THE RELATIONS OF THE INTERSTITIAL CELLS OF LEYDIG TO THE PRODUCTION OF AN INTERNAL SECRETION BY THE MAMMALIAN TESTIS.*

By FREDERIC M. HANES, M.D.

Associate in Pathology, Columbia University.

(From the Department of Pathology, Columbia University, New York.)

PLATES XXX–XXXVI.

The questions considered in this paper are, "Does the mammalian testicle, in addition to the formation of sperm, furnish an internal secretion essential to the normal development of the organism?" and, "If so, which cells of the testicle have this function?"

The testicle is well suited for such a study, both from its anatomic relations and from its histologic structure. It is easily accessible to experimental manipulations, and natural abnormal placements of the organ are not uncommon, entailing characteristic changes of structure which greatly aid in analyzing the possible functions of its several component elements.

Historical.—Despite the statements to the contrary by several writers (von Lenhossèk, 1897, Plato, 1896, Bouin et Ancel, 1903), Leydig (1850), and not Kölliker (1854), first described the presence of characteristic cells lying within the interstices of the seminiferous tubules. Kölliker's careful histological study of the interstitial cells appeared four years later. He characterized these structures as clear round cells, analogous to embryonic connective tissue, vacuolated, and containing fat and pigment granules. An important fact pointed out by Kölliker, and one too little considered in certain theories which have been advanced to explain the functions of the interstitial cells, is the constant presence of these cells not only in the interstices of the seminiferous tubules, but in the

* Received for publication, January 5, 1911.

mediastinum and connective tissue septa of the testis, and under the tunica albuginea.

The work of von Ebner (1871), confirmed and extended that of Leydig, Kölliker, Henle, and others. He described the interstitial cells as irregularly polygonal, the nucleus vesicular and sometimes double, the protoplasm granular and containing fat and pigment. Von Ebner agreed with Kölliker that the interstitial cells were of connective tissue origin and this has come to be the prevailing view with later writers. It is true that Hofmeister (1872) was inclined to view the interstitial cells as epithelial structures. He noted their great variability in various species and in the same species from time to time. He found in the fetus that their number diminished progressively until birth, and that after birth they were scantily present until the onset of puberty.

Von Hansemann (1895), studying the testicle of the hibernating marmot, found that spermatogenesis ceases during the hibernating period, and that during this season the interstitial cells almost completely disappear; with the resumption of spermatogenesis in the spring the interstitial cells reappear, and indeed in such quantity that upon section the testicle presents a sarcoma-like picture. He also pointed out that, whereas the sperm-forming cells disappear in patients suffering from febrile and wasting diseases (tuberculosis, cancer, etc.), the interstitial cells increase in number.

Reinke (1896), discovered in the testicle of an executed criminal the crystals which bear his name. He found them in the interstitial cells and lymphatics, and this led him to advance the tentative opinion that they represent the internal secretion of the testicle. Reinke's crystals have been found only in the human testicle.

The papers of Bouin et Ancel (1903, 1904) are the most important which have appeared since the work of Leydig and Kölliker. From a few experiments and a thorough study of normal and cryptorchid testes from various mammalia, they have concluded that the interstitial cells represent a definite organ, "la glande interstitielle," which furnishes an internal secretion to the body, and probably serves in addition as a trophic organ for the Sertoli and sperm-forming cells. They bring forward convinc-

ing evidence that the cells of Leydig are gland cells which in all probability furnish an internal secretion to the body. They do not, however, satisfactorily eliminate the Sertoli cell as a possible source of an internal secretion, merely stating that in animals with one testicle removed and the vas deferens on the other side ligated, the Sertoli cells show " des signes de dégénérescence," presumably fatty degeneration, since this is the only evidence of degeneration mentioned. They did not succeed in any case in causing the disappearance of the Sertoli cells from the seminal tubules, and nothing short of this can be admitted as morphological evidence. It will be shown later in this paper that the presence of an excessive amount of fat in the Sertoli cells is not a sign of degeneration, but rather an evidence of functional activity on the part of these cells. It should be insisted upon that as long as any cells with well preserved nuclei remain in the seminal tubules they can not be positively eliminated as possible producers of an internal secretion. The important work of Bouin and Ancel will be referred to hereafter, as well as the contributions of other writers, which can be more profitably reviewed in connection with the special parts of the subject of which they treat.

Embryology.—There is general agreement among recent investigators upon the mesenchymal origin of the interstitial cells. Whitehead's (1904) very clear description may be partially quoted. " The intertubular tissue of the testis of the pig embryo in stages immediately preceding the appearance of Leydig cells is a mesenchymal structure derived from the mesothelium of the genital ridge. Histologically, it is a connective tissue syncytium, consisting of cells and an exoplastic network of fibrils. The cells are scarcely more than naked nuclei, though some have a small collection of cytoplasm at one pole. From the cells of this tissue Leydig's cells are developed by growth of cytoplasm. At first they are markedly branched; some of the branches are connected with the general exoplastic network, so that the cells retain the syncytial arrangement of their ancestors. They increase in number and size very rapidly and soon lose their branches.

"Leydig's cells pass through two phases of growth, between which a phase of atrophy intervenes. Growth is very rapid from their appearance in the embryo 2.4 centimeters long, until the length of 3.5 centimeters is reached. This is followed by the phase of atrophy, during which the cells return almost to their first state of nearly naked nuclei. This process reaches its acme in the embryo 14 centimeters long. Synchronous with it there is extensive growth of the seminal tubules, particularly in length, so that they are much convoluted, and the inter-tubular spaces are correspondingly narrowed. In the embryo 20 centimeters long, the cells enter upon the second phase of growth, which attains its maximum in the pig of 28 centimeters very near to term. Here the cells are enormously increased in size, so that they constitute the predominating feature of the microscopic picture."

During the period from birth to puberty, which may be regarded as the resting period of the testicle, the interstitial cells are scanty; at the time of puberty they again increase markedly in size and number.

Histology.—The microscopic appearance of the interstitial cells varies markedly in the same species from time to time, and in different species. They have been observed in all the classes of vertebrates, and analogous structures exist in the testes of invertebrates. They are found in all parts of the testicle except the epididymis, and are frequently very numerous just beneath the tunica albuginea. The capillaries of the testicle, on their way to supply the seminiferous tubules, are surrounded by interstitial cells. A striking analogy in this respect exists between the testicle and the adrenal, where the cortical cells surround the capillaries leading to the medulla. The interstitial cells vary greatly in size. They are irregularly polygonal in shape, frequently resembling liver cells or the cells of the corpus luteum. The nucleus, which may be double, lies eccentrically, and is surrounded by a condensed endoplasm which in turn merges peripherally into a thinner, vacuolated ectoplasm (plate XXX, Fig. 1). A certain small proportion of the cells are homogeneous throughout.

