THE DEVELOPMENT OF THE UROGENITAL SYSTEM IN THE MARSUPIALIA, WITH SPECIAL REFERENCE TO TRICHOSURUS VULPECULA

PART I

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EDITED WITH AN INTRODUCTORY NOTE BY
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INTRODUCTORY NOTE

The present memoir is the result of the independent investigations of the two authors whose names appear in the title. The research was originally undertaken at my suggestion by Miss Buchanan during her tenure of a travelling scholarship of the University of Melbourne in 1914, and was intended to afford as complete an account of the morphogenesis of the marsupial urogenital system as the available material would permit. Miss Buchanan devoted her attention more particularly to the earlier stages of development, concerning which practically nothing was known, and embodied her results in a thesis which, along with other contributions, was accepted for the degree of D.Sc. by the University of Melbourne. This thesis has not been published and is incorporated in the present memoir. Owing to the

limited time at her disposal, Miss Buchanan was unable to deal in any detail with the later stages. As these seemed worthy of detailed study, notwithstanding the extensive investigations of van den Broek, I invited Miss Fraser to continue the research. In order to do so, Dr Fraser found it necessary to make an independent study of the earlier stages, and with the observations, models, and drawings of Miss Buchanan before her, she has been able to supplement the latter's account of these stages, whilst, with much additional material at her disposal, she has provided an extended account of the later development.

The work has been carried out under my direction, and wherever the two observers have arrived at a different interpretation, I have exercised my editorial functions, and the view expressed is that which seemed to me most in accordance with the evidence.

The authors desire to express their thanks to Miss E. A. Steele for the beautiful drawings of the models represented in figs. 23, 24 and 25.

PRONEPHROS AND MESONEPHROS

Our three earliest embryos of *Trichosurus vulpecula* (Stages I, *a*, *b*, and *c*) measure 5 mm. in greatest length (*G.L.*). The anterior end of the Wolffian duct in (*a*) first appears on the right side on a level with the third spinal ganglion, but, after running for ·05 mm., it is absent for ·08 mm., again reappearing in the sections between the third and fourth spinal ganglia at the same time as the duct of the left side. In (*c*) we find the anterior end of the duct opposite the fourth spinal ganglion on both sides, but on the right, after only three sections (·03 mm.), it is again discontinuous for ·07 mm. From this region down to between the fifth and sixth spinal ganglia are seven or eight degenerate excretory tubules on each side. In (*a*), the first four of these are the most rudimentary; they are smaller than the remainder and more or less solid, whilst the hinder ones are partly tubular. One or two are isolated—for example, the first on the right, which lies medially to the anterior end of the first portion of the Wolffian duct—but the majority extend out from the dorso-medial side of the duct, and are not connected with the coelomic epithelium (plate I, fig. 1, *p.t.*). On the left, however, two, and on the right, one, are united with the latter by a solid cellular cord. In (*c*) the conditions are similar, although the excretory remnants are still more degenerate, seven being exceedingly small, and all solid. As in (*a*), there are no definite ciliated funnels, but on the left side two of the cords are connected with the coelomic epithelium by a solid strand of cells. There is no metamerism. Plate I, fig. 2 shows a longitudinal section through the anterior end of the left Wolffian duct in (*b*). In all probability these anterior degenerate tubules are vestiges of the pronephros.

From the level of the interspace between the fifth and sixth spinal ganglia backwards, there follow about fifty more tubules, the first of which, especially

in (*c*), is distinguished from the more anterior remnants by its larger size and tubular structure; behind these, down to the level of the twenty-fourth spinal ganglion, are still others in process of formation. All the tubules are united with the dorso-medial or medial side of the Wolffian duct, and do not appear to be connected with the coelomic epithelium. The majority communicate with the cavity of the duct, but some, especially at the anterior end, are only joined with the latter by a solid cord. The largest tubules are simple and almost straight, and have a narrower portion, the future collecting duct, uniting them with the Wolffian duct; glomeruli are not yet developed. In (*b*), at the anterior end of the left side, two of the collecting ducts have each two tubules running into them. In this same embryo, there appear to be four or five tubules to a segment at the posterior end, but further anteriorly there are apparently only three, or rarely two. As is known, the number of mesonephric tubules to a segment presents great variation in different mammals. In an embryo of the bat, *Rhinolophus hipposideros*, with twenty-one somites, Van der Stricht (34) describes two to a somite towards the posterior end of the body, and three further anteriorly. In the human embryo, the mesonephric tubules are also dysmetameric from the first, and four may occur to one segment (Felix (14)). Neither is there any strict metamerism in the early mesonephros of the Spermophilus embryo (Janosik (21)), nor in the rabbit, according to Schreiner (31), where three or four tubule primordia are usually found to a segment.

Stage II (*a*, β '98 and *b*. γ '99) measures only 4·5 mm. *G.L.*, but is distinctly older. In embryo (*b*). the first excretory tubule is solid, and arises from the dorso-medial side of an isolated portion of the Wolffian duct; it curves round medially and ventrally to meet the coelomic epithelium, at which place there is a definite nephrostome (plate I, fig. 4, *c.f.*). Altogether in (*b*), there are about seven rudimentary tubules at the cranial end of the excretory organ, the hinder ones being on the whole less well developed than the more anterior. They all arise from the dorso-medial side of the duct, and most of them are connected with the coelomic epithelium; the majority are small, and all are solid, with the exception of the fourth on the right side, which is especially well developed, and consists of a hollow proximal portion, arising from the dorsal side of the Wolffian duct, followed by a narrow solid piece running medially, and a wider solid distal section extending up dorsally between the aorta and the cardinal vein. In (*a*) much the same conditions prevail, but in this embryo, the degenerate remnants are not so easily recognised, and are less sharply marked off from the more definite posterior tubules. Almost all the cords are tubular, and several, most noticeably on the left side, are made up of a medial tubular portion, united, on the one hand, by a narrow solid cord with the Wolffian duct, and, on the other, by a narrow or wider solid connection with the coelomic epithelium, where, in a few cases, a rudimentary nephrostome may be seen (plate I, fig. 3, *c.f.*).

From the sixth spinal ganglion backwards occur a further series of tubules,

which are much larger than the preceding ones. In (*a*) for example, on the left side, they number approximately fifty-seven. The majority are hollow, have roughly an S-shaped form, but four or five at the anterior end are more simple. At about the thirty-seventh tubule, the coiling becomes very slight, and the thirty-eighth to the forty-seventh tubules are joined to the Wolffian duct by a solid or only partially hollow cord of cells. The forty-eighth is isolated, being altogether separated from the duct. On the right side, the first two or three tubules, starting from the sixth spinal ganglion, exactly resemble those occurring anterior to this ganglion; they are small, and apparently united by solid epithelial cords with the coelomic epithelium. Posterior to these, are four longer but simple tubules, and behind again, we find about fifty-one more, of which the thirty-sixth to the forty-seventh have only a solid connection with the Wolffian duct, and the last eight or nine are quite isolated.

