NOTES ON MARSUPIALIAN ANATOMY.

II. ON THE FEMALE GENITAL ORGANS OF A VIRGIN SARCOPHILUS SATANICUS.

Pl. XXIII., XXIV., XXV., XXVI., XXVII.

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In a former communication to the Linnean Society of N.S.W. (10) I described the genital apparatus in a female specimen of Sarcophilus satanicus which had borne young, and in that paper drew attention to various phenomena, especially in connection with the median vaginal apparatus, which could only be satisfactorily explained by the examination of similar organs in the virgin animal. In the ordinary course of events, such an undoubted virgin would be difficult to procure, and, even if obtained, would be hard to recognise as such in our present knowledge of these organs in this genus. Nevertheless, since my last paper was written, through the kindness of Mrs. Roberts, C.M.Z.S., of Hobart, such a virgin has been placed at my disposal. Mrs. Roberts has had for some time past in her gardens at Beaumaris a number of Tasmanian "devils," among which was the specimen in question. It came to the gardens as a baby attached to the teat about August, 1909, and had lived ever since in a cage with its mother and an old male. Last March quarrels occurred through the young female resenting the attentions of the male, with the result that she was severely handled, and had to be removed to another cage. Soon after she seemed to rally, but after a little while sank and died. A post-mortem showed that external injuries were not the sole cause of death, for the internal organs were very much diseased.

I wish to place on record my grateful appreciation of the kindness of Mrs. Roberts in putting this specimen at my disposal.

My best thanks are due to the following gentlemen for the loan of literature:—Professors J. T. Wilson and W. A. Haswell, and Acting Professor Johnston.

I am able to confirm and considerably augment my account of the genital organs in this genus.

I have nothing to add to my previous account of the external form of the female organs, except that in this specimen the Fallopian tubes seem slightly more convoluted than in the former specimen.

STRUCTURE OF THE VIRGIN UTERUS.

Fig. 1 represents a transverse section of the uterus of the virgin. From side to side the whole uterine body is flattened, containing consequently a correspondingly flattened lumen. Each dorsal and ventral wall of the uterus is raised into a rounded fold, separated from the lateral uterine wall by deep grooves. With the exception of these grooves the inner surface of the uterus is quite smooth.

A somewhat enlarged section of the uterus is shown in Fig. 2. The inner covering of the uterine wall is a thin epithelium consisting partly of cells with rounded nuclei partly of columnar cells mixed indiscriminately. It measures in thickness about .012 mms. The uterine glands have an epithelium continuous with the inner uterine epithelium, but no columnar cells are present in it. The glands are slightly convoluted tubes with an average diameter of .009 mms. The mucosa is well defined and sharply marked off from the muscularis. It contains large numbers of uterine glands, but in no case did I find these to penetrate the muscularis. The tissue of the mucosa is of the nature of a loose network, the nuclei of whose cells become aggregated for the most part round the uterine glands, the vessels, and just below the uterine epithelium. The vessels which supply the mucosa, after piercing the muscularis, lie for the most part in the lower portion of this layer, although fine capillary branches supply the inner portion of the mucosa. For the most part the vessels have extremely thin and ill-defined walls. The average thickness of the mucosa is .71 mms.

The muscularis is a definite layer, .032 mms in average thickness, consisting of circularly running plain fibres only.

It is interrupted only by the incoming blood vessels. There is a well marked serosa continuous with the broad ligament.

A comparison with the uterus of a female with pouch young (described in my last paper) shows that the chief difference in the latter is the greater growth of the mucosa, it being about one and a half times as thick. This increase in growth takes place for the most part on the lateral walls of the uterus, forming here two large cushion-shape masses. Other points noticeable in the multiparous uterus are:—The inner epithelium is entirely columnar, the mucosa is considerably more vascular, almost the entire space between the uterine glands being taken up by vessels, the uterine glands have increased in number and diameter, the muscularis has increased in thickness, and has become invaded to some extent by uterine glands.

MEDIAN VAGINAL APPARATUS (SINUS VAGINALIS).

