throughout life. Potentia and libido are not marked as a rule but are not necessarily absent. Psychic disturbances related probably more to the trauma than to endocrinology may be present. Vasomotor disturbances such as "hot flushes," tachycardia, etc., similar to those of the female menopause, are not uncommon in some individuals. (Cf. Selye[1])

The pre-pubertal female castrate. Very few cases of castration of the female before puberty are reported in the literature. Agenesis of the ovaries is also rare. True ovarian agenesis is usually accompanied by shortness of stature, infantile development of the mammary glands, uterus and vagina and diffuse osteoporosis. Urinary excretion of gonadotrophins is increased. Often other congenital anomalies exist. (Cf. Selye[1])

The post-pubertal female castrate. Castration of women after puberty is common, and the picture resulting from such an operation is well known. Usually, the younger the patient the more severe are the subjective symptoms such as "hot flushes," tachycardia, vertigo, nervousness, excitability, irritability, and headache. Gradual regression of the development of the accessory sex organs follows. Menstruation ceases. Symptoms may reappear as the patient enters the fifth decade, at the time when climacteric would have been expected

to occur.

Pre-pubertal female. Maturity must reach a certain stage before the gonads become responsive to the gonadotrophins of the anterior lobe of the pituitary. FSH and LH stimuli gradually increase causing enlargement and growth of more and more Graafian follicles, which produce more and more oestrogens. Between the ages of three to seven years, both boys and girls excrete small and constant amounts of oestrogens and keto-steroids in the urine. From eight to eleven years the excretion of oestrogens by girls increases. Pituitary FSH has been detected in the urine of girls as early as eleven years of age. About one and one-half years before the menarche, the excretion of oestrogens becomes cyclic, each cycle increasing in intensity. The stimulation is eventually sufficient to produce rapid growth of a sufficient number of follicles which are able to produce an effective quantity of oestrogens. One of these follicles ruptures and a corpus luteum is formed. (Cf. Cameron[2])

With the first ovulation, the whole system is launched into a new era in which reproduction is possible. The waxing and waning, growth and regression, control and reciprocal control, augmenting and depressing continues in a fairly definite cyclic pattern once established. Anovulatory cycles are apparently common in the teen-age girl.

Menstruation. While bleeding is the most striking feature of the menstrual cycle, it is but only a phase of a series of events

which affect the body as a whole. It may be considered the end of one short chapter of the reproductive history. A co-ordination of the whole cycle is briefly given.

The hormones. Immediately following menstruation, FSH of the anterior lobe of the pituitary, stimulates a large number of follicles to develop in each ovary. FSH stimulates the morphological response, but not the production of hormones[1], for this a small amount of LH is required[2]. As the follicles develop they produce increasing amounts of oestrogens[3]. The oestrogenic hormones of the follicles have their greatest effect upon tissues of Müllerian origin such as the oviducts, uterus, and upper part of the vagina. In all these tissues, the response is growth, the greatest change being noted in the endometrium[3].

As a rule, only one follicle reaches maturity and discharges an ovum. The unruptured follicles continue to produce hormones, but soon undergo atresia and as they do so, produce less and less oestrogens.

After rupture of the follicle and extrusion of the ovum, the corpus luteum forms from the granulosa cells under the influence of LH of the anterior pituitary[2]. Production of LH from the anterior pituitary is initiated by FSH[1]. The corpus luteum continues to produce oestrogens in smaller quantities and, as well, begins to produce a second hormone, progesterone. Production of progesterone from the corpus luteum is under control of LH and prolactin[2].

Progesterone is produced only after ovulation[3], since corpus luteum formation follows ovulation. Under the influence of progesterone, the endometrium enters the secretory phase and ovulation is inhibited[3].

After ovulation numerous growing follicles undergo atresia resulting in a decreased production of oestrogens. The corpus luteum continues to produce oestrogens in lesser amounts and after maturity when regression begins, produces less and less oestrogens as time goes on. Less progesterone is produced as regression of the corpus luteum proceeds[2]. Degeneration of the granulosa-lutein cells of the corpus luteum begins from nine to eleven days after ovulation[9] or from four to six days before the onset of menstruation[4]. The degeneration is slow and gradual, and is reversible should pregnancy occur[5]. The gradual withdrawal of progesterone and oestrogens has a two-fold effect. Onset of the menstrual flow is the most striking effect. The ovarian hormones causing growth are no longer sufficient to maintain the endometrium in a growing state. The second effect is the removal of the reciprocal action of the ovarian hormones depressing the production of FSH in the anterior lobe of the pituitary. With inhibiting influence removed, the pituitary produces increasing amounts of FSH to stimulate more follicles in the ovary to grow, and thus a new cycle is initiated. (Cf. Cameron[2])

Withdrawal of oestrogens is the prime cause of the endometrial regression, although there are probably other factors[2]. Progesterone administration will delay the onset of menses, and the removal of a recently formed corpus luteum will result in menstrual bleeding within

forty-eight hours. The onset of bleeding after such removal can be delayed by use of progesterone, while oestrogens do not have this effect. (Cf. Cameron[2])

An anovulatory cycle differs little from the ovulatory cycle, and yet no corpus luteum has formed. There must be occasions when a functioning corpus luteum has not formed after ovulation[6]. Still the corpus luteum is indispensable to menstruation, for there are none found in the ovaries of individuals who do not menstruate[7].

Newer Concepts of Menstruation. Daron[11] emphasized that the vasculature of the endometrium undergoes cyclic changes as well as the glands and stroma. Markee[12] observing endometrial implants in the anterior chamber of a monkey's eye, described the bulk of the hemorrhage as arteriolar in origin and therefore the coiled arterioles control the amount of the menstrual hemorrhage. The explanation of menstruation as a consequence of vascular changes is based primarily on these observations[6].

Kaiser's description[6] of the vasculature changes of the endometrium are excellent. This author outlines the two main concepts of menstruation as found in the literature: (a) the coiled arterioles increase rapidly and extend as the secretory phase of the cycle progresses and this increasing complexity is supposed to reach a point where it impedes the flow of blood to produce ischemia which in turn sets off the chain reaction of menstruation; (b) the pharmaco-dynamic concept which supposes the prolonged periods of vaso-

constriction observed prior to menses to be due to vasomotor substances produced and the sequence of vaso-constriction, ischemia and menstruation. (Cf. Kaiser[6])

According to Kaiser[6], both of these concepts assume that the complex coiled arterioles are necessary precursors of menstruation. The hypotheses bypass and actually contradict earlier work on hormonal withdrawal as a common precursor of menstrual flow. Nor do the concepts explain menses in the anovulatory cycle in which the coiled arterioles are considerably less complex. In some experimental animals which menstruate, there are no coiled arterioles. Certainly there must on occasion, be a failure to form a functioning corpus luteum, and if such should happen, this lessens the complexity of the arterioles. (Cf. Kaiser[6])

Time of ovulation. With inevitable variations, the mass of evidence indicates that ovulation in the human female occurs 14 to 15 days prior to the onset of menstrual flow, in a cycle which is 28 to 30 days in length. Numerous methods of investigation have been followed to determine this time of ovulation. During the cycle, should ovulation occur, a slight rise in body temperature corresponds to the time of ovulation. Temperature recordings over a number of cycles thus gives a good indication if and when ovulation has occurred. Studies of corpora lutea and ovaries removed at operation have given some information. The recovery of ova from the oviduct and endometrial studies are used. During the menstrual cycle, there

are variations of electrical potentials, differences in the motility of the uterus and oviducts. These have been used in determination of times of ovulation. Pregnancy occurring from a single coitus in cases are known. The sudden rise in the amount of pituitary gonadotrophins is said to be a sensitive indicator, the rise corresponding closely to the ovulation time.

Characteristics of the normal menstrual cycle. Goldzieher et al.[8] have recently reported data of 524 cycles of 109 selected normal women. Of these 524 cycles, 500 were ovulatory. No cycle was less than 19 days when the anovulatory cycles were excluded. Over half of the normal cycles were from 26 to 29 days in length and 92.8 per cent of them fell within the 23 to 36 days interval. The oestrogenic phase in the majority of cases was from 10 to 16 days in length. Of 490 cycles, 69.5 per cent were 11 to 14 days during the progesteronal period, and 94 per cent fell within the 10 to 16 day period. The duration of bleeding in 95.4 per cent was from 3 to 7 days, and in 68.5 per cent from 3 to 5 days. (Cf. Goldzieher et al[8])

Irregularities from established cycles are associated with minor disturbances in many cases. Minor illnesses, changes in environment, travelling, fear or desire of pregnancy may all influence the time of onset of menstruation, as well as many other external environmental factors.

Cyclic changes in the accessory organs of reproduction. Cyclic changes in the epithelia of the organs of reproduction occur

in response to hormonal stimuli throughout the menstrual cycle. Papanicolaou, Traut and Marchetti[9] have recently reported their studies in the form of an excellent monograph. A brief summary of the cyclic changes is included here for the sake of completeness. Much of the material is taken from their monograph. The epithelium covering the surface of the endometrium, and lining its glands, as well as the epithelium of the endosalpinx and endocervix have the same embryological derivation from the epithelium of the Müllerian duct which in turn is derived from coelomic epithelium. It is reasonable to expect that these tissues exhibit similar responses to the same hormonal stimuli.

The endometrium shows cyclic morphological variations more pronounced than any other part of the reproductive system. Three types of cells are found in the endometrium, namely the secretory, the ciliated and the "rod" cells. During the follicular phase, the glandular epithelium is low and columnar; there are few ciliated cells, and rod cells are rare. The glandular epithelium increases in height, and at the time of ovulation reaches its maximum height. The nuclei tend to become ovoid or oblong and assume a central position in the cell. The ciliated cells on the surface are more numerous. The transition between follicular and secretory phases is not strictly synchronized with ovulation. The interval between ovulation and secretory activity varies greatly. A regional variation from one area to another is also present. Soon after ovulation, changes characteristic of the luteal phase are noted. The nuclei of the

secretory cells become more basal in position and small blebs of secretion project beyond the limits of the terminal bars. Glycogen becomes abundant. As the luteal phase advances the cytoplasm becomes paler staining, the nuclei of the cells more basal, and the secretion more abundant. The glands show an increased tortuosity and the stroma has a looser texture. Increased tortuosity of the blood vessels and hypertrophy of the stroma cells is also noted. The picture is most marked at a period 7 to 10 days after ovulation. Regressive changes in the endometrium mirror the regression changes in the corpus luteum. The glands collapse and show even greater tortuosity. Disintegration of the mucosa, degeneration of mucosal cells, resorption of the edema, infiltration with leucocytes, extravasation of red blood cells, especially in the compact layer, congestion, engorgement of the vascular bed, and a greater tortuosity of the spiral vessels complete the picture of the late luteal phase. During menses, the necrosis of the mucosa usually extends to the basalis. The necrotic portion is cast off in cell clusters and fragments. The epithelium of the basal glandular remnants grows along the surface to meet proliferating epithelium from adjacent glands. The endometrium is now ready to respond to further oestrogenic stimuli from the now increasing and growing follicles of the next cycle. (Cf. Papanicolaou, Traut and Marchetti[9])

Snyder[10] demonstrated that the tubal epithelium showed variations during the normal menstrual cycle. The most characteristic morphological variation in the epithelial cells of the mucosa

is the change in their height. The cells are tallest during the follicular phase. In the luteal phase, secretion and the nuclei of the non-ciliated cells protrude into the tubal lumen beyond the limits of the cells. The nuclei of the ciliated cells remain practically unchanged during the luteal phase. In pregnancy, the features of the tubal mucosa are essentially the same as during the luteal phase. During the menstrual phase the epithelium is at its minimal height. The ciliated cells are low columnar cells with large round or oval vesicular nuclei which are centrally placed. The relatively abundant cytoplasm is pale staining. The cilia are easily identified. The non-ciliated cells are more compact, with less distinct cell boundaries. Their nuclei are oblong and the cytoplasm of the cells is relatively reduced. (Cf. Papanicolaou, Traut and Marchetti[9])

Cyclic modifications of the endocervix are most distinctly expressed in the glandular epithelium. The most distinctive change is found in the late follicular phase when the cells lining the endocervical glands are tall and columnar, and show increased secretory activity. The nuclei are elongated and the papillary folds protruding into the glands grow larger during the late follicular phase. (Cf. Papinicolaou, Traut and Marchetti[9])

Pregnancy. During the normal menstrual cycle, there are quantitative and qualitative variations in the amount of gonadotrophins excreted. The greatest increase of gonadotrophins excreted lasts from the tenth to the fifteenth day of the cycle, with the peak

amounts of oestrogens.

The subjective symptoms of the climacteric are varied in nature and in severity from case to case, and the onset and cessation of these symptoms are insiduous and ill defined. Menopause or the cessation of menses, however, is fairly well defined, and according to most authors occurs during the fifth decade of life, with many exceptions.

After the menopause, the reproductive organs undergo retrogressive changes. The ovaries decrease in weight due to atrophy and non-formation of new structures. The irregular pitted surface gradually becomes relatively smooth. Follicles in atresia are found for a varying period of time after the menopause, but soon these and the primordial follicles disappear. A corpus luteum may or may not be found in one of the ovaries for a few months following the last menstrual period[7]. The ovarian vessels show a marked degeneration of the Mönckeberg type.

The accessory organs share in the atrophic changes. The uterus becomes smaller due to atrophy of the myometrium. The thin endometrium measures only 0.2 to 2.0 mm in thickness. No differentiation into basal and functional layers is possible. The glands decrease gradually in number and many become cystic with a low lining epithelium. The cervix shows a diminution in size at a later age, and eventually has fewer glands which produce only a scanty secretion. (Cf. Fluhmann[13])

Atrophic changes of the oviducts, vagina and vulva are noted

at about the twelfth and thirteenth day. This peak occurs after the oestrogens have shown their peak. FSH predominates during the first half of the cycle. Increased amounts of luteinizing hormone is associated with the time of ovulation. At the peak of gonadotrophin excretion about 15 RUU (rat uterine units) are found in each litre of urine.

With conception, the gonadotrophin level remains elevated and continues to rise. When excretion of 400 to 600 RUU per litre of urine is present, pregnancy tests are positive. This additional amount of gonadotrophins is due to the presence of large quantities of chorionic gonadotrophins. (Cf. Goldzhier[14])

A peak phenomenon occurs in all normal pregnancies. About the sixth week there is a sudden rise, the maximum is quickly reached, and by the twelfth week a decline. The daily output at the peak is variable, ranging from 75,000 to 100,000 RUU. After the peak, the rate of excretion is from 1500 to 5000 RUU per litre of urine.

After parturition and expulsion of the placental tissue, there is a precipitous drop in the curve of excretion. Only 52 per cent of women have a positive pregnancy test 24 hours after delivery, and none have a positive pregnancy test after 4 days. (Cf. Goldzhier[14])

Menopause and climacteric. Growth of follicles in the ovaries wanes markedly as the fortieth year of life is reached and in some cases even before this time[18]. The climacteric and menopause are associated with the fewer follicles and the gradually lessened

as well. There is diminution of elastic fibres, an increase of fibrous tissue, and a decrease in the size of muscle fibres. The epithelium becomes thin and the blood vessels show degenerative changes. The amount of glandular tissue in the breast decreases. (Cf. Fluhmann[13])

The menopause and climacteric are fundamentally endocrine manifestations[13], although the factors which bring about these phenomena are not clearly established. Control does not appear to be within the gonads for the symptoms of the climacteric are frequently present before menopause, while there is still functioning ovarian tissue. Exhaustion of suitable ovarian tissue may well be the cause of the phenomena (Zondek--quoted by Cameron[2]). With cessation of ovarian response to pituitary stimulation, there is rapid cessation of oestrogens due to lack of follicles and with this lack of oestrogens there is atrophy of the sex organs. The regression of the ovaries removes the inhibition they normally exert on the anterior lobe of the pituitary. (Mazer and Israel--quoted by Fluhmann[13])

Hormone integration in the male. During the early years of life there is little difference in the excretion of oestrogens and androgens by either sex. Later, girls excrete more oestrogens than boys, and conversely boys excrete more keto-steroids than girls. The increased excretion of keto-steroids becomes marked about the eleventh year when the secondary sex characteristics begin to appear. The endocrines leading to sexual development become evident about

five years before sexual maturity.

There is no evidence of cyclic fertility in man. FSH controls the spermatogenic elements and ICSH (LH) controls the development of the interstitial cells. Secondary sex characteristics are dependent upon the formation of the androgens which are the secretions of the interstitial cells. (Cf. Cameron[2])

The relationship between the interstitial cells and the seminiferous tubules is unknown[15].

The male climacteric. Some males experience subjective symptoms such as nervousness, depression, decline in memory and power of concentration, fatiguability, disturbed sleep, and other symptoms similar to those of the female climacteric. The number of individuals having such symptoms is considerably less than in the case of females and much more severe in intensity. As a rule, the onset of symptoms is later in life in the case of males. As in the case of the female, the climacteric syndrome in males is attributed to hormone imbalance. (Cf. Cameron[2])

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C H A P T E R S I X

THE PARATHYROID GLANDS

CHAPTER VI

THE PARATHYROID GLANDS

Introduction. The parathyroid glands which are probably the smallest endocrine organs of the body[1], escaped attention until 1880, when Sandström recognized them and considered them as embryonic vestiges of thyroid tissue[2]. Kohn in 1899 recognized the parathyroids as bodies independent of the thyroid. It was not until the beginning of the present century that removal of the glands was known to cause death[2]. Hanson[3] produced a weakly active extract of the parathyroid glands in 1923. Collip[4] simultaneously and independently produced a much more active extract which, when injected into a parathyroid-ectomized animal, would prevent the post-parathyroidectomy syndrome.

Gross description. The parathyroid glands are small, soft, flabby, round or oval bodies. The surface is smooth and covered with a thin capsule. A hilus has been described, but is poorly defined and difficult to identify. The colour varies with the age of the subject. In infancy, the glands have a yellowish-red colour, which gradually darkens to yellowish-brown or reddish-brown. The darkening of the colour corresponds to an increasing deposition of interstitial and intracellular pigment associated with increasing age. (Cf. Morgan[5])

Size and weight. The size of the glands varies to some extent according to the number present[5], with age[1], whether or not there is

disease in one or more of the glands[2], with the presence or absence of kidney disease[6]. With so many normal and abnormal factors influencing the size and weights of the parathyroids, the definite figures frequently quoted mean very little.

Normally there are no great variations in size. The glands measure from 4 to 9 mm in length and 2 to 4 mm in diameter[5]. The inferior pair of glands are usually larger than the superior pair. The size of the glands is actually little indication of the amount of functioning parenchyma present, since adipose tissue is a normal variable component.

Selye[2] gives the range of 20 to 50 mgms as the average weight of the glands, while Shelling[1] gives his figures as 35 to 55 mgms. The factors influencing the size also influence the weights. In the female[5], the glands weigh approximately 20 per cent heavier than those of the male. After the menopause, the difference in weights is less apparent in corresponding age groups of males and females. The inferior pair of organs is approximately one fifth heavier than the superior pair. (Cf. Morgan[5])

Position. The glands are situated, as a rule, between the posterior borders of the lobes of the thyroid gland and its capsule. The parathyroids are designated according to their situation as inferior and superior pairs.

The superior glands are usually two in number and are more constant in their position than the inferior pair. The superior

glands are situated one on either side, at the level of the lower border of the cricoid cartilage, behind the junction of the pharynx and the oesophagus[15].

The inferior pair of parathyroid glands are very variable in their position. Indeed, Crotti[16] states that the concept of two superior and two inferior parathyroids is far from being correct.

Number of glands. Four or more glands were found in over 99 per cent of over one thousand bodies examined by Pepere (quoted in Gray's Anatomy[15] and by Crotti[16]). Reports of as few as one and two glands and as many as eight are found in the literature according to Morgan[5], who lists five possibilities for such variations. The glands may be congenitally absent, or buried in the thyroid or thymus. Abnormal positions are common. Finally asymetrical distribution may add to the difficulty of finding the glands. The number found at autopsy or operation varies directly with the experience of the operator. (Cf. Morgan[5])

Blood supply. The inferior parathyroid arteries are branches of the inferior thyroid arteries which have rich anastomoses with the arteries of the larynx, trachea, oesophagus, and also anastomoses with the superior thyroid arteries. Interruption of the thyroid arteries at thyroidectomy does not necessarily imperil the parathyroids. (Cf. Curtis[14])

Nerve supply. According to Rhinehart[13], the nerves of the

parathyroids have the same origin as those of the thyroid. Fibres from the cervical sympathetic ganglia accompany the branches of the thyroid arteries which supply the parathyroids. The nerves form a perivascular plexus, and they branch with the vessels, so that the smaller arteriole twigs are usually accompanied by a single nerve fibre. He states that no nerves are found around the veins or capillaries. There are fewer nerves in the parathyroid gland than are present in the thyroid. No ganglion cells are found in the parathyroids. Unfortunately, Rhinehart does not give the source of his material, although his work is subsequently quoted in the literature as being on human material. (Cf. Rhinehart[13])

Lymphatic drainage. The lymphatics of the parathyroids are not well known, although it is fairly certain that they are associated with those of the thyroid gland[1].

Note on embryology. The development of the inferior pair of parathyroid glands is intimately associated with the development of the thymus. The third pharyngeal pouch grows out from the pharynx as a hollow pear-shaped structure, the stalk of which is attached to the pharynx. The pouch becomes solid by proliferation of the endodermal cells of the wall. At the 8 mm stage, a small group of cells on the dorsal aspect become differentiated and later develop as parathyroid III which is the inferior parathyroid of adult anatomy. The remaining and larger part of the stalk separates from the parathyroid and eventually forms approximately one half of the thymus gland.

The fourth pharyngeal pouch also separates from the wall of the pharynx. It becomes solid by a proliferation of the endodermal wall and its dorsal portion shows a differentiation similar to the case of the parathyroid III. This dorsal portion shows the differentiation about the 8 mm stage also, and gives rise to parathyroids IV or the superior parathyroids of the adult anatomy. (Cf. Hamilton, Boyd and Mossman[17])

I. HISTOLOGY

Capsule. Each gland has a very thin capsule consisting of collagenous connective tissue in which a few small blood vessels may be seen. Bands of connective tissue pass from the capsule into the gland and separate the parenchymatous cells into irregular groups. The capsule is frequently torn away from the gland during dissection.

Reticulum. The reticulum of the parathyroids forms a fine meshwork which supports the groups of cells. The fibres are well demonstrated by silver impregnation or periodic acid methods.

Fat cells. Adipose tissue cells are present at all ages and in almost all glands. In general, the adipose tissue increases from infancy onwards, although the amount is not marked before the age of thirty years.

Blood vessels within the organ. The parathyroids are classed amongst the most vascular organs of the body. The sinus-like

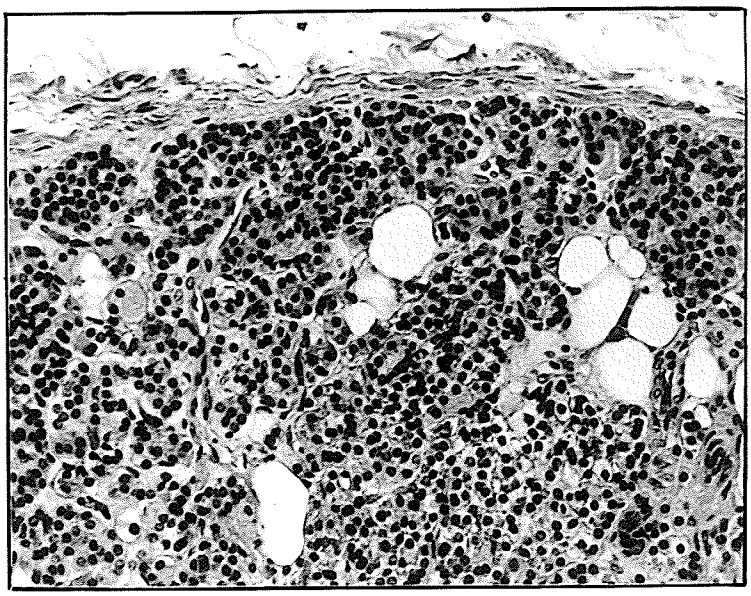


FIGURE 62

The thin collagenous capsule of the parathyroid gland. The general arrangement of the parenchyma is compact in type. A 6577. H & E. 250 x.

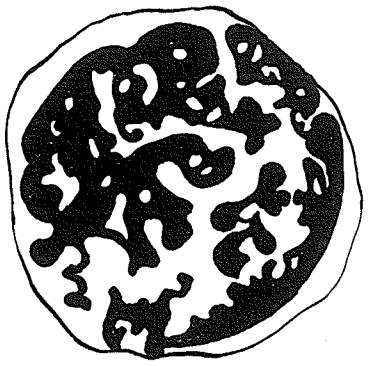
capillaries are in close relationship with the epithelial cells[8] and the reticulum. The vessels traverse the interstitial connective tissue which forms bands arising from the capsule.

Pigments. Small irregular granules of pale yellow to brownish pigment are found in many cells of all glands. The granules of pigment vary in size from 0.5 to 4 micra in diameter, and are most conspicuous in the oxyphil cells. In the younger age groups they are very fine, pale in colour and difficult to demonstrate. Although the exact nature of the pigment is not known, it is not lipochrome. (Cf. Gilmour[7])

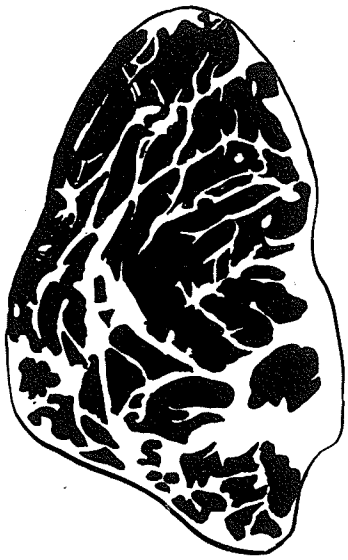
Lipoids. Lipoids can be demonstrated with osmic acid methods and are found in variable amounts in the form of minute granule or globules which may measure up to 6.5 micra in diameter. Vacuoles are found in some of the globules. There is a close association between the lipoids and pigments, although the nature of this association is not known. (Cf. Gilmour[7])

General Arrangement of the Parenchyma. According to Marine[9], Welsh in 1898 first suggested three types of gross structure of the parathyroid glands. Gilmour[7] suggests a similar classification, adding a fourth group. The four groups, i.e., compact, coarsely trabecular, lobular and partly large acinar, according to Gilmour, are illustrated on page 202. No functional significance has been attached to the general arrangement of the parenchyma of the

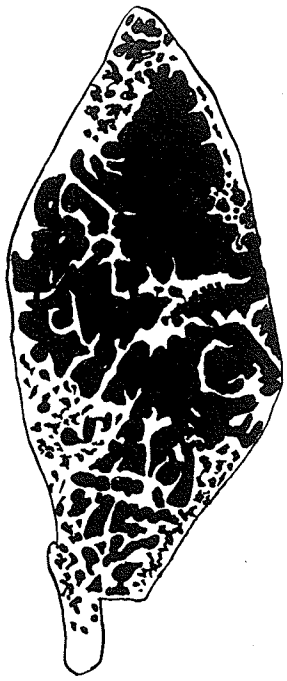
Compact gross structure.