Stage III (α '97), which measures 7 mm., shows a further degeneration at the anterior end of the excretory organ, the first tubule occurring on a level with the fifth spinal ganglion, close behind the cranial end of the Wolffian duct. The latter is again discontinuous on both sides. The first one or two rudimentary epithelial cords are solid, whilst the following three are tubular, and all, except the fourth on the right, which is very small, arise from the Wolffian duct, and have more or less degenerate nephrostomes. Behind these, the tubules are larger and more definite, and curve up from the Wolffian duct on the medial side of the cardinal vein, being no longer united with the coelomic epithelium. Just behind the fifth spinal ganglion on the left side, and on a level with the second epithelial cord, is an outbulging into the coelom, situated ventro-medially to the primordium of the suprarenal body. We probably have here a rudimentary external glomerulus. Near the eighth spinal ganglion, Malpighian corpuscles are beginning to develop, and further back, where the tubules are larger and more curved, they become more definite. Tubules are still forming at the posterior end, the last running into the mesoderm of the metanephric blastema.

The next stage (Stage IV, XIX '04) is an embryo measuring 7·5 mm., and here, rudimentary tubules are first found between the fifth and sixth spinal ganglia on the right, and opposite the sixth on the left side. From this level down to the eighth spinal ganglion, opposite which the first glomeruli are seen to be forming, are ten degenerate tubules on the right side, and eight on the left. They are either partly tubular or altogether solid, and many are short; only one on each side is not joined with the Wolffian duct, the majority taking their origin from the medial side of the latter, but there is a certain amount of variation, some arising from the duct more dorsally. Four tubules on the right and two on the left are each connected with the coelom c epithelium by a solid cellular cord.

The posterior tubules are well developed at this stage, and possess well marked glomeruli (plate I, fig. 6). They are differentiated into (1) a narrow

collecting duct (*c.d.*), (2) a median larger portion (*m.p.*), and (3) a thinner segment (*t.s.*) connected with (4) the Malpighian capsule, containing the glomerulus (*gl.*). Towards the posterior end, however, they are less well developed, and the last few are isolated from the Wolffian duct; more tubules are still forming.

Stage V (II '01) measures only 6 mm., the body of the embryo being greatly curved. Here, from the level of the sixth spinal ganglion to the eighth, are nine degenerate tubules on the left side, and seven on the right. With the exception of two on the right and one on the left, which are quite isolated from the Wolffian duct, they are all united with the dorso-medial side of the latter. The greater number are partly tubular, and several run up round the medial side of the cardinal vein (plate II, fig. 8). On the right, all except the first one are joined with the coelomic epithelium by a more or less solid cord of cells, sometimes closely adjacent to the suprarenal primordium (plate II, fig. 8, *c.f.*), but on the left, all are separated from it. On a level with the second tubule and with the sixth spinal ganglion, we again find, on the left side, in the same position as in Stage III, what may, with great probability, be regarded as a rudimentary external glomerulus (plate II, fig. 7, *e.gl.*).

Posterior to the eighth spinal ganglion, follow roughly forty-five tubules, the best developed much resembling those of the last stage. At the hinder end, at the level of the twenty-fifth spinal ganglion, tubules are still forming.

By Stage VI (XX '04), an embryo of 7·75 mm., we find the anterior end of the Wolffian duct still further reduced, and it now lies on a level with the eighth spinal ganglion. From here to the eleventh spinal ganglion, are twelve atrophied excretory tubules on the left, and nine on the right. Of these, the anterior ones are mostly small isolated fragments whilst the more posterior are better developed, being longer and partly tubular, but only one or two are connected with the coelomic epithelium by a solid cord. The eighth tubule on the left is elongated, arises from the dorsal side of the Wolffian duct, and is connected with the coelomic epithelium close to the suprarenal body, recalling the third tubule on the right in Stage V (plate II, fig. 8).

Posteriorly, the tubules are considerably advanced in development as compared with the last stage, and possess well-marked Malpighian corpuscles. The hindermost are small, the last on the left being especially so; it is isolated from the Wolffian duct, and runs into the metanephric blastema. On the right, the last tubule is united with the duct, and has no connection with the blastema; on this side, where new ones have ceased to form, we counted about forty-two tubules connected with the Wolffian duct.

In our next series of embryos, which measure 7·25 mm. (Stages VII, III '01, and VIII *a* and *b*, XII '02), there is continued reduction at the anterior end of the mesonephros. The Wolffian duct, which is again discontinuous, first appears between the ninth and tenth spinal ganglia, except on the left side in Stage VII, where it is not present until between the tenth and

eleventh. The anterior end of the duct on the left side lies in an indentation on the ventral or ventro-medial side of the cardinal vein.

From the anterior end of the Wolffian duct down to between the eleventh and twelfth spinal ganglia, where glomeruli first appear, we find from five to seven atrophied mesonephric tubules. They are mostly degenerate, being partly tubular or altogether solid, and arise, as usual, from the dorso-medial or medial side of the Wolffian duct.

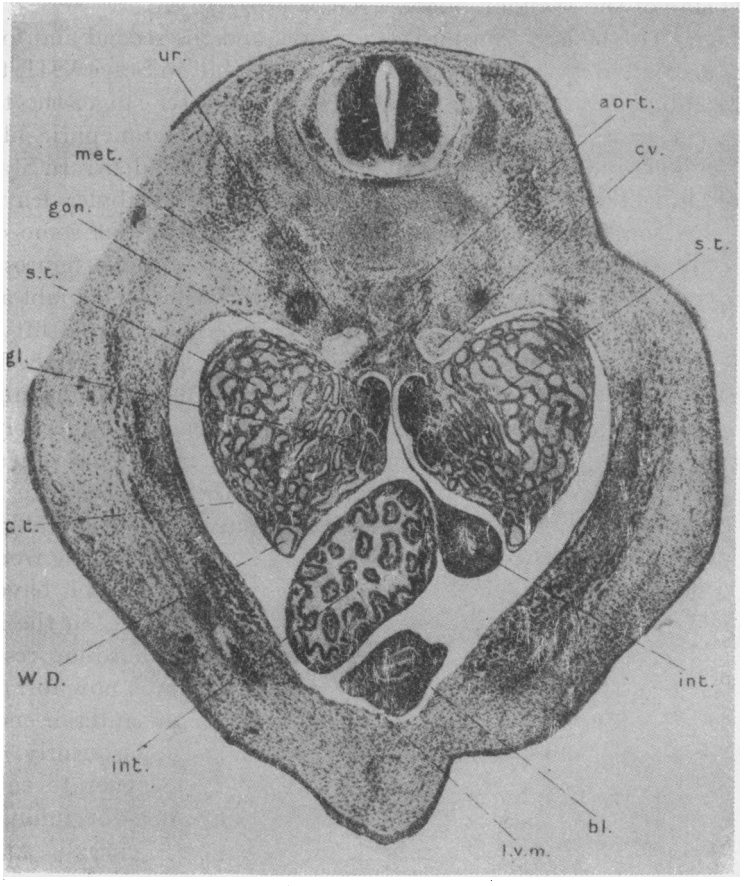
In Stage VII, the first four on the left side, and the second and fourth on the right, are united with the coelomic epithelium, but in Stage VIII, they are apparently all separated from the latter. In the latter stage, the terminal tubule of the left side is still continuous with the metanephric blastema, but on the right, and in Stage VII, they are quite independent. In Stage VII, there are altogether about forty-nine well-developed tubules leaving the Wolffian duct on the right side, and forty on the left, but some of these subdivide immediately after leaving the duct, so that these figures do not represent the maximum number of tubules. This branching no doubt accounts for the fact that the number of glomeruli, of which we counted fifty on the right, the first two being small, and fifty-five on the left, where the last two are only partially developed, is greater than the number of collecting ducts.