The foregoing description is true of material fixed and stained in the usual way, that is to say, without special effort to place in evi-

dence the granular structure of the protoplasm (Altmann's granules). If, however, small pieces of testicle be fixed by the method of Altmann (Schmorl, 1909), or some of its modifications (Benda, Schridde), methods which depend essentially upon the use of oxidizing agents such as potassium dichromate or osmium tetroxide, and then stained according to the methods of Altmann, Benda, or Weigert (Fauré-Fremiet, Mayer, Schaeffer, 1910), one obtains an entirely different protoplasmic picture from that which has become classical. It is now seen that the dense endoplasm around the nucleus is composed of closely packed granules of various sizes (plate XXXI, Fig. 2), and that the relatively clear ectoplasm contains fewer granules. In the ectoplasm a second kind of granule is seen, which, I think, is identical with certain granules described by Whitehead (1906) in the ectoplasm of the interstitial cells. With formol fixation and paraffin sections one can stain these granules with methylene blue. With the acid fuchsin of Altmann they stain a distinctly clearer red than the other cell granules (Fig. 2). They usually lie in a small vacuole. Treated by Weigert's myelin-staining method, or Heidenhain's iron-hematoxylin they are colored black.

Fat is quantitatively a very variable constituent of the interstitial cell, though rarely entirely absent. In human beings and in some animals (dog and cat) it is present in relatively large amount, whereas in the interstitial cells of the pig, fat droplets are usually very scanty. In discussing the functions of the interstitial cells we shall have occasion to consider further the significance of their fat content.

Pigments, soluble and insoluble, are common constituents of the interstitial cells of many animals. The testicle of the pig is colored brown by them, and they are in part soluble in water. Other pigments are present as lipochromes—pigmented complexes of a lipoid nature. Nothing is known of the structure or possible functions of the pigments of the testicle. Crystals or crystalloid substances, such as those described in the human testicle by Reinke and Lubarsch, seem to be very rare in the testicles of the lower mammalia.

THE FUNCTIONS OF THE INTERSTITIAL AND SERTOLI CELLS OF THE TESTIS.

It is one of the oldest and most commonly observed phenomena that castration of the young animal profoundly alters the normal growth and development of the body (Tandler and Gross, 1907, 1908, Griffiths, 1895). We speak of the organs of generation as primary sexual characters, for they differentiate the sexes in early embryonic life. With the maturation of the sexual life of the individual, other characters become evident which further separate the sexes. Thus at puberty the larynx grows rapidly and the voice changes, the growth of hair increases upon characteristic areas, the external genitalia develop more rapidly, the sexual instinct becomes manifest, skeletal differences develop in the male and female—in a word, under the influence of some new and powerful stimulus the body proceeds to sexual maturation. All these delayed manifestations of sex are grouped together as secondary sexual characters.

Whether or not the primary sexual characters of the male are guided in their development by a testicular secretion, remains to be proven. Their origin, at any rate, would seem to rest upon somatic inheritance. It is certain, on the other hand, that secondary sexual characters depend entirely upon some function of the testis, for castration prevents their appearance. The problem is not, then, whether the testes furnish a secretion essential to the normal development of the body, but what element of the testicle has this function, and what the nature of the secretion really is.

The testicle is a glandular structure, one function of which is the secretion of sperm. It contains three parenchymatous elements; namely, the series of sperm-forming cells (spermatogonia, spermatocytes, and spermatids), the Sertoli cells, and the interstitial cells. Which of these three elements is responsible for the production of a secretion which governs the development of secondary sexual characters?

A study of the condition of cryptorchidism will partially elucidate the problem. The material for such a study may be obtained very readily from slaughter-houses, and the advantages of such material

as compared with the difficult study of the rare condition of cryptorchidism in man, or in cryptorchid animals experimentally produced in the laboratory, will be easily appreciated. It is the custom of stock raisers to castrate male pigs at an early age. At the time of castration, in the great majority of the animals, both testicles have descended and are consequently excised. Occasionally one testicle descends before the other, and in such cases only one organ is removed, the other descending later. The condition of permanently retained testes, cryptochidism, is not uncommon in the pig, and may be unilateral or bilateral. Finally, a certain number of normal animals with two scrotal testes come to slaughter. An almost unlimited amount of healthy experimental material is thus available, Nature and the stock-raiser relieving us here of the drudgery and uncertainties of laboratory experimentation.

In the present study I have examined fifty-six cryptorchid testes from the pig, seven from the sheep, and six from man. In no single instance have I found spermatozoa, and only in very young cryptorchid pig testes have spermatogonia been demonstrable. This is in accordance with the well established clinical fact that bilaterally cryptorchid animals are invariably sterile (Bouin et Ancel, 1904, and Whitehead, 1908). Conflicting statements upon this point are met with, but in a careful search of the literature no well authenticated case of a fertile cryptorchid has been found, whereas most writers agree upon the infertility of doubly cryptorchid animals. It should be borne in mind that a truly cryptorchid testicle lies completely within the internal abdominal ring; confusion has arisen through the lax application of the term to funicular and incompletely descended scrotal testes.

All writers agree that the doubly cryptorchid animal develops every attribute of the normal male, save the power of propagation. Secondary sexual characters are well developed and the male passion is normally or even excessively evident (Whitehead, 1908, Bouin et Ancel, 1904). Sections from the cryptorchid pig testicle reveal a truly remarkable picture, due to the excessive number of Leydig cells present. One observes that whereas in the normal organ (plate XXXII, Fig. 3) the spermatic tubules greatly predominate

over the interstitial cells, in the cryptorchid testicle the reverse is true (plate XXXII, Fig. 4). The interstitial cells of the cryptorchid testis are usually larger than those of the normal organ, but otherwise their histological structure is the same. The seminiferous tubules, on the contrary, present marked abnormalities. The basement membrane of the tubule is thickened, and it is lined only by a single layer of Sertoli cells (plate XXXIII, Fig. 5). *No trace whatsoever of sperm-forming cells can be found in the adult cryptorchid testicle.* In very young cryptorchids, large clear primary sperm cells (Ursamenzellen) are seen (plate XXXIII, Fig. 6), but they apparently do not develop further, and soon disappear.