Coarsely trabecular gross structure.



Lobular gross structure.



Partly large acinar gross structure.

FIGURE 63

The general arrangement of the parenchyma of the parathyroids, redrawn after Gilmour[7], 1939. The black areas represent the parenchyma of the gland.

parathyroids.

Compact. The gland, or part of it, consists of a solid mass of cells. The microsection has a uniform appearance and shows sheets of cells. Relative to the other types, there is little vascularity, perivascular connective tissue, or adipose tissue in the gland.

Coarse trabecular. This type corresponds to Welsh's anastomosing strand or column type. The parenchyma is arranged in trabeculae which branch and anastomose. Compared with the compact type, the perivascular connective tissue and adipose tissue are more abundant.

Lobular. The parenchyma of the whole gland or parts of it is split into angular masses by loose areolar tissue.

Large Acinar. This arrangement is uncommon. The arrangement of the parenchyma resembles that of the lobular type, except that the masses are rounded instead of being angular in shape.

The gross structure of the parathyroids varies in different age groups. From birth until ten years of age, the arrangement is usually trabecular or compact. Between the years of ten and twenty the structure is more variable, the majority of glands still being trabecular or compact. After twenty years, there are extreme variations. In the glands of most adults[9], more than one type of arrangement may be seen in the same gland. (Cf. Gilmour[7])

The cells of the parenchyma. There are two main types of

parenchymal cells[7,11,10], the principal and the oxyphil, each with varieties, i.e., dark, pale, and waterclear principal cells, and dark and pale oxyphil cells.

Some authors[5,10] believe that the different types of cells are to be regarded as stages in the life cycle of a single kind of secretory cell. The differences of opinion regarding this concept have lead to considerable confusion in texts and papers in the literature, especially with respect to nomenclature of the cells. Cowdry[10] states that "the oxyphil cells, particularly the dark ones, are senile." Morgan[5] supports the concept that the stages of transformation go from pale principal to dark principal to dark oxyphil to pale oxyphil. The theories that the principal cells can be transformed into oxyphil cells and that the two morphological types merely represent different stages of activity lack proof[2]. The physiological significance of the various morphological cell types is not known.

Principal cells. The greater portion of the parenchyma consists of principal cells (chief cells). The majority of these cells are polygonal, however, they may be spherical, cuboidal, or cylindrical when found in special formations. After fixation in watery fixatives, three varieties of principal cells may be recognized, i.e., dark, pale, and waterclear (wasserhelle)[7].

Dark principal cells. The dark principal cells have darkly staining homogenous cytoplasm which fills the cell. The depth of

staining, although dark and basophil, varies in intensity. The cells vary in diameter from 5 to 12 micra (average 8 micra). The spherical or oval nuclei have distinct nuclear membranes. The nuclei of the dark principal cells stain darker than those of the pale principal cells. One or two, occasionally three or four nucleoli are present. Dark principal cells are found in most glands, but are not abundant.

Pale principal cells. Pale principal cells are called the transitional type of principal cells by some authors. The cell body has a clear zone adjacent to the nucleus. The peripheral cytoplasm is dark and basophilic and indistinctly granular. In general this type of cell is the predominant type, and together with the dark principal cells make up the bulk of the gland. With advancing years[5], there is a slight relative decrease in the number of pale principal cells.

The pale principal cells measure from 7 to 15 micra in diameter. The nucleus which is slightly larger than that of the dark variety, measures from 5 to 6 micra in diameter, and is irregularly spherical in shape. Some of the cells show vacuolization of the cytoplasm, but this is variable.

Waterclear (wasserhelle) principal cells. These cells have a clear zone which extends from the nucleus to the well defined cell membrane. The dark staining spherical nucleus appears to be situated in an otherwise empty space. This appearance is due to the loss of glycogen from the cell in the preparation of the section. The

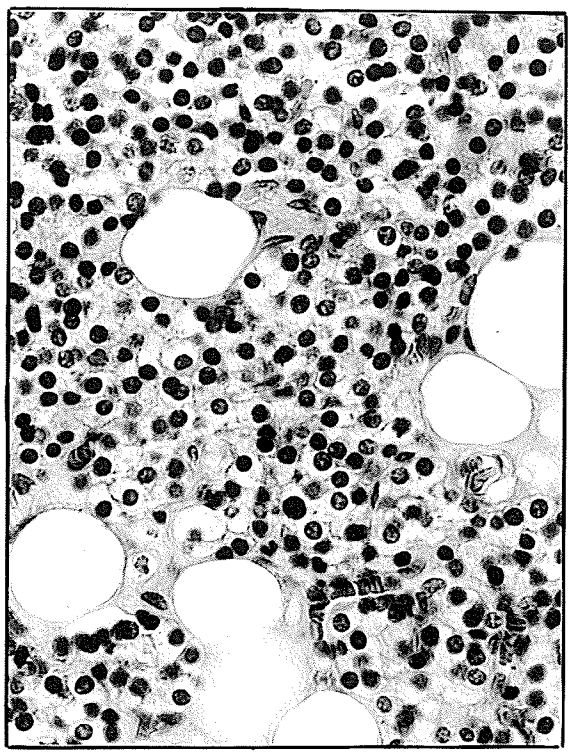


FIGURE 64

Normal parathyroid tissue. The majority of the cells in the field are transitional principal cells and dark principal cells. Some waterclear principal cells are seen scattered throughout the field. A 6597. H & E. 450 x.

diameter of the cells varies from 7 to 15 micra with an average of 10 micra.

Oxyphil cells. Oxyphil cells may be identified from principal cells in that they are larger, take an eosinophilic stain, and have larger granules which may be packed in the cell. It is customary to describe two types of oxyphil cells, i.e., the dark oxyphil and pale oxyphil cells.

Dark oxyphil cells. Dark oxyphil cells are difficult to find in some specimens. Rarely, if ever, are these cells present in the glands of infants. The cells may be large, measuring from 6 to 14 micra in diameter. The cytoplasm is almost or entirely filled with granules of fairly uniform size. These granules, stained with eosin, are dark red in colour. The cytoplasm of the cell is pink in hematoxylin and eosin preparations. The nuclei are similar to those of the dark principal cells and measure from 4 to 5 micra in diameter.

Pale oxyphil cells. Pale oxyphil cells are more numerous than the dark variety. They are large, irregularly polygonal cells with distinct cell outlines. The cells measure on an average from 13 to 15 micra in diameter. The cytoplasm stains reddish-pink and shows a varying amount of vacuolization. According to Gilmour[7] these vacuoles are filled with lipoid material in the fresh state. Granules whose number and staining intensity vary considerably, almost completely fill the cytoplasm. In some cells the granules are abundant, densely

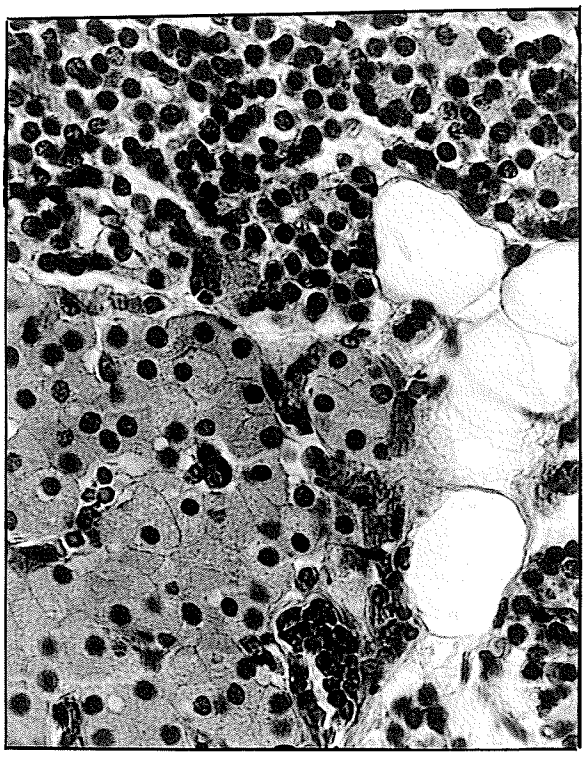


FIGURE 65

Normal parathyroid tissue. Numerous large pale oxyphil cells with finely granular cytoplasm and well defined cell membrane occupy one corner of the field. A 6597. H & E. 500 x.

packed and brightly stained, while in other cells the granules are smaller, sparse, and less deeply stained.

Pale oxyphil cells may be found as single cells or in irregular groups as shown in Figure . In some glands, the cells are numerous. Some subjects may have several pale oxyphil cells in one gland, and very few in the remaining glands.

Inconstant structures. Under this heading, Gilmour[7] describes the columnar-celled alveoli, colloid vesicles (true colloid and cystic vesicles) and Kürsteiner's canals. This author gives an excellent detailed account of these structures.

Columnar-celled alveoli. A columnar-celled alveolus has a lumen which varies from 3 to 40 micra in diameter and which is lined with short or tall columnar cells, usually of the transitional type. The lumen is empty or contains a little pale staining eosinophilic granular coagulum. The structure resembles an alveolus of a secreting gland. Gilmour[7] found them in random sections in 24 per cent of normal subjects occurring as single or multiple groups. This author considers that they are transformed into true colloid vesicles. (Cf. Gilmour[7])

Colloid vesicles. True colloid vesicles are present in almost all adult glands and are found at an early age in life. They are small, spherical or slightly oval, and are lined with a regular row of sharply defined cells, which are usually transitional in type. The

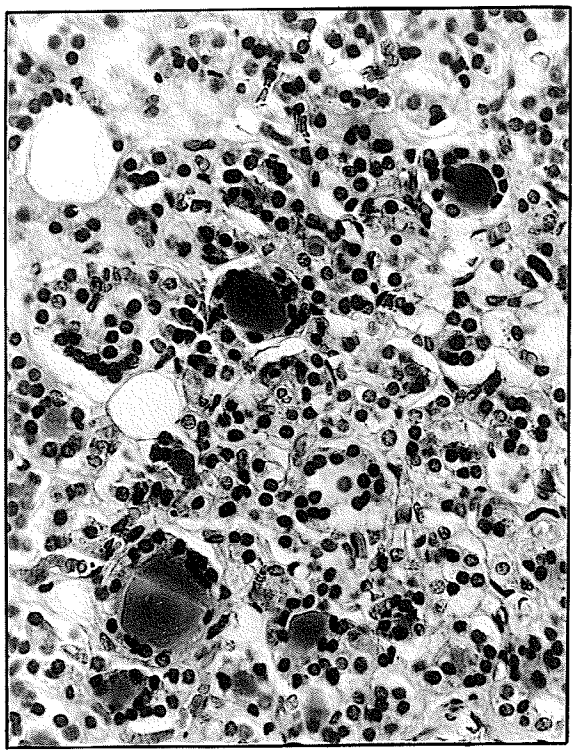


FIGURE 66

Colloid filled vesicles in the parathyroid gland. A 6597. H & E. 350 x.

lumen contains a deeply staining homogenous eosinophilic coagulum which has the appearance of colloid material. The diameter of the vesicles varies from 30 to 40 micra as a rule, but there are many exceptions. They are considered to be normal structures. The vesicles may be scattered throughout the whole gland, but tend to be arranged in groups at the surface or at one pole of the gland. (Cf. Gilmour[7])

Cystic vesicles. Cystic vesicles differ from true colloid vesicles in many features. The cystic variety are usually much larger, although their diameter seldom exceeds 300 micra; their shape is irregular and their lining cells show greater variations. A coagulum, which may have a reticular, globular, finely or coarsely vacuolated, or a homogenous appearance, may or may not completely fill the vesicle. The coagulum, compared with that of the true colloid vesicle, stains less intensely with eosin. Cystic vesicles are found at all ages and are more common in the inferior parathyroids than in the superior pair. Their position within the glands is very variable. The nature of these structures is obscure. There is no evidence that they have a physiological significance. (Cf. Gilmour[7])

Other cysts. Gilmour[7] describes vestigal structures and cysts arising from the parathyroids themselves, the thymic cords and the thymus. Collectively these structures are called Kürsteiner's canals. Apparently the structures are not common and are seldom of clinical importance.

The parathyroid hormone. The hormone of the parathyroid glands has never been isolated in the pure state, but it is almost certainly a simple protein[2]. The preparations at present available behave as a protein. These preparations are hydrolyzed in boiling acids and alkalis, and are rendered inert by trypsin and pepsin, explaining why they cannot be administered by mouth[12]. There is no conclusive evidence that the parathyroids produce more than one hormone, according to Selye[2], who also states that "the mechanism through which the parathyroid hormone exerts its diverse actions is still not fully understood." Probably most but not all of the actions of the hormone are secondary to its effect upon calcium and phosphorous metabolism[2]. (Cf. Selye[2])

Calcium metabolism. Normal values of blood calcium range between 9.5 to 11 mgms per 100 cubic centimetres of whole blood. Practically all of the calcium is found in the plasma, since little or none is found in the red blood cells[2,12]. Only one half of the blood calcium is in the form of Ca ions and thus diffusible through the tissue membranes. The remainder of the calcium is loosely combined with serum proteins[12].

The daily requirements of calcium in man ranges from 0.7 to 1.0 grams. Most of the calcium is absorbed in the upper intestine. About 70 to 90 per cent of the ingested calcium is eliminated in the feces, partly by excretion in the lower gastro-intestinal tract and partly by incomplete absorption. Only small quantities of calcium are

excreted in the urine, and if the blood calcium is less than 6 mgms per cent urinary excretion ceases.

Approximately 2 per cent of the adult body is calcium, of which 99 per cent is found in the osseous skeletal system. The main calcium reserve of the body is thus found in the bones. (Cf. Selye[2])

Phosphorous metabolism. Phosphorous is present in the blood partly as an acid soluble (inorganic) form and partly as organic phosphoric acid esters. The inorganic phosphate of the serum of adults ranges from 2.5 to 3.5 mgms per cent, and in children from 4.5 to 6.0 mgms per cent. About 1.25 grams of phosphorous are ingested daily, of which about 75 per cent is excreted in the urine as acid phosphates. (Cf. Selye[2])

Magnesium. The metabolism of magnesium is similar to that of calcium. About 50 to 80 per cent of the ingested magnesium is excreted via the gastro-intestinal tract[2].

Other factors influencing calcium metabolism. While it is not the purpose of this discussion to include all those factors influencing calcium and phosphorous metabolism, we may mention the following to indicate the complexity of the subject. Besides the parathyroid hormone, other factors which play a part are: (1) the calcium requirements of the skeletal system, (2) the pH of the blood, (3) the proportion of calcium to phosphorous in the blood, (4) presence of vitamin D in the food, (5) the absorption of calcium and phosphorous

from the intestine, and (6) the rate of excretion of these substances.

Present theories regarding the function of the hormone. Selye[2], in his monograph, reviews the theories regarding the function of the parathyroid hormone. One theory suggests that the hormone lowers the renal threshold for phosphates. With the increased urinary excretion of phosphates, there follows a mobilization into the blood of inorganic phosphates from the bones, where it is present as calcium phosphate. An increase of calcium in the blood results when the phosphate ions are excreted. According to Selye, experimental evidence does not support this theory. A second theory proposes a calcium compound in union with the hormone, resulting in calcium X. A third theory proposes that the hormone acts primarily upon the osteoblasts. If the hormone is present in large quantities the osteoblasts are transformed into osteoclasts, resulting in bone destruction. If the hormone is present in small quantities the osteoblasts are stimulated to proliferate and form new bone. Finally Selye[2] suggests that there is a possibility that the hormone acts both on the skeletal system and the kidneys.

The general effects of the parathyroid hormone can be studied following the injection of excessive doses of the extract of the glands, or indirectly by removal of the glands.

Following excessive doses of the parathyroid hormone, there is an almost immediate increase in phosphate content of the urine, a decrease in the blood phosphates, and a rise in the blood calcium levels. (Cf. Selye[2])

Following extirpation of the glands in animals, or removal of too much parathyroid tissue in human thyroidectomy, there is a rapid depression of blood plasma calcium, elevation of plasma inorganic phosphates, and a diminution of urinary excretion of phosphates. Tetany supervenes which leads to death, if not treated. (Cf. Cameron[12])

Hypoparathyroidism. Experimentally, hypoparathyroidism is accomplished usually by the excising of the whole thyroid-parathyroid apparatus. Clinically most cases develop after operative procedures on the neck, where parathyroid tissue is removed accidentally. On rare occasions, the condition develops for some unknown reason[18].

The symptoms usually appear within seven days after the thyroidectomy, but may be delayed as long as two to three months. The signs and symptoms of hypoparathyroidism may be active or latent, and are similar in both the post-operative and idiopathic cases. The acute symptoms and signs may be sudden in onset and be manifest in a number of ways such as generalized convulsions simulating idiopathic epilepsy, acute psychotic symptoms, generalized rigidity, or stridor and dyspnoea. Such extreme phenomena are uncommon. More commonly, the attack is less sudden with early signs of numbness, tingling, sensation of stiffness about the face and mouth, and painful contractions of the muscles of the extremities. (Cf. Rose[18])

Following parathyroidectomy, the blood calcium level falls to as low as 5 mgms per cent and the inorganic phosphates rise to levels as high as 5.5 mgms per cent in adults and to 7 mgms per cent in

children[2]. There is decreased urinary excretion of phosphate, and a decreased urinary output of calcium[12].

The disturbances following parathyroidectomy are due to disturbances in calcium and phosphorus metabolism[2] as a result of which there is an increased irritability of the neuromuscular system, the slightest stimulus producing painful, tonic spasms or tetany.

Hyperparathyroidism. In hyperparathyroidism, there is an increased elimination of phosphate through the kidney, a lowering of plasma inorganic phosphate, mobilization of bone mineral, and increase of calcium in the blood. There is an exaggerated action of the hormone. (Cf. Cameron[12])

Neoplasia and hyperplasia of the parathyroids are listed by Cope[19] as the chief causes of hyperparathyroidism. In discussing his series of 70 cases from the Massachusetts General Hospital, Cope[19] states that 63 per cent of 70 cases of hyperparathyroidism were due to benign adenoma of the parathyroid. The parathyroid tumor is almost always a benign adenoma. The few malignant tumors reported in the literature are mainly in patients who did not have hyperparathyroidism[12]. In the primary type of hyperplasia, the cells of the glands are mostly of the waterclear type[19]. Secondary hyperplasia results from the physiological need for greater amounts of the hormone and may be present also in cases of vitamin D deficiencies and in some cases of nephritis[19].

Depending on whether the urinary system or the skeletal system

involvement predominates and the degree of change present in each system, it is possible to describe several types of disease. The symptomatology can be divided into three groups, i.e., those symptoms due to (a) hypercalcemia, (b) osteoporosis, and (c) hypercalcinuria and hyperphosphaturia. Hypotonia, lassitude, constipation, fatiguability, weight loss, and anorexia may be attributed to the hypercalcemia. Bone pain, tenderness, joint pain, fractures and deformities may be associated with the osteoporosis, cysts and giant cell tumors accompanying the depletion of minerals from the skeletal system. Polyuria, polydipsia, nocturia, calculi formation, colic and hematuria are related to the hypercalcinuria and hyperphosphaturia. (Cf. Albright et al.[20])

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C H A P T E R S E V E N

THE THYROID GLAND

CHAPTER VII

THE THYROID GLAND

Introduction. The thyroid gland accounts for only 0.5 per cent of the body weight[2] and yet its hormone exerts an influence over all other tissues. No organ or tissue in the body is able to function optimally in the total absence of the thyroid hormone, although not all organs are impaired to the same degree[1].

The normal histological picture varies with factors such as the age of the patient, sex, food, state of nutrition, geographical location, climate, season, and psychic stimuli. Rienhoff[5] believes that there is "perhaps no other organ in the human body which presents such functional and histological lability as the thyroid." Marine and Lenhardt[11] remarked in 1911 that it was difficult to define the normal picture of the thyroid gland, and such is true at the present because of the wide normal variations. Most textbook descriptions follow a rather standardized style but the figures given for the normal ranges vary according to the observations of the author in his own locality.

The normal adult human thyroid weighs between 20 and 25 grams which is not more than 0.35 grams per kilo of body weight. The gland is slightly larger in the female and is relatively larger for infants (approximately 1:850 for infants and 1:2250 for adults). The amount of iodine available in the diet is the most important factor influencing the size of the gland. (Cf. Marine[4])

Gross description. The thyroid gland is named from the fact that it superficially resembles a shield (thyreos = shield). The gland is shaped rather more like the letter "H" than a shield, the two lateral lobes representing the vertical elements of the letter, and the isthmus the transverse portion.

The lateral lobes lie anteriorly toward the right and left of the trachea and larynx, each resembling a three-sided pyramid. The lower tip or pole reaches to within two cm of the suprasternal notch (sixth tracheal ring). The isthmus is very variable in size and shape. In size it may almost equal a lateral lobe or it may be a thin narrow band and in some cases may be absent. Connected with the upper border of the isthmus is the pyramidal lobe, which is a very variable elongated process with its base below and apex extended superiorly for a variable distance. (Cf. Joll[3])

The thyroid gland has two capsules, i.e., a false and a true capsule. The false capsule is derived from the pretracheal layer of the deep cervical fascia. It is not uniform in thickness, nor does it completely surround the thyroid, being strong and thick superiorly and laterally, thin and imperfect inferiorly. Fine fibrous strands run between the false and true capsule forming the main support of the gland. The true capsule is thin and transparent and cannot be detached from the gland without injury to the parenchyma. Thickened areas of the capsule are in continuity with the connective tissue found between the irregular masses of parenchyma. (Cf. Joll[3])

Rienhoff[5,10] claims that distinct and true lobules do not exist.

This investigator used wax plate reconstruction and microdissection methods and found that the mass of each lobe of the thyroid is divided and subdivided "... into many connecting or annectant bars, bands and plates of parenchyma ...." The entire lobe is irregularly traversed by septa of connective tissue which carry blood vessels, nerves and lymphatics. The irregular masses of parenchyma are joined to each other at one or more points. In no instance did Rienhoff find a portion of the parenchyma completely surrounded and separated from the rest of the gland. On the surface the areas are arranged in a leaf-like manner, while deeper in the lobe the arrangement is more complex than at the periphery. Rienhoff supports his descriptions with excellent drawings and photographs. (Cf. Rienhoff[5,10])

Thyroid tissue is translucent, pale amber red to bright amber red in colour, and is firm and elastic in consistency. On cross section, the larger follicles can be seen with the naked eye. The follicles contain a clear, viscid, amber-coloured protein substance or colloid.

Blood supply. The thyroid is one of the most vascular organs of the body[4,6,7]. A volume of blood equal to that of the whole body passes through the thyroid once an hour[9]. The gland, in proportion to size, receives nearly four times as much blood as the kidney[8].

Four main arteries supply the thyroid gland; the paired superior thyroid arteries arising usually from the external carotid trunk, and the paired inferior thyroid arteries arising usually from

the thyroid axis which is a branch of the subclavian artery. A fifth artery, the thyroideaima, is inconstant and may arise from a number of sources. Besides the above mentioned there are numerous unnamed, irregular arteries which are small in the normal gland but which may attain great size in some goiters. (Cf. Joll[3])

Venous drainage. The veins begin as a perifollicular plexus. They follow the smaller arteries as far as the periphery of the gland where they form a conspicuous plexus on the surface which covers the whole gland but is more obvious on the anterior. From this plexus a variable set of trunks which may be divided into three main groups: (a) superior, (b) lateral or middle, and (c) inferior. These veins drain mostly into the internal jugular veins although there are many variations. (Cf. Joll[3])

Lymphatics. The lymphatics of the thyroid originate as a closed system in the form of an anastomosing plexus of capillaries in the interfollicular spaces of the gland. The rich plexus consists of communicating capillary lymph channels which form rather dilated endothelial sacs or small pockets which vary in size. According to Reinhoff[5], there are three main plexuses, i.e., interfollicular, intraglandular, and extraglandular. The interfollicular plexus, mentioned above, which unlike the blood capillary system is not devoted to separate and distinct follicles but rather to spaces between the groups of follicles. The second division or intraglandular plexus is composed of rather large connecting trunks situated along the course

of the septa and forming frequent anastomoses with each other and with the interfollicular plexus. The extraglandular vessels on the outer surface of the gland lie external to the blood vessels and internal to the fibrous true capsule enclosing the thyroid. From the latter plexus originate the still larger collecting trunks which accompany the superior thyroid vessels to the cervical lymph nodes and also to the trunks which form a plexus in the pre-tracheal fascia draining into the mediastinal lymph nodes. (Cf. Rienhoff[5])

Nerves. The nerve supply to the thyroid is chiefly sympathetic. Fibres from T1 and T2 pass through the inferior cervical ganglion, where some are interrupted, others passing on to the middle and superior ganglia where they are interrupted. Accompanying the vessels to the gland and in the gland, the nerves form a rich perivascular and perifollicular plexus. (Cf. Joll[3])

Vagus fibres reach the gland via the superior and the recurrent laryngeal nerves[3].