The next stage (Stage IX *a*5 '97, and *b*, IV '01) is considerably older, and measures 8·5 mm. (*G.L.*). The cranial end of the mesonephros has undergone still further atrophy, and now extends from between the fifteenth and sixteenth spinal ganglia down to the level of the twenty-fifth spinal ganglion. The Wolffian duct runs slightly cranially to this level, and, arising from it, are four or five very small tubular remnants, one or two of which have a thin solid connection with the coelomic epithelium. One tubule, on the left side in (*b*), is united with the latter close to the suprarenal primordium, resembling some we have seen in preceding stages. The mesonephros now forms a conspicuous and well-marked organ, which is narrower at the anterior end, where the tubules are less well developed, and increases in size posteriorly.

By Stage XI (*G.L.* 10 mm., VI '01) we find no degenerate tubules at the anterior end of the mesonephros, well developed ones beginning almost directly. In this embryo we counted about forty-eight glomeruli. At 11 mm. *G.L.* (Stage XII), the organ extends from the nineteenth spinal ganglion down to the twenty-fifth, its posterior portion, especially behind the genital ridge, being thickest and containing large glomeruli.

In the new-born foetus of 14·5 mm. *G.L.*, 6·5 mm. *H.L.* (Stage XV *b*), the mesonephros (text-fig. 1) extends from the twentieth to the twenty-fifth spinal ganglion, and has altogether a length of 1·9 mm. with a dorso-ventral measurement of ·59 mm. at its posterior widest end. It is now attached by a narrow mesentery on the medial side, and possesses a convex surface on the dorsal and lateral aspects as far as the level of the Wolffian duct (*W.D.*), whilst its ventro-medial surface, along the medial side of which runs the primordium of the gonad (*gon.*), is only slightly convex or almost flat. This

is well seen in a transverse section (text-fig. 1). The gland is composed of many coiled secretory tubules (*s.t.*), separated from each other by very thin layers of connective tissue, and each secretory tubule runs into a collecting or terminal tubule (*c.t.*), which communicates with the Wolffian duct. The outlines of the cells of the secretory tubules are not always clearly marked; in the collecting



Text-fig. 1. Stage XV *b* (XIV '02, new-born), *G.L.* 14·5 mm. Microphotograph of a transverse section, showing the mesonephros towards its posterior end. The hinder end of the metanephros (*met.*) is also seen, lying dorsal to the mesonephros. *aort.* aorta; *bl.* bladder; *c.t.* collecting tubule; *c.v.* cardinal vein; *gl.* glomeruli; *gon.* gonad; *int.* intestine; *l.v.m.* ligamentum vesicae medium; *met.* metanephros; *s.t.* secretory tubule; *ur.* ureter; *W.D.* Wolffian duct. (Sl. 23–4–6.)

tubules, the epithelium is lower, stains less deeply with eosin, and possesses much more distinct cell outlines; in general it resembles that of the Wolffian duct. Malpighian corpuscles (*gl.*) are large and conspicuous, and lie in one or two rows near the ventro-medial surface. The majority are considerably

compressed dorso-ventrally, and are widest in the transverse direction. In the several newly born young, which we examined, the larger glomeruli had an average diameter of ·19 mm. (transversely) × ·15 mm. (antero-posteriorly) with a dorso-ventral measurement of only ·08 to ·1 mm. They numbered thirty-nine to forty-eight on each side, the first few being usually very small.

In pouch-young with head lengths of 6·5 to 7·5 mm., the mesonephros is still fully developed, and is evidently actively functional. It extends over the same number of spinal ganglia, and the average size and number of the glomeruli show little variation compared with that of the new-born foetus. The posterior end, however, has become bent round ventrally. This end is especially well developed, having now a dorso-ventral measurement of ·79 mm. in one of our specimens, and here the largest glomeruli are formed.

The later history of the mesonephros, which, after this stage, begins to degenerate at the anterior end, will be considered in Part II of this paper.

DISCUSSION

Pronephros. Turning to the consideration of the cranial portion of the excretory system in Stages I to VI, the question at once arises as to how much of it may be regarded as pronephros and how much as mesonephros. From the material at our disposal we are not in a position to discuss the mode of origin of these anterior tubules, or how many unite together to form the Wolffian duct. At Stage I, the most anterior portion of the Wolffian duct is already interrupted and in process of degeneration; the pronephros, therefore, has probably undergone a certain amount of atrophy. In our first three stages, anterior to the sixth spinal ganglion, are a number of very small and degenerate tubules, which are distinguished from those posterior to this level by their small size and more rudimentary character. We regard it as exceedingly probable that these anterior structures represent the pronephros. Another fact in favour of this view is the occurrence, near the sixth spinal ganglion on the left side, of a rudimentary external glomerulus in both Stages III and IV; Felix (14), however, maintains that this does not conclusively prove that the tubule opposite this point is a pronephric one, as an external glomerulus may first appear when the pronephros is in full degeneration, and at a time when a mesonephric tubule has taken its place. We must confess that, in Trichosurus, there is absolutely no evidence of any such substitution of mesonephric for pronephric tubules.

At Stages III and IV we find the first indication of an internal glomerulus opposite the eighth spinal ganglion, therefore we conclude that all the degenerate tubules found behind this level in later stages must be mesonephric. The region, then, between the sixth and eighth spinal ganglia, is one of doubtful significance. The least rudimentary of the tubules occurring within this area are very similar to the degenerating mesonephric ones posterior to it. Both may extend up from the Wolffian duct round the dorso-medial side of the cardinal vein to become attached to the coelomic epithelium close to the

suprarenal primordium. The nephrostomes of the tubules cranial to the sixth spinal ganglion, when present, appear to be situated more ventrally, on the ventro-medial side of the urogenital fold. Mesonephric nephrostomes, occurring on the lateral side of the fold, such as are described in Echidna by Keibel (24), are never found in Trichosurus, whilst the pronephric tubules and nephrostomes, figured by this author, correspond almost exactly with apparently mesonephric structures in Trichosurus. In the human embryo, according to Felix (14), all the mesonephric tubules develop an internal glomerulus before they undergo degeneration; if this may be taken as holding good also for Trichosurus, then the tubules between the sixth and eighth spinal ganglia must be part of the pronephros, which would thus consist of approximately fourteen to sixteen tubules. The most anteriorly situated glomeruli, which we have observed in this marsupial, lie opposite the eighth spinal ganglion; they are very imperfect and almost certainly never become functional.