If one compares now, as a useful criterion of the development of secondary sexual characters, the external genitalia of normal, castrated, and cryptorchid pigs, one finds that whereas the genitalia of castrated animals are markedly undeveloped and atrophic in appearance (plate XXXIV, Fig. 7), no distinction whatever can be made between the genitalia of normal (plate XXXIV, Fig. 8) and cryptorchid animals (plate XXXIV, Fig. 9). Since, then, in the absence of the sperm-forming cells secondary sexual characters develop quite normally, one may safely eliminate these cells from the problem of determining which element of the testicle furnishes an internal secretion to the organism.

It is unfortunately not so easy to eliminate similarly either one or the other of the two remaining parenchymatous elements, interstitial cells and Sertoli cells. No method has yet been found which will cause the disappearance of one while leaving intact the other. Various methods have been tried, such as occlusion of the vas deferens (Bouin et Ancel, 1904, Griffiths, 1895), x-ray injury to the testicle (Simmonds, 1907, Herxheimer and Hoffmann, 1908), freezing (Cevalotto, 1909), etc., but, whereas the spermatogenic cells disappear after any sufficient injury to the testicle, the Sertoli and interstitial cells persist. Artificially produced cryptorchid testes in dogs present essentially the same histologic picture as that described above in cryptorchid pigs; both Sertoli and interstitial cells persist, the sperm-cells disappearing. I have successfully transplanted pieces of testicle under the rectus muscle of castrated dogs (auto-transplants), but here too the Sertoli cells and

the interstitial cells seem equally viable, both persisting in the transplant (plate XXXV, Fig. 10). I have not been able to cause the disappearance of the Sertoli or interstitial cells in dogs by ligating the vasa deferentia. Such ligations in young dogs do not prevent the normal maturing of the sperm-cells, but subsequently these disappear.

Confronted, then, with the seeming impossibility of solving the question in hand by the direct method of physically eliminating either the Sertoli or the interstitial cells, after the manner in which the spermatogenic cells are eliminated in the cryptorchid testicle, one is forced to adopt the more usual method of determining, from a study of the facts obtainable, which of two possibilities is the better supported by these facts. We may first examine the evidence for and against the interstitial cell as the elaborator of an internal secretion, and then seek to establish from the facts at hand the true function of the Sertoli cells.

We have seen that embryologically the interstitial cell is of mesenchymal origin. Histologically it presents the type of a gland cell—a plentiful cytoplasm rich in cell granules. The histological resemblance of the interstitial cells to the cells of the liver is worthy of emphasis, and especially is this true when both are stained to demonstrate their rich content of cell granules. The interstitial cells stand in the closest possible relation to the rich capillary meshwork of the testicle; they surround the capillaries in their course to the tubules, and are bathed in the large inter-tubular lymph spaces.

A compensatory hypertrophy can be demonstrated in the interstitial cells under certain conditions. In a pig from which one testicle has been removed at an early age, the interstitial cells of the remaining organ are present in markedly increased numbers (plate XXXV, Fig. 11). So striking is this increase that one can easily tell microscopically the monoscrotal from the biscrotal organ (Bouin et Ancel, 1903). But a more convincing proof of such compensatory hypertrophy is seen on comparing the monocryptorchid testicle (plate XXXVI, Fig. 12) of an animal in which the other organ has descended and been removed, with the testes of a bilaterally cryptorchid animal (plate XXXVI, Fig. 13). In the former the predominance of the interstitial cells over the atrophic seminifer-

ous tubules is much more striking than in the latter. At the same time no hypertrophy of the Sertoli cells can be noted. Indeed they are present in relatively far smaller numbers than in the normal organ. If the Sertoli cells really function as producers of an internal secretion, one would certainly expect to find evidence of their increase in monocryptorchid testes, as well as in animals possessing only one scrotal testis, especially in view of the striking augmentation of the interstitial cells under these conditions.

It has been suggested by Plato (1896) that the interstitial cells have for their function the furnishing of nutriment, in the form of fat, to the Sertoli and spermatogenic cells. Quite apart from a total lack of evidence in support of such a view, Plato's claim to have demonstrated the passage of droplets of neutral fat from the interstitial into the Sertoli cells through certain preformed canaliculi of the tubular wall has been thoroughly discredited (Beissner, 1898). Moreover, the conditions existent in the cryptorchid testis remove any doubts of its falsity, for here we find an enormous relative increase of the interstitial cells, with atrophic seminiferous tubules and a complete absence of spermatogenic cells.

Von Lenhossèk (1897) advanced the view that the interstitial cells furnish a pabulum for the use of the cells of the seminiferous tubules. The discussion of such a theory is profitless, based as it is on slight and equivocal evidence. The same may be said of Harvey's (1875) belief that the interstitial cells represent nerve cells.

Von Bardeleben (1897) noticed a fancied resemblance between the Sertoli and Leydig cells, and thought the "worn-out Sertoli cells" were in time replaced by young Leydig cells. Such theories are, in the main, only of minor historical interest, but Goldmann (1909), using the vital stains of Ehrlich and Mesnil, has recently revived interest in the view of von Bardeleben by claiming the demonstrable passage of Leydig cells through the walls of the seminiferous tubules, and indeed he figures quite beautifully such an event. I have repeated with care the work of Goldmann and can say that in the course of an enumeration of 1,000 Leydig cells in sections from the testicles of rats which had been vitally stained with trypan-blue and pyrrol-blue, no suggestion of the phe-

nomenon described by Goldmann was observed. It must be very rare, for among the many careful histological studies by various observers of the easily stainable Leydig cells, no one has described a similar picture.

In cryptorchid animals, the findings, both in the testicles and in the external genitalia, demonstrate clearly enough that the interstitial cells represent a structure which has a general bodily function, and is not in any reasonably thinkable way connected with the nutrition of the spermatogenic cells.

Such a nutritive organ does exist, however, within the seminiferous tubule in the form of the very specialized Sertoli cells. The greatest divergence of opinion has existed in regard to the function of these cells since their discovery by Sertoli in 1865. This is evident from the various names applied to them by different investigators, such as "sustentacular cells" (F. Merkel), "follicle cells" (La Valette St. George), "Nährzellen" (Peter), and "sperm-nourishing-cells" (Grobben). The Sertoli cells form a single-layered lining for the membrana propria of the seminiferous tubules. The cell boundaries are indistinguishable in ordinary preparations, and Regaud (1910) describes them as forming a syncytium. Von Ebner (1902) states, however, that in physiological salt solution they can be isolated as discrete cells. In the non-functioning tubule they assume the form of cylindrical epithelial cells, but during spermatogenesis, filmy protoplasmic processes reach far out into the lumen of the tubule. Their cytoplasm always contains fat droplets in very considerable amount, a finding to be described in more detail presently, and this fat appears in the Sertoli cells before the embryonic differentiation of the cells of Leydig (Whitehead, 1904, Allen, 1904).