Embryological note. The thyroid begins at the 1.37 mm stage[2] in the endoderm of the floor of the pharynx as a thickening which soon evaginates and by the 5 mm stage has the shape of two flask-like lobes attached to a common hollow stalk which connects with the pharynx and is known as the thyroglossal duct[19]. The thyroid primordia has an intimate relation with the aortic sac. The thyroglossal duct is the most important part of the thyroid primordia, small contributions being received from the ventral components of the fourth

pharyngeal pouch, which later fuse with the main primordia. With the development of the neck, the heart and great vessels become widely separated from the thyroid, although remnants of thyroid may be found attached to the arch of the aorta in adult life. Normally the thyroglossal duct is reabsorbed. Rarely it may persist, in whole or in part, caudal or cranially, as a cyst or a duct. Occasionally the caudal portion persists as the pyramidal lobe.

Initially the glandular tissue consists of a solid mass of cells. Branching plates one or two cells thick soon develop and these are further separated by an ingrowth of connective tissue and blood vessels. The formation of the follicles is a complicated series of changes. By the 24 mm stage the definitive follicles are surrounded by mesenchyme[7]. Colloid appears in 60 mm embryos[19], although the colloid is not abundant until after birth[2]. (Cf. Hamilton, Boyd and Mossman[19])

The microscopic anatomy. The ultimate histological unit of the thyroid gland is the follicle, groups of which make up the mass of parenchyma. The follicles are entirely discrete separate individual units[10]. The follicles are bound together closely by interfollicular connective tissue which carries arterioles, capillaries, venules, nerves and lymphatics.

The tissue of the thyroid may be divided for descriptive purposes into the parenchyma (follicles) and the stroma (interfollicular tissue). Occasionally small portions of thymus or parathyroid may be found in a section. In certain pathological conditions, lymphatic

tissue, either diffuse or in the form of lymphatic nodules may be found throughout the gland.

The vascular bed about the follicle is very rich[10]. The smaller capillaries arising from the arterioles in the interfollicular connective tissue form an intimate network about each follicle similar to a basketwork around a ball[7]. The lymphatics do not form a similar network and are external to the blood vessels[5]. Details of the lymphatic system were given on page 224.

I. THE FOLLICLES

Size. Many of the follicles vary in size from 0.2 mm to 1 mm in diameter, the great majority being small and in many scarcely any lumen is present[10]. Most normal follicles range from 0.3 to 0.8 mm in diameter[11]. There is great irregularity of size of the follicles. Large and small may be indiscriminately mixed throughout the gland.

Shape. The shape of the follicles varies as much as the size. No two follicles are similar. Their shape ranges from almost a perfect sphere to almost a perfect cube. No psuedopodial outpouchings of the wall were noted by Rienhoff, who found that all major irregularities in the epithelial wall occur inside the follicle. No true indication of the shape of the follicle can be gained from the studies of single sections. (Cf. Rienhoff)

Colloid. All the follicles are filled with clear, amber yellow viscid material, termed colloid, which is essentially insoluble in

water, alcohol or ether and when coagulated is easily stainable with eosin or acid dyes[6].

In the fixed state the colloid is frequently separated from the epithelium by clear spaces, giving the appearance of a spiny border in the most extreme cases. The clear spaces are artefacts because we know that the lumen of the follicle is completely filled during life[12]. Vacuolation does, however, vary with the degree of activity of the gland, being most marked in hyperthyroidism, and less marked in hypothyroidism or in the resting gland. Thus it appears that the artefacts are more easily produced in the colloid of the hyperactive gland.

The colloid is chiefly acidophilic but may exhibit patchy or diffuse basophilia[12]. The difference in staining reactions of the colloid in the follicles varies with the degree of functional activity at the moment[15]. There may be differences of staining in the colloid of the same follicle. The most acidophilic staining is found in states of hypoactivity.

The amount of colloid accumulating in the vesicles at one time denotes the degree of activity of the gland[17]. During hyperactivity of the gland, there is decreased amount of colloid, increased peripheral vacuolation and basophilia, while during hypoactivity there is accumulation of colloid, decreased vacuolation and acidophilia[18]. It is generally agreed that the colloid is a storage product and contains large quantities of thyroid hormone[2].

Epithelium. The epithelial cells form a continuous lining in

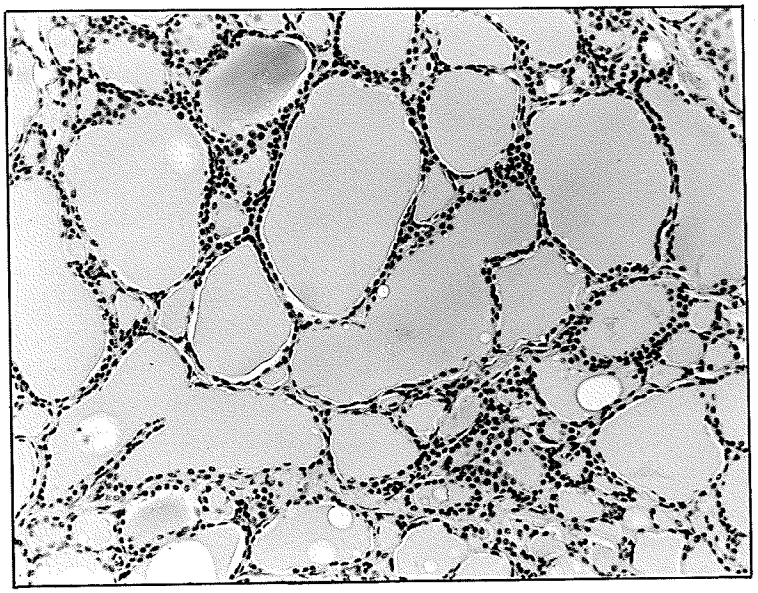


FIGURE 67

Normal thyroid tissue, showing the normal irregularity of size and shape of the follicles. H & E. 150 x. (5028-46).

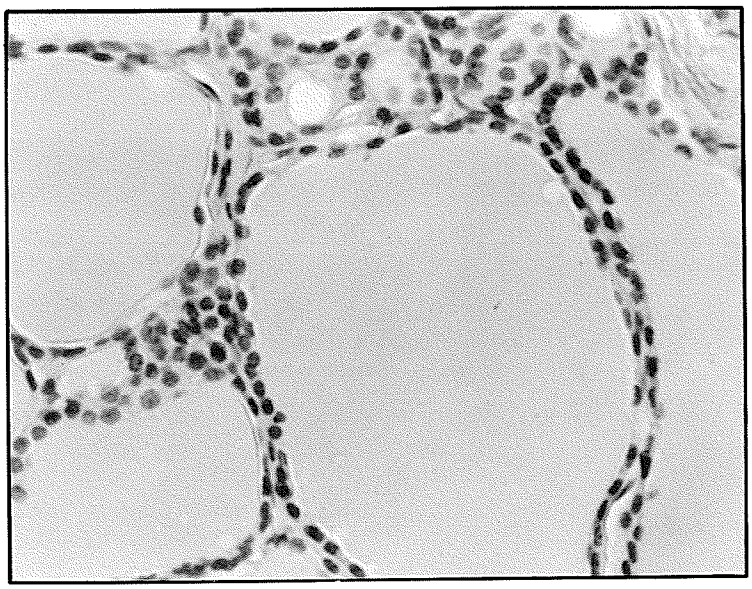


FIGURE 68

Same tissue as Fig. 67 to show more detail of the epithelium. 400 x. H & E.

the follicle arranged in a single layer. No true basement membrane is present[6,7]. The epithelium rests on a fine layer of condensed connective tissue fibrils.

Numerous types of cells have been described, most of which require special methods for their demonstration. Not all investigators are in agreement regarding the types of cells. Most authors think that the different cells represent variations of a single cell type. Others state that some of them are definitely degenerating.

Most of the cells have large vesicular nuclei, prominent nucleoli and pale staining cytoplasm which often contains granules which stain like colloid and which are located in the pole next to the lumen. Occasionally a cell shows a dark, shrunken nucleus and stains homogenously with acid dyes, similar to the colloid[13].

The follicular cells vary in height, depending on the degree of physiological activity. Under normal conditions, the cells are cuboidal or low columnar[14]. During hypoactivity the cells become flattened and during hyperactivity they become tall and columnar.

The variations. From the foregoing remarks, it is seen that the picture is extremely varied. The size of the follicle, the amount of its colloid, the staining reaction of the colloid, the amount of vacuolation, the height of the epithelium and the amount of interfollicular tissue, especially the lymphoid tissue, all vary with the activity of the gland.

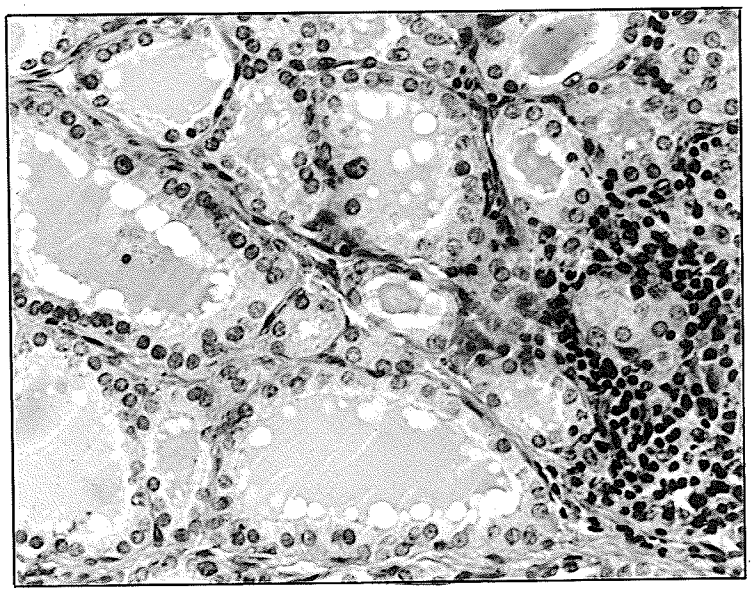


FIGURE 69

The thyroid in hyperthyroidism. H & E. 300 x. (3709-49).

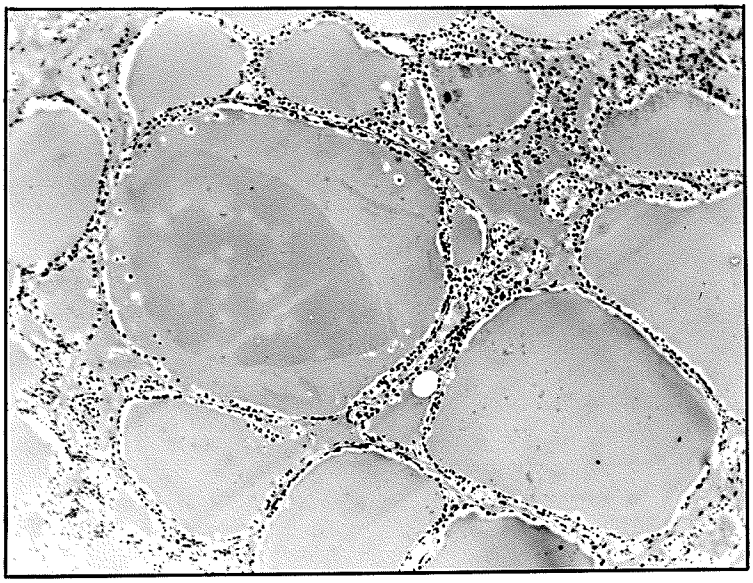


FIGURE 70

The thyroid gland in colloid goitre.
H & E. 75 x. A 6041.

The following table, given in more detail by Cowdry[18], summarizes the variations of the histological components of the thyroid gland in states of hypoactivity and hyperactivity.

	Hyperactivity	Hypoactivity
Colloid		
amount -	decreases (absorption)	increases (retention)
density -	decreases (numerous vacuoles appear)	increases (few vacuoles)
homogeneity -	marked	absent
cells in -	occasional	quite numerous
Follicles		
size -	small	very large
shape -	very irregular	more uniform
Follicular cells		
size -	increased	decreased
shape -	columnar	greatly flattened
multiplication -	mitoses occasional	mitoses rare
Interfollicular tissue		
blood vessels -	engorged	less dilated than normal
lymphatics -	distended	less active than normal
cells -	histiocytes more noticeable, lymphocytes considerably increased.	histiocytes less noticeable, lymphocytes slightly increased.

II. THE FUNCTION OF THE THYROID

Introduction. Most theories of thyroid function recognize that the secretion of the gland is linked with the oxidative processes of all tissue cells. However, it is not possible to explain all the manifestations of hypothyroidism and hyperthyroidism on this assumption[16]. The hormone (or hormones) produced under physiological conditions and its mode of action are not known[2]. More than one compound which is physiologically active can be obtained by extraction of thyroid tissue, and although the number is considerably smaller than that obtained in extractions of the adrenal cortex, there exists here a somewhat parallel situation. Whether these compounds represent the hormone, a part of it, or its precursor has become a moot question. The already voluminous literature on all the aspects of thyroid physiology continues to increase rapidly, and in the discussion to follow, the monographs of Cameron[16] and Selye[2] have been used almost exclusively.

The hormone. By a series of fractional extractions of thyroid tissue, three iodine containing compounds are obtained; diiodotyrosine and thyroxine which are iodized amino acids, and thyroglobulin which is a psuedoglobulin.

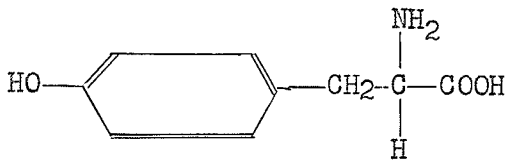
Diiodotyrosine contains 58.7 per cent iodine and has been isolated from thyroid tissue, several sources other than thyroid tissue, and from enzymic hydrolysis of thyroglobulin. It has been isolated in crystalline form and has been found to have only a trace of hormone activity, if any.

Thyroxine contains 63.5 per cent iodine and was isolated first by Kendall in 1919, and in 1927 Harrington and Barger established its formula. Thyroxine, which is in the form of colourless crystals, has two isomers. Only the l-thyroxine has physiological activity. l-thyroxine closely imitates all the actions of functional thyroid but it probably does not represent the form of the hormone which is normally secreted by the gland. Of the substances now available in pure form, thyroxine possesses the greatest biological activity[16]. Any change in the structure of the thyroxine molecule reduces its physiological activity[9].

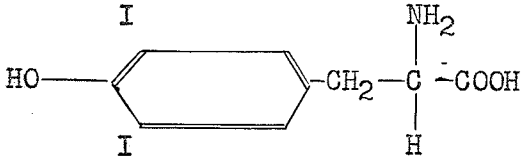
Thyroglobulin is a pseudoglobulin[16] and is the only protein in the colloid[9]. It has a molecular weight[16] of approximately 700,000. The substance possesses greater physiological activity than its thyroxine content would indicate.

A number of compounds are important in the synthesis of thyroxine. Thyronine is a compound which results when the iodine is removed from thyroxine. It has no physiological activity. Tetrabrom-thyronine is similar to thyroxine, except that bromine replaces the iodine of thyroxine. It has only slight physiological activity, suggesting the specificity of the iodine fraction. Tyrosine, the mother substance of thyroxine, is inert. Diiodotyrosine, resulting from the substitution of iodine at 3' and 5' for hydrogen, has only a very slight trace of activity, so slight that some deny any.

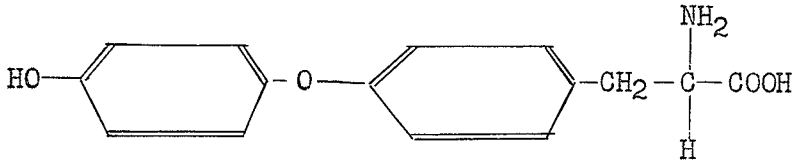
The following structural formulae are given by Selye[2]:



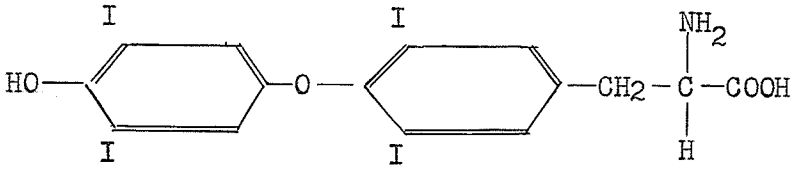
TYROSINE



DIIODOTYROSINE



THYRONINE



THYROXINE

Cameron reviews the problem of the nature of the true hormone. According to this reviewer, some authors consider that the hormone is neither thyroxine nor thyroglobulin but an iodized albumin. Another group, Cameron states, consider that the thyroid hormone circulates as an integral part of the plasma proteins in much the same way as antibodies. The highest concentration of iodine is found with the alpha and beta-globulins, while the major part of it is present in the plasma albumin fraction. (Cf. Cameron[16])

There is little evidence that the thyroid produces normally several hormones[2]. The exact nature of the hormone in the blood is not known. It is estimated that the human thyroid produces daily amounts of the hormone equivalent to one-third mgm of thyroxine, the daily intake of iodine required for this production being between 0.1 to 0.2 mgm. (Salter - quoted by Cameron)

The actions of the hormone. The results of thyroidectomy and feeding dessicated thyroid tissue have given an indication of some of the functions of the thyroid hormone. The rate of response to thyroidectomy, substitution therapy after thyroidectomy, or feeding of thyroid to excess, is not rapid when the action of thyroid is compared with the action of insulin or adrenalin. If an organizm is deprived of all thyroid tissue, the basal metabolic rate falls from 40 to 45 per cent below normal in 60 to 80 days. The decline in the basal metabolic rate follows a predictable curve. In the normal adult, 1 grain of desiccated thyroid by mouth, or 1 mgm thyroxine

intravenously causes a rise of 2.8 per cent in the basal metabolic rate. Upon the administration of a single dose, the rise in the basal metabolic rate does not begin until seven hours later and this rise reaches its maximum after one or two weeks. The single dose may continue to exert influence for a period up to two months after administration. (Cf. Selye[2])

The effects of thyroidectomy and the administration of thyroid extract are listed in the following table which is based on material from Selye[2] and Cameron[16].

Organ or System	Effects of Thyroidectomy	Effects of Feeding Dessicated Thyroid
Body temperature	lowered	slightly raised
B.M.R.	drops to -40%	slightly raised excessive dose rise up to plus 100%
Glucose tolerance	increases	decreases
Total blood lipid especially cholesterol	rise	decreased
Urinary excretion N_2	decreases	increases
Blood iodine level	decreases	increases
Calcium-phosphorus excretion	decreased	increased
Growth in young	retarded	if toxic - retarded
Diuresis	tendency to accumulate HOH	increases

Organ or System	Effects of Thyroidectomy	Effects of Feeding Dessicated Thyroid
Cardio-vascular		
heart size	decreased	increased
pulse rate	decreased	increased
systolic BP	decreased	increased
peripheral	vasoconstriction	vasodilation
Lymphoid system		
lymph nodes	show moderate involution	small doses - stimulate
spleen		
marrow		
thymus		toxic doses-involution
Muscle system	decreased strength	loss of strength
Central nervous system	early operation - impedes develop. of intelligence late operation - decreased activity	increased activity
Accessory sex organs both sexes	involution	involution
Menstruation	amenorrhoea	amenorrhoea

Iodine. The element iodine is widely distributed in nature, although few materials are rich in it. The iodine content of plant life, animals existing on it, and their products vary with the iodine content of the soil of a district. (Cf. Cameron)

Most endocrine glands contain relatively more iodine than non-endocrine organs. The thyroid is the richest source in the body[16]. The total iodine content of the human body is approximately 50 mgms, and of this the thyroid contains 10-15 mgms and the muscles 25 mgms[2].

Iodine is present in both the cells and the colloid of the thyroid[16]. Forty per cent of the thyroid iodine is thyroxine iodine, the other 60 per cent corresponds to diiodotyrosine[2].

Iodine is absorbed mainly from the intestine and is excreted mainly by the kidney. Only a part of the iodine liberated from the decomposition of the hormone is excreted, the remainder of it is re-utilized[2].

The normal blood iodine level is from 2.4 to 5.5 per cent and in the plasma from 4 to 10 per cent[2].

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C H A P T E R E I G H T

THE PANCREAS

CHAPTER VIII

THE PANCREAS

Introduction. Next to the liver the pancreas is the largest gland connected with the gastro-intestinal tract and consists of exocrine and endocrine tissue.

The gland lies in the retroperitoneal space at approximately the level of the second and third lumbar vertebrae. It is subdivided into three parts, the head, body and tail. The right extremity of the gland, being thicker and wider, forms an enlarged bulbous mass which is called the head. The mid portion is the body, and the left extremity which extends almost to the spleen is called the tail. The divisions are arbitrary, no sharp lines of demarcation being present.

In the adult human, the gland measures from twenty to twenty-five centimetres in length, and weighs from 65 to 160 grams[1]. Ogilvie[2] gives the average figure of 2.6 grams at birth, and at twenty-one years it is stabilized at approximately 66 grams. There is a wide range of normal values.

The gland is soft in consistency, and on cross section is pale pink in colour due to the vascularity. The lobules vary in size, the larger lobules being visible to the naked eye. A thin layer of connective tissue covers the gland, but does not, however, form a true capsule.

I. THE EXOCRINE PANCREATIC TISSUE

The pancreas is a compound acinous gland whose lobules are bound together by loose connective tissue through which run excretory ducts, blood vessels, nerves and lymphatics. The acini vary from spherical structures to short tubules, and are made up of a single layer of pyramidal cells resting on a delicate reticular membrane[1]. The secretions empty into a duct system which penetrates the whole gland. (Cf. post)

The acini. The acinar cell has two zones when seen in the stained section. The apex of the cell next to the lumen is closely packed with coarse, round, highly refractile bodies or granules. These are the zymogen granules which stain brilliantly with acid dyes such as eosin, phloxine and fuschin. Their size is fairly uniform, although some variation is found. The number of granules varies with secretory activity of the gland. During fasting they accumulate to fill more and more of the apex of the cell; while during secretion of pancreatic juice, their number decreases. This observation was first described by Heidenhain[9] in 1875, and observed in the living animal by Covell[10], whose work is discussed in connection with the physiology of the acinar cells on page 260. There is a variation in the number of granules found in the cells of different acini, since the whole gland does not secrete at one time, some acini producing pancreatic juice while others are in a resting state[11].

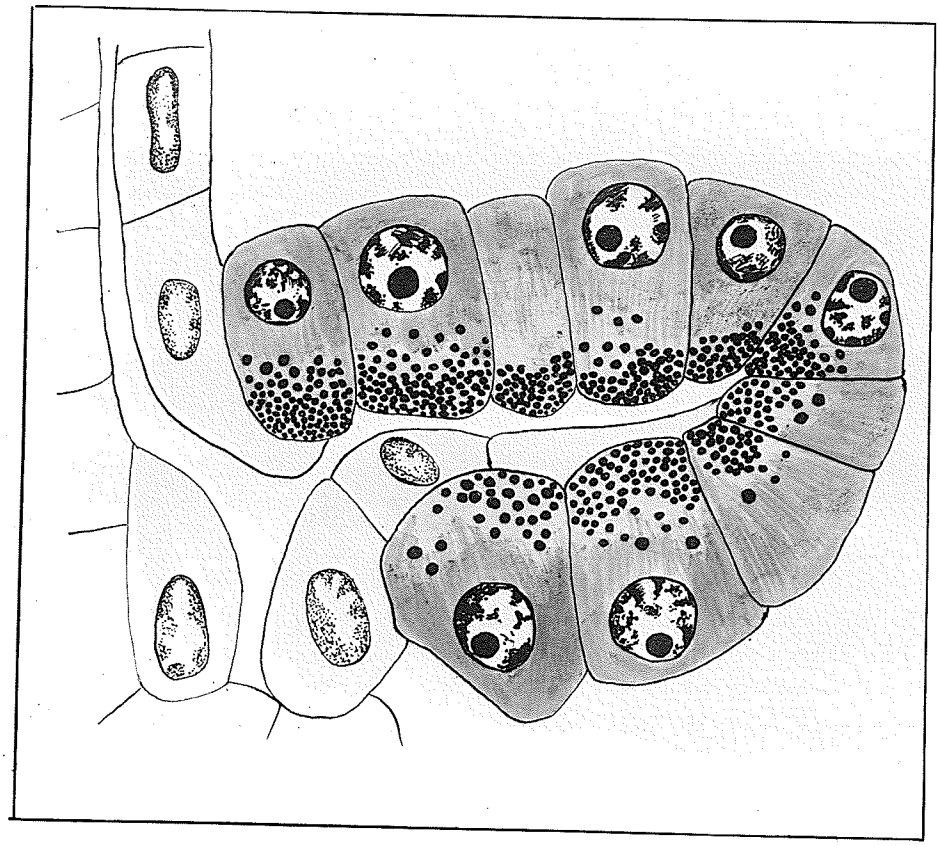

FIGURE 72

Drawing to show the relation of the ducts, acinar cells, and the centro-acinar cells. Redrawn and modified after Bensley, 1911.

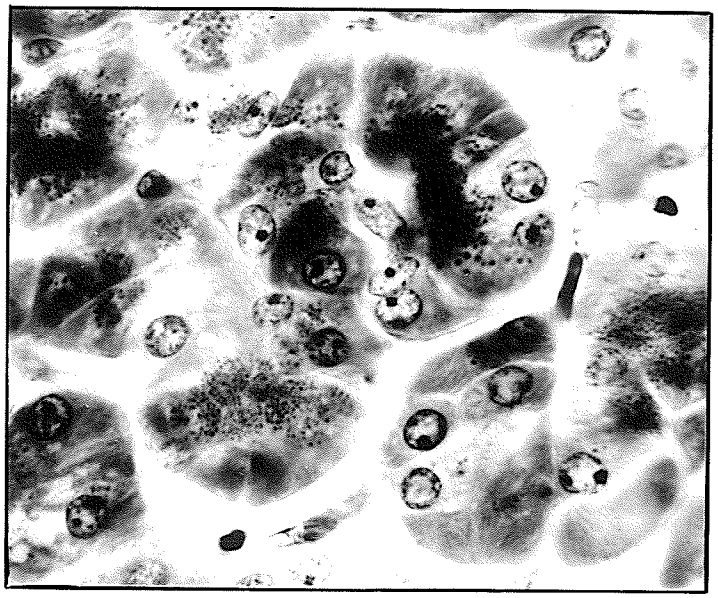

FIGURE 73

The zymogen granules in the acinus cells. Oil immersion. 1000 x. Masson Trichrome Stain.