In the embryo of Perameles (*2PA*), with fifteen to sixteen somites, excretory tubules are connected with the Wolffian duct as far back as the beginning of the thirteenth somite, after which level the duct extends back for some distance quite free. If the conditions in Trichosurus are similar to those in Perameles, and if we reckon on three somites to the head, then the pronephros in the former animal would reach almost as far as the tenth spinal ganglion.

This conclusion coincides fairly closely with the results obtained for other mammals. In her observations on the rabbit, Kerens (25) finds that the pronephric region—i.e. the region which gives rise to the Wolffian duct—extends from the eighth to the tenth myotomes in the mole, and from the seventh to the tenth in the rabbit. In both these cases, however, the canals are segmental, three being present in the mole and four in the rabbit. They may possess nephrostomes, but never become vesicles, and the Wolffian duct is only a solid cellular cord. In Trichosurus, the presumably homologous canals show no signs of metamerism, and often contain a cavity, whilst the Wolffian duct is always hollow, even at its anterior end. In man (Felix (14)), the pronephros reaches as far as the fourteenth somite, whilst the mesonephros, according to this author, begins at the ninth. Here, again, both tubules and Wolffian duct are more usually solid. In Echidna (Keibel (24)), the pronephric region lies opposite the fourth to the sixth spinal ganglia, and there appear to be about eight tubule primordia. In Spermophilus (Janosik (21)), the pronephros extends from the distal end of the sixth mesoblastic somite to the distal end of the twelfth; there are, however, no external glomeruli, and the six or seven canals develop without any signs of metamerism. In an embryo of the bat, ***Rhinolophus hipposideros*** (van der Stricht (34)), two pronephric vestiges are present between the seventh and eighth somites, and one between the ninth and tenth on the left side, whilst on the right, one is found opposite the seventh somite and two opposite the eighth and ninth.

We may therefore conclude that the pronephros in Trichosurus, although a rudimentary organ as in other mammals, is nevertheless at least as well marked as in man and in Echidna, and better developed than in the rabbit, the mole, Spermophilus and Rhinolophus.

Mesonephros. In early stages we have seen that the mesonephros undergoes a progressive degeneration at the anterior end; this begins before the hindermost tubules have completed their development, and continues up to Stage XI (*G.L.* 10 mm.), when the cranial limit of the organ lies opposite the seventeenth spinal ganglion. The mesonephric tubules apparently develop in the same way as in other mammals, and have a similar histological differentiation, each tubule consisting of a collecting portion and much coiled secretory portion, which is connected with a Malpighian corpuscle. The most anterior are incompletely formed, with rudimentary glomeruli; they quickly atrophy, and disappear without leaving a trace. Mesonephric nephrostomes, more usually in the form of solid cellular cords uniting the tubule with the coelomic epithelium, are often present, but they are either found in connection with rudimentary tubules or appear after the glomerulus has been lost. After Stages VI to VIII, no new tubules are formed at the hinder end, the mesonephros having reached its posterior limit at the level of the twenty-fifth spinal ganglion.

As already mentioned, the number of glomeruli on the left side at Stage VII exceeded the number of tubules by fifteen. In several places, two tubules were seen to originate from one collecting duct, so that new tubules evidently arise by budding from this duct; no division of the Malpighian corpuscles was observed. Van den Broek (10) has referred to the formation of sprouts from the mesonephric tubules in Trichosurus and Macropus, and states (p. 362) that the same process continues throughout life in these forms, as well as in Didelphys. In *Macropus ruficollis* (*D.C.L.* 34 mm.) this author describes and figures a short outgrowth arising from the secretory portion of a mesonephric canal near to a Malpighian corpuscle. The occurrence of such sprouts has been described in other orders of mammals by several authors. The number of tubules in Trichosurus apparently varies not only in different embryos, but on opposite sides of the same embryo. A corresponding variability has been recorded by Schreiner (31) in the rabbit.

Between the embryonic stage of 10 mm. *G.L.* and the foetal stage of 7·5 mm. *H.L.*, no further reduction of the mesonephros occurs; a few of the glomeruli, however, at the anterior end, always remain small, as do one or two at the posterior end. The number of glomeruli in these older stages varies from forty-one to fifty-one, including the smaller ones at the anterior end, and their diameters vary slightly, being always widest transversely and narrowest in the dorso-ventral direction. On the whole, they are largest at 10 mm. *G.L.*; here they numbered about forty-eight, many of them having a transverse measurement of ·20 mm. whilst some reached a diameter of ·27 mm. Although the mesonephros attains its maximum development during this

period, it possibly begins to function as early as Stage IV, when many of the glomeruli are apparently working, and the allantois is fairly well marked.

Van den Broek (10) has described and figured the mesonephros in *Didelphys cancrivora* (*D.C.L.* 38 mm.), in *Macropus ruficollis* (*D.C.L.* 34 mm.), and in *Dasyurus viverrinus* (*D.C.L.* 19·6 mm.). In the stages of the different marsupials examined by this author, the measurement of the glomeruli varied from a length of ·133 mm. (Didelphys, *D.C.L.* 38 mm.) to ·2 mm. (Macropus, *D.C.L.* 34 mm.), and from a width of ·08 mm. (Didelphys) to ·1 mm. (Macropus). In the foetus of Macropus with a length of 34 mm. thirty-two canals opened into the Wolffian duct, whilst there were altogether thirty-one glomeruli. In a male pouch-young of Trichosurus with a dorsal contour length of 32 mm., examined by van den Broek, the glomeruli measured ·160 mm. in length by ·066 mm. in breadth, but our observations show that the glomeruli in Trichosurus are still larger in younger stages, and appear to reach their maximum size in embryos with a length of 10 mm. Compared with the figures obtained in other mammals, of which Bremer (4) has given a rough summary in his table on p. 192, we find that, in proportion to the size of the animal, the mesonephros in Trichosurus is a large and exceedingly well developed organ, and we see no reason to doubt that it functions for a considerable period both before and after birth, as it is held to do in reptiles, in Didelphys (Selenka (33)) and in Echidna (Keibel (24)).