A brief review at this point of the process of spermatogenesis will facilitate an understanding of the functions of the cells of Sertoli. Lying partially embedded in the protoplasm of the Sertoli cells, close to the basement membrane of the tubule, are small round cells with a more or less richly chromatic nucleus. These are the sperm-mother cells or spermatogonia. They initiate a generation of sperm by dividing into two cells, known as spermatocytes of the

first order, which in turn undergo mitotic division, giving rise eventually to four spermatids. Shortly after the formation of the spermatids, the old generation of spermatozoa are cast off into the lumen of the seminiferous tubule, and now the spermatids and Sertoli cells enact a scene which is quite without analogy elsewhere in the body. A group of spermatids (eight to twelve in the rat), form a true protoplasmic union with the cytoplasmic prolongation of a Sertoli cell, so that together they present the picture of an axially elongated, multinucleated protoplasmic mass.

During this development of the spermatids from the spermatogonia, and their subsequent conjugation with the Sertoli cells, important changes have taken place in the fats in the Sertoli cells. Von Ebner (1902) has described a "circulation" of the fat of the Sertoli cell, during the course of a spermatic generation, from the base of the Sertoli cell toward the lumen of the tubule, and noted that *pari passu* with the development of the sperm, the fat diminished. He concluded that it was utilized as nutriment for the developing spermatozoa. His clear and convincing descriptions are based upon histological changes in the normal testes, but no evidence of a physiological nature has, so far as I am aware, been brought forward in its support. Such evidence, obtained from a microchemical and chemical study of normal and cryptorchid testes, is presented elsewhere in this journal (Hanes and Rosenbloom, page 355) and may be summarized very briefly at this point. If seminal tubules in various stages of spermatogenesis are stained for fat, it will be seen that as spermatogenesis proceeds the large fat droplets which are present in the basal portions of the Sertoli cells in the beginning, divide into very much smaller droplets and pass centralward in the protoplasmic prolongations of the Sertoli cells, and thence into the spermatids after their union with the Sertoli cells. During its migration the fat can be shown by appropriate staining methods to have changed in its chemical character from a neutral fat to a lipoid. Using Weigert's method for the staining of myelin, Regaud (1910) has also demonstrated lipoids in the Sertoli cells and spermatids. The exact manner in which the fat furnished by the Sertoli cells is eventually utilized by the spermatids and sperm, shares the obscurity which envelops the question of the ultimate

metabolism of fats by the cells throughout the body. In cryptorchid testes it has been pointed out that the sperm-forming cells have completely disappeared (Fig. 5). If the fat of the Sertoli cells is destined for the nutriment of the spermatids and sperm, the absence from the seminal tubules of these elements should result in the presence of an excessive amount of fat in the Sertoli cells. That such is strikingly the case will be seen from a section of a cryptorchid testis stained to demonstrate fat (Figs. 4 and 5). The Sertoli cells are seen to be loaded with large neutral-fat droplets. Upon chemical analysis of normal and cryptorchid testes, the microchemical findings are corroborated. Of the dried weight of normal pig testes, 19 per cent. was found to be fatty material, whereas the percentage of fatty substances present in cryptorchid testes was found to amount to 31 per cent. of their dried weight. In other words, the fat content of the cryptorchid testicle is almost double that of the normal. We conclude, therefore, that fat accumulates in the Sertoli cells of the cryptorchid testis because of the absence of the spermatogenic cells which normally utilize this fat.

The classical researches of Miescher (Burian, 1906) upon salmon sperm, and upon the sperm of the bull, fully support the above conclusion. He found that, whereas the head or nucleus of the sperm is almost devoid of fatty matter (1 to 2 per cent.), the tail, or cytoplasm, is extremely rich in fatty substances, containing 58 per cent. of ether-soluble material, as compared with 42 per cent. of protein. Upon analysis of the 58 per cent. of fatty matter of salmon sperm, 50.4 to 53 per cent. was found to be lecithin; 17 per cent., cholesterin; and 31.3 to 35.4 per cent., true fat, very rich in oleic acid. Very similar results were obtained from analyses of bull spermatozoa (Miescher), and the sperm of the herring (Matthews).

The general state of nutrition of the body has no demonstrable bearing upon the fats of the testicle. It is impossible to cause their disappearance by starvation, and patients dying from wasting diseases with extreme cachexia show no diminution in the testicular fat (Cordes, 1898, Thaler, 1904, Traina, 1904).

From the facts at hand it may be concluded that one function of the Sertoli cell is the providing of nutriment to the peculiarly situated spermatogenic cells. Histological, chemical, and physiological

evidence, amply support this conclusion. Whether or not the Sertoli cells furnish in addition an internal secretion to the body, we cannot say with absolute certainty. There is no evidence whatever that they elaborate an internal secretion, whereas an abundance of facts points to their functioning solely as sperm-nourishing cells. The interstitial cells of Leydig, on the other hand, present the characters of active gland cells, and the evidence which I have presented has lead me to believe that they furnish an internal secretion to the male body necessary for its normal development.

CONCLUSIONS.

1. The mammalian testicle elaborates an internal secretion necessary to the normal development of secondary sexual characters.

2. The spermatogenic cells play no part in the formation of this secretion.

3. One function of the Sertoli cells is to supply fat to the developing sperm-cells. In the absence of the sperm-cells from the mammalian testis, fat accumulates in large amount in the Sertoli cells. The Sertoli cells seem to take no part in the production of an internal secretion.

4. The interstitial cells of Leydig are, in all probability, responsible for the production of the internal secretion of the mammalian testis.

BIBLIOGRAPHY.

Allen, B. M., The Embryonic Development of the Ovary and Testes in Mammals, *Am. Jour. Anat.*, 1904, iii, 90.

von Bardeleben, Beiträge zur Histologie des Hodens and zur Spermatogenese beim Menschen, *Arch. f. Anat. u. Physiol., Anat. Abt*, 1897, Supplement, 193.

Beissner, Die Zwischenzellen des Hodens und ihre Bedeutung, *Arch. f. mikr. Anat.*, 1898, li, 794.

Bouin et Ancel, Recherches sur les cellules interstitielles du testicule des mammifères, *Arch. de zool. expér.*, 1903, series 4, i, 437.

Bouin et Ancel, Recherches sur la signification physiologique de la glande interstitielle du testicule des mammifères, *Jour de physiol. et de path. gén.*, 1904, vi, 1012.

Bouin et Ancel, Sur la ligature des canaux déférents chez les animaux jeunes, *Compt. rend. Soc. de biol.*, 1904, i, 84.