No granules are found in the basal portion of the cell which is occupied by the chromidial substance. This substance stains intensely blue with the basic stains such as hematoxylin and toluidine blue. The staining is most intense next to the reticular membrane and gradually fades in intensity as the apex is approached. The chromidial substance is homogenous in the living state[10] and after certain fixatives. After fixation in fluids containing osmic or chromic acids, no striations are demonstrable. After fixation in fluids containing acetic acid, such as Bouin's or Zenker's fluids, fibrillary structures are seen and it can be shown by special methods that they correspond to the large filamentous mitochondria and are due to them[12]. The striations are vertically placed on either side of the nucleus, and horizontal below it[13].

The nucleus of acinus cell is characterized by the richness in chromatin which forms a fine network of threads and granules. Granules are in contact with the inner surface of the nuclear membrane. One or more large spherical, strongly acidophilic bodies are found within the nucleus and are known as the plasmosomes or nucleoli. Applied to the surface of these bodies are granules of chromatin. Cells with two nuclei are rare in man[13].

The duct system. Typical pancreatic ducts are accompanied by a system of tubules. The two pancreatic ducts, main (Wirsung's) and accessory (Santorini's) carry pancreatic juices to the duodenum. The tubules which have no lumen, according to Cowdry[14], consist of a cord

of cells.

The duct of Wirsung traverses the pancreas from the tail to the head. It begins in the tail as a fine delicate structure and gradually becomes greater in diameter as it approaches the head and receives in its course numerous tributaries which open into it practically at right angles. The duct is situated closer to the anterior surface than the posterior surface. It opens into the duodenum by obliquely penetrating the coats of the duodenum side by side with the common bile duct. They end in a papilla-like elevation of the mucous membrane, the short common cavity of which is the ampulla of Vater[11].

In the head of the majority of glands there is a shorter duct, the accessory pancreatic duct or the duct of Santorini, the relations of which are very variable. It usually opens by a separate orifice into the descending portion of the duodenum and is always connected with the main duct by a transverse branch. More rarely it empties into the main duct or may be entirely absent[15].

The ducts at their proximal end are lined with epithelium closely resembling that of the duodenum. The cells are tall and columnar with distally placed elongated nuclei. True goblet cells occur with varying frequency and are well demonstrated by staining with periodic acid. Near the duodenum, the epithelium and the basement membrane are thrown into folds. This appearance disappears as the ducts become finer. The epithelium has a striking similarity to that of the extrahepatic biliary system. The ducts are supported by relatively thick bands of connective tissue, chiefly collagen fibres

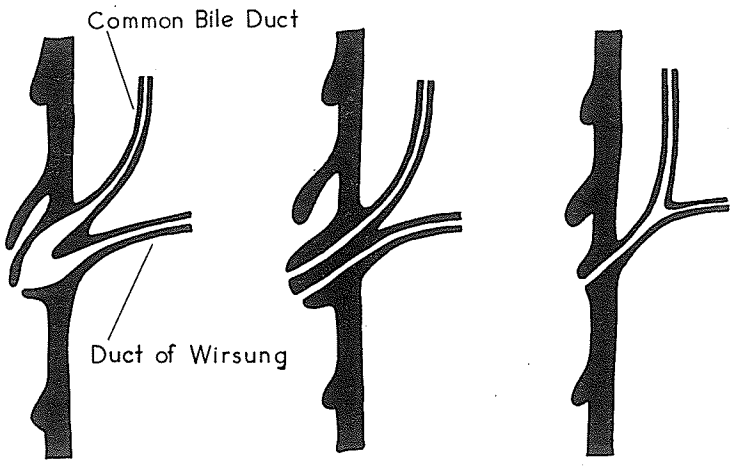

FIGURE 74

Diagram showing the common variations of the relations between the common bile duct and the duct of Wirsung.

with a few elastic fibres. The epithelium changes from the columnar type to the cuboidal type as the ducts become smaller in calibre, and the mucous secretion becomes less and less.

As the ducts enter the acinus, there is noted a type of cell which is distinct from either the acinous cell or the duct cell. These are the centro-acinous cells, so called by Langerhans. Their shape is fusiform, often flat, and in some instances have short projections which penetrate between the secreting cells. Many authors feel that they represent the terminal portion of the duct as it meets the acinus. The cento-acinar cells have pale staining acidophilic cytoplasm and possess no zymogen or other specific granules and no chromidial substance. The nucleus is usually oval, rich in chromatin and similar to that of the acinus cell. The acidophile nucleolus, so prominent in the acinus cell, is not conspicuous[13].

Tubules. Much less obvious in a stained section is that system of tubules described by Bensley[17]. By injecting vital dyes, such as pyronin and neutral red, into the aorta of an animal killed by bleeding, Bensley was able to demonstrate these tubules which escape detection by ordinary injection methods. This system of tubules forms a tortuous, intricate series of anastomoses which connect the duct and branches of the duct. The tubules vary in thickness from 12 to 27 micra in the guinea pig, and are sometimes thicker where small islets are attached. Bensley described the lumen as continuous throughout, although it may be as narrow as 1.5 micra in diameter in places.

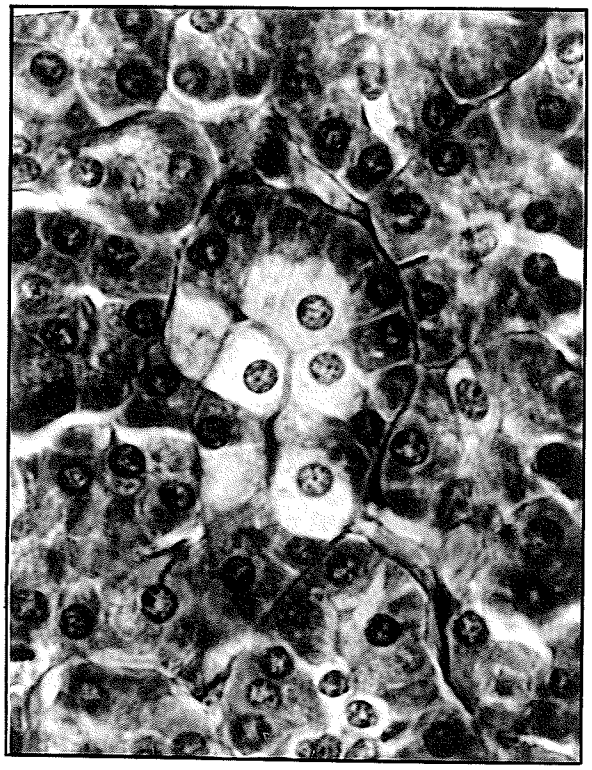

FIGURE 75

A group of centro-acinar cells in the pancreas of an adult male. 800 x. Hematoxylin and eosin stain.

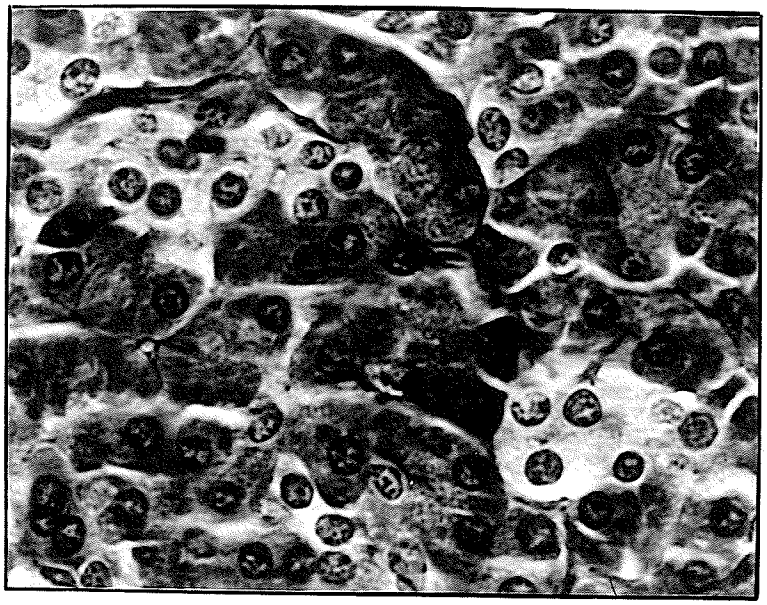

FIGURE 76

Another two groups of centro-acinar cells in the same tissue as Figure 75. 800 x.

Single islet cells are seen here and there. Also attached to the tubules, although less frequently than the attachment of islets, are small acini, which vary from a single acinous to a group of acini. In some cases these acini arise from the ducts which lead to the islets. (Cf. Bensley[17]) According to Cowdry[14], these tubules do not carry secretion. A further discussion of the tubules is given in connection with the islets on page 274.

The blood vascular supply. Pierson[3] gives an excellent account of the arterial supply of the pancreas. The following table and quotation are from his paper.

The arterial supply of the pancreas, as found by Pierson[3] in the study of fifty cases.

Anterior superior pancreaticoduodenal in 100 per cent of cases

Anterior inferior pancreaticoduodenal in 100 per cent

form the anterior arcade

Posterior superior pancreaticoduodenal in 96 per cent

Posterior inferior pancreaticoduodenal in 100 per cent

form the posterior arcade

Inferior pancreatic in 100 per cent (Wharton)

Superior pancreatic in 54 per cent

Pancreatic branches of the splenic in 100 per cent

Pancreatic branches of the gastroduodenal in 62 per cent

Pancreatic branches of the hepatic in 10 per cent

Typically the pancreas is supplied by two arteries from the gastroduodenal, two from the superior mesenteric, and one from either the splenic, hepatic, or celiac arteries. In addition to these a variable number of small arteries arise from the splenic, gastroduodenal, and hepatic. The larger vessels arising from the gastroduodenal artery are the superior pancreaticoduodenal arteries (right pancreaticoduodenal of some authors). These are two in number, an anterior and a posterior. They anastomose with corresponding anterior and posterior inferior pancreaticoduodenal arteries (left pancreaticoduodenal) from the superior mesenteric. These vessels form two arterial arcades, one on the posterior surface of the head of the pancreas, the other, except for its lowermost part, on the anterior surface. They are respectively the posterior and the anterior arcades of the pancreas. The two inferior pancreaticoduodenal arteries usually arise in a common trunk from the superior mesenteric, the common inferior pancreaticoduodenal artery. The superior pancreatic artery is the least constant of any of the large arteries to the pancreas. When present, it arises from the splenic, hepatic, or directly from the celiac arteries. In addition to the arteries mentioned, an inferior pancreatic artery, which passes along the inferior margin of the body to the tail, may take origin from the superior mesenteric, the anterior superior pancreaticoduodenal, the inferior pancreaticoduodenal, or the superior pancreatic artery.

The relationship of the arteries to the veins is fairly constant. Every artery here described has a concomitant vein. According to the writer's observations, as well as Petrén's, the veins usually lie nearer the surface of the gland than the arteries. The anterior superior pancreaticoduodenal vein drains constantly into the right gastroepiploic vein, which is a tributary of the superior mesenteric. The posterior superior pancreaticoduodenal vein almost always drains directly into the portal vein. The lower veins empty directly into the superior mesenteric or one of its tributaries. The posterior inferior pancreaticoduodenal vein may empty into the inferior mesenteric. The splenic vein, which is sometimes imbedded in the gland, receives several veins from the body and tail. (Pierson[3])

The intralobular pancreatic circulation has been reported by Oppenheimer and Mann[4], who used neoprene injection and corrosion methods. These investigators found that:

1. Anastomoses exist between intralobular arterioles and also between acinar capillary fields fed by different arterioles within any one lobule.

2. Blood may reach acinar capillaries via islets of Langerhans or directly without passing through islet tissue.

3. The potential intralobular capillary circulation is very large.

The blood supply of the islets is discussed on page 279, in connection with the islets of Langerhans.

Nerve supply. Sympathetic preganglionic fibres arise from cells in the intermediolateral column in the spinal cord and reach the celiac plexus via the splanchnics. Post ganglionic sympathetic fibres from the celiac ganglia reach the pancreas via the hepatic, superior mesenteric and splenic plexuses. Stimulation of these fibres has no marked effect upon pancreatic secretions. The parasympathetic preganglionic fibres arise in the dorsal motor nucleus of the vagus and reach the pancreas via the vagus nerve. The postganglionic fibres arise from cells, probably in the pancreas or closely associated with it. The function of augmenting both the external and internal secretory activity of the pancreas has been ascribed to these fibres (Cf. Kuntz[5]). The fibres accompany the arteries into the gland and end about the acini in fine terminals[1]. The islets have an abundant supply of nerve, both myelinated and unmyelinated[6]; however, it is difficult to assess the effects of this nerve control, since at the present time there is no method for estimating the amount of insulin in the blood[7].

The lymphatic drainage. The lymph drainage of the pancreas is

adequately described to date[21]. Callander[8] described the lymphatic drainage as being extensive, the vessels draining into the pancreatico-splenic nodes at the hilus of the spleen, and into the pancreatico-duodenal and preaortic nodes near the origin of the superior mesenteric artery.

The Physiology of the exocrine pancreas. Within a few minutes after the ingestion of food, the flow of pancreatic juice increases, reaches a maximum in two to three hours, decreases slowly to reach a basal rate in five to nine hours[18]. Both nervous and humoral mechanisms control the flow of pancreatic juice. Secretory fibres from the vagus and sympathetic innervate the acini. The vagal secretion is small in amount and has a high content of organic substances and ferments[19]. Pilocarpin produces the same effect when injected. Stimulation of the sympathetic fibres causes the production of a scanty secretion which is similar to that caused by vagal stimulation[20].

While reflex nervous control of pancreatic secretion occurs, more important are the chemical humoral mechanisms. These apparently play the chief role in the production of the pancreatic digestive juices[18].

Bayliss and Starling[22], in 1902, showed that if an acid extract of the epithelium of the duodenum and the upper portion of the small intestine were injected intravenously into an animal, there resulted a copious flow of pancreatic secretion. Injection of the acid alone

did not produce the same results. They postulated the presence of an inactive substance which they called prosecretin in the intestinal mucosal cells. This mother substance was changed into secretin in the presence of acid, and this absorbed into the blood stream acted as a chemical stimulant to the pancreas. In this connection, they proposed the term hormone which was used for the first time. Secretin[23] has now been prepared in crystalline form and has a molecular weight of 5000.

A second hormone of the duodenal mucosa and upper small intestine mucosa has been isolated and called pancreomyzin by Harper and Raper[24] in 1943. This hormone stimulates the secretion of the enzymes trypsin, amylase and lipase, and does so without increasing the volume of the pancreatic juice. These authors suggest that this hormone is responsible for the enzymic content of the pancreatic juice.

The secreting mechanism depends largely on the stimulus from the acid chyme as it enters the first part of the duodenum. A certain amount of secretin is quickly carried by the blood to the acinar cells of the pancreas and there excites the secretion of a strongly alkaline pancreatic juice. As soon as enough of the juice is present to neutralize the acid chyme, secretin formation and hence further pancreatic secretion stops. As long as the duodenal contents are acid the pylorus remains closed: therefore as soon as these contents are neutralized the pylorus relaxes and allows more acid chyme to enter. Thus the formation of secretin proceeds afresh and the whole chain goes on until the stomach is empty. (Cf. McCaughan and Purcell[25])

The secretion of the zymogen granules has been observed in the living animal by Covell[10]. Mice were given intravenous injections of neutral red and then an intraperitoneal injection of pilocarpin or secretin. Seven minutes after the pilocarpin, large vacuoles were noted in the distal pole of the acinus cell. These vacuoles increased in size and passed out of the cell into the lumen of the acinus. Several zymogen granules were also extruded. The vacuoles had the appearance of being pinched off after they were extruded through the cell membrane. The intracellular vacuoles appeared to be formed through the liquifaction of the zymogen granules. (Cf. Covell)

Pancreatic juice. Pancreatic juice[18] is a clear alkaline secretion having a pH from 8.7 to 8.98. The daily quantity is estimated from 510 to 860 cc's by Babkin, and between 1000 - 1500 by Ivy. Trypsin, chymotrypsin, amylase and lipase are among the enzymes in the secretion. The enzymes are not secreted in their active forms, but as protein proenzymes such as trypsinogen and chymotrypsinogen. Interokinase[19], an enzyme of the intestinal mucosa, has an optimum pH between 5.2 - 6.0 and in the presence of the acid chyme, changes the inactive trypsinogen into the active trypsin, and chymotrypsinogen into chymotrypsin, which have an optimum pH of about 8. Trypsinogen may also be activated by trypsin or by a slightly acid medium. The enzymes are mainly responsible for the proteolytic activity of the pancreatic juice. The final breakdown is brought about by other enzymes. Amylase is a starch splitter, active in a neutral, slightly

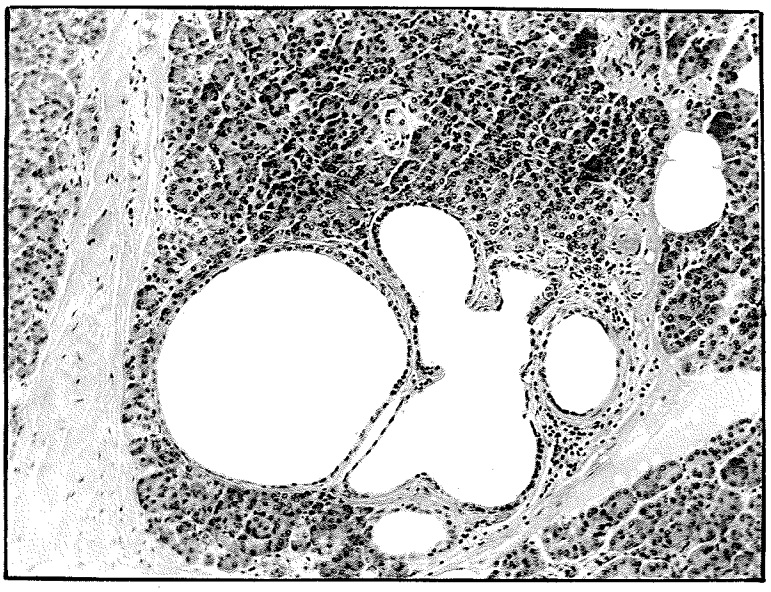

FIGURE 77

The pancreas in old age, showing the locules, some fat and wide trabulae. A 7261. Hematoxylin and eosin stain. 125 x.

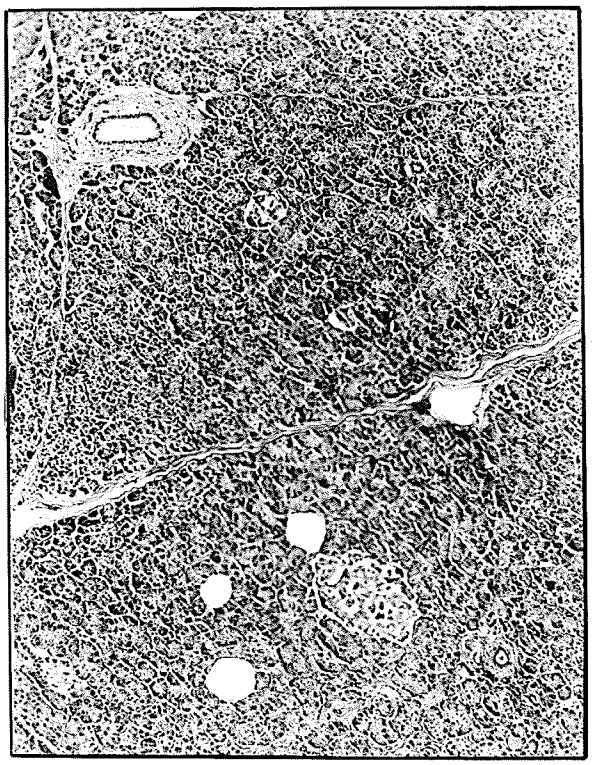

FIGURE 78

Low power field of adult pancreas showing lobules, acini, islets, ducts and septae. A 7261. 50 x. Hematoxylin and eosin stain.

acid or slightly alkaline medium. Lipase is a fat splitter, breaking the fat molecule into fatty acids and glycerine[19].

McCaughan and Purcell[25] give the following analyses of pancreatic fluid obtained from pancreatic fistulae:

pH	8.2 to 8.5
Water (per cent)	98.45 to 98.86
Total solids (%)	1.13 to 1.86
Nitrogen (%)	0.075 to 0.098
Albumen (%)	0.099 to 0.174
Ash (%)	0.580 to 0.950
Specific Gravity	1.005 to 1.014
Enzymes -	lipase, amylase, trypsin

Changes with age. Proliferation of the cells of the interlobular and intralobular ducts is the first and most conspicuous sign of the process of ageing, according to Warren[23]. Expansion of the ducts distal to this proliferation leads to flattening of the epithelium and finally the formation of cavity. These spaces so formed, Warren[23] terms locules, which are cavities lined with very flat epithelium and which may be quite large, at times involving a whole lobule. They are present in most subjects after the third decade, and become more numerous in the senile group. Degenerative changes also occur in the alveoli, in the form of loss of basophil substance, increase or decrease in the size of the nuclei, and multiple nuclei in a cell. The islets frequently atrophy, perhaps secondary to some of the above changes. True adipose tissue may be present in excess. (Cf. Warren[23])

II. THE ENDOCRINE PANCREATIC TISSUE

Scattered throughout the pancreas in an irregular manner are the islets of Langerhans. They are pale in a section stained with hematoxylin and eosin, for the islet cells contain no zymogen granules and no chromidial substance. After good fixation and staining they are contrasted against the darker background of acinar tissue.

The structures were discovered by Langerhans in 1869. In the same year, subsequent to his announcement, the name 'Les ilôts de Langerhans' was applied to them by Laguesse. They have been called 'interlobular cell clumps,' 'secondary cell groups' and 'pointes folliculaires' by various authors, and by American anatomists, the Islands of Langerhans. (Cf. Lane[26])

The size of the islets varies considerably, ranging from a single cell to a compact group of cells measuring up to 300 micra in diameter, the average being from 70 - 175 micra[27,16].

Amount and distribution of islet tissue. Many estimations of the number, total weight and distribution of the islets have been made. Authors agree on only a few points; that there is a wide variation in the total weight and number of islets; that there is a wide variation in these factors in sections from the same block, from area to area in the same specimen, from individual to individual in the same species, and from species to species; that the count depends much upon the method used in its determination.

Opie[11] counted the islets in stained sections from the head,

tail and body of ten specimens. Since he was studying only one plane he squared the counts and concluded that the islets were more numerous in the tail portion of the gland than elsewhere in the gland. Writing in 1935, Opie[13] was still of the same opinion. Bensley[28], using the guinea pig and intravital staining found that the islets were more numerous in the tail than in the remainder of the gland. His figures obtained for the dog pancreas are less convincing. Shields Warren[16] states that the islets are from two to four times more numerous in the tail than in the other parts of the gland. Waters and Best[58] point out that the number of units of insulin obtained per unit weight of tissue is greater in the tail than elsewhere. Further, many of the islet adenomas arise in the tail portion, an argument used by some writers to infer an unequal distribution of the islets. Gomori[27] does not believe that the notion that the islets are more numerous in the tail is substantiated. Susman[30] agrees that the distribution is fairly uniform.

Clarke[29] found in seven subjects ranging from the age of one-half year to 45 years in age, the islets varied from 120,000 to 1,760,000 in the organ. Ogilvie[2] gives the figures of 284,000 average at birth, 960,000 average up to the third year, and from the ages of 5 to 64 years the range of 618,644 to 2.30 million. Susman[30], using planimetric methods expresses the islets in percentages of the total organ, and gives the normal range as 0.9 - 2.7 per cent for adults, and 0.9 - 3.6 per cent for infants. Maximow and Bloom[1] give the range of 208,369 - 1,760,000 as normal. Gomori[27] gives the range

of 250,000 - 2,500,000, with an average normal around 500,000. Shields Warren[16] gives the average figure of around 1,000,000. These figures are best summarized in the table given below.

SUMMARY OF THE AMOUNT OF ISLET TISSUE AND THE NUMBER OF ISLETS AS CALCULATED BY DIFFERENT AUTHORS

Opie[11] (1900)	head - 18 islets per 0.5 square centimetre body - 18 tail -
Bensley[17] (1911)	@ figures for the guinea pig
Clarke[29] (1913)	120,000 - 1,760,000 in the whole gland
Ogilvie[2] (1937)	birth average, 284,000 in organ by third year, 960,000 in organ range - 118,110 to 2.35 million 5 to 64 years, range - 618,644 to 2.3 million
Susman[30] (1942)	Average normal percentage of weight of whole gland - in the infant - 0.9 to 3.6% in the adult - 0.9 to 2.7%
Gomori[27] (1945)	normal range - 250,000 to 2,500,000 islets percentage (weight) of total gland - 1 to 1.5% average normal number of islets - 500,000
Warren[16] (1938)	average total number of islets 1,000,000
Maximow & Bloom[1]	normal range - 208,369 to 1,760,000

Brief mention of the methods of counting and weighing the islets should be given at this point. The first method, used by Opie[11] and early investigators, consists of taking several blocks of tissue and counting the islets found in areas of stained sections. The number of islets counted in a unit area was squared or the thickness of the section was taken into account and the number estimated. This method assumes a more or less even distribution of islets, and is inferior to the method used by Bensley[17], in which an animal was killed by bleeding and the aorta injected with a solution of vital dye such as neutral red 1 in 15,000 or janus green 1 in 15,000. Both the islets and acinar tissue take up the dye, which however is reduced more slowly in the islets. After an interval the acinar tissue is colourless or almost so, and the islets remain stained and well demarcated. Pyronin (1 in 1000) injected in a similar manner stains the duct system, enabling Bensley to study the relation of the ducts to the islets. By these methods, Bensley was able to count small islets which escape detection by ordinary methods of staining. Unfortunately, the method is not as successful with human material as in some animals. The planimetric method is a third technique, used by Susman[30] and also others, to obtain the relative weights of the islets to the weights of whole glands. In this method, the stained sections are projected upon a sheet of paper and the islets are then pencilled in outline. The areas are then cut out of the sheet and weighed. The relative weight of islets to acinar tissue is thus estimated.