The question as to the functional significance of the mesonephros in the Amniota has not received very much attention. Weber, from his studies on various mammals, and Felix (14) in the case of the human embryo, consider that the mesonephros possesses no excretory function at all; they maintain that, if it had, there would necessarily be an interval during which there was no excretion, since the Wolffian body degenerates before the metanephros becomes active. It should be remembered, however, that in man the mesonephros is essentially a greatly reduced organ. On the other hand, on the ground of the histological appearance of the tubules, and the abundance of glomeruli, von Winiwarter (38), like most other workers, holds that the mesonephros in mammals does act as an excretory organ, and suggests that even when it has a rudimentary development, with few incompletely formed glomeruli, as, for example, in the case of the mole, the excretory function might be performed by the tubules alone, or partly with the aid of the placenta. He refers to the work of Jacqué[1] in 1902, who, from his experiments on the composition of the allantoic and amniotic fluids in the sheep and other mammals, concluded that the mesonephros secreted urine, and that the fluid passes, at the beginning of development, into the allantois, later, into either the allantois or amniotic cavity, and finally, exclusively into the latter. Oertel (28), in the absence of any definite evidence in favour of the excretion of urine by the mesonephros in mammalian embryos, for he is apparently unaware of Jacqué's work, has discussed the possibility of the

[1] *Mém. cour. Acad. roy. de Belg.* T. 63, 1902.

organ being one of internal secretion. For our part, we are strongly of opinion that the mesonephros in Trichosurus and other marsupials is actively excretory. The suggestion as to the possible participation of the placenta in embryonic excretion has been put forward by several investigators, and has received confirmation recently in the very interesting paper by Bremer (4) to which we have already referred. This author has considered in some detail the relations of the mesonephros, permanent kidney and placenta in different mammals, and concludes that in those mammals with a well developed mesonephros (e.g. pig, sheep, cat), the fluid excreted is discharged into the allantois, and that it does not usually pass through the urethral opening, even if the latter is present. In mammals, such as the rabbit, guinea-pig and man, in which the Wolffian body degenerates early, or in those in which a fully developed Wolffian body is absent, such as the rat and mouse, the urinary function is exercised by the placenta, which, in these forms, is found to be provided with what he terms "thin plates of epithelium, covering the foetal capillaries[1]." He holds that these "plates," through which an interchange takes place by osmosis between the foetal and maternal blood, functionally replace the glomeruli of the mesonephros, and states that they are not found in those mammals with a continuous embryonic excretion.

Like Selenka (33), Bremer notes the retention of an active Wolffian body in the opossum for some time after birth, before the kidney can replace it, and states (p. 187): "This in itself would prove that for this class of mammals, as in the lizard, the mesonephros is certainly functional as a urinary organ." He points out the close relationship in different mammals between the size and duration of the mesonephros and the size of the allantois. There is little doubt that in marsupials, the allantois, which is invariably present in this group, is used as the receptacle for urine during embryonic life. It is least developed in Dasyurus (Hill (20)), a circumstance which may possibly be correlated with the fact that, in this genus, the young when born are in certain respects in a more immature condition than in other marsupials, and therefore the length of time, during which the mesonephros functions in the embryo, is shorter. In Dasyurus, the cloacal membrane is not ruptured until birth, but in Trichosurus, and apparently in most of the other Didelphia, the cloaca opens out to the exterior some time before birth (*G.L.* 9·5 mm., *v.p.* 56), so that the possibility of some urine finding its way into the amniotic cavity in later stages is not entirely excluded.

METANEPHROS AND URETER

The primordium of the ureter first appears in Stage III (*G.L.* 7 mm., *a* '07) as a hollow bud-like outgrowth from the dorso-medial side of the Wolffian duct (plate II, fig. 9, *u.b.*) shortly in front of the opening of the

[1] Whether these "plates" are the thin layers of syncytio-trophoblast which enclose the lacunae filled by maternal blood, and between which the foetal capillaries are situated, or something quite distinct, is not clear from the author's account (J. P. H.).

latter into the cloaca, and on a level with the hind-limb. The metanephric blastema (plate II, fig. 9, *m.bl.*) is already well marked; it forms a condensed mass of mesenchyme surrounding the ureteric bud, and extending forwards to run into the last incompletely developed mesonephric tubule. It is thus continuous with the nephrogenic cord.

At Stage IV (*G.L.* 7·5 mm., XIX '01), the outgrowth from the Wolffian duct is apparently less well developed, but at Stage V (*G.L.* 6 mm., II '01), it is more advanced (text-fig. 3, *u.b.* and plate II, fig. 10, *u.b.*), and here the metanephric blastema more definitely encircles the distal end of the bud, appearing horse-shoe-shaped in cross section (plate II, fig. 10, *m.bl.*).

At Stage VI (*G.L.* 7·5 mm., XX '04), the distal end of the bud, destined to become the kidney pelvis, is enlarged, whilst the proximal portion, which develops into the ureter, is reduced in diameter. The metanephric blastema surrounds the primordium of the pelvis as a compact mass of mesenchyme, which becomes slightly less dense as it stretches up, on the right side, on a line with and very close to the last mesonephric tubule, and, on the left, directly into the latter. Many of the cells of the blastema are undergoing mitosis.

At Stage VIII *b* (*G.L.* 7·25 mm., XII '02), the ureter, immediately after its origin from the dorso-medial side of the Wolffian duct, turns medially and then sharply forwards, gradually enlarging as the distal end is approached (text-fig. 4, *u.b.*). The latter is roughly pear-shaped, has thickened walls, and a well-marked cavity, and is surrounded by the metanephric blastema. The much narrower and less dense anterior portion of the latter is prolonged forwards in Stage VII (*G.L.* 7·25 mm., III '01) dorsally to the last mesonephric tubule on each side, but in Stage VIII, at least on one side, it is still continuous with the small and incompletely developed last tubule at the hinder end of the mesonephros (plate III, fig. 11, *m.bl.*).

In an embryo with a length of 8·5 mm. (Stage IX), the ureter takes its origin from the Wolffian duct nearer to the cloaca (text-fig. 5, *ur.*), and has increased considerably in length. It now consists of a long and narrow tube, extending up dorsally to the hinder end of the mesonephros; its expanded distal end, forming the pelvis, lies on a level with the twenty-fourth spinal ganglion, some distance cranially to the posterior end of the mesonephric gland. The metanephric blastema is, at this stage, an independent formation, having no longer any connection with the hinder mesonephric tubules, which have now almost completed their development. It forms a thick layer of mesenchyme covering the pelvis; near the walls of the latter, its cells are dense and darkly staining, but near the periphery of the mass, they are looser and paler. A division into the inner and outer zones of Schreiner (31) can perhaps be distinguished, but is as yet not very definite. In the transverse series (Stage IX *b*, IV '01), the pelvis is expanded and elongated, and is surrounded by thick undulating walls, the first indication of the future primary collecting tubules.

At Stage XI (*G.L.* 10 mm., VI '01), the ureter now arises from the

medial side of the Wolffian duct (text-fig. 7, *ur.*). The pelvis is distinctly lobed, and cranial and caudal prolongations, destined to develop into the first collecting tubules, are now formed, the latter curving round ventro-laterally. The ureter at this stage appears to run out from about the mid-region of the medial side of the pelvis. The blastema is differentiated into an inner zone, which surrounds the pelvis as a thin dense cap, and an outer broader zone, which is more lightly staining.