Burian, R., Chemie der Spermatozoen, *Ergenbn. d. Physiol.*, 1906, v, 768

Cevalotto, G., Ueber Verpflanzungen und Gefrierungen der Hoden, *Frankfurter Ztschr. f. Path.*, 1909, iii, 331.

Cordes, Untersuchungen über den Einfluss acuter und chronischer Allgemeinerkrankungen auf der Testicle, speciell auf die Spermatogenese sowie Beobachtungen über das auftreten von Fett in den Hoden, *Virchows Arch. f. path. Anat.*, 1898, cli, 402.

von Ebner, Untersuchungen über den Bau der Samenkanälchen, etc., *Untersuchungen a. d. Institut für Physiol. und Hist. in Graz*, 1871, No. 2, 200.

von Ebner, Kölliker's Handbuch der Gewebelehre des Menschen, Leipzig, 6th edition, 1902, iii, pt. 2, p. 451.

Fauré-Fremiet, Mayer, and Schaeffer, Sur la microchimie des corps gras; application à l'étude des mitochondries, *Arch. d'anat. micr.*, 1910, xii, 19.

Goldmann, E. S., Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Färbung, *Beitr. z. klin. Chir.*, 1909, lxiv, 192.

Griffiths, Three Lectures upon the Testis, *Lancet*, 1895, i, 791.

von Hansemann, Ueber die sogenannten Zwischenzellen des Hodens und deren Bedeutung für pathologische Veränderungen, *Virchows Arch. f. path. Anat.*, 1895, cxlii, 538.

Harvey, R. J., Ueber die Zwichensubstanz des Hoden, *Centralbl. f. d. med. Wissensch.*, 1875, xiii, 497.

Herxheimer and Hoffmann, Ueber die anatomischen Wirkungen der Röntgenstrahlen auf den Hoden, *Deutsch. med. Wchnschr.*, 1908, xxxiv, 1551.

Hofmeister, Untersuchungen über die Zwischensubstanz in Hoden der Säugetiere, *Sitzungsber. d. math.-naturw. Kl. d. k. Akad. d. Wissensch.*, 1872, lxv, Abt. iii, 77.

Kölliker, Mikroskopische Anatomie oder Gewebelehre des Menschen, Leipzig, 1854, pt. ii.

von Lenhossèk, Beiträge zur Kenntniss der Zwischenzellen des Hodens, *Arch. f. Anat. u. Physiol., Anat. Abt.*, 1897, 65.

Leydig, Zur Anatomie der männlichen Geschlectsorgane und Analdrusen der Säugetiere, *Ztschr. f. wissensch. Zool.*, 1850, ii, 1.

Plato, Die interstitiellen Zellen des Hodens und ihre physiologische Bedeutung, *Arch. f. mikr. Anat.*, 1896, xlviii, 280.

Regaud, Cl., Études sur la structure des tubes spermatogenese chez les mammifères, *Arch. d'anat. micr.*, 1910, xi, 291.

Reinke, Ueber Kristalloidbildungen in den Interstitiellenzellen des menschlichen Hodens, *Arch. f. mikr. Anat.*, 1896, xlvii, 34.

Schmorl, (for Altmann's method with modifications), Die pathologisch-histologische Untersuchungsmethoden, Leipzig, 1909, p. 120.

Simmonds, M., Ueber die Einwirkung von Röntgenstrahlen auf den Hoden, *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1908, xiv, 229–238.

Tandler, J., and Gross, S., Ueber den Einfluss der Kastration auf den Organismus, *Wien. klin. Wchnschr.*, 1907, xx, 1596.

Tandler, J., and Gross, S., Untersuchungen an Skopzen, *Wien. klin. Wchnschr.*, 1908, xxi, 277.

Thaler, H. A., Ueber das Vorkommen von Fett und Kristallen im menschlichen Testikle unter normalen und pathologischen Verhältnissen, *Beitr. z. path. Anat.*, 1904, xxxvi, 528.

Traina, R., Ueber das Verhalten des Fettes und der Zellgranula bei chronischem Marasmus und acute Hungerzuständen, *Beitr. z. path. Anat.*, 1904, xxxv, 1.

Whitehead, R. H., The Embryonic Development of the Interstitial Cells of Leydig, *Am. Jour. Anat.*, 1904, iii, 167.

Whitehead, R. H., A Peculiar Case of Cryptorchidism and its Bearing upon the Problem of the Function of the Interstitial Cells of the Testis, *Anat. Rec.*, 1908, ii, 177.

Whitehead, R. H., The Presence of Granules in the Interstitial Cells of the Testis, *Anat. Rec.*, 1906, i, 60.

EXPLANATION OF PLATES.

Plate XXX

Fig. 1. × 1,000. Zenker's fluid, hematoxylin and eosin. Section of cryptorchid pig testicle showing the interstitial cells surrounding a capillary. Some of the cells are homogeneous throughout, but most of them show a dense endoplasm and a vacuolated peripheral ectoplasm containing definite granules.

Plate XXXI.

Fig. 2. × 1,000. Altmann's stain. The granules of the cells are stained red, fat droplets black. As in Fig. 1, the endoplasm and the ectoplasm are sharply contrasted, due to their granular contents

Plate XXXII.

Figs. 3 and 4 are microphotographs taken under the same magnification In the normal testicle (Fig. 3) the actively functioning seminal tubules dominate the picture; the interstitial cells occupy the interstices of the nearly contiguous tubules. In the cryptorchid testicle (Fig. 4) the picture is exactly the reverse; the seminal tubules are shrunken, and the interstitial cells greatly predominate. Both sections are stained to show fat (Sudan III). The round droplets of fat form a peripheral fringe in the normal seminal tubules (Fig. 3), while in the tubules of the cryptorchid testis (Fig. 4), the fat droplets are very abundant, filling the Sertoli cells.

Plate XXXIII.

Fig. 5. High power drawing of two seminal tubules of a cryptorchid pig testis, surrounded by interstitial cells. The basement membranes of the seminal tubules are thickened, and they are lined by Sertoli cells which contain large fat droplets. The interstitial cells appear normal in every way, contrasting sharply with the obviously atrophic, aspermatic seminal tubules.

Fig. 6. High power drawing of a section of a single seminal tubule from a very young cryptorchid pig testis. The tubule is lined by Sertoli cells containing large fat droplets (shown as vacuoles in the drawing). The primordial sperm cells (Ursamenzellen) with deeply staining nuclei lie nearer the lumen.

Plate XXXIV.

Fig. 7. Photograph of the urino-genital apparatus of an adult castrated pig.