Variation of amount of acinar and islet tissue with age. The islets appear to be more numerous per unit area in a stained section of the pancreas of an infant than in the same unit area of adult gland. Laguesse[31] considered that the islets were more numerous during fetal life than at birth, and after birth they undergo further diminution in number. Opie[11](1900) thought that after birth the islets remain constant in number and merely became separated from each other by growth of acinus tissue. Ogilvie[2] has studied the growth of the acinus and islet tissue. According to Ogilvie[2], both acinar and islet tissue increase after birth and exhibit a curve similar to that of the body weight except that there is no increase after the sixty-fourth year. There is a rapid rise in the total number of islets in the period after birth, this increase being maintained during the first two years of life. During the third year, the rate becomes less marked and rapidly becomes stabilized at 960,000. Thus the total number of islets increases after birth, being 3.4 times during the first three years. Expressed in terms of weight, the islet tissue increases from the average of 0.12 grams at birth to the average of 1.07 grams at the age of twenty-one years. The curve of islet tissue weight plotted against age is similar to the weight of the body and pancreatic acinar tissue, the increase being less marked during childhood than in the case of the body and the acinar tissue. (Cf. Ogilvie) Susman[30] agrees with Ogilvie that there is an increase in the total number of islets and as well an increase in the absolute weight of them after birth.

The islets. The islets form well circumscribed groups of cells, consisting of anastomosing short cords only one or two cells thick[27]. There is no capsule in the strict sense of the word, the basement membranes of the acini and ducts, and the interacinar connective tissue delimiting the islets from adjacent tissue. Some are in direct contact with acini. Islet cells embedded in the duct epithelium are often overlooked unless specific stains are employed. Frequently islet cells are seen wedged in amongst the acinar cells. Some of these are probably peripheral processes of larger groups, while in other areas, the individual islet cells are obviously outside all insular relationship. Extrainsular islet cells are found in varying numbers in all species (Cf. Gomori[27]).

The islet cells are more or less characteristic in appearance with almost any staining technique. Their shape is cuboidal, sometimes columnar or wedge-like. With routine staining, the cytoplasm is homogenous, although with good fixation of fresh tissue faint differences in staining qualities can be detected. The most distinctive feature, however, is the presence of specific granules.

The granules were first detected in the islet cells by Laguesse and Diamere independently in the rabbit pancreas. Schulze described similar cells in the guinea pig pancreas. (Cf. Lane[26])

Lane[26] using differential fixation found two types of cells in the islets of the guinea, according to the fixing properties of the granules. To those cells whose granules were precipitated by 50 - 70 per cent alcohol, he applied the term 'A' cells. Those whose granules

were fixed in chrome-sublimate solutions, he termed β cells. The terms alpha, α, and A cells are now used interchangeably for Lane's 'A' cell, and beta, β, B for his β type of cell. The granules of the two types of cells have different chemical properties, and at the same time differ from the granules of the acinar cells[26]. The differential staining is due to the oxyphilia of the A cells, and the basophilia of the B cells[32].

Bensley used differential staining instead of differential fixation and was able to demonstrate the presence of the three types of cells in the same preparation. He used a 'neutral' stain in which a basic dye such as genetian violet is completely precipitated by an acid dye such as orange G. A weak solution of the neutral precipitate is used for staining, the differentiation done with clove oil. After fixation in chrome-sublimate solution, to which is added a minute quantity of acetic acid, the granules of the A cell stain blue, the granules of the B cells stain red. The C cells do not have granules. Other combinations to produce neutral stains are possible[33,34] and are discussed on page 277, together with some of the difficulties encountered in islet staining.

Bloom[35] has described a third type of cell containing very small blue granules when stained with mallory stain, which he calls the D cell. These are reported to be universal, although Gomori[27] has found them to be definitely present only in man and guinea pig.

In most species, the B cells greatly outnumber the alpha cells. In normal human islets between 60 to 90 per cent of all cells are

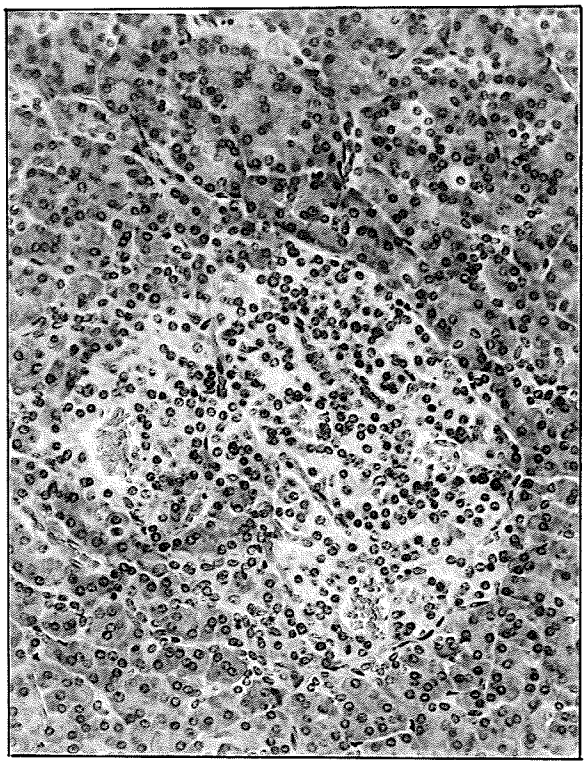

FIGURE 79

An islet of Langerhans, stained with hematoxylin and eosin. The pancreas is congested so that the vascular sinuses are dilated. WGH A 7174. 200 x.

B cells, 2 to 8 per cent are D cells, and the remainder are A cells. There is considerable variation from islet to islet. The extrainsular cells are mostly A cells. (Cf. Gomori[27])

There is considerable variation in the granularity of the islet cells. Opie[13] suggests that B cells are the parent cells for the A cells on the basis of the shape of the nucleus. Gomori[27] considers that the process is one of degranulation and notes that the finding is common in the B cells in apparently normal human material. This author describes three types of granulation: (a) diffuse, (b) discontinuous, and (c) margination. The significance of this arrangement is not known.

Besides the differences in staining of the granules of the islet cells, there are certain morphological differences.

The A cells are comparatively larger than the B cell, and the nucleus usually elliptical, although may be frequently circular in outline. The nucleus is large, vesicular and with very little chromatin which is distributed throughout in small spherical masses. The granules are packed throughout the entire cytoplasm in some cells; in others they are gathered at one end, the remainder of the cytoplasm being devoid of granules. The cells are polygonal and stand out against the background of B cells.

The B cells are smaller in appearance, the nucleus is smaller than that of the A cell, has more chromatin in the form of heavy masses and is circular in outline. The B cells are more numerous than the A cells, and appear to be arranged in continuous cords,

"uninterrupted by the presence of A cells"[26]. Actually the cells occur without any definite arrangement, although the A cells do occur a little more frequently about the periphery of the islet[16].

The D cells were demonstrated by Bloom[35] in 1931, using the Mallory Azan method (Heidenhain's modification of Mallory's aniline blue stain) after Zenker-formol fixation. With this stain the islets are predominately yellow-orange contrasted against the deeper reds and blues of the acinar tissue. The majority of the cells are filled with very minute grayish yellow granules; these are the B cells. The A cells are scattered throughout the islet and around the periphery and are filled with larger, brilliantly stained red granules. These granules are much smaller than the zymogen granules of the acinus cells. As with other stains, the granules in some of the A cells are seen occasionally throughout the cytoplasm, and in other cells accumulated at one pole. The D cells have pale blue cytoplasm, and are filled with closely packed fine blue granules. In some the cytoplasm is homogenous and no granules are seen.

Gomori[41] believes that the A cells arise from the duct epithelium. He presents evidence that the D cells are actually aged A cells. Both A and D cells are found in the acini, and in the islets have much the same distribution and predilection for the periphery and around vessels. There is a low D:A ratio in the young islets and in the lining of the ducts, while in older islets the D:A ratio is much higher.

The alpha cells arise from duct epithelium, the D cells are

probably aged A cells, and the origin of the B cells is unknown. (Gomori[41])

The origin of the islets. Most histologists agree that the acini and the islets are formed from the ducts[27]. The duct connections are vestiges showing the origin of the islets. The position of the islet is determined by its developmental history. If the islet has arisen from the main duct, one of its branches, or from small tubules in immediate vicinity of the main duct, the position of the islet will be interlobular and will have the appearance of having a capsule. If, however, the islet originated from several intralobular ductules, the islet will everywhere be surrounded by acini (Cf. Bensley[12]). Islet cells, which are mostly A cells, are found scattered throughout the epithelium lining the ducts. Bensley[17] interprets these as having differentiated from the epithelial lining of the tubules and also from the columnar epithelium of the larger ducts.

The islets form from ducts during adult life, although there is very little formation of new islets in the adult human pancreas. The epithelium of the small ducts becomes multilayered, to form buds which become detached after enlarging. (Cf. Gomori[27])

The relation of ducts to islets. By vital staining, Bensley has shown that in the guinea pig there exists an intricate series of tubules, which are not detected in ordinary stains. This author has shown that with few exceptions the islets are all connected at some place with the duct system. There may be more than one connection,

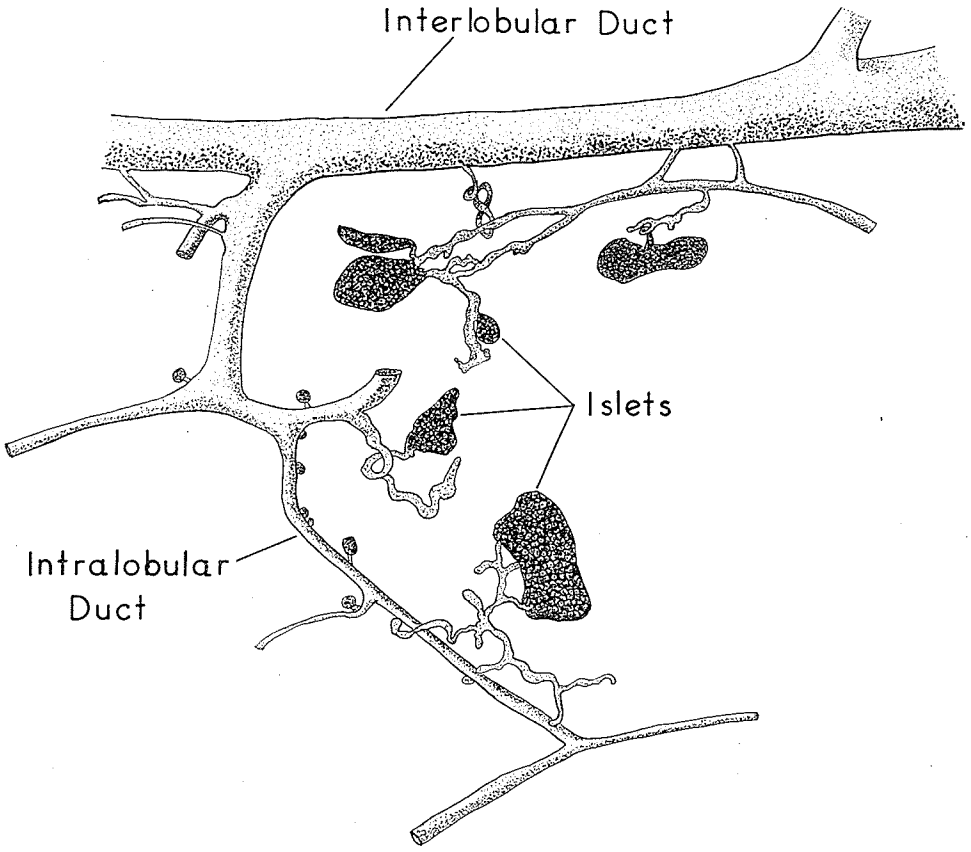

FIGURE 80

The relation of the islets of Langerhans to the ducts. Redrawn and simplified after Bensley, 1911.

especially in the larger islets which appear to have arisen from more than one duct, and later coalesced.

Bensley[17] has grouped the islets into four classes, according to their relation with the ducts:

(a) Islets in the interstitial tissue, connected with the duct or its primary branches directly by short ducts or by tubules. These islets are all sizes, varying from a single cell to the largest in the pancreas, and have no connection with acinus tissue.

(b) Islets in the pancreatic lobules, connected with the interlobular system by tubules or branches. They have apparently become secondarily surrounded by acinus tissue.

(c) Islets within the lobules in continuity with the acini or ducts or both. Most of the islets are of this type, and are similar to those described in man by Laguesse. There is no connective tissue between the islet and the acinus.

(d) Islets connected with neither ducts nor acini, and found in the interstitial tissue or in the substance of the lobules. These are few in number. (Cf. Bensley[17])

The relations of acini to islets. The question of whether islets are formed from acini, and whether acini are formed from islets has been the grounds of bitter contention since Laguesse put forward his "theorie de balancement[36]," and there are two camps. One group, consisting of Lane[26], Bensley[17,12], Ukai, Allen, champion the theory that once formed the islets and the acini are specific, non interchangeable, and each tissue is capable of growth by division of its own cells and by new formation from the ducts. The other group, consisting of Saguchi, Vincent, Otani and Sergeyeva say that the relation is dynamic rather than static and that one tissue can change one to the other as the demands to various stimuli arise. Gomori[27]

has never seen a cell intermediate between an acinus and islet cell. Hard[36] has had the same experience. The second of the above theories is contrary to laws of the growth elsewhere in the body. While it is true that glands such as the thyroid change their morphology in response to various stimuli, the basic cell type of the epithelium does not change. One type of epithelium may change to another type in some mucous surfaces as from columnar to squamous, but this metaplasia has definite limits. Other examples may be given, but the more highly specialized glandular epithelia appear to be incapable of true metaplasia[28]. Both acinus cells and islet cells are highly specialized types of cells, each with specific granules and complex secretions (exocrine and endocrine respectively) and it would appear unlikely that transitions from one type to another should occur. This is a dangerous type of reasoning and falls into the category of those whose work is based on too lax criteria of what constitutes islet tissue, and their failure or refusal to use specific stains. Gomori[27] has stated the situation by saying much the same as Bensley who wrote some thirty-three years before him, "If this criteria (specific stains) is accepted, the overwhelming majority of papers championing acinar-insular transformation must be dismissed because the authors failed to demonstrate such cells." Gomori[27], however, does feel that there is no doubt that the islets are formed in embryonic life by the budding of acini.

Staining of the islets. Neutral stains of the type described

by Bensley have formed the basis of most investigations of islet tissue since the method was announced. The principal of neutral staining has been outlined previously on page 270. Martin[33] has given a detailed account on the preparation of neutral dyes and the various combinations of dyes which may be used. Bowie[34] preferred Biebrich scarlet and ethyl violet when working on the principal islets of the teolost, Neomaenis griseus. With this stain he was able to demonstrate the presence of a third type of cell in the islet tissue of the teolost.

Unfortunately, the neutral stains of the type described by Bensley and Lane work well for the pancreas of the guinea pig, the tissue for which the stain was perfected, but for tissues of other animals, similar results are difficult to obtain. It is possible to obtain reversal of staining reactions and much of the controversy found in the literature probably finds its origin in this fact[41]. Many factors enter into the staining process, such as pH of fixation, pH of the stain itself, and the obtaining of ultrafresh material. Frequently investigators have used lots of dyes which are impossible for other workers to obtain.

Bloom in 1931 recommended the use of Mallory-Heidenhain azan stain for the differential staining of the islet tissue. Differentiation is good for the D cells and A cells, but the B cells are not brilliantly stained.

In 1939, Gomori[32] noting that in well fixed material a good hematoxylin and eosin stain shows a difference in the alpha and beta cells, suggested a modified hematoxylin and eosin stain, designed to

bring out these staining differences. The method may be used after any fixative, although a modified Bouin's fluid is recommended. Best results are obtained with tissues obtained within four hours after death, although some good preparations are possible with tissue fixed as long as ten hours after death. Bell[42] confirms this latter point. Gomori[32] recommends refixing of the sections in the modified Bouin's fluid, after the paraffin has been removed and the sections taken to water. After refixing, the sections are then treated with potassium permanganate and potassium metabisulphite for oxidation. Staining is done with a ripened chromium hematoxylin and either phloxine or ponceau de xylidene. The alpha granules are blue, the beta granules red and the D cell granules are not demonstrated. This method has been tried successfully in this study and although the results are not as brilliant as those depicted by different authors working with ultra-fresh animal material and other stains, nevertheless the granules are to be seen.

The insular blood supply. The vascularity of the islets of Langerhans was early recognised, and because of this feature, early investigators suggested that the islets had some internal secretion. Several extensive studies have been made, in which injection methods and congestion methods are the most popular.

Beck and Berg[43] came to the conclusion that the vascular pattern of the islets of the guinea pig, white rat, monkey and man were similar to that of the white mouse which they used in their

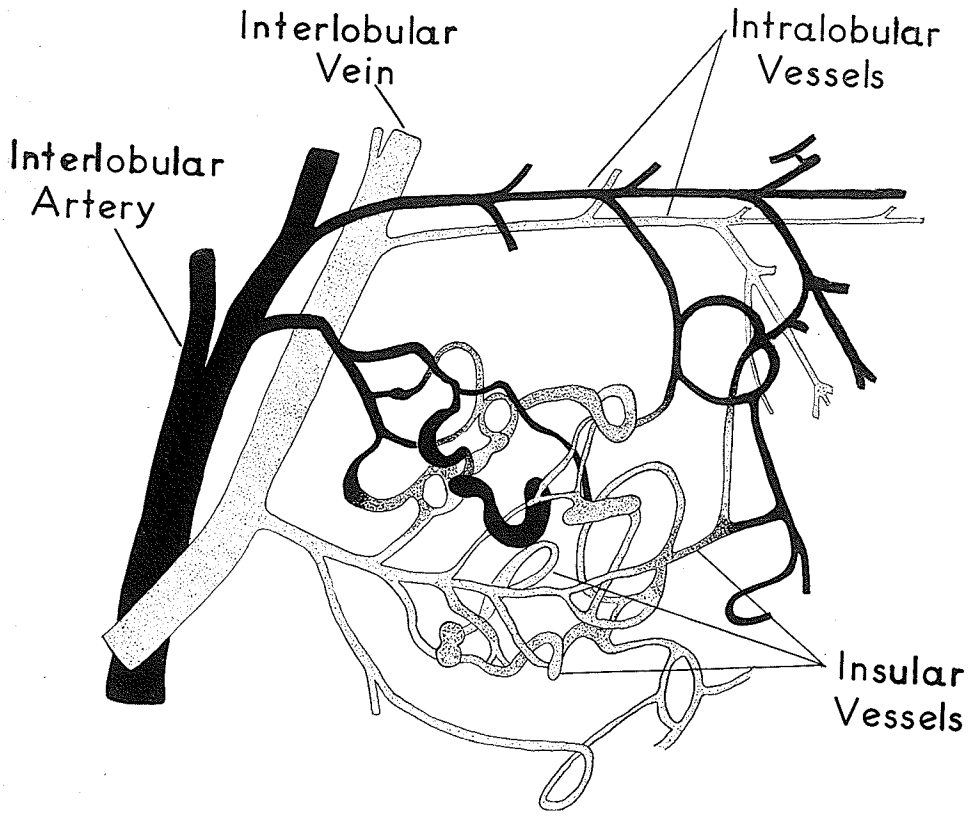

FIGURE 81

The blood vascular supply of the islets of Langerhans, according to Beck and Berg. Redrawn after Beck and Berg, 1931.

investigation. The afferent arterioles are short, slightly wider in diameter than capillaries and arise in most cases directly from the intralobular arteries to supply the islets in the central zone of the primary lobules. The islets situated in the periphery of the lobules and in the interlobular connective tissue receive short branches from the interlobular arteries. Islets of the larger order, over 0.15 mm, receive two or more arterioles; those smaller than 0.15 mm in diameter receiving usually only one afferent vessel. Upon entering the islet, the arterioles divide into a tortuous, anastomosing plexus of capillaries. Anastomoses between the capillaries of the islets, and those in the interacinar rete are common. Usually more than one efferent venule is present, depending on the size of the islet. These venules are short, emptying directly into the intralobular veins. Occasionally the venules join the interlobular and larger pancreatic veins. After leaving the islet, the venules often course over its surface and often receive tributaries along the way. Tributaries are also received from the adjacent interacinar capillaries. (Cf. Beck and Berg[43])

Wharton[44], using single injection methods, agrees essentially with Beck and Berg[43]. His illustrations which do not show the venous system, are similar in their arterial pattern.

Warren[16] points out that the islet capillaries are thin-walled and without the usual perivascular fibrous tissue.

The nerve supply of the islets. The nerve supply of the islets

and acini has been given on page 257.

The neuro-insular complexes of Simard. Intimate association of nervous and epithelial elements are found to be constant structures in the interlobular and intralobular septa by Simard[45]. The complexes have both insular and acinar cells, intimately mixed with the nerve tissue. Simard suggests that the complexes may contribute to chemical transmission of nerve impulses.

The lymphatic drainage of the islets. The lymphatic drainage of the islets is not known[21].

III. THE PHYSIOLOGY OF THE ISLETS

Introduction. Since the exact mechanism of the production of insulin and its mode of action are not known, any complete discussion of experimental work and the theories concerning such mechanisms is too long to be included in this brief summary. Waters and Best[57], Selye[40], and Cameron[39] have discussed many of the details. Only a brief summary of some of the aspects is attempted here, although it is necessary to include some experimental work which forms some of the basis of the present theories concerning the physiology of the islets.

Source of insulin. The pancreas influences the metabolism of carbohydrates through the medium of a hormone, since pancreatectomy causes diabetes in the experimental animal. A transplantation by vascular anastomosis to the neck of an animal which has been

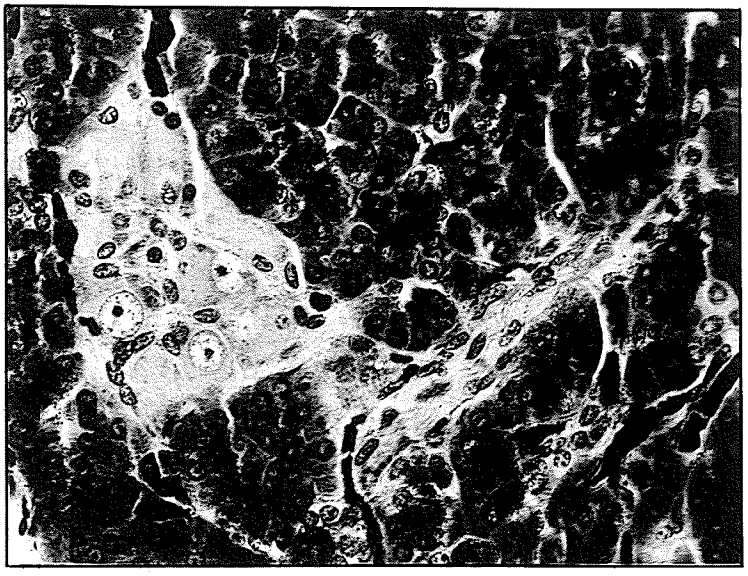

FIGURE 82

A group of ganglion cells and a bundle of unmedullated nerves in close proximity. Masson Trichrome stain. 400 x.

pancreatectomized, will prevent the development of diabetes[40].

It is generally agreed that insulin is produced only by the islets of the pancreas[39]. Histologically the islets are glandular structures which have the features of an endocrine gland. Relatively large amounts of the hormone are found in the principal islets of teolost fishes in which few enzyme producing cells are present. The hormone is present in those glands where the ducts have been ligated and the exogenous gland tissue has undergone atrophy leaving the islets intact. After extensive partial pancreatectomy (nine-tenths) has been done, the islets in the remainder of the gland prevent the sudden onset of diabetes. Soon, however, diabetes develops in proportion to the progressive degeneration of the islets. Islet cell adenomata and even some carcinomas of islet cells are rich in insulin. After removal of the adenomata, the symptoms of hyperinsulism disappear. (Cf. Selye[40])

It is generally accepted that the islet function is not related to that of the acinar tissue and the sole function of the islets is the production of insulin. (Cf. Cameron[39])

There is considerable evidence that the beta cells are the producers of insulin. The development of diabetes after partial pancreatectomy is proportional to the degenerative changes in the remaining islet tissue, particularly in the beta cells. Diabetogenic anterior-pituitary substances exert specific damaging effects upon the beta cells and at the same time decrease the amount of insulin which is present in the pancreas. The beta granules, like insulin, are

soluble in alcohol. A high carbohydrate diet causes degranulation of the beta cells. Insulin causes degranulation and involution of the beta cells, a process which is considered to be compensatory atrophy. Alloxan is a drug which has a rather selective action upon the beta cells resulting in diabetes which rather parallels the destruction of that type of cell. (Cf. Waters and Best[57], and Selye[40])

While the beta cells are thought to be the producers of insulin and the evidence presented above supports this, the function of the other types of cells in the islets is not known.

Nerve endings of the vagus have been located in the beta cells of the islets[57]. Vagus control is not essential but an additional mechanism. Hyperglycemia stimulates the islets to produce insulin. No adequate evidence is presented that the islets are under control of any pituitary hormone although the pituitary hormone producing hyperglycemia stimulates the islets indirectly. (Cf. Cameron[39])

The insulin is secreted into the circulation, the vascularity of the islets facilitating this mechanism. Under the action of physiological stimuli, the islets secrete insulin in minute amounts either continuously or spasmodically[39].

Pancreatectomy. Removal of a dog's pancreas is soon followed by glycosuria and ketonuria. The series of metabolic disturbances that follow the pancreatectomy are similar to those in a severe case of diabetes mellitus in the human subject. The dog is unlikely to survive more than two weeks following the operation, even with the

greatest care, unless insulin is administered in the form of injections. (Cf. Waters and Best[57])

Pancreatectomy decreases the amount of glycogen in the liver while there is less effect upon the glycogen of the muscle. Insulin replenishes the depleted glycogen stores in both the muscles and liver[40].

Pancreatectomy disturbs lipid metabolism. There is a transient marked hyperlipemia and hypercholesterolemia with fat deposition in the liver. Part of this disturbance may be due to the loss of the exocrine secretion of the pancreas[40].