The general arrangement and structure of this portion of the female sexual organs of Sarcophilus is worthy of considerable discussion. In my last communication, I ventured the remark that the structure may not be present in the virgin, but may arise at the time of the first pregnancy. I was led to this conclusion by the fact that in the comparatively large and late pouch young described in that communication no sign of the median vaginal apparatus, as such, was present; while in a pouch young of Perameles, a section of whose organs was figured by Hill (5, p. 77) there are indications that the foundation of the median vaginae have already been laid. Van den Broek, again, describes the presence of a well marked median vaginal apparatus in a pouch young of Didelphys, for he says:—"Dagegen fand ich bei Beuteljungen von Didelphys schon sehr fruh den Sinus Vaginalis in einen Entwicklungsgrad, der jenem des erwachsenen Tieres relativ wenig nach steht." this being quite the opposite to what had been already described by Brass. I was also led to the above-mentioned conclusion by the peculiar irregular arrangement of the two median vaginal cul-de-sacs with regard to the vaginae, and again by the fact that the anteriorly directed portion in each median vaginal cul-de-sac is quite narrow and canal-like, an occurrence which led me to give it, for the time being, the special name of the median

vaginal neck. The presence of these median vaginal necks distinctly marks off the arrangements of the female organs in Sarcophilus from that of any other described marsupial. At the time of my last paper I had not seen Van den Broek's paper on the marsupials (3), and did not know that he had already called that portion of the sinus vaginalis (median vaginal apparatus) into which the uterine necks opened, the fornix. In Sarcophilus the fornix is drawn out on each side into a narrow tube. It had been pointed out (3) that the position of the uterine openings into the median vagina may vary; for example, in the Didelphidae the uterine necks penetrate the median vagina from the anterior side, in Phascolomys they are placed at the antero-lateral corners, in Antechinus apicalis quite laterally, while in the Macropods they enter the median vagina from the dorsal side. In Sarcophilus each uterine neck opens at the apex of a large papilla into each median vaginal neck. This papilla differs from all other marsupials except Phascolomys (3) in having lobed edges. Since this opens into the anterior prolongation of each cul-de-sac, the entry of the uterus is quite from the cranial end, and it can be placed in the same group, therefore, as the Didelphidæ.

Van den Broek, who examined a single specimen of Sarcophilus, but not apparently in detail, says (3, p. 51):—"Dasyurus ursinus und Antechinus apicalis kommen in der Gestalt ihres Sinus vaginalis Phascolomys sehr nahe, doch unterscheiden sich, besonders Antechinus vom Letztgenannten dadurch, dass die Uteri mehr horizontal verlaufen und folglich mehr von der Seite her in den Sinus vaginalis ausmunden." This statement indicates that Van den Broek had not observed the presence of the median vaginal necks in this genus.

Morphologically, the median vagina of the virgin differs from that of the multipara mainly in the fact that the cul-de-sacs are much smaller and considerably shorter. The median vaginal apparatus in Sarcophilus has a number of peculiar features which mark it off from the similar portions of the sexual organs in other marsupials: it is considerably less developed in proportion to the vaginae than is usual, its shape is irregular, it cannot be said to be triangular, as in the Didelphidae, nor tongue-like as in Phascolomys, nor cylindrical as in Macropods. It retains more than any other—having as it does a complete and strong septum containing muscle fibres—the character of

two separate outgrowths approximating, but hardly sufficiently to form a homogeneous structure. Van den Broek has said:—"Man konnte in dieser doppelten Herkunft Anlass finden statt von einem 'Sinus vaginalis' von 'Sinus vaginales' zu sprechen," and this statement applies above all to the condition in Sarcophilus.

MICROSCOPIC STRUCTURE.

Muscularis. — In common with Hill and Van den Broek, I find that, as in the rest of Marsupials, the muscular tissue of the median vaginal apparatus consists of circularly running nonstriate fibres. On this point, however, the latter says (3, p. 60): "Muskelfasern kommen niemals in Septum vor, die. . . Muscularis circularis in der Wand des Sinus umschliesst immer die beiden Hohlraume des Sinus als einen einheitlichen Raum." Hill, however, (5, p. 53) says of the two cul-de-sacs in the virgin Perameles that "they are separated by a common partition wall, and each is surrounded by a delicate layer of circular nonstriate fibres." He refers to Fig. 7, but the presence of these fibres in the septum is more plainly and unmistakably shown in Fig. 10 of a section through the median vaginal cul-de-sac of a specimen with two 17.5 young in the pouch.