Fig. 8. The urino-genital organs of a normal pig.

FIG. 9. The urino-genital organs of a monocryptorchid pig (the other testicle had descended normally and had been removed).

The photographs are taken to scale and show the bladder, prostate, seminal vesicles, glands of Cowper, and penis. The organs of the castrated pig (Fig. 7) are small and atrophic, whereas the organs of the cryptorchid pig (Fig. 9) are as well developed as in the normal animal (Fig. 8).

PLATE XXXV.

FIG. 10. Section from a piece of dog's testicle transplanted under the rectus muscle. The spermatogenic cells have disappeared from the seminal tubules, leaving only the Sertoli cells. Many interstitial cells have survived.

FIG. 11. Section from the normal testicle of a pig having only one scrotal testis, the other having been removed in early life. This figure is to be compared with Fig. 3, which represents the normal relation of seminal tubules and interstitial cells in pigs having two scrotal testes.

Both sections have the same magnification. In Fig. 11 the relative increase of the interstitial cells is well seen (compensatory hypertrophy).

PLATE XXXVI.

FIG. 12 shows the relative proportion of interstitial cells to seminal tubules in a pig having only one cryptorchid testis and no scrotal testis.

FIG. 13 shows the same in a pig having two cryptorchid testes. In Fig. 12 the interstitial cells are relatively far more abundant than in Fig. 13 (compensatory hypertrophy).

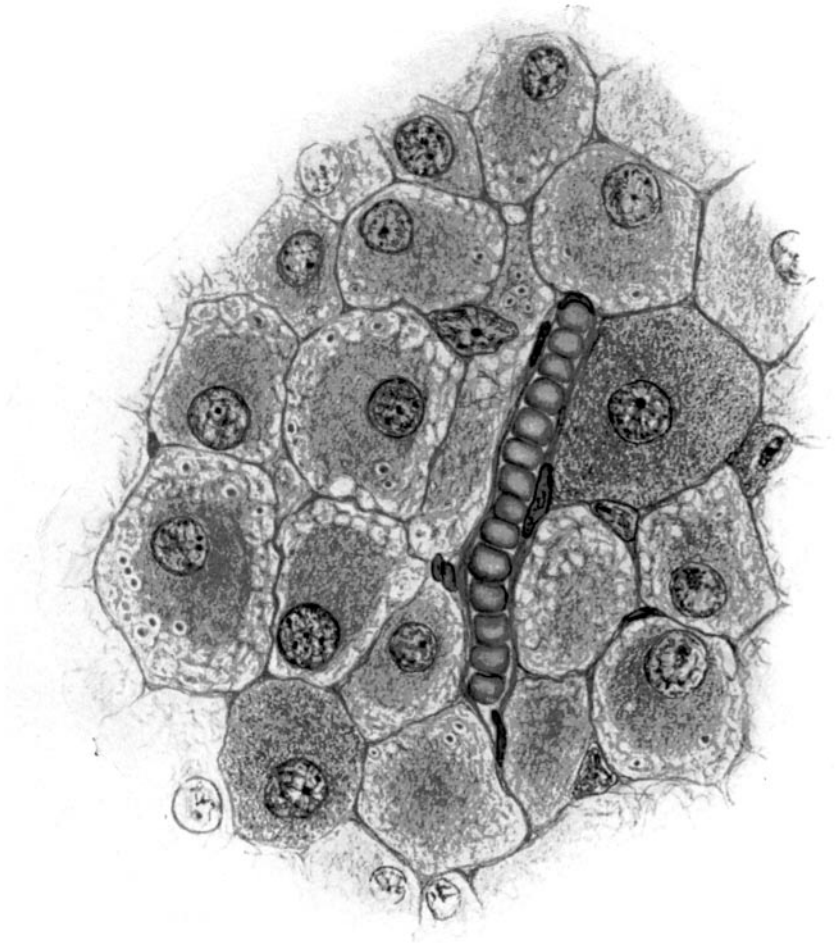

FIG. 1.

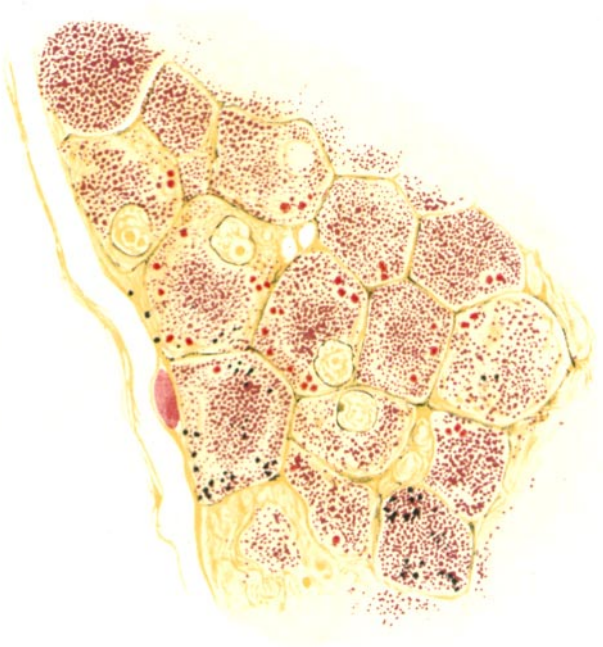

FIG. 2.

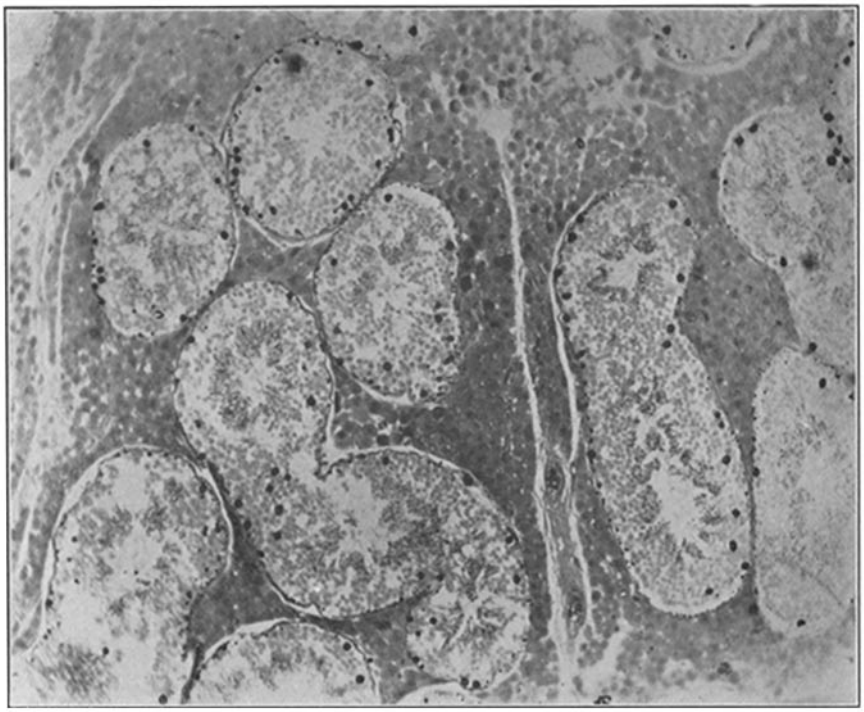

FIG. 3.