In the embryo with a length of 11 mm. (Stage XII), the ureter leaves the medial side of the Wolffian duct by a narrow opening, just at the point of junction of the latter with the urogenital sinus (text-fig. 9, *ur.*). This opening appears to be closed in some of the embryos[1]. The ureter curves round dorsally, and runs anteriorly, almost parallel with and dorso-medially to the Wolffian duct, to enter the pelvis at a distance of about one-third from its hinder end. The pelvis now lies dorsally to the mesonephros on a level with the twenty-third spinal ganglion, but stretches in front of and behind this level. It is elongated in the antero-posterior direction, and, owing principally to the ventral curvature of the well-developed caudal pole, appears arc-shaped in sagittal section. On the right side, besides the well-marked cranial and caudal prolongations, are four other small primary collecting tubules, three arising from near the ventral side of the main body of the pelvis and one from the dorsal side; one of these already shows signs of division into two secondary tubules. The metanephric tissue, closely surrounding the pelvis, is becoming pushed outwards by the developing tubules, and is assuming a slightly lobulated appearance. By Stage XII *b* (*G.L.* 11·5 mm.) we find two more primary tubules arising from the dorso-medial side of the pelvis, whilst the inner zone of metanephric tissue, consisting of a thin investing layer of darkly staining cells, has now become divided up round the best developed tubular outgrowths, into isolated caps of cellular tissue. In the transverse series of this stage, the margin of that part of the cap, situated on the pelvic side of the secondary tubules, has thickened to form metanephric spheres, and at Stage XIII (*G.L.* 12 mm.) these spheres have become transformed into small metanephric vesicles. In the next embryo (Stage XIV, *G.L.* 13 mm.), there are eight well-developed primary collecting tubules, the secondary tubules are more marked, and the metanephric vesicles more numerous; the gland now reaches as far forwards as the twenty-first spinal ganglion. Plate III, fig. 12 shows a section through a primary collecting tubule (*p.c.t.*) in an embryo of 13·5 mm. *G.L.*, from which two secondary tubules (*c.t.*) have arisen; the margin of the metanephric cap (*m.bl.*) on the side towards the pelvis is thickened, and becoming transformed into metanephric vesicles (*m.v.*).

In the new-born foetus (Stage XV, *G.L.* 14·5 mm.), the whole kidney has increased considerably in size, and now lies opposite the twentieth and twenty-first spinal ganglia, on a level with the suprarenal body. In a trans-

[1] Beard (1) finds the ureter solid in a Trichosurus embryo with a length of 14 mm.

verse series of this stage, we find that the metanephric vesicles have already united with the collecting ducts of the pelvis, and have elongated to form the secretory tubules which are beginning to curve round, and Bowman's capsules are becoming differentiated. In the longitudinal series (plate III, figs. 13 and 14), development has progressed still further; the secretory tubules (*s.t.*) are definitely S-shaped, and Bowman's capsules (*b.c.*) more invaginated by the ingrowing glomeruli. The ureter runs out directly from the pelvis, dorsally to the gonad, and dorso-medially to the mesonephros. Near the cranial end of the urogenital sinus, well behind the Wolffian ducts, the ureters (text-fig. 13, *ur.*) curve round ventrally, and then run antero-ventrally, to open, no longer into the Wolffian duct, but directly into the base of the bladder primordium. Their apertures are situated close together on a distinct papilla (*p.*), which lies medially to, and a short distance in front of, the openings of the Wolffian ducts (*W.D.*) into the sinus (*u.g.s.*), and which is directed towards the fundus of the bladder. The formation of this papilla, medial to the openings of the Wolffian ducts, recalls the condition in Echidna (Keibel(24)) (cf. text-figs. 11 and 13); in the latter, however, the papilla projects from the dorsal wall of a widened part of the urogenital sinus immediately below the bladder.

In pouch-young with a head length of 6·5 mm. (*G.L.* 16·5 mm.) glomeruli are becoming more definite, and soon afterwards (*H.L.* 7·5 mm.), numerous fully formed Malpighian corpuscles are present.

As the bladder, the formation of which is described later (p. 70), increases in size—it is already well established in pouch-young with a head length of 7·5 mm.—the apertures of the ureters become drawn forwards, so that the characteristic bend, in the posterior region of each, comes to lie wholly in front of the hinder portions of the Wolffian ducts (*H.L.* 15 mm., text-fig. 15, *ur.*). The ureters pass forwards through the tissue of the genital cord to open as before on a papilla, the bifid tip of which projects into the base of the bladder a long way in front of the opening of the Wolffian ducts (text-figs. 14, 15 and 17)[1]. A definite urethra is thus developed. As the female approaches the adult condition, the terminal bend of the ureter, though still retained, becomes less marked, the duct passing forwards between the lateral and median vaginae on each side to enter the bladder.

[1] In a male pouch-young of Trichosurus *with a dorsal contour length of* 32 *mm.*, van den Broek (10, p. 330) describes and figures the posterior curved region of the ureters as lying altogether behind the terminal portions of the Wolffian ducts. The ureters pass forwards to penetrate obliquely through the tissue of the dorsal wall of the urogenital sinus between the orifices of the Wolffian ducts, and open into the bladder on two papillae lying just cranially to these orifices. In our series a somewhat similar condition occurs in pouch-young with a *head length of* 7·5 *mm.* (*G.L.* 17 mm.). In slightly older stages the whole of the posterior curved portions of the ureters come to lie in front of the Wolffian ducts, as stated above.

DISCUSSION

The development of the metanephros in Trichosurus agrees very closely with that of other mammals—e.g. rabbit, man, pig—as described by Schreiner (31), and therefore has not been considered in great detail. As in these forms, so in Trichosurus, the metanephric blastema arises from the nephrogenic tissue, which is the caudal continuation of the blastema out of which the mesonephric tubules develop. As in the rabbit, the primordium of the hindermost tubule, with which the metanephric tissue is still connected in Stages VI, VII and VIII, on one or both sides, is often in the form of a small vesicle, which probably never develops into a fully formed excretory tubule. The metanephric tissue surrounds the distal end of the developing ureter, and moves with the latter as it grows forwards dorsally to the mesonephros; the secretory tubules undergo the usual mode of development.

Glomeruli first begin to develop in the foetus of *G.L.* 16·5 mm. (*H.L.* 6·5 mm.), and are more marked in the foetus of *G.L.* 17 mm. (*H.L.* 7·5 mm.), at a time when the anterior end of the mesonephros first shows signs of degeneration (cf. Part II of this paper). For some time after birth, therefore, the metanephros is incapable of functioning, and the excretory activity must be carried on by the mesonephros, which is at its highest development in the new-born foetus. Although in the foetus with a head length of 7·5 mm. (*G.L.* 17 mm.) the excretory canals of the kidney are still very imperfect, they develop very rapidly, and the permanent kidney must soon begin to excrete. As the hinder end of the mesonephros still appears functional, having well-marked glomeruli, in a foetus with a head length of 12·5 mm., the two excretory organs evidently function together for a considerable period. Van den Broek (10) has observed similar conditions in Macropus, where he states (p. 366) that the existence of glomeruli in the primordium of the kidney of Macropus (*D.C.L.* 34 mm.), when the mesonephros is apparently at the height of its development, and undoubtedly functional, is an appearance, which carries with it the possibility of the two kidneys acting simultaneously. The mere presence of glomeruli, he holds, is not by itself evidence of functional activity, since the formation of these structures precedes the union of the excretory canals with the collecting ducts. In this particular, marsupials (and Monotremes), he points out, agree with reptiles, which are also born with a functional mesonephros, the latter remaining for a long time active together with the kidney. In Trichosurus, however, it should be noted that the formation of glomeruli does not precede the union of the excretory canals with the collecting ducts of the pelvis.