In Sarcophilus I find that each sinus is contained in its own portion of circular muscle, which wholly surrounds it, and is, therefore, present in the septum. The presence of these muscle fibres in the septum of both Perameles and Sarcophilus gives the two median vaginal cul-de-sacs an independence which is absent in other marsupials. In Sarcophilus I find that the epithelial lining of the vaginae, median cul-de-sacs, and of the necks is of practically the same character throughout, consisting of an overlying layer of flattened cells which is not at all continuous, below which is a layer one or two cells thick of cells containing rounded nuclei. The epithelium of the urogenital sinus is similar. I have already (10) shown that the posterior ends of the median vaginal cul-de-sacs are embedded in a mass of deeply staining connective tissue, continuous with a central mass of similar tissue passing backwards between the lateral vaginae, and coming into relation with them about the point where the two lateral vaginae meet the urogenital sinus. This rod of connective tissue is similar to that already described by Hill in Perameles (5, p. 54). This mass of tissue is distinctly defined in the specimen under

discussion, being first a rhomboidal mass situated between the two inpassing ureters. Later, in company with the two vaginae, it comes to lie just outside the circularis of the urethra as a deeply stained triangular mass. The circularis of the urethra develops an upper portion, which grows over the two vaginae, and encloses them together with the deeply staining tissue. The muscular layer here, then, is in section of a somewhat elliptic shape, crossed by another muscular layer, the dorsal portion of the original circularis of the urethra. In this way two spaces are formed, an upper and smaller containing the vaginae with the intervening deeply staining tissue, and a lower large one containing the urethra. The muscular layer between these two spaces disappears, and so is formed the circularis of the urogenital strand. The vaginae have up to this time each kept its own circular muscle layer. The muscle layer of the vaginae consists each of an outer and inner circular layer portion, with an intervening mass consisting of connecting tissue with a few circular fibres. Just before the vaginae enter the urogenital sinus their muscular layer dwindles away. The two vaginae enter the two dorsal grooves of the urogenital sinus, the presence of which gives the sinus somewhat the shape in section of a V, the limbs of the letter being formed by the grooves just mentioned. The cloaca is covered with a stratified epithelium.

REMAINS OF THE WOLFFIAN DUCT.

In sectioning there was found in the course of the left vagina round the bladder, embedded in its wall, between the inner and outer portions of the circularis, a short, blind discontinuous tube, representing, I take it, the remains of the Wolffian Duct (Fig. 3). It extends through a total length of 450 micra, thirty sections of 15 micra each. Here and there it shows a lumen, at other places it is a solid cell strand. It is thickest in the middle, and tapers off at each end, leaving no trace. It is not found in the similar position in the right vagina, nor in the multiparous specimen, nor have I found it in the pouch young. It is to be regarded as an individual variation. Although remnants of the Wolffian duct have been found persistent in the papilla uteri, it has never, so far as I know, been found in the position above described.

THE CLITORIS.

Extremely little has been done on the clitoris of Marsupials. Those who early described the genital organs in

the Marsupialia were content to describe its external form, without paying any attention to its minute structure. Home made the statement that the clitoris in marsupials is comparatively large. Owen is responsible for the assertion that in those genera of marsupials in which the male has a divided penis, the female possesses a correspondingly split clitoris. Brass concurred in this view, but it was shown by Hill not to be the case in Perameles. Van den Broek later showed in detail that a relation exists between the sinus vaginalis (median vaginal apparatus) and the clitoris, in that a split clitoris is found in those marsupials in which the median vaginal cul-de-sacs are completely separated, while in those forms possessing a single median vagina the clitoris is correspondingly simple and undivided. This latter case is characteristic of the Macropods.

As regards the minuter structure of the clitoris, it was first attempted in the case of Perameles by Hill, and was continued by Van den Broek for the genera Phascolarctos, Phascolomys, Halmaturus, Didelphys, and others. As a result, the latter writer was able to deduce some generalities on the structure and insertion of the clitoris in this group. Histologically, he found that all marsupials possessed a median septum clitoridis complete or incomplete, and that the erectile tissue of the clitoris formed either a single or a paired mass. The insertion of the clitoris may take place by means of an intervening septum clitoridis, which gradually disappears cranially, or, as in Didelphys, no such preputium may be present, when the insertion takes place by mere approximation of the ventral wall of the clitoris on the wall of the cloaca, the epithelial septum so formed afterwards disappearing cranially.