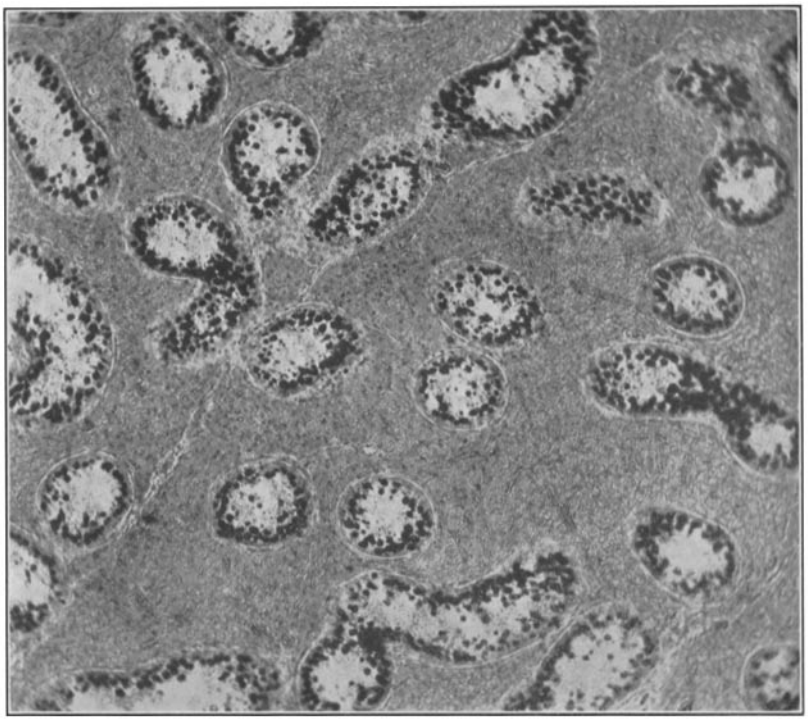

FIG. 4.

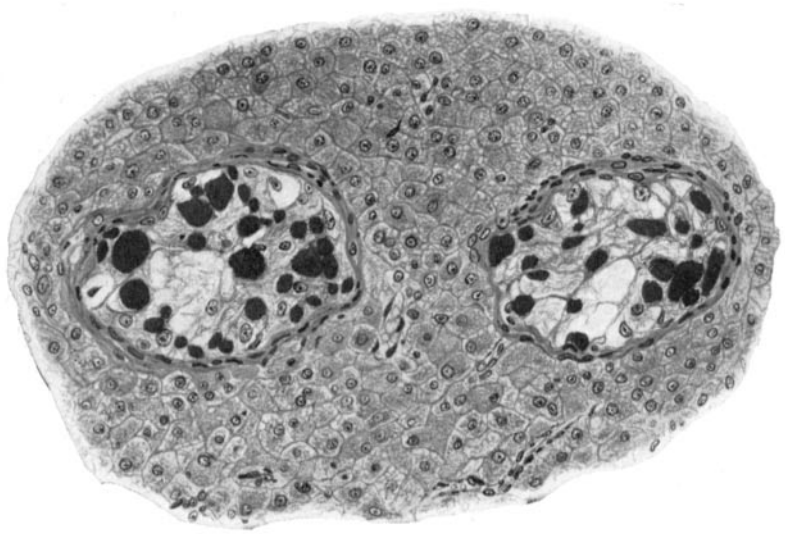

FIG. 5.

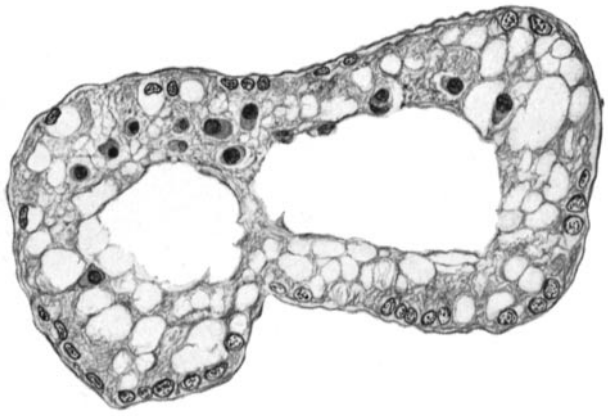

FIG. 6.

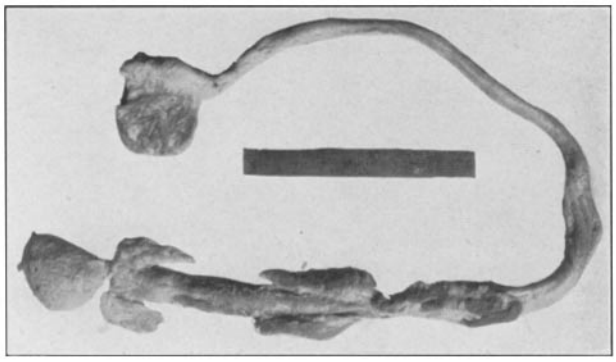

FIG. 7.

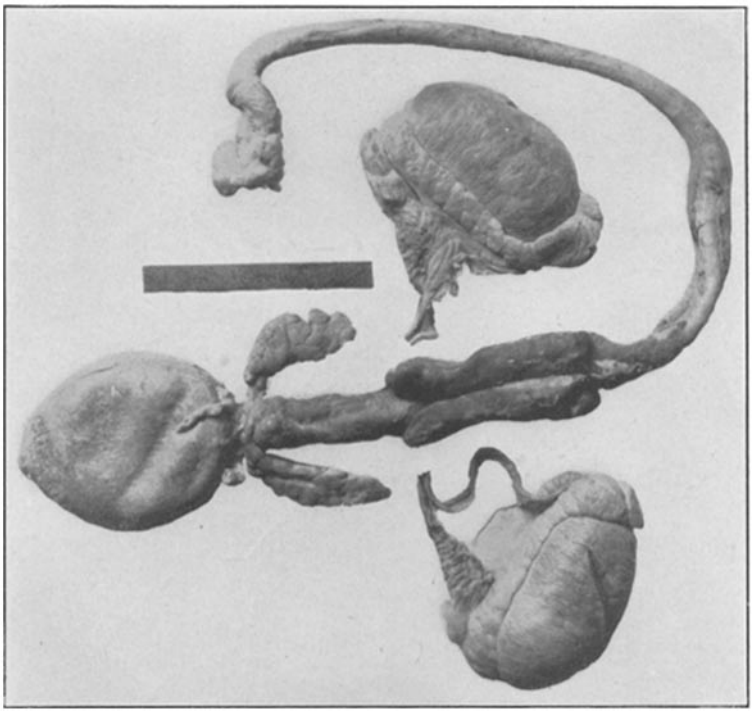

FIG. 8.