Acetone bodies (acetone, acetoacetic acid, and betahydroxybutyric acid) are normally present in the blood in small quantities and originate from the metabolism of amino acids and fatty acids in the liver. Hepatectomy causes their disappearance. Acetone bodies are utilized to some extent in the body normally. In the absence of insulin, glucose is not utilized, and acetone bodies accumulate in concentrations too large for normal metabolism, resulting in the clinical condition known as ketosis. Presumably the increase of the acetone bodies in the blood is a reflection of the rate of increased utilization of depot fat and proteins for energy requirements of the body. The old theory that fat burns in the fire carbohydrates must be abandoned. (Cf. Selye[40] and Waters and Best[57])

Injection of Insulin. Following the injection of insulin, there is a lowering of the concentration of blood glucose, and if the dose is

large enough, hypoglycemia and convulsions result[39]. In the intact animal there is considerable deposition of glycogen in the muscles without any very definite change in liver glycogen. In the pancreatectomized animal, depleted glycogen stores are replenished in both the liver and muscle[40]. The precise chemical actions of insulin have still to be determined and the fate of insulin is not known[39].

Only the liver participates appreciably in the formation of glucose. The liver can make blood glucose from its own glycogen stores and from non-sugars. The process is termed gluconeogenesis, the mechanisms of which are not fully understood. Insulin inhibits gluconeogenesis while pancreatectomy augments it. Large quantities of glucose are excreted in the urine of fasting pancreatectomized animals, the gluconeogenesis not being controlled. (Cf. Selye[40])

All experimental observations indicate that the most important mechanisms through which insulin influences carbohydrate metabolism are by: (a) decreasing the rate of sugar formation from noncarbohydrate sources (gluconeogenesis), (b) increasing the rate of sugar mobilization and (c) increasing the rate of sugar storage in the form of muscle and liver glycogen. (Cf. Selye[40])

Hyperinsulinism. The condition of hyperinsulinism was first recognized by Harris (Cameron). Approximately one hundred cases are now reported[40]. A group of conditions with symptoms of hypoglycemia are not all associated with hyperinsulinism. Cameron lists these as: (a) those cases of hypoglycemia due to hyperinsulinism and caused by a

functioning tumor of islet tissue, either a benign adenoma or malignant neoplasm, (b) those not associated with hyperinsulinism, and (c) those cases where the cause of the hypoglycemia is not known.

The symptoms of hypoglycemia vary at different blood concentrations of glucose. Fatigue, lassitude, restlessness and malais are common complaints. The accompanying compensatory secretion of adrenine produces such symptoms as pallor, cold clammy perspiration, palpatation, tremor and often hunger or thirst. In hypoglycemia the senses are clouded, and frequently the behaviour of the patient resembles that of alcoholic intoxication, bravado, negativism and hallucinations. Finally there may be convulsions and paralysis with loss of memory, coma and even death in extreme cases. (Cf. Cameron[39])

Note on carbohydrate metabolism. The normal blood sugar is an equilibrium mixture of two isomers, alpha and beta glucose, both dextrorotatory[40].

Absorption of carbohydrates is mostly from the small intestine, traces only being absorbed from the stomach and large intestine. Complex carbohydrates are broken down into monosaccacharides or simple sugars during digestion process. Following the absorption of large quantities of glucose, some of it is oxidized and the remainder is deposited in the liver or muscles as glycogen. (Cf. Selye[40])

The storage of carbohydrates is almost exclusively as glycogen in the liver and muscles. The decidua and the placenta are the only other tissues known to have significant amounts. Muscle glycogen comes

chiefly from blood sugar, while liver glycogen may come from blood glucose, lactic acid and nonsugars.

In the post absorptive state, the sole source of blood glucose is the liver[57].

The "Cori" cycle or lactic acid cycle appears to be most important during exercise and is given together with a discussion of the various chemical steps by Selye[40].

There is a normal balance of regulating endocrine factors which control carbohydrate metabolism. Insulin from the islets is counteracted by the hormones of the pituitary, thyroid and adrenal cortex. If, for example, there is an increase of pituitary hormone effect without the corresponding increase of insulin output, a relative deficiency of insulin is present which may lead to the production of the diabetic state. (Cf. Cameron[39])

That the pancreas, liver and pituitary are among those organs playing a major role is demonstrated by the Staub-Traugott phenomenon. If two doses of glucose are given to an animal in rapid succession, the second causes a less pronounced hyperglycemia. The islets have been alerted. The Staub-Traugott phenomenon is absent in the pancreatectomized animal and as well in the hepatectomized or hypophysectomized animal. (Cf. Selye[40])

Diabetes. Cameron's excellent short description of the sequence of events occurring in the untreated diabetic patient is quoted below, directly from his monograph.

> In the untreated diabetic the course of the abnormal metabolic changes is (i) hyperglycemia, increasing until it is accompanied by (ii) glycosuria, (which at first may occur only after meals, and later may be continuous). As the glycosuria increases, since excretion of glucose must be accompanied by excretion of extra water, this causes (iii) polyuria, which induces (iv) polydipsia. Distorted carbohydrate metabolism is followed by distorted protein metabolism, so that an undue amount of protein is catabolized to glucose and excretory nitrogenous compounds and so causes (v) an increased azoturia. The carbohydrate and protein wastage may be sufficient to lead to (vi) loss of weight and (vii) polyphagia. Ultimately fat metabolism becomes distorted, acetoacetic acid is formed in too large an amount for its correct catabolism, and so (viii) the "acetone bodies," acetoacetic acid, beta-hydroxybutyric acid, and acetone, appear in the urine, and acetone may appear on the breath. Finally, through the large efflux of these two acids into the blood, its mineral balance is disturbed, and (ix) the plasma base becomes inadequate to carry the normal amount of carbon dioxide from tissues to lungs, so that the accumulation of carbon dioxide in the tissues stimulates the respiratory centre to "air-hunger," coma ensues, and the patient dies. (Cameron[39])

The history of the pancreas. The older anatomists believed that the pancreas existed to support and protect more important organs in contact with the gland. Wirsung in 1643 described the main duct which traverses the length of the gland. With recognition of the duct, a physiological significance was then possible. Santorini described accurately the small duct which now bears his name. His drawings were published in 1775, some thirty-eight years after his death. (Cf. Opie[11])

Claude Bernard in 1856 recognized the zymogen granules in the acinar cells. Heidenhain, in 1875, showed the changes in number of zymogen granules during the process of secretion of the gland.

Langerhans, in 1869, gave the first good description of the microscopic anatomy of the pancreas. He described the histology of the

acinar cells, of the centro-acinar cells which he named. He also recognized that when pancreatic tissue was stored in Muller's for two or three days, small groups of cells appeared under low magnification as intensely yellow flecks. Under higher magnification, these groups consisted of irregularly placed polygenal cells. He also recognized the vascularity of these areas. Of the nature of these areas, Langerhans claimed total ignorance. Subsequent to Langerhan's announcement, the name "les ilots de Langerhans" was applied to the structures by Laguesse. (Cf. Lane[26])

Twenty years after the discovery of the islets, von Mering and Minkowski found that total pancreatectomy resulted in severe and fatal diabetes[46]. The hypothesis that the pancreas possessed an anti-diabetic function which was dependent upon an internal secretion was accepted by the majority of physiologists after these classical experiments[47]. In the decade that followed, it was shown that the pancreases of all vertebrate classes had islets[12]. In 1893, Laguesse suggested that the search in cases of human diabetes would reveal changes involving the islets of Langerhans[12]. Schafer[48], in 1895, suggested that on the basis of such experiments as those of Schiff and Thiroloix, and on anatomical grounds, the islets furnished an internal secretion which had some control over carbohydrate metabolism. Diamere[49] made a similar suggestion. Ssobolew[50] reported that after ligating the duct of Wirsung in dogs, the islets were not involved in the initial sclerosis, and believed that this explained the absence of glycosuria after the ligation. Ssobolew's 1902 paper, quoted by

Barron[51], using the pancreas of dogs, cats and rabbits reports similar findings. In a period from 30 to 120 days after ligation, the islets did become involved in a sclerotic process, and with this glycosuria made its appearance.

Opie[52] in 1901, reported a series of cases of diabetics the chief or only lesion found in which was in the islets. Reports to the contrary were not long in appearing, however, for other investigators were unable to find such a high percentage of diabetics with lesions in the islets[12]. According to Macloed[47], several investigators after the turn of the century succeeded occasionally in demonstrating substances in extracts of the pancreas capable of diminishing one or other of the symptoms of diabetic laboratory animals. Carlson[53] reported that pregnant bitches near term did not develop severe glycosuria after complete pancreatectomy until the young were born. Bensley[12], in 1915, described in detail the pathological changes that followed the ligation of the ducts. Sharpey-Schafer[6], in 1916, introduced the term insulin for the then hypothetical hormone, unaware that de Meyer had also suggested the same term in 1909 (Arch. di fisiol, vii, 1909).

Barron[51] discussed in a paper the pathology of the rare condition of pancreatic lithiasis, and the changes that followed the obstruction of the ducts, either spontaneously or experimentally. He reviewed the work of many others, and was in agreement that the islets secreted a hormone into the blood or lymph stream and this hormone had some control over carbohydrate metabolism.

"There could be little doubt of the existence of the hormone, the problem was to obtain it in extracts of the gland" - Macleod[47].

After Barron's paper, it occurred to Banting[54] that the failure to obtain active extracts of the whole gland may depend upon the fact that the hormone was destroyed by the activation of the pancreatic enzymes, also present in the extracts. With Best as an assistant, Banting took advantage of the acinar degeneration of the ligation of the ducts[55]. In dogs, seven to ten weeks after the duct ligation, they found an abundance of healthy islet tissue and complete replacement of the acinar tissue by fibrous tissue. The animals were then given a lethal dose of chloroform, the fibrous pancreas placed in a chilled mortor with Ringer's solution, partially frozen, macerated and filtered. The filtrate when injected into diabetic dogs lowered the level of the blood sugar. The diabetic animal was able to retain a much greater percentage of injected sugar than it could otherwise. Interesting enough, these investigators called the new hormone insulin, entirely ignorant of Sharpey-Schafer's earlier suggestion. In another paper[56], the same year, Banting and Best concluded from Carlson's report[53], that the fetal animals provided the de-pancreatized pregnant bitches with the necessary hormone to prevent a severe diabetes. Extracts of the pancreas of fetal calves of five months gestation or less contained no enzymes. The filtrate produced the same effect as the extract of degenerated pancreas. There remained only the purification and large scale production of insulin for clinical purposes, much of which is due to Collip and Best (Cf. Banting[54]).

Unfortunately, the discovery of insulin and the isolation of the hormone eventually in crystalline form by Abel in 1926 did not settle many contentions. Papers such as that of Vincent[37] continued to appear. In 1924, Vincent did not believe that the islets were separate and distinct organs, representing instead temporarily modified portions of the secretory tubules. Otani[38] in 1927, admitted that there was evidence that the islets had an internal secretory activity, but could not be accepted, however, as constituting an organ sui generis.

Note on Embryology. The pancreas develops from two separate primordia which arise from the duodenal endoderm. The first primordia to appear is the dorsal pancreatic bud (at the 3 mm stage). The ventral bud appears a little later. The two pancreatic primordia fuse to form a single organ at about the 12 mm stage. The lower part of the head represents the ventral bud, while the upper part of the head, the body and tail represent the dorsal bud.

Both the duct and the acinar arise from the hollow pancreatic buds. As the buds increase in length, they extend into the surrounding mesenchyme. Epithelial "sprouts" arise from their solid tips, become canalized and form the collecting ducts. The acini arise from the tips of the collecting ducts. The islets develop in much the same manner and in the third month of fetal life some of them begin to separate off from the collecting tubules. Secretion granules appear later in the islet cells. Pancreatic enzymes are produced by the acini after the fifth month. (Cf. Hamilton, Boyd and Mossman[58])

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C H A P T E R N I N E

THE HYPOPHYSIS CEREBRI

(THE PITUITARY)

CHAPTER IX

THE HYPOPHYSIS CEREBRI

Introduction. The hypophysis cerebri has the most complex structure of any of the endocrine organs. Its origin from two anlagen accounts for the composite structure of epithelial, glial, nerve fibre elements and connective tissue. Changes occur in the organ with relation to age, but these are not marked. Thus, concretions which vary considerably in size and shape are found in the pars anterior of the newborn. Within the first few months of postnatal life these concretions disappear. Many changes that occur with age are associated with the partial or complete obliteration of the hypophyseal cleft, the remnants of which are variable. Occasionally extraneous material, even bone and cartilage may be found in the gland. The size and weight of the different lobes vary according to age and sex, and in the female the size and weight of the pars anterior are influenced, at least in some, by pregnancy.

The hypophysis is an ovoid, somewhat flattened appendage of the hypothalamus and is attached to the tuber cinereum in the floor of the third ventricle by the neural portion of the stalk. It closely resembles a small cherry to which the stem is attached. The stalk extends through the aperture of the diaphragm of the sella tursica in a downward and forward direction. The bulbous portion of the gland is situated in the sella tursica of the sphenoid bone and is separated from bone by the periosteum of the bone, the dura and the capsule of the gland, all

of which are blended together imperceptibly. The normal size and weight are discussed on page 310, where it will be seen that there is a wide range of normal variation.

Several functions have been ascribed to the gland. These functions are performed through the elaboration of several hormones which exert influences over other tissues and endocrine organs to earn for the hypophysis the title of "Master Gland." At present, six hormones have been prepared in either pure or almost pure state from extracts of the anterior lobe. The process of purification is difficult since the hormones are all protein substances. Several other "actions" have been ascribed to crude and purified extracts of the pars anterior of the hypophysis. In most instances these actions are apparently not due to specific hormones. Hormones have also been extracted from the pars nervosa and pars intermedia.

Many questions regarding the nature of the gland, its structure and its functions are still to be answered and these questions form the basis of considerable research at the present. Up to 1936 more than five hundred significant histological studies had been published[34] and there have been many more since that time. When the number of papers dealing with physiological, experimental and biochemical work are included, the literature is voluminous.

I. TERMINOLOGY

The terminology used in the descriptions of the hypophysis varies greatly. The nomenclature of Bailey[2] and Bucy[1] has been adopted in this discussion. The following table is taken from Bucy[1]:

A. Pars Buccalis:
   1. pars anterior
   2. pars tuberalis
   3. pars intermedia

   } anterior lobe

B. Pars nervosa:
   1. processus infundibuli
   2. infundibulum or stalk

   } posterior lobe

Brief reference to the many synonyms used in discussions of the hypophysis is included here, together with a brief description of the location and extent of the lobes, which are illustrated in Figure 83.

Pars anterior. The pars anterior is also called the anterior lobe, glandular lobe and pars distalis. The lobe represents approximately three-fourths of the mass of the gland and is the largest part of the pars buccalis, representing practically all of the stomodeal portion. The lobe is situated a little farther from the brain, hence the name distalis. It consists of epithelial cells which are arranged in cords or alveoli, accounting for the term glandular lobe. The pars anterior is bounded superiorly by pars tuberalis and posteriorly by the hypophyseal cleft or its remnants. Inferiorly, anteriorly and laterally it is bounded only by its capsule which is continuous with dura of sella.

Pars tuberalis. The pars tuberalis derives its name from its rather inconstant association with the tuber cinereum. The part is small and insignificant in man. The extent of the pars tuberalis has been well described by Green[32] who found it constantly associated with the anterior surface of the stalk, where it may extend superiorly up to the optic chiasma. Posteriorly, the relations were variable, except in the angle between the pars nervosa and the stalk. The pars tuberalis may be found as a thin layer over the superior surface of the pars anterior, pars intermedia and the pars nervosa.

Pars intermedia. The pars intermedia represents another very small part of the hypophysis (2 per cent of the epithelium at birth and 1.5 per cent in adults). The structure is very irregular and may be represented by a single layer of cells, or it may be many cells in thickness. The lobe is bounded anteriorly by the cleft or remnants of it and posteriorly by the capsule of pars posterior, except at the fenestrations of the capsule. Lateral and inferior boundaries are the capsule of the hypophysis. The pars intermedia may become important on account of its epithelial cells which invade the pars nervosa and because of cysts and locules that may develop in later years.

Infundibulum. The infundibulum is commonly referred to as the stalk of the hypophysis. Vessels and non-medullated nerve fibres connecting the hypothalamus and the different parts of the hypophysis are present in the infundibulum.

Pars nervosa. The pars nervosa has been referred to as the neurohypophysis, the neural lobe, the posterior lobe and the pars posterior. The lobe is bounded everywhere by its capsule except anteriorly at the fenestrations and where it is continuous with the stalk. Epithelial elements are present in the neural lobe, but these originate from the pars intermedia. The pars nervosa represents about one fifth of the hypophysis.

Embryological note. Rathke[3] first stated that the hypophysis arises from two anlagens. The oral ectoderm through the formation of Rathke's pouch gives rise to the anterior or glandular lobe of the hypophysis[31]. The neural ectoderm forming the infundibular region of the third ventricle gives rise to the posterior or neural lobe[31]. The evagination of Rathke's pouch begins in the human embryo at the 3 mm stage[4] and grows upwards towards the ventral surface of the neural lobe[1]. The anterior surface of the pouch gives rise to the pars anterior[1]. Growth of connective tissue, trabeculae and blood vessels into the central core and peripheral zone of the pars anterior occurs at a later time. The posterior wall of the pouch comes into contact with the neural lobe at an early stage and remains relatively thin and epithelium-like to give rise to the pars intermedia[6]. The fate of the cleft of Rathke's pouch has been given in the discussion of the pars intermedia.

According to Atwell[6], Tilney first showed that the pars tuberalis was derived from a pair of lateral lobes, although this origin had been suggested by others working with reptiles. The earliest appearance of

the paired lateral lobes which form the pars tuberalis was observed by Atwell[6] in a 10.5 mm embryo, when the lobes appear as ridges. By the 45 mm stage, the pars tuberalis grows forwards and backwards surrounding the infundibulum and spreading out under the tuber cinerem. (Cf. Atwell[6])

Melchionna and Moore[40] in a study of 54 subjects at autopsy found that 51 of these unselected cases had a small mass of typical or atypical pituitary tissue in the pharyngeal wall. These masses were essentially of undifferentiated epithelial cells and differentiated cells similar to those of the pars anterior. In 25 per cent of the cases, chromophilic cells were absent. Acidophils and basophils when present were few in number and made up less than 1 per cent of the cells present. Melchionna and Moore[40] who review the earlier work of Haberfield (1909) and Christeller (1914) feel that because of the size, growth and histology these pharyngeal pituitary glands contribute little physiological activity, and explain their presence by the embryological origin of the pars anterior. (Cf. Melchionna and Moore[40])

Meningeal relations. Until recently, the meningeal relations to the hypophysis were not definitely established. Atwell[6] in his text-figure does not attempt to represent the arachnoid pia. Bailey[2] modified Atwell's drawing and illustrated the arachnoid surrounding the whole gland except at the extreme posterior pole where the blood vessels enter the posterior lobe. General textbooks seldom make definite statements[12] and most illustrations and discussions are based

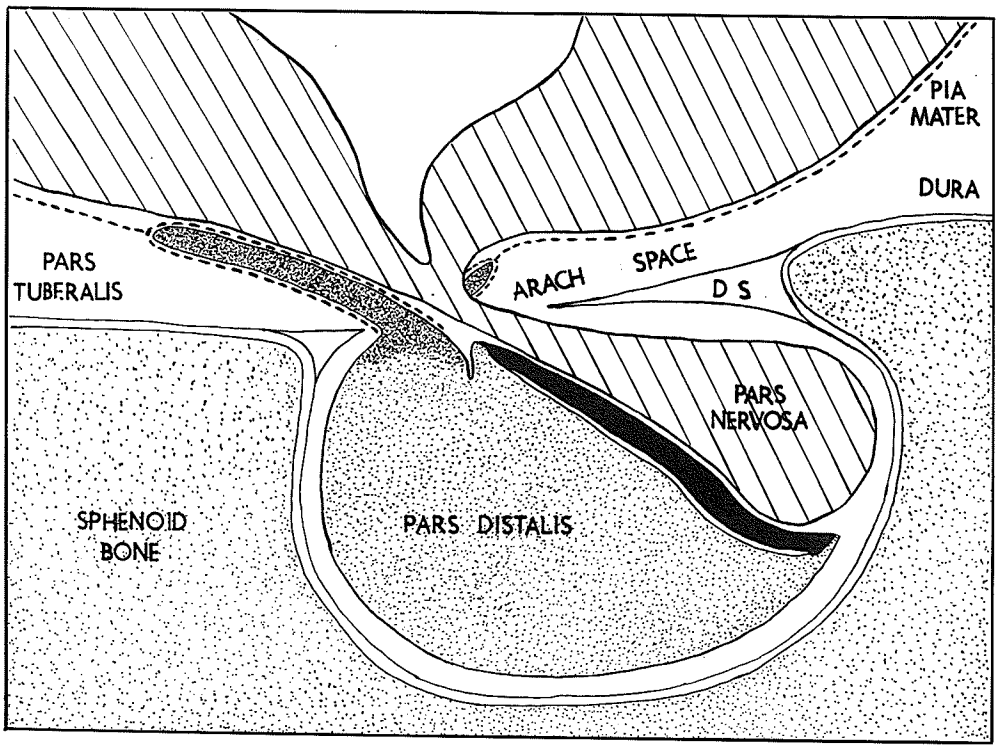

FIGURE 83

The meningeal relations of the hypophysis according to Atwell[6]. Redrawn from Atwell, Amer. J. Anat., 1926, 37:159.

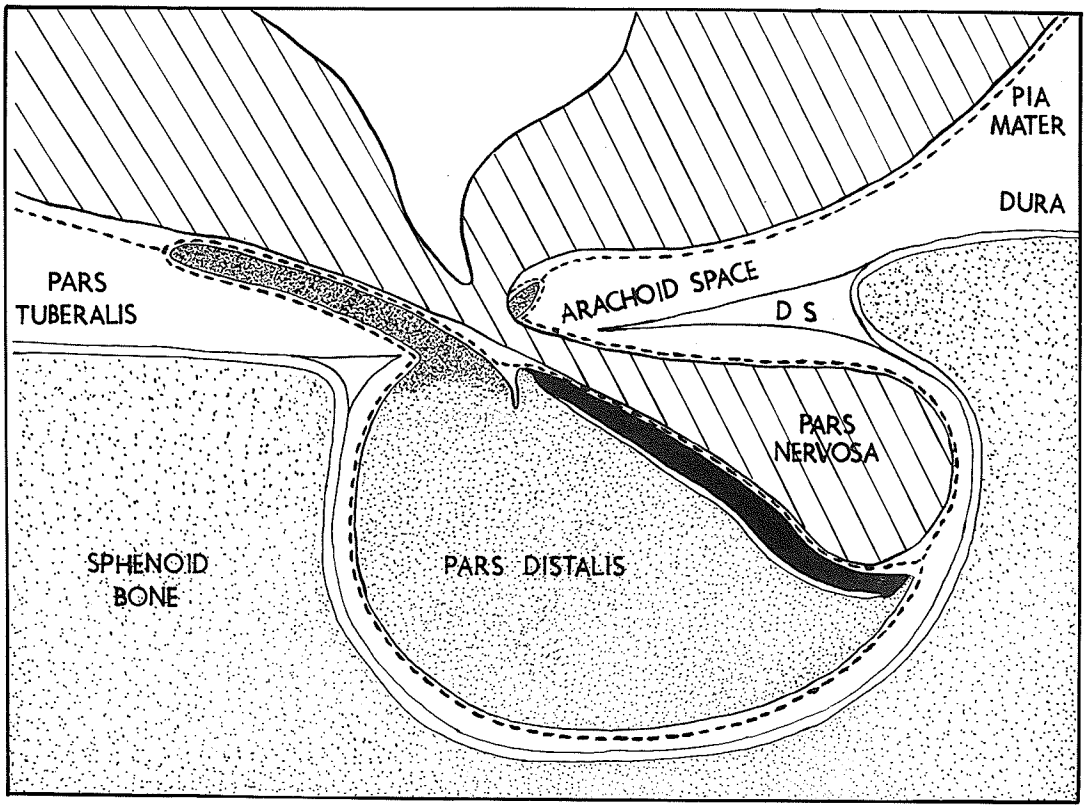

FIGURE 84

Diagrammatic representation of the meningeal relations of the hypophysis according to Bailey[2], slightly modified.

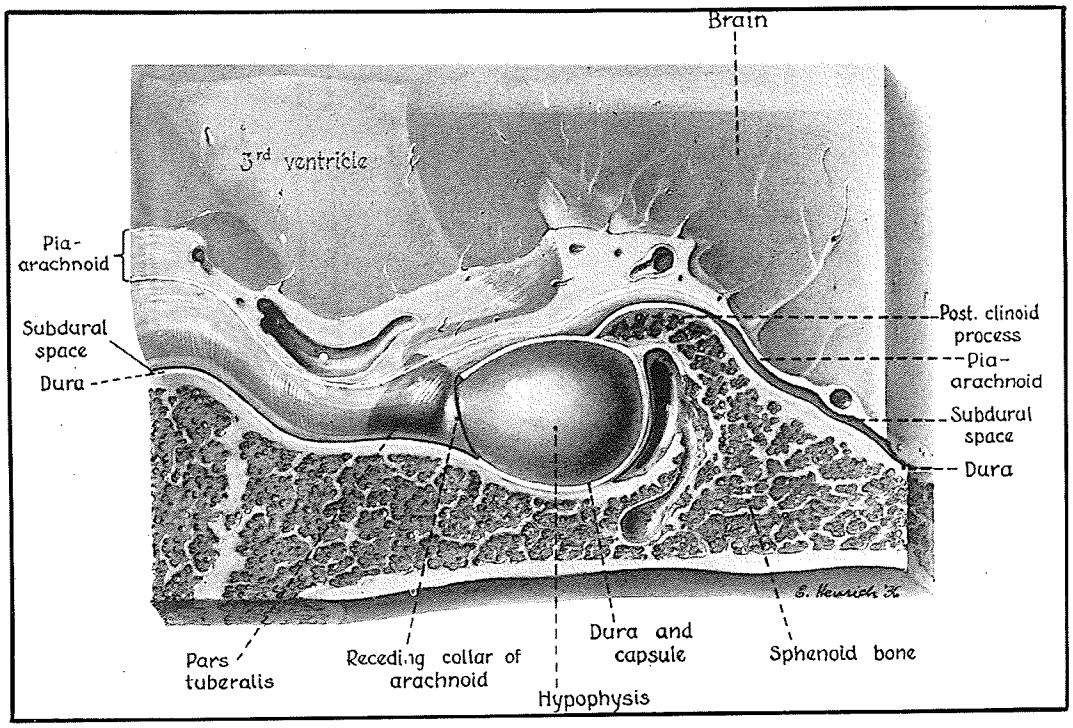

FIGURE 85

The meningeal relations of the hypophysis of the dog. Reproduced from Schwartz, Anat. Rec., 1936, 67:35, with written permission from the publishers, The Wistar Institute of Anatomy and Biology.