In Sarcophilus, I find that the clitoris possesses structurally features of interest which, in the present early state of our knowledge of this organ in marsupials, make it worthy of description.

EXTERNAL FEATURES OF THE CLITORIS.

The organ with its surroundings is shown in Fig. 4.

Looked at from above the clitoris presents two well-marked lateral portions, united towards the apex, and diverging considerably towards the base. The line of union is shown just in front of the apex by a fine, narrow,

superficially placed longitudinal groove. Apically, this groove bifurcates so that the clitoris here seems to consist of three lobes, which at the apex are slightly free from one another. The two grooves separating them pass backward and downwards towards the ventral side of the clitoris, and pass forward underneath, being gradually lost. Ventrally, therefore, the clitoris also presents the appearance of being divided into three lobes by two shallow grooves.

The two main portions of the clitoris (looked at from above) become widely separated cranially, and fuse with the ventro-lateral wall of the cloaca. Arising between these from the clitoris floor, and separated from the lateral clitoris folds by somewhat deep grooves, which extend ventro-laterally below the lateral clitoris folds, is a fold which, being caudally weak and of no great height, on being traced in a cranial direction, becomes considerably stronger, but not so strong as the lateral clitoris folds. Sections show that this fold is continuous with the median apical lobe of the clitoris This fold splits anteriorly into two divisions, separated by a deep narrow groove, which, passing forward, loses itself in a very deep pouch, downwards into which also pass the folds enclosing it. Into this pouch two other pairs of folds pass; one pair is continuous with two from the urogenital canal, and themselves secondarily folded, lose themselves in the pouch. Bounding the pouch laterally and passing into it is a pair of transverse folds, one on each side. Each of these arises from about half-way up the lateral side of the urogenital canal, and forms a swollen fold of mucous membrane which almost touches its fellow of the opposite side. The floor of this pouch is much sunk below the general level of the floor of the urogenital canal, and is considerably thinner.

MICROSCOPIC STRUCTURE OF THE CLITORIS.

The following account is based upon the examination in serial sections of 15 micra in thickness, both transversely and horizontally, of the clitoris in the virgin, and in an old adult female with pouch young:—

Immediately behind the apex of the clitoris, the cloacal wall is raised into a median cloacal fold. This is separated from the clitoris by a narrow but deep transverse groove, curved in accordance with the shape of the apex of the clitoris. Histologically this fold is found to be ex-

tremely vascular, containing a small proportion of erectile tissue, and is fairly abundantly supplied with nerve tissue. It is partially divided by epithelial septa similar to those of the clitoris.

Sections through the caudal portion of the clitoris a little in advance of the apex show that the organ is here divided into three lobes by two complete septa. Each of these septa is caudally converted into the shallow grooves separating the three free apical portions of the organ. These septa, as will be shown later, correspond to the lateral septa, such as occur, e.g., in Phascolarctos (3, Pl. 4, Fig. 4). This portion of the clitoris is shown in Fig. 5. From each of these main lateral septa passes off into the lateral clitoris lobes, secondary lateral septa, each passing ventro-laterally to end in a slight swelling, making the septum somewhat club-shaped. No such secondary septa occur in the central lobe. In this central portion we find in the mid-line extending upwards a short distance from the ventral clitoris wall a short epithelial septum representing what is left of the original median septum of the clitoris which is found in the pouch young of this genus, and which persists in those marsupials (e.g., Perameles, vide 5, p. 56, Pl. 5, Fig. 9), in which the clitoris remains in the adult in a somewhat primitive condition.

The clitoris wall is seen to be composed of a stratified epithelium continuous with that of the cloaca. This epithelium consists of a basal layer of cells with large nuclei arranged at right angles to the surface, these being overlain by four or five layers of flattened pavement cells.

The lobes of the clitoris are extremely vascular, containing numerous large thin-walled vessels. Where the rudiment of the median septum appears an alteration occurs in the main lateral septa. Each of these consists of two portions—a dorsal portion, and a ventral, the division between being marked by the origin of the secondary lateral septum. A change takes place in such a way that the ventral portion gradually disappears, leaving only the dorsal portion in connection with the secondary lateral septum. For a time the lowest portion of the ventral division persists, but in time also disappears, the clitoris possessing now two diverging septa composed mainly of the secondary lateral septa, which now extend somewhat in length ventro-laterally. Round the ends of these septa the vessels of each lobe enter into communication.