In Perameles, the condition of the kidneys at birth is very similar to that of Trichosurus. In the new-born Dasyurus, however, the metanephros shows a remarkably poor development. In a recently born foetus, measuring 5·5 mm. *G.L.* and 2·5 mm. *H.L.* (Stage A), the primordium of the ureter and pelvis is in the form of a very short bulbous outgrowth from the dorso-medial side of the Wolffian duct, a short distance in front of the opening of

the latter into the cloaca. It is surrounded by a dense mass of mesenchyme, which stretches forwards as far as the last mesonephric tubule, and which, on the right side, appears to be connected with the latter, the hindermost tubule of this side being very incompletely formed. In a pouch-young with a length of 8·5 to 9 mm. (*H.L.* 5 to 5·5 mm.) and about 7 days old (Stage F), glomeruli are just beginning to develop, whilst in one with a length of 10 mm. (*H.L.* 6·5 mm.), about 10 days old (Stage G), they are comparable to the glomeruli in young of Trichosurus with a head length of 7·5 mm. The growth of the kidney is thus very rapid.

The ureter takes its origin from the dorso-medial side of the Wolffian duct as is the case also in Monodelphian mammals. In marsupials, in the course of further development, it becomes shifted ventrally until it eventually arises from the medial side of the duct, and this medial position is permanently retained. In the Monodelphia, on the other hand, the shifting is such that it finally arises from the lateral aspect of the Wolffian duct. The medial position, occurring as it does in Echidna (Keibel (24)) and in the lizard, is apparently the more primitive one, and in the Didelphia is doubtless the cause of the non-fusion of the Müllerian ducts in the female, as Keibel (23) has emphasised. With the gradual incorporation of the posterior ends of the Wolffian ducts into the urogenital sinus (cf. section on Cloaca), the ureters come to open directly into the latter, a condition which is attained shortly before birth. In the newly born young (*G.L.* 14·5 mm.), they open independently into the base of the bladder primordium on a well-marked papilla, medially to and just in front of the Wolffian ducts. With the growth of the bladder, the apertures of the ureters, and with them the curved portions of the ducts, are carried forwards, so that the papilla, which develops a bifid tip, comes to lie in the neck of the bladder some distance in front of the Wolffian ducts.

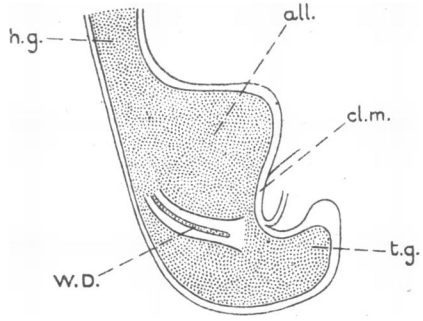
Van den Broek ((9) p. 332) originally suggested that the caudally convex bend of the ureters in marsupials is due to the early development of the bladder, by which the openings of the excretory ducts are drawn forwards, but, as the tissue round the genital ducts of each side is connected across the middle line in the form of a bridge, the ureters are bound to arch round the caudal end of this bridge. In a later paper (11), he refers to the similar bend in the ureters of the male, and arrives at the conclusion that it cannot be due to the formation of the bridge above mentioned, i.e. the genital cord. Our observations confirm that conclusion. In Trichosurus, the ureters begin to acquire their characteristic terminal curvature just before they attain independent openings into the urogenital sinus, in embryos of both sexes with a length of 12 to 13 mm., at a time when the primordium of the bladder is only present as a swelling of the allantoic stalk, and long before the genital cord is developed. Moreover, as we have pointed out in our section on the Müllerian duct, the genital cord does not form an isolated bridge of tissue as figured by van den Broek (9) (p. 331), but develops as a continuous band of mesoderm extending forwards from the openings of the Wolffian ducts into the sinus, and

dorsally to the bladder. The ureters actually pass through the tissue of the cord, in the hinder portion of which are embedded the base of the bladder together with the genital ducts.

This bend at the posterior ends of the ureters is much less marked in later stages and is practically absent in the adult. It is due to a combination of circumstances; in part to the more rapid growth of the ureters at about the time of birth, but mainly to the lengthening of the urogenital sinus and the growth of the bladder. It may be noted that the same curvature is found in the ureters of the Monodelphia, but it is less pronounced than in the marsupials (cf. Keibel's(23) figure of a 25 mm. human embryo, plate IV, fig. 9).

CLOACA

In our youngest embryo (Stage I, *G.L.* 5 mm.) the cloaca appears as a wide cavity (text-fig. 2), its longest diameter being antero-posterior. The hind-gut (*h.g.*) opens into the dorsal side of its anterior end, whilst the primordium of the allantois (*all.*) forms a wide outbulging more ventrally. The Wolffian ducts (*W.D.*) lie close against its ventro-lateral walls, dorsally and posteriorly to the allantois, and do not as yet open into the chamber (plate III, fig. 15, *W.D.*). The cloacal membrane, which begins immediately behind the allantoic primordium, is well developed; ectoderm and entoderm are fused together in (*b*) (text-fig. 2, *cl.m.*), but are apparently distinct in (*a*) and (*c*) (plate III, fig. 15, *cl.m.*). In a transverse section of this region the cloaca is almost rectangular in shape (plate III, fig. 15, *cl.*), but more cranially it becomes narrowed transversely, and widened dorso-ventrally. A short wide tail gut (text-fig. 2, *t.g.*), having a length of ·025 mm. in Stage I *c*, runs out from the posterior side of the cloaca; towards the end of the tail, its dorsal wall fuses with the notochord, and the solid tip of the common cell mass so formed unites with the ventral wall of the medullary tube. All three structures thus terminate in the persisting remnant of the tail bud. There is no neurenteric canal.

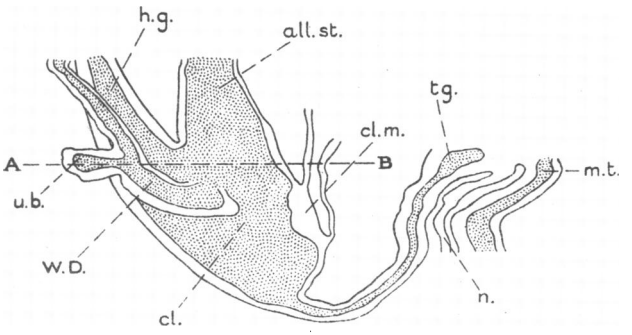


Text-fig. 2. Stage I *b* (I *a* '01), *G.L.* 5 mm. Reconstruction of the cloacal region. *all.* allantoic primordium; *cl.m.* cloacal membrane; *h.g.* hind-gut. *t.g.* tail gut; *W.D.* Wolffian duct. ×50.