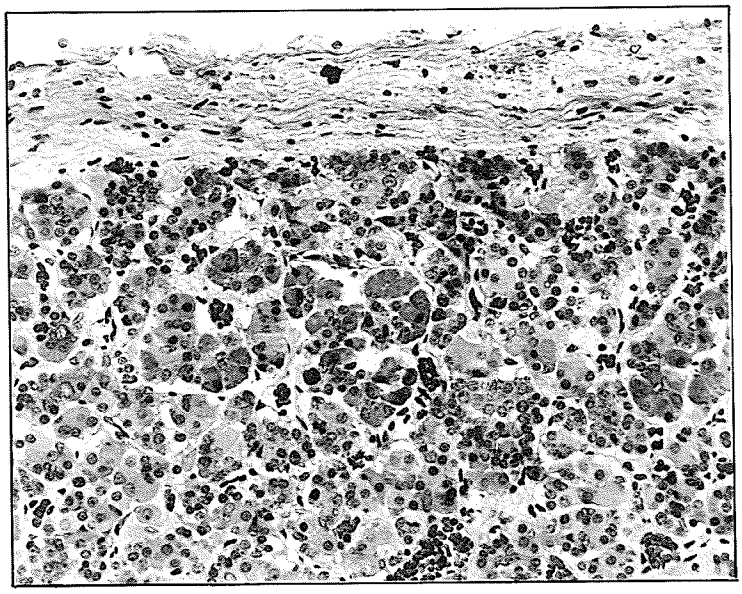

FIGURE 85 a

The capsule of the pars anterior of the hypophysis of a twelve-year-old boy. In this section, only part of the capsule is adherent to the gland, the remainder being left in the sella tursica with the periosteum. Hematoxylin and eosin. WGH A 7911. 200 x.

upon the studies of animal material[10]. Schwartz[12] published an excellent article in 1936 on the meningeal relationships of the dog and the following year Wislocki[11] published his studies of the meningeal relations based on the study of human embryos. In 1945, Sunderland[10] described the meningeal relations in human infants and adults and gives a good review of the problem.

From his studies of human embryos, Wislocki[11] concluded that the epithelial hypophysis loses its connection with the mouth and comes to lie in diffuse mesenchyme at the base of the brain before the meninges in this region are differentiated. He further concluded that the arachnoid space never surrounds the body of the hypophysis within the sella tursica, and the space when completely differentiated forms a cistern which encloses the stalk of the hypophysis in the form of a collar. The dura develops around the body of the hypophysis and forming the sella diaphragm, fuses with the superior surface of the pars anterior and the pars posterior, preventing the development of the pia arachnoid or subdural space around the body. (Cf. Wislocki[11])

Sunderland's description[10] is similar in most respects to that of Wislocki[11] in connection with infants and children, although variations occur in the adult. According to Sunderland[10], the shallow sella of infancy and childhood is lined with dura which also provides the sella diaphragm. The aperture of the diaphragm is usually larger than the infundibulum and the pars tubera is passing through it. If the anterior rim of the aperture is not closely applied to the infundibulum and the pars tuberalis, the superior surface of the pars

anterior is covered by the pia which descends along the stalk onto the surface of the pars anterior and is reflected forwards to blend with the margin of the aperture. The same arrangement holds if the posterior rim of the aperture is not applied to the stalk. Normally no arachnoid is found below the level of the dural aperture in infants and children.

In the adult, the same arrangement may exist, although it is often modified in some measure by disproportionate growth between the sella and the gland. In adult life, especially in advancing years, the gland sinks into the fossa. This has the effect of enabling the pia, arachnoid and the subarachnoid space to descend through the aperture and ultimately extend over the entire superior surface of the gland and even further should the factors responsible for the altered relationships continue to operate. (Cf. Sunderland[10])

Weight of the hypophysis. The average weight of the main body of the hypophysis is approximately 570 milligrams with extreme values from 400 to 855 milligrams in normal adults[14]. The size and weight of the hypophysis is influenced not only by pathological processes but also by such factors as age, pregnancy, castration, body length and the influences of other endocrine organs. Simmonds[13], in a study of 800 glands, concluded that the weight of the hypophysis increased up to the sixtieth year and then gradually decreased. The average weight of the gland of women who have born children is somewhat higher than those of males of a corresponding age group[13]. The size and weight of the organ has a fairly wide range.

Rasmussen[15] found that when age was plotted against weight for 122 specimens obtained from subjects between the ages of birth to nineteen years, the growth curve was approximately a straight line. Growth is faster in the female gland than in the male during the teens, the sex difference being due to the growth of the anterior lobe which represents three fourths of the gland. The individual variations are moderate and the same as for adults[15].

The epithelial portion of the pars intermedia increases relatively less rapidly than the rest of the gland, since at birth the intermedia represents 2 per cent of the gland and at age nineteen years slightly less than 1.5 per cent. The weight of this lobe is less variable than the rest of the gland. The processus infundibuli represents one fifth of the gland and its growth is represented also by a straight line. (Cf. Rasmussen[15])

The arterial supply and venous drainage. The arterial supply of the hypophysis arises from two sources, the internal carotid arteries as they course through the cavernous sinus and from the circle of Willis[1]. The two hypophyseal arteries form the main arterial supply. They are branches of the internal carotid arteries, arising from the left and right sides respectively. Occasionally there are more than two hypophyseal arteries[1]. The arteries turn medially, leave the cavernous sinuses, and reach the stalk. A twig to the pars nervosa is given off as the arteries approach the gland. The arteries enter the gland in a plane between the pars intermedia and the pars nervosa, and

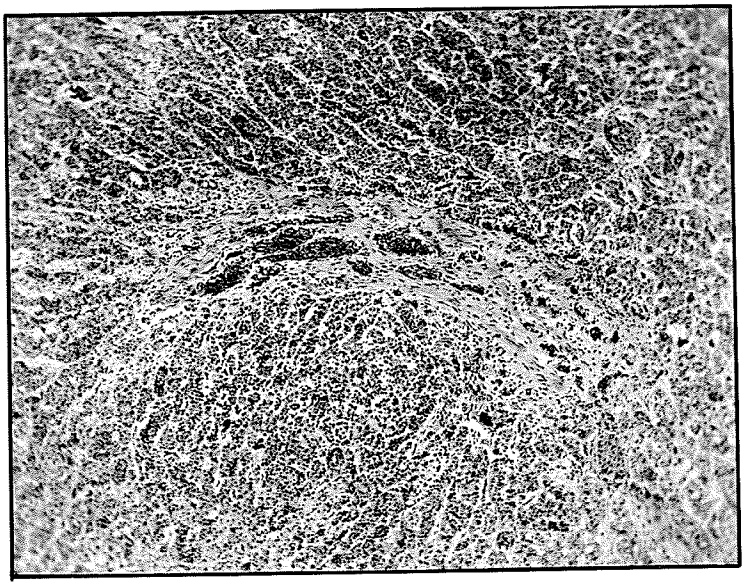

FIGURE 86

The vascular connective tissue trabeculum of the pars anterior of the hypophysis of a twelve-year-old boy, who died of accidental electrocution. Hematoxylin and eosin stain. WGH A 7911. 50 x.

then turn anteriorly to enter the pars anterior following the rather large connective tissue trabeculae which occupy the central portion of each lateral half of the lobe. Numerous branches are given off in every direction to the many sinusoids within the lobe.

The second and lesser arterial supply is formed by the numerous arteries from the posterior and the anterior communicating arteries of the circle of Willis[1]. According to Nickolskaia[8], these vessels number from 20 to 25 in the human and go either to the tuber cinereum or the stalk. The arteries converge on the stalk and pars tuberalis[1].

The vascularity of the pars anterior, pars intermedia and pars nervosa is not the same. Of the three lobes, the pars anterior has the greatest vascularity. The pars intermedia has the least vascularity, receiving its blood supply from the capillary network at the line of separation between the pars intermedia and the pars nervosa[1].

The venous drainage of the hypophysis consists of two systems, the systemic system which follows the arteries that supply the gland, and the hypophyseo-portal system described by Popa and Fielding[7,30]. There is still considerable speculation regarding the direction of blood flow in the hypophysis[9], especially in connection with the hypophyseal-portal system. According to Popa and Fielding[7,30], who first described the portal system, veins which arise in the different lobes follow the course of the arterial twigs and drain into the cavernous sinuses. The hypophyseo-portal veins arise from the sinusoids of the pars anterior, the capillaries of the pars nervosa and a few branches from the pars intermedia. The veins proceed upwards in the stalk

independently of each other, giving off no branches or having no communication with each other. After a short course in the stalk, the veins acquire a thick neuroglial sheath. Beneath the infundibular process, the veins lose their neuroglial sheaths and open into a network of fine channels which forms a secondary distributing net. (Cf. Popa and Fielding[7,30]) Wislocki[36], Green[32] and Rioch[35] are among others who contend that the direction of the blood flow is from the hypothalamic region to the anterior lobe in the portal system, that is, the system is afferent and not efferent. Wislocki[36] gives a good review of the problem.

Innervation. A large tract of non-medullated nerve fibres arising in the supra-optic nucleus in the floor of the third ventricle descends through the tuber cinereum, down the stalk and into the infundibular process. Rasmussen[22] estimates that there are from 54,000 to 70,000 nerve cells in the supra-optic nucleus which is about 5 mm long, and that these cells give rise to at least 50,000 fibres. The innervation appears to be disproportionate to the number of cells in the pars nervosa[22]. Many of the fibres pass through the posterior lobe to terminate on the connective tissue capsule. Some enter the pars intermedia through fenestrations of the anterior wall of the capsule. Still others supply the posterior lobe[1].

The fibres are grouped together in dense bundles in the infundibulum and are well demonstrated by Cajal's silver-pyridine method of impregnation. As the fibres reach the infundibular process,

they spread out in a diverging manner. According to Bucy[1], individual fibres separate from the others and form a network about the cells of the pars nervosa. Frequently the fibres terminate in what have been called "end-bulbs." Some authors, including Bucy[1], feel that the end-bulbs are degenerated hyaline bodies of Herring. Rasmussen[22] states that they are nothing more than the close association of the nerve fibres to nuclei and the appearance is due to heavy deposition of silver.

Lymphatics. As may be expected, no lymphatics have been demonstrated in the hypophysis cerebri.

II. PARS NERVOSA (THE POSTERIOR LOBE, NEUROHYPOPHYSIS)

The pars nervosa represents approximately 25 per cent of the bulk of the hypophysis cerebri. It is ectodermal in origin and arises from the evagination of the neural tube. The cells making up the pars nervosa then have morphology which in many respects resemble those of other cells of the central nervous system. The blood supply has been discussed on page 311, where it was noted that the vascularity was considerably less than that of the pars anterior.

Pituicytes. The tissue of the pars nervosa consists of a network of special cells and their processes. Hematoxylin and eosin stains give little indication of the true histology of these cells which are well demonstrated by Penfield's modification of Hortega's silver-carbonate method of impregnation[1]. Their morphology differs from other

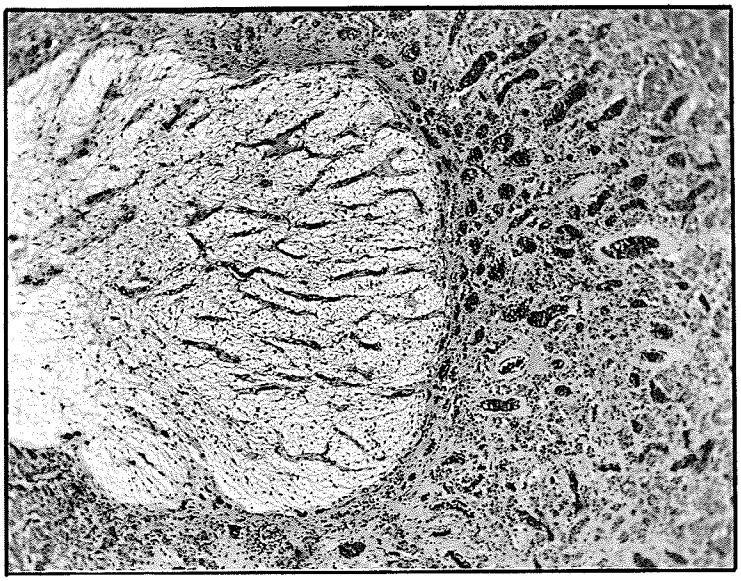

FIGURE 87

Horizontal section of the hypophysis of a twelve-year-old boy killed accidentally by electrocution. The lighter staining pars nervosa is almost completely surrounded by pars anterior in this field. The vascularity of both lobes is well shown. H & E stain. 50 x. A 7911.

cells of the central nervous system in many respects and Bucy[1] has named them pituicytes.

Each pituicyte has one or more processes which arise from the cell body and extend for long distances into the tissue of the pars nervosa. The processes are longer than those of the glial cells seen in the central nervous system. The cells are irregular in shape, almost no two are alike. The processes vary in length and number. The cell may have one or two long processes with several short processes or only numerous short ones. Numerous processes divide dichotomously, but more often short branches are given off. The processes usually terminate on the connective tissue of blood vessels, the septa or the capsule of the pars nervosa. (Cf. Bucy[1])

Pigment. Yellow to yellowish-brown pigment is usually found in the pars nervosa. Characteristically, this pigment is not abundant but it may be found in sufficient quantity to give the lobe a brownish colour[13]. In ordinary preparations, the pigment appears in the form of coarse granules found in the cytoplasm of both pituicytes and elongated ovoid or polygonal cells along the connective tissue septa. The nature of the pigment is not known[1].

The connective tissue of the pars nervosa. The pars nervosa is surrounded by a dense connective tissue layer which is intact at the junction between the pars nervosa and the pars intermedia. Nerve fibres pass from the pars nervosa to epithelial tissue and the epithelial tissues invade the pars nervosa through small fenestrations in the

capsule[1]. Posteriorly the capsule is intact and is continuous with the dura[11,12,10]. A dense network of connective tissue fibrils is scattered throughout the lobe. The fibrils are mostly reticular with some collagen. The network is densest along the posterior wall of the lobe, while anteriorly in the lobe less connective tissue is present. (Cf. Bucy[1])

Epithelial cells. Simple or branched tubular glands continuous with the pars intermedia extend posteriorly into the pars nervosa[17] through fenestrations of the connective tissue capsule separating the two lobes[1]. The glands are usually found in subjects less than four years of age. They vary from compound glands to relatively short tubules[17]. The compound glands have numerous alveolar pockets communicating by means of a narrow duct with the cleft of the pars intermedia[16]. In general, the glands are situated in the anterior or lateral portions of the posterior lobe[16] and are relatively short. No tubular glands extend entirely across the posterior lobe and none penetrate the anterior lobe. After the fourth year the typical glands were not found in the series studied by Lewis and Lee[16]. Rasmussen[17] found the glands more frequent in females (approximately 78 per cent) than males (approximately 49 per cent). They are lined with cylindrical epithelial cells, the nuclei of which are usually round, basal and lightly staining since only a moderate amount of chromatin is present. Fine granules are scattered throughout the cytoplasm which is stained light blue in hematoxylin and eosin preparations. The terminal portion

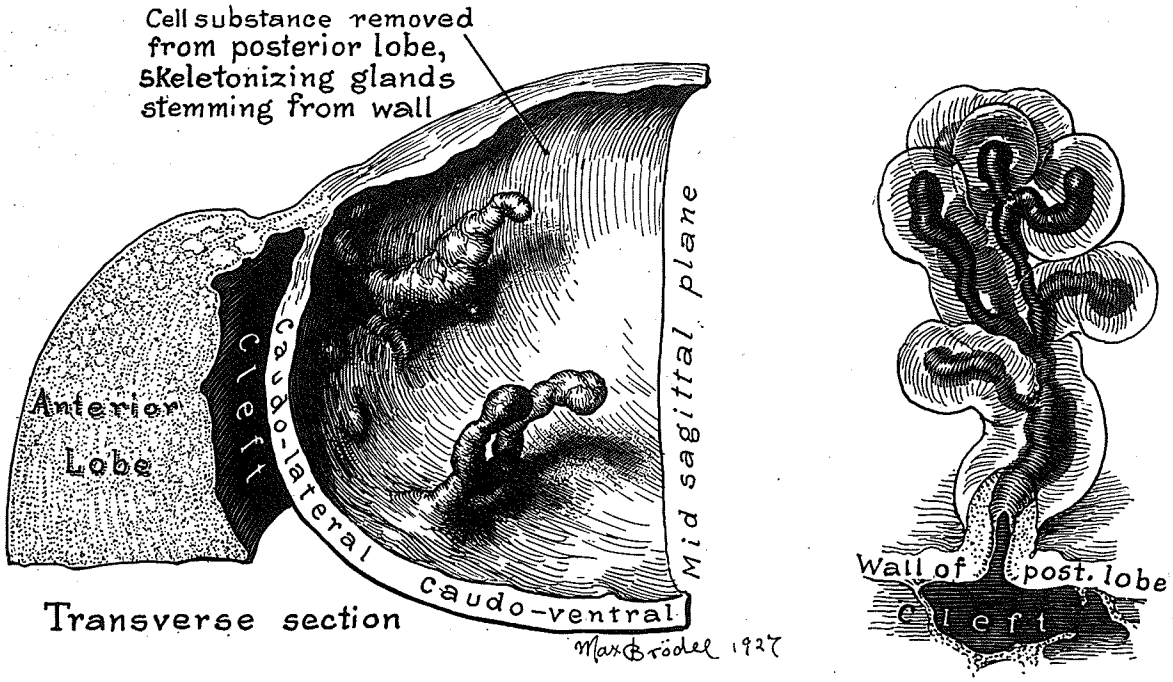

FIGURE 88

Drawings from the hypophysis of a three-and-a-half-year-old girl. Left hand figure, magnified 29 times, was made partly from a wax model reconstruction to show the extent of the projection into one posterior lobe of the tubulo-racemose glands. The neurogliar tissue was removed from the posterior lobe, leaving the glandular tissue projecting as two large glands and several smaller elevations. The position of these glands is quite typical. The cleft is practically intact.

Figure on the right, magnified 185 times, represents an individual gland from the same specimen. The duct empties into the hypophyseal cleft.

Reproduced from Lewis, D., and Lee, F. C., "on the glandular elements in the posterior lobe of the human hypophysis," Bull. of the Johns Hopkins Hosp., 1927, 41:241, with the written permission of the publishers.

of the duct is small and lined with small cuboidal cells. (Cf. Lewis and Lee[16])

III. PARS INTERMEDIA

Although the pars intermedia develops from Rathke's pouch, it is usually described in connection with the pars nervosa, since most of the epithelial elements of the pars nervosa are derived from evaginations of the pars intermedia. The pars intermedia represents such a small proportion of the adult human hypophysis that some authors deny its existence in the human. Rasmussen[25] has estimated the size and weight of the pars intermedia in man. In the human newborn, this part represents approximately 2.5% of the epithelial portion of the hypophysis. There is considerable variation. The parenchyma of the part averages 0.9 per cent of the hypophysis (range from 0.13 to 3.6 per cent), in the adult the ratio being about 2/5 of that found in the newborn. In the adult, the weight is about twice that found in the newborn, the average being 4.6 milligrams (range from 0.5 to 20.1 milligrams). The minimal weight of 0.5 milligrams is the equivalent of a mass not more than 0.8 cubic millimetres, while the average weight of 4.5 milligrams is equivalent to a mass slightly less than 1.7 cubic millimetres. (Cf. Rasmussen[25])

The pars intermedia of the fetus, after the hypophysis has assumed its general form, consists of a fairly uniform epithelial layer several cells in thickness. The layer is situated between the cleft and the pars nervosa. This arrangement which usually persists until the time of birth is similar to the arrangement found in the usual laboratory

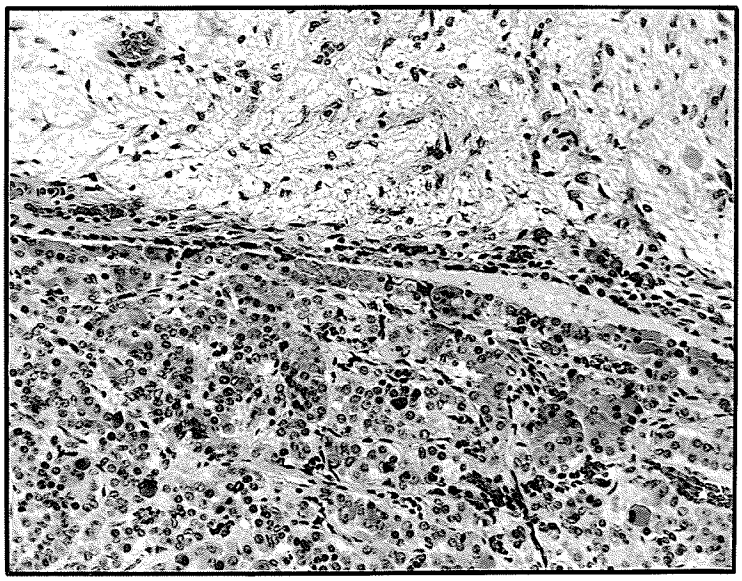

FIGURE 89

Remnant of the hypophyseal cleft and pars intermedia of the hypophysis of a twelve-year-old boy. H & E. 175 x.

animals[25]. During childhood, considerable irregularity occurs due to the formation of diverticulae and gland-like outgrowths from the pars intermedia into the pars nervosa. The cleft may persist or be finally obliterated. A variable amount of colloid accumulates in the cleft and the diverticulae. Because of its smallness and the great variability many authors do not regard the pars intermedia as an important functioning lobe, at least in the human. The formation of the cysts, trabeculae, racemose glands and colloid masses from the epithelial elements of the pars intermedia, make knowledge of the lobe important to the student of histology and pathology. The pars intermedia may be represented by only a single layer of cells. The lumen of the cleft may be obliterated and represented by only a few small cystic spaces. At the other extreme, the abundant epithelial cells may form a prominent layer, usually very irregular in thickness. The colloid is also very variable in amount and is independent of the quantity of the epithelial elements present. (Cf. Rasmussen[25])

Many types of cells lining the remnants of the cleft have been described. The tubulo-racemose glands and the basophilic cells masses have been described in connection with the pars nervosa on page 318. The basophils which have invaded the pars nervosa and those present in the pars anterior are essentially alike. Those in the pars nervosa do not as a rule attain the size or degree of vaculation as seen in those of the anterior lobe. With some techniques, a slight difference in the tone of the colour reaction is noted. (Cf. Rasmussen[26])

Gillman[29], Rasmussen[27] and Shanklin[28] report the presence of

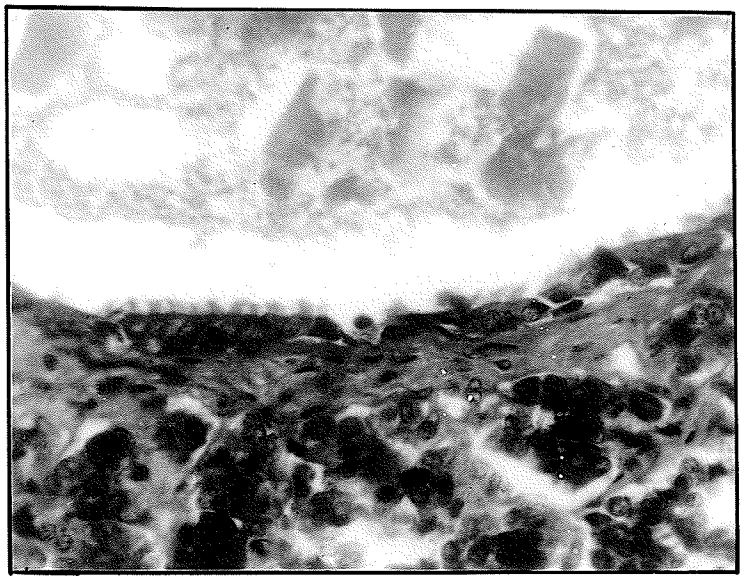

FIGURE 90

The cilia found in the pars intermedia of an adult hypophysis, as seen with high power lens. Hematoxylin and eosin stain. 600 x.

ciliated cells in the pars intermedia of some glands. Gillman gives a detailed account of the ciliated cells and mucous cells which he found in 20 per cent of 80 Bantu hypophyses. Ciliated cells are usually found associated with mucous cells although the latter may be present independently. The ciliated cells occur in groups of 5 to 80 cells. They vary from cuboidal or low columnar to a typical pseudo-stratified form of ciliated epithelium. The cilia measure from 7 to 10 micra in length and terminate intracellularly in a distinct acidophilic body. The cytoplasm is faintly acidophilic. The mucous cells when filled with mucous measure from 25 to 35 micra in height. The cytoplasm is compressed against the base of the cell. The nucleus is homogenous and irregular in shape. Other mucous cells are low columnar cells. (Cf. Gillman[29])

Summary of the epithelial cells of the pars intermedia. The epithelial tissue of the pars intermedia may be sub-divided into several groups, which are summarized below:

(a) The tubulo-racemose glands which are present for the most part during the first four years of life. These glands have been described in connection with the pars nervosa.

(b) The evaginations of the hypophyseal cleft into the pars nervosa.

(c) The basophilic cells of the pars nervosa, also described in connection with that lobe.

(d) The basophilic cells and nongranular cells which form the posterior wall of the hypophyseal cleft.

(e) The ependyma-like cells which are best demonstrated by the method of Cajal, according to Bucy[1]. Two processes extend from the cell body. One process reaches the anterior border of the pars intermedia, and the other process reaches the posterior border. The nuclei of these cells are small and ovoid. (Cf. Bucy[1])

(f) Ciliated cells which have been described in connection with the pars intermedia.

(g) Goblet and mucinous cells (according to Gillman[29]).

(h) Eosinophilic cells which are similar to those of the anterior lobe.

The "colloid" bodies of the hypophysis. Many refractile bodies and deeply staining masses have been grouped under the term "colloid" bodies. Whether or not the masses are true colloid cannot be determined with present methods. Not all these bodies are related to the pars intermedia, but since many are associated in some way with the epithelial elements of the pars intermedia, this is a convenient place to list them. Bucy[1] gives an excellent account, which forms the basis of the following discussion.