In close proximity to the ends of these septa appear the masses of erectile tissue. These are somewhat more differentiated than is the case in any marsupial as yet described. Each mass is caudally somewhat cylindrical, tapering off to a point, and consists of large thin-walled cells with muscle fibres, the whole surrounded by fibrous tissue. Pursued cranially they become somewhat flattened, and then semi-lunar surrounding the ends of the two septa. Relations have now been established between the ventral clitoris wall and the epithelium of the cloaca. This relation, in the absence of a preputium clitoridis, is extremely simple, and consists of a mere approximation and fusion of the two epithelia, the result being the formation of an epithelial septum, similar to the other septa of the clitoris (Fig. 7).

This fusion commences from the mid-ventral line. This process of formation of relations between the clitoris and cloaca differs from that of any marsupial so far described. As Van den Broek points out in Halmaturus and Phascolarctos, the fusion takes place caudally through the medium of a preputium, which disappears cranially, while in Didelphys, in which there is no preputium, the clitoris wall being placed laterally upon the cloacal epithelium, the former dwindling away, and only leaving the latter, the result being the formation of a median ventral space between clitoris and cloaca, bounded laterally by the lines of combination of the two.

The points where the septa of the clitoris meet the upper surface of that organ are indicated by longitudinal shallow furrows. These now invade the septa, gradually converting them by the diverging of their epithelial walls into deep narrow grooves.

A glance at Fig. 7 will show the internal structure of the clitoris at this height. The organ has lost its superficial trilobed structure, the two lateral lobes having overgrown the central one, which has correspondingly diminished in size. The latter is separated from the former by incomplete septa, which are being converted into grooves. These grooves pass ventro-laterally from the bottom of the single longitudinal groove of the upper surface of the clitoris, this representing the upper portion of the median septum of other marsupials. The ventral portion of that septum remains in the section as evidence of the combination of two lateral portions of the clitoris. For a fair distance on either side of this latter portion of the median

septum the approximation of the clitoris on the cloacal wall is evident. Here and there small evanescent septa are formed as ingrowths of the clitoris epithelium. Erectile tissue is present in two curved bands round the ends of the two septa. Outside these latter the vessels are mainly arranged in somewhat horseshoe-shaped masses. Ventral to the vessels on either side of the ventral median septum is abundance of nerve tissue.

As the fusion of the two approximating epithelia continues, the clitoris sinks by degrees further and further into the submucosa of the urogenital canal. As soon as this is complete, absorption of the septum so formed takes place, beginning at the upper end, the last remaining remnants being on the mid-ventral side.

The general clitoris tissue and that of the urogenital canal form now a homogeneous mass, except that the clitoris is immediately recognisable by its vascularity. (Fig. 8).

The erectile tissue here consists in section of two lateral well-marked curved bands, separated in the midline by a septum consisting of fibres crossing from the upper to the lower sides of the outer fibrous sheath. This septum is by no means continuous, but is interrupted at intervals to allow the lateral portions of the corpora cavernosa to communicate. (Fig. 9).

The lateral portions of the clitoris, up till now the most important, begin to lose their identity in the urogenital canal, and there is a corresponding increase in growth of the central clitoris fold. The vessels of the lateral portion pass into the main vessel of the central fold. This fold now becomes longitudinally divided by a groove, caudally shallow, but cranially deepening considerably. The two portions of erectile tissue diverge considerably, and sink deep into the wall of the urogenital canal. Anteriorly they are found to end in the bulb-shaped swelling illustrated in Fig. 11.

In sectioning the clitoris of a pouch young of Sarcophilus, I find that apically the organ is completely divided by a septum. The septum followed cranially in sections bifurcates, and thus divides the clitoris into three parts. The lower ends of the two lateral septa so formed become withdrawn, and the arrangement is that shown in Fig. 10. From this the adult structure is developed. The arrange-

ment of middle portion of the adult clitoris (Fig. 7) is directly derivable from that in the pouch young by the conversion of the upper portion of the median septum, and the lateral septa into grooves. The growth of the middle fold so formed, the completion of the lateral septa, and the appearance of secondary lateral septa, lead to the arrangement at the apex of the adult organ, while the further development of the median septum, and after it a groove, in the middle portion, with the dwindling away of the lateral portions, presents us with the arrangement shown in the cranial portion of the adult clitoris (Figs. 5 and 6).