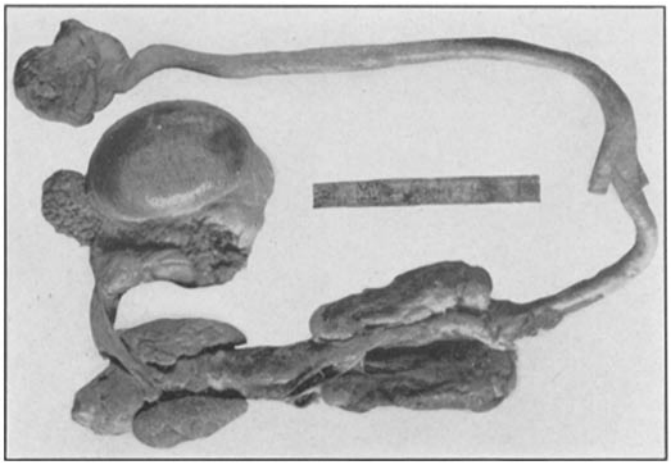

FIG. 9.

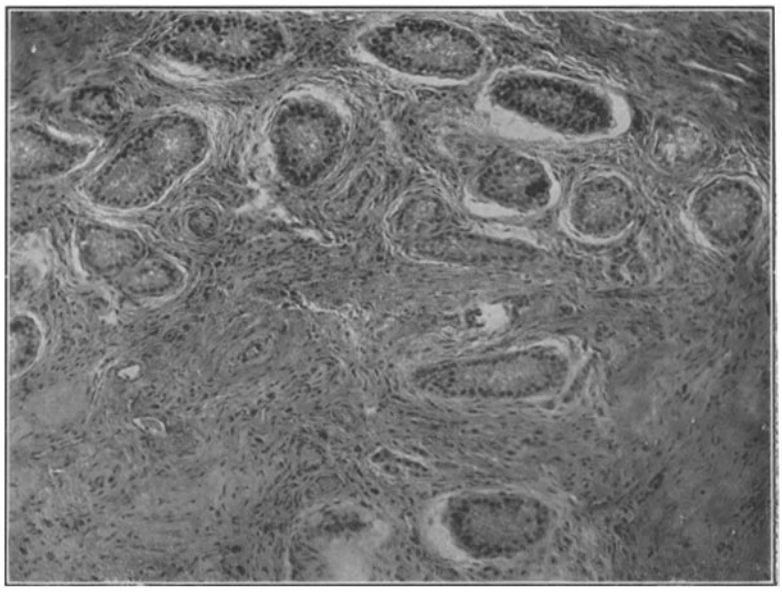

FIG. 10.

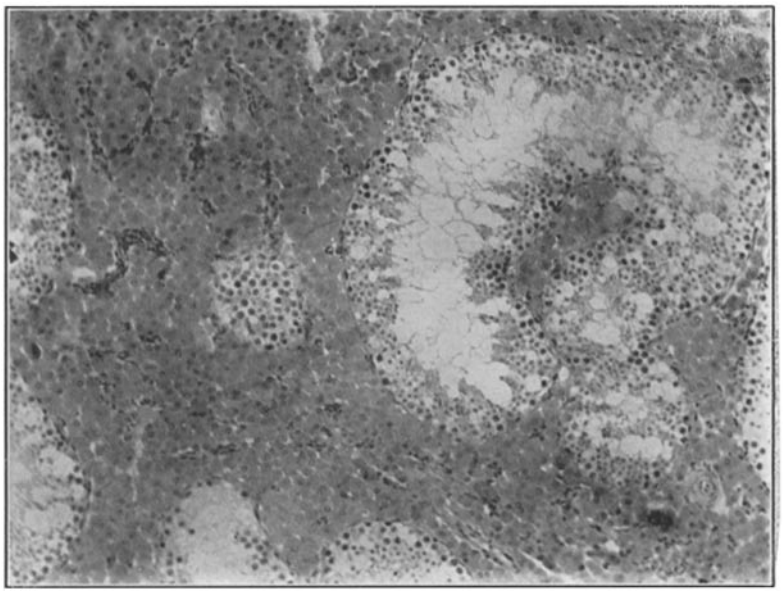

FIG. 11.

FIG. 12.

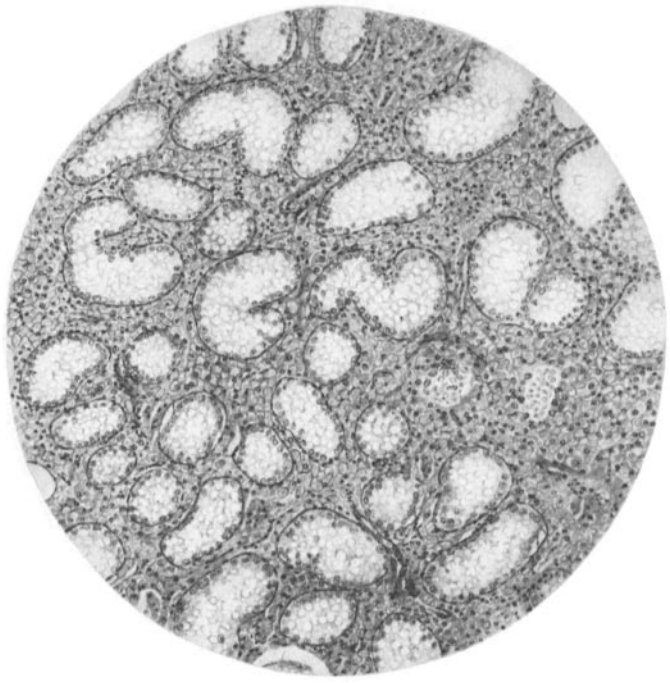

FIG. 13