The small hyaline or eosin bodies of Herring of the pars nervosa and less commonly in the pars intermedia are considered by Bucy[1] to be degenerated end bulbs. Not all authors agree with this theory, and recently Rasmussen[22] has shown that the nerve fibres pass right through the hyaline bodies or beside them. The appearance that the nerve fibres end in the hyaline bodies is an artifact, produced in silver

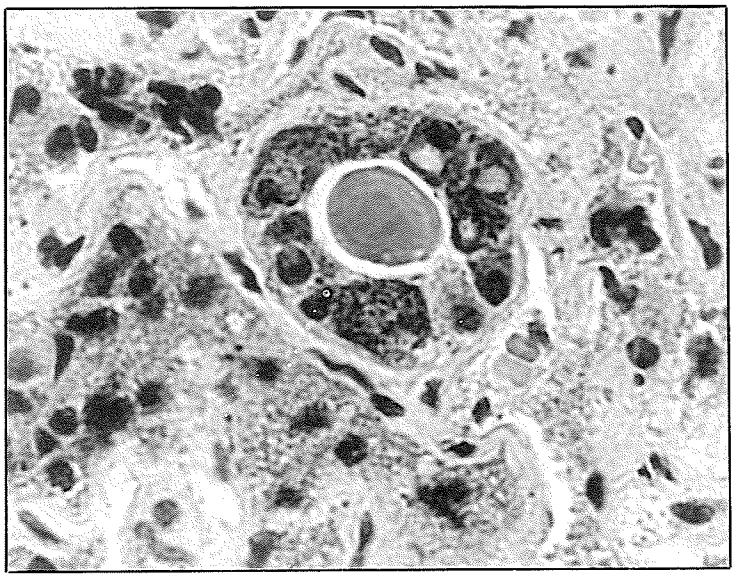

FIGURE 91

A colloid body surrounded by chromophils in the pars anterior of an adult female. Hematoxylin and eosin. 800 x.

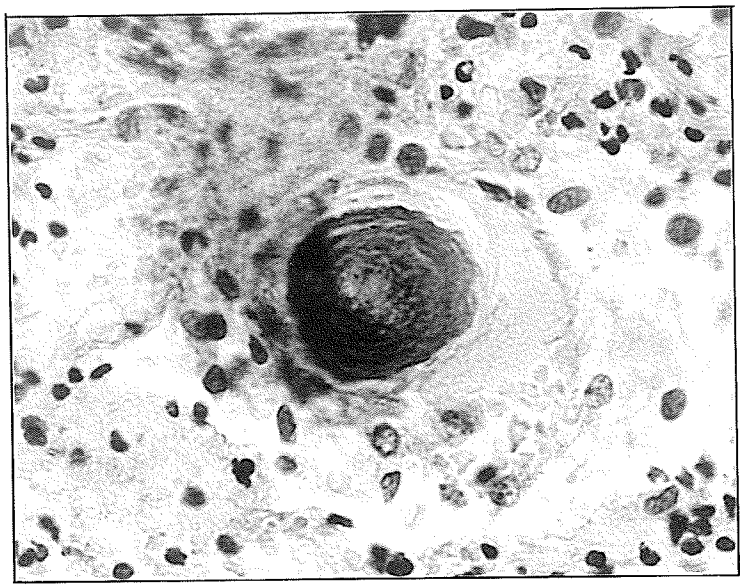

FIGURE 92

A lamellated calcified body in the hypophysis of an adult female. Hematoxylin and eosin stain. 600 x. WGH A 7285.

impregnation methods[22].

Other masses of colloid-like material are seen in the pars intermedia and the pars nervosa near the epithelial-neural junction. These masses are present in cysts which are lined by low cuboidal epithelium, and are formed by distension of the tubulo-racemose glands.

In the remnants of the cleft, homogenous masses which usually are the largest seen in the hypophysis, are observed. These masses represent the secretion of the glands into the region.

Homogenous masses of material are found in the centre of the cell masses in the pars anterior and the pars tuberalis. According to Bucy[1], these are due to degenerative changes and are more numerous in the posterior part of the pars anterior. The material is very similar to that seen in the cleft.

About the cephalic end of the cleft in both the anterior and posterior lobes are small cysts and gland-like structures which are lined with ciliated epithelium. These structures contain mucinous material entirely unlike the colloid seen in the cleft and the tubulo-racemose glands.

Shanklin[37] has described the formation of certain concretions which are found for the most part in the capsule. At certain stages, according to this author, the developing concretions take an eosinophilic stain.

Plaut and Galenson[39] found colloid concretions or masses in 100 per cent of fetuses and newborn infants examined by them. Most of these concretions disappear in the first few postnatal months. The concretions

vary in size and shape. Practically all were found in the pars anterior and only a few in the pars intermedia or pars nervosa. The staining of the concretions varies from blue to pinkish-blue in hematoxylin and eosin preparations. About one half of the bodies are lamellated and the majority of them are surrounded by epithelial cells in an irregular manner. (Cf. Plaut and Galenson[39])

IV. THE PARS ANTERIOR

The pars anterior is the largest lobe of the hypophysis, accounting for approximately seventy-five per cent of the weight[14]. The lobe is the best known and is probably the most important[2].

Cell types. The polygonal cells of the pars anterior are arranged in columns[2] or alveoli[1] which are separated from one another by numerous vascular sinuses and a small amount of connective tissue. The cells have been classified into two groups, the chromophils and chromophobes, each group having two subdivisions. The chromophils may be either basophilic or eosinophilic, and the chromophobes either chief cells or the large variety of chromophobes, viz.,

(a) Chromophils - i. basophils (beta, cyanophils)
ii. eosinophils (alpha, acidophil, oxyphyl)

(b) Chromophobes - i. chief cells (reserve, principal)
ii. large chromophobes.

Bailey[2] and Bucy[1] suggest the terms alpha and beta cells for the eosinophils and basophils respectively, since the granules of the cells are not consistent in staining reactions. However, Cowdry[18] points out

any eosinophilic granule may be induced to take up basophilic dye and prefers to retain the original terms.

Distribution. In the human, the basophils and eosinophils show no characteristic distribution which appears to be haphazard throughout the whole of the pars anterior[2]. A single section through the midsagittal plane does not give a true picture of the distribution of the cells. Several sections should be studied and Rasmussen[33] suggests that the horizontal plane is more representative than the sagittal plane in which sections are usually taken. However, there is a tendency for the acidophils to be concentrated in a large area located somewhat posterior in each lateral half of the pars anterior. This arrangement leaves a large area near the mid-sagittal plane and an anterior and marginal zone where the acidophils are less numerous and where there are more basophils and chromophobes. The basophils tend to collect about the two vascular connective tissue trabeculae that extend into the pars anterior. A distinct eosinophilic area usually surrounds these basophilic accumulations. (Cf. Rasmussen[33])

Percentage of cell types. The relative number of the three cell types varies according to age and sex. These figures would have little more than academic interest if it were not for the fact that the percentages change in pathological states[34]. The literature is contradictory but the important thing is that the changes do occur. There are many reports in the literature which have no statistical value. The careful studies of Rasmussen[19,20,21] who has given the percentages

of the different cells in both males and females at various ages, should be taken as a standard[34]. These figures are summarized in the following table:

The relative number (per cent) of the different types of cells in the adult human hypophysis, according to Rasmussen (19,20,21,33)

	MALE		FEMALE	
	Range (%)	Mean	Range (%)	Mean
Chromophobes	34-36	52	33-74	49-50
Acidophils	23-59	37	17-59	44
Basophils	5-27	11	3-16	7

Rasmussen[20] found essentially no difference in the averages of the cells in the glands of pregnant and non-pregnant women. The glands of females show a higher percentage of eosinophils than the glands of males.

In a recent paper, Rasmussen[21] has given the variations in the proportions of the cell types in the pars anterior during the first 19 years of life. These figures are given below.

Table to show the variations in the different cell types in the pars anterior of the human male and female, as demonstrated during the first nineteen years of life, according to Rasmussen[21]. Figures are approximate averages and are given in percentage.

	Birth		Late Teens	
	male	female	male	female
Chromophobes	65%	61%	48%	43%
Acidophils	25%	29%	40%	49%
Basophils	9%	9%	(11%)	(7%)

At birth the number of basophils is about 9 per cent. As age increases, a slight increase in the relative number of basophils occurs in males while a slight decrease occurs in females. There is a wide normal variation in the percentage of basophils.

In the newborn, approximately 29 per cent of the cells in the female anterior lobe are eosinophils while in the male there are approximately 25 per cent. A noticeable increase occurs in both sexes with age, the increase being more noticeable in females who have approximately 9 per cent more eosinophils than males in the late teens.

Chromophobes represent approximately 61 per cent in the female and 65 per cent in the male at birth. There is a decline in their numbers to approximately 43% in the female and 48 per cent in the male at 19 years of age (Cf. Rasmussen[21]).

In both sexes, over the age of 50 years, there is a relative decrease in the number of eosinophils and an increase of chromophobes. The basophils show a relatively higher percentage in the older age groups of females[33].

A wide range in the normal variations is present in the relative number of cell types of the pars anterior. The usual and approximate mean values of chromophobes (50 per cent), eosinophils (40 per cent) and basophils (10 per cent) are not as useful as the normal variations when one is attempting to assess whether or not a given gland is normal.

Histology of the cells. The nuclei of the chromophils and chromophobes are of two types. Some are vesicular with scattered granules of chromatin, while others have a heavy network of chromatin[2]. Mitotic figures are rare. A few fat globules can usually be demonstrated with the common techniques[1]. A clear area, the macula, is seen near the nucleus of the chromophil cells. This area is the negative image of the golgi apparatus and is most easily seen in the basophils[2].

The eosinophils. The granules of the eosinophils are large, spherical and very distinct. Usually the cell is closely packed with granules and the other details are obscured[2]. The granules appear in the first part of the third fetal month when a few eosinophils are present[23]. As fetal life advances, the cells become more numerous and form groups[23].

The basophils. The basophils are larger than the eosinophil cells[2]. The granules are finer, more numerous and much less distinct than those of the eosinophils, although with proper light they may be distinguished. The basophils appear in the pars anterior slightly later than the eosinophils between the three and one quarter to fourth month of fetal life[23].

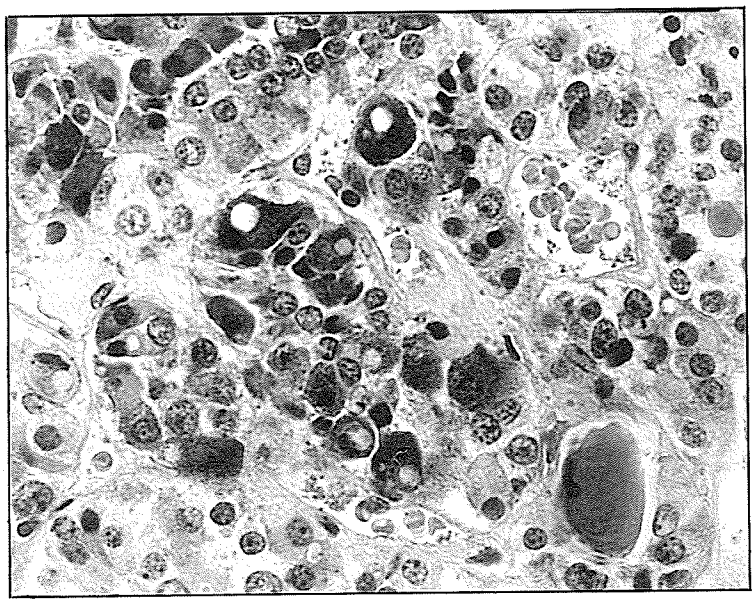

FIGURE 93

The pars anterior of an adult woman. There is marked vacuolization of the basophils. The gland is hyperemic. A colloid body surrounded by chromophobes is seen in the right-hand corner of the photomicrograph. Hematoxylin and eosin stain. WGH A 7283. 500 x.

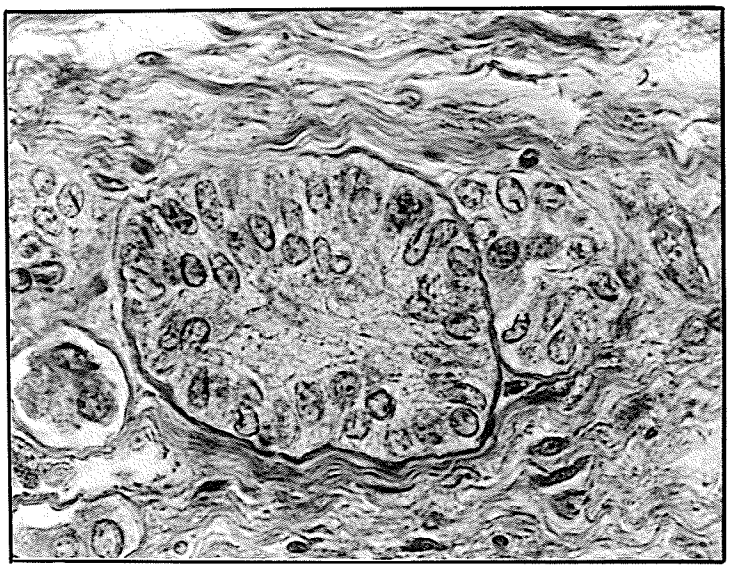

FIGURE 94

Groups of squamous cells in the stalk of a hypophysis. Tissue section from the collection of Dr. D. J. Bowie. H & E. 600 x.

The chromophobes. The chromophobes have relatively small amounts of agranular cytoplasm and indistinct cellular boundaries. Often the chromophobes are found in the centre of cell columns, although they may constitute entire columns, especially near the stalk. There are two cell types described, i.e., the larger chromophobe which is said by some to be a chromophil which has lost its granules and the small chromophobe or chief cell. According to Severinghaus[34], the chromophobes in the embryonic gland develop into the small chromophobes which will later develop into acidophils (acidophilic chromophobes) and those which will develop into basophils (basophilic chromophobes). Severinghaus[34] bases his argument upon his discovery of two distinct types of Golgi nets in chromophobes. These correspond to the Golgi networks of the acidophils and basophils respectively. The same author reviews the other theories of chromophobe-chromophil relationships (Cf. Severinghaus[34]).

V. PARS TUBERALIS

The pars tuberalis is so called because of its association with the tuber cinereum. When the infundibulum is cut across in removing the hypophysis, most of the lobe remains adherent to the base of the brain. The small lobe has received little attention and is a very insignificant part of the human hypophysis, although it is well developed in some animals such as the cat and dog[2]. The location and extent of the lobe is indicated in Figure 83.

A collar of pars tuberalis has been frequently described in man and is said to surround the lower part of the tuber cinereum[32]. As

mentioned earlier, Green[32] found that the tuberalis was constant in its distribution over the anterior aspect of the hypophyseal stalk, extending upwards as far as the optic chiasma. Posteriorly the pars tuberalis was less constant and found only constantly in the angle between the neural stalk and the neural lobe. The lobe may be found as a thin layer over the upper part of the pars distalis, pars intermedia and even the pars nervosa[2].

The cells of the pars tuberalis are similar to some of the cells of other parts of the pars buccalis with the exception that they are somewhat smaller and have no stainable granules. Colloid degeneration may be found within the cell masses[1]. Simmonds[13] found squamous cells in about 80 per cent of adults. These cells are supposedly the remnants of epithelium from Rathke's pouch[1]. Numerous blood vessels and vascular sinuses are present in the pars tuberalis. The vessels pass through the pars tuberalis en route to the pars anterior and from the pars anterior and pars nervosa upward to the stalk and the hypothalamus.

VI. THE HYPOPHYSEAL STALK AND MEDIAN EMINENCE

Green[32] has recently published a detailed description of these structures in man. Since the structures are seldom included in sections of routine material a detailed discussion of them is not included here.

The relationship of the pars tuberalis and the size of that lobe has been described on page 336. A transverse section of the hypophyseal stalk is illustrated in Figure 95.

Green[32] describes what he terms a neurovascular zone which measures

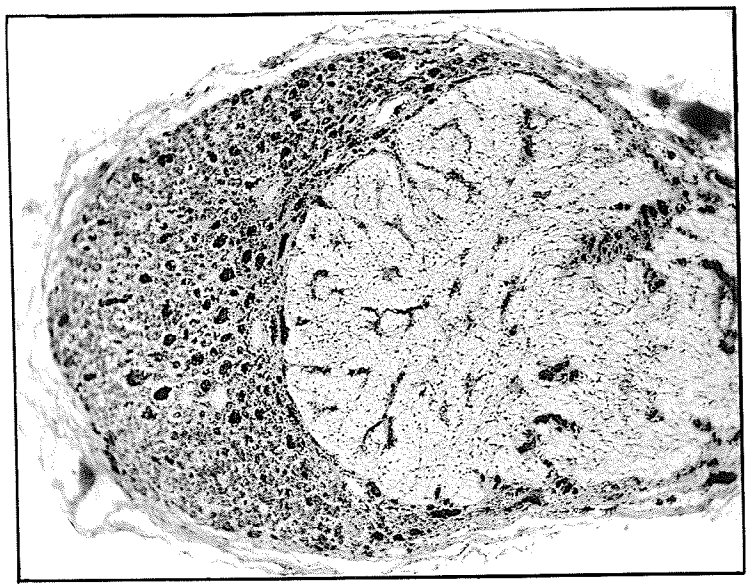

FIGURE 95

Transverse section of the stalk of the hypophysis. The pars tuberalis almost completely surrounds the nerve tissue.

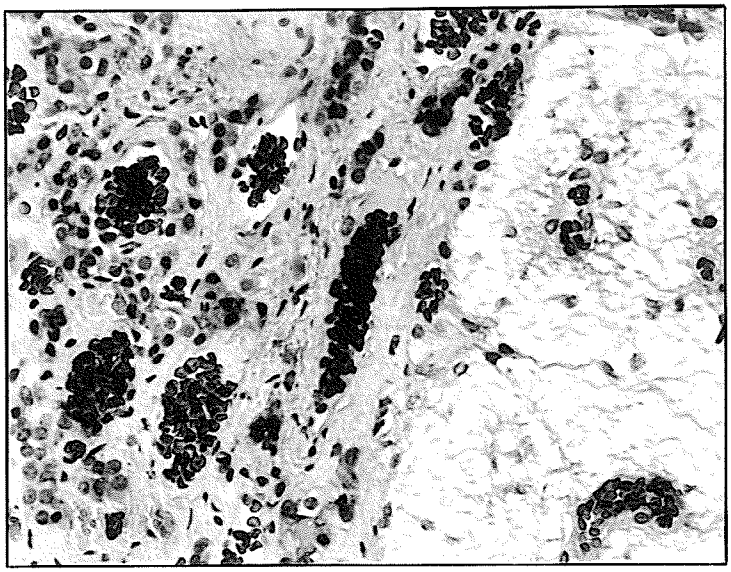

FIGURE 96

The neuro-vascular zone of the stalk of the hypophysis. Masson's trichrome stain. 300 x.

up to 1 millimetre in thickness and which is continuous with the pars tuberalis on the posterior aspect of the hypophyseal stalk. The zone extends upwards almost to the mamillary bodies. Inferiorly it is continuous with the pars tuberalis (Cf. Fig. 96). In the zone are blood vessels, nerve fibres, collagen fibres and islands of glandular cells similar to those seen elsewhere in the pars tuberalis. The nerve fibres of the zone are derived chiefly from the perivascular plexuses and are extremely complex in the pattern formed (Cf. Green[32]).

The bulk of the nerve fibres within the neural stem pass without interruption from the tractus hypophyseus to the neural lobe of the hypophysis[32]. The vessels of the stalk have perivascular sheathes[7] which occupy 30-40 per cent of the volume of the median eminence and neural stalk[32]. The sheathes of these hypophysio-portal vessels consist of ordinary connective tissue in which smooth muscle fibres, collagen and reticular fibres may be demonstrated (Cf. Green[32]).

Concretions and extraneous material. Many of the extraneous elements present in the hypophysis are mesothelial in origin. Shanklin[38] reports cartilage and bone. Calcified bodies with lamellated structure are usually observed in older subjects. They are found in the capsule and neurohypophysis[38] and are derived from mesothelium[37] (Cf. Shanklin[38]).

Reference to the colloid bodies of the hypophysis has been made on page 325.

VII. THE HORMONES OF THE HYPOPHYSIS

The following discussion is a brief summary of the material found in the monographs by Cameron and Selye. No attempt is made to discuss the hormones and their properties, since such is beyond the scope of the present work and the subjects are well treated in the considerably more authoratative works mentioned.

The hormones of the pars anterior. Although the anterior lobe of the hypophysis has only two types of cells, as many as fifteen different hormones have been postulated to be produced by the lobe. The existence of these hormones has not been substantiated and within the last ten years a more rational view has been held[41]. Only four hormones of the anterior lobe have been isolated in pure form--the luteotrophic, somatotrophic, adrenocorticotrophic and luteinizing hormones. The follicular-stimulating and thyrotrophic activities are probably due to separate hormones[9]. They are all proteins[41]. Possibly the target glands have secondary actions not understood or appreciated. These actions may be responsible for some of the results obtained when crude extracts of the pars anterior are injected into experimental animals. Certain of the actions appear to be produced in part by combinations of known hormones.

The following list of the accepted hormones of the pars anterior have been isolated in pure or almost pure form. The synonyms are given together with a few of the properties and actions of each hormone. The

experimental basis and clinical observations relating to their use are not included, but are found in the works of Selye and Cameron.

Follicle-stimulating hormone. (Syn.--FSH, follicle-stimulator, thylakentrin). Although this substance has not been isolated, the highly purified preparations stimulate growth of the granulosa cells in the ovaries of hypophysecomized animals. The hormone also stimulates the seminiferous tubules of the testes of intact or hypophysectomized animals. The role of FSH has been discussed on pages 171, 172, 179 and 180.

Luteinizing hormone. (Syn.--LH, Interstitial-cell-stimulating hormone, ICSH, chorionic gonadotrophin, metakentrin). This hormone has been isolated in pure form[9] and is a glycoprotein[41]. When injected, the hormone stimulates the transformation of mature ovarian follicles into corpora lutea and stimulates the growth of the theca cells and their production of the folliculoid hormone[9]. In the male, the hormone stimulates the development and the production of hormones by the Leydig cells of the testes. Further discussion is found on pages 173, 179, 180 and 188.

Luteotrophic hormone. (Syn.--LTH, luteotrophin, mammotrophin, prolactin, galactin, lactogenic hormone). This hormone helps to maintain fully developed corpora lutea and stimulates them to produce the luteoid hormones. It stimulates the mammary glands to secrete milk after the mammary glands have been brought to full development during pregnancy.

A further discussion is found on page 174.

Corticotrophic hormone. (Syn.--ACTH, adreno-corticotrophic hormone, adrenotrophin, corticotrophin, adrenotrophic hormone, cortico-trophic hormone). This hormone has been prepared in pure form and is a protein-like substance. The hormone stimulates the growth and hormone production of the adrenal cortex. It tends to deplete the adrenal cortex of its lipid and ascorbic acid content.

Thyrotrophic hormone. (Syn.--thyrotrophin, thyreotrophic hormone). This hormone has not been isolated in pure form. It stimulates the growth and the hormone production of the thyroid gland.

Somatotrophic hormone. (somatotrophin, growth hormone) This principle has been isolated. As long as the epiphyseal junction cartilages are still not fused, injections of the hormone cause skeletal growth, both in length and thickness[9]. Injections will produce gigantism, if given long enough, and if prolonged will lead to enfeebled gigantism[41].

Other "actions" of anterior lobe extracts. Several other "actions" are noted with injections of crude extracts of the anterior lobe of the hypophysis. As Selye points out in his monograph, there has not been a claim put forth for a separate hormone to account for each action. Simply these are the effects of the injection. Some of the actions have reasonable explanations, others have to wait further investigation and data. The following is only a list of these actions, based on Selye's monograph.

1. The ovulation-inducing action
2. The antagonistic action--antagonistic to certain of the gonadotrophins
3. The synergistic action--alleged to augment the action of LH preparations
4. The antiluteogenic action
5. The thymotrophic action
6. The renotrophic action
7. The nephrosclerotic action
8. The gluco-corticotrophic, mineralo-corticotrophic action, lipocorticotrophic action, and testo-corticotrophic actions
9. The adrenomedullotrophic action
10. The parathyrotrophic action
11. The mammogenic action
12. The glycotropic and anti-insulin action
13. The glycostatic action
14. The pancreatotrophic action
15. The diabetogenic action
16. The anti-diabetic effect
17. The contra-insular action
18. The ketogenic action
19. The fatty-liver producing action
20. The preputial gland-stimulating action

The hormones of the pars nervosa. Crude extracts of the pars nervosa exhibit three chief activities: (a) they produce a rise in blood pressure (vasoconstrictor), (b) they cause uterine contractions (oxytocic), and (c) they diminish diuresis[9]. By differential fractionation, two non-crystalline fractions have been obtained. One fraction has pressor potency and very low oxytocic potency, while the other has very high oxytocic potency and only negligible pressor activity. No crystalline compounds have yet been obtained but these amorphous fractions are usually considered as almost pure hormones[41]. Thus it is generally agreed that there are at least two distinct principles of the pars nervosa, the oxytocic and vasopressor hormones[9]. The anti-diuretic action is probably due to the vasopressor principle[9].

Vasopressin. (Syn.--vasopressor principle, postlobin-V, pitressin, vasopressor-anti-diuretic principle). The hormone has not been isolated. Some preparations, however, are very potent. The anti-diuretic hormone of the pars nervosa is probably identical with vasopressin[9].

Oxytocin. (Syn.--Oxytocic hormone, oxytocic posterior-lobe principle, postlobin-O, pitocin). The chemical and physical properties of oxytocin and vasopressin are similar and make separation difficult. The hormone has not been isolated.

The pars intermedia. Aqueous extracts of the pars intermedia produce dispersion of black pigment granules of amphibia. It has been suggested that this is due to a hormone of the pars intermedia, to which the name intermedin has been assigned. Some of the synonyms are--middle-lobe hormone, meanophore-expanding principle, B-hormone. The function of intermedin, should it exist, is not known in the case of birds and mammals. (Cf. Cameron and Selye[1])

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