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EXPLANATION OF PLATES.

Fig. 1.—Photomicrograph of a transverse section of the virgin uterus of Sarcophilus.

Fig. 2.—Photomicrograph of the section of uterus a little enlarged.

Fig. 3.—Photomicrograph of the left lateral vagina, showing the remains of the Wolffian duct. The larger open space towards the right of the figure is the vagina, while the much smaller canal towards the left represents the persisting Wolffian duct.

Fig. 4.—Reproduction of a drawing (from photograph) of the clitoris of Sarcophilus and the region surrounding it.

Figs. 5, 6, 7, 8, and 9.—Photomicrographs of transverse sections of the clitoris of Sarcophilus at various heights, from the apex forwards.

Fig. 10.—Photomicrograph of a transverse section of the clitoris of a pouch young of Sarcophilus.

Fig. 11.—Photomicrograph of a horizontal section of the terminal cranial portion of the clitoris in Sarcophilus.

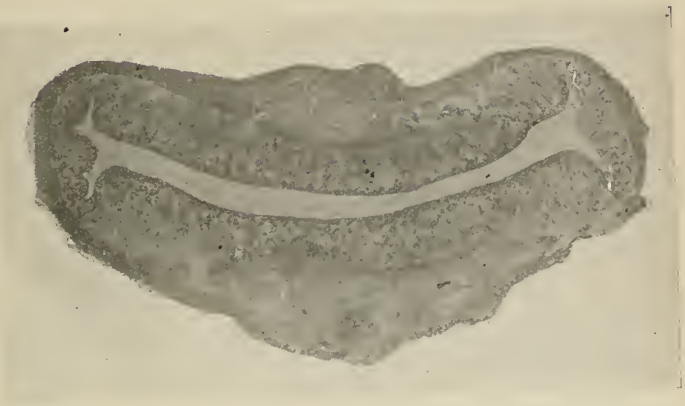

FIG. 1

FIG 2.

Fig. 3

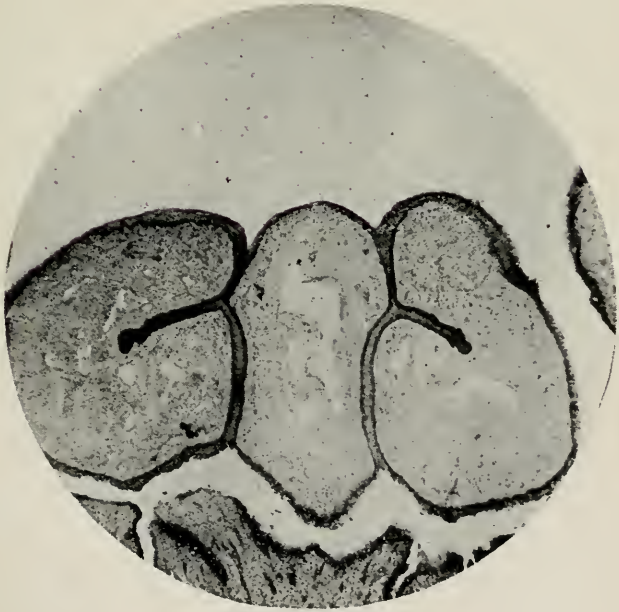

Fig. 5

FIG. 11

FIG. 4

Fig. 6

Fig. 7.

FIG. 8

FIG. 9

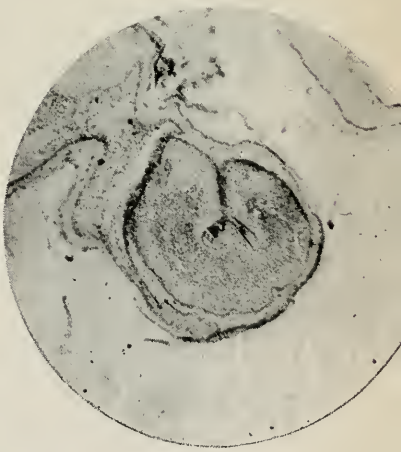

FIG. 10