At Stage III (*G.L.* 7 mm.) the left Wolffian duct has just acquired an opening into the cloaca, but the junction on the right side is still solid. The entrance of the gut, dorsal and anterior to the Wolffian ducts, is bounded by a ridge on each side. The allantois is now more definite, and the cloacal membrane consists of fused ectoderm and entoderm. The tail gut is longer, but has still a wide connection with the cloaca; towards the tip of the tail its

walls become thicker, and its solid posterior end fuses as before with the notochord, and with the ventral wall of the neural tube.

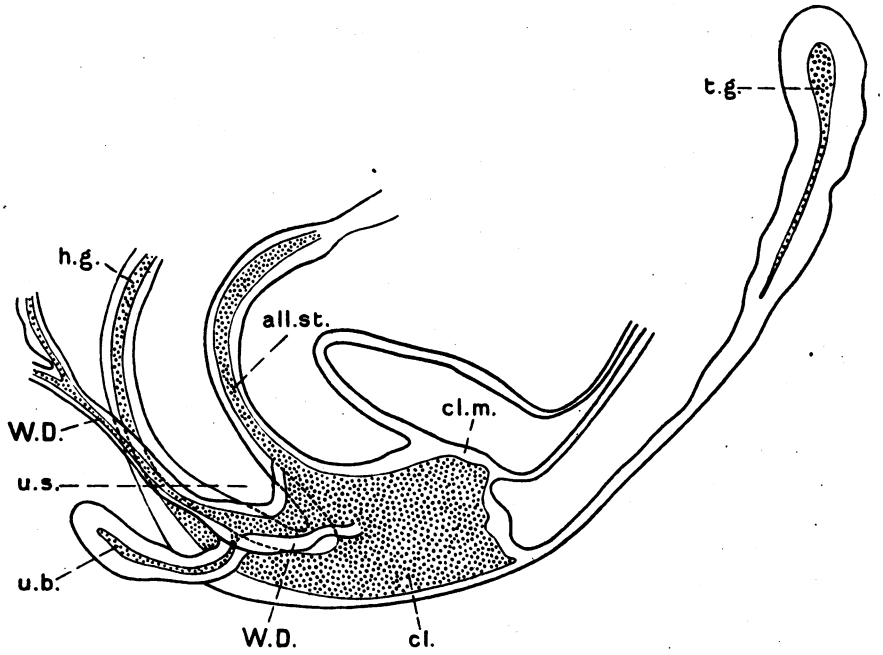
By Stage V (*G.L.* 6 mm.), the cloaca (text-fig. 3, *cl.*) has enlarged considerably, as a result of the growth of the embryo as a whole. The allantois is now a thin-walled sack, communicating with the cloaca by a wide duct. Owing to this development of the allantois and to the formation of a definite allantoic stalk (*all.st.*), the ventral wall of the cloaca is now of much greater extent, and therefore the cloacal membrane (text-fig. 3 and plate III, fig. 15, *cl.m.*), although fusion of ecto- and entoderm is more extensive than formerly, does not begin directly posterior to the entrance of the allantois as in the earliest stages, but some distance behind that point. The Wolffian ducts open on the ventro-lateral walls of the chamber, posterior to the allantoic stalk (text-fig. 3, *W.D.* and plate II, fig. 10, *L.W.D.*). The tail gut (text-fig. 3, *tg.*) leaves the cloaca as a narrow tube, which, towards the tip of the tail, increases greatly in diameter; its walls thicken, and, fusing with the ventral side of the notochord (*n.*), it terminates, as in the preceding stages, in the solid mass of cells in which notochord and medullary tube (*m.t.*) also end.



Text-fig. 3. Stage V (II '01), *G.L.* 6 mm. Reconstruction of the cloacal region. *all.st.* allantoic stalk; *cl.* cloaca; *cl.m.* cloacal membrane; *h.g.* hind-gut; *m.t.* medullary tube; *n.* notochord; *t.g.* tail gut; *u.b.* ureteric bud; *W.D.* Wolffian duct. ×50. (For section across A. B. see plate II, fig. 10.)

By Stage VIII *a* (*G.L.* 7·25 mm.) the cloaca has become narrower, both dorso-ventrally and transversely, and, in a median longitudinal section (text-fig. 4, *cl.*), is somewhat rectangular in shape. The newly formed portion of the ventral wall of the body, lying immediately cranially to the cloacal membrane (*cl.m.*), already present at Stage V, has increased in extent. The allantois communicates with the antero-ventral side of the cloacal chamber by a narrow dorso-ventrally flattened duct (*all.st.*). At the same level on each side open the Wolffian ducts (*W.D.*), the bases of which have widened out considerably in the sagittal direction, so that their openings extend over a greater area of the cloacal wall (cf. text-figs. 3 and 4). The wide hind-gut (*h.g.*) now enters the cloaca dorsally and just posteriorly to the openings of the allantois (*all.st.*) and Wolffian ducts (*W.D.*); the cloaca has thus begun to separate into a dorsal or rectal segment, and a ventral or urogenital segment, the latter representing the future urogenital sinus. The mesoderm between the two segments, forming the so-called urorectal septum (*u.s.*), or, as we

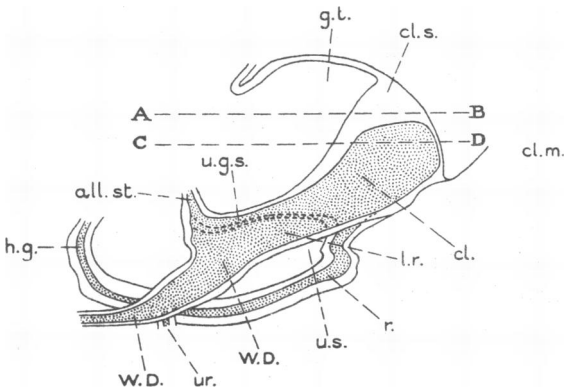
prefer to term it—the urorectal fold—is penetrated by a narrow prolongation of the coelom. The tail gut (*tg.*), which is longer and has become drawn out with the elongation of the tail, stretches out from the postero-dorsal wall of the cloaca as a thin cord of cells, which gradually increases in thickness and becomes tubular, as it runs into the tip of the tail, where it is enlarged and has a wide cavity. The posterior wall runs into the solid mass of cells, in which notochord and medullary tube still terminate. By Stage VIII *b*, its narrow connection with the cloacal wall has disappeared.



Text-fig. 4. Stage VIII (XII *a* '02), *G.L.* 7·25 mm. Reconstruction of cloacal region. *u.s.* urorectal septum. Other lettering as in text-fig. 3. ×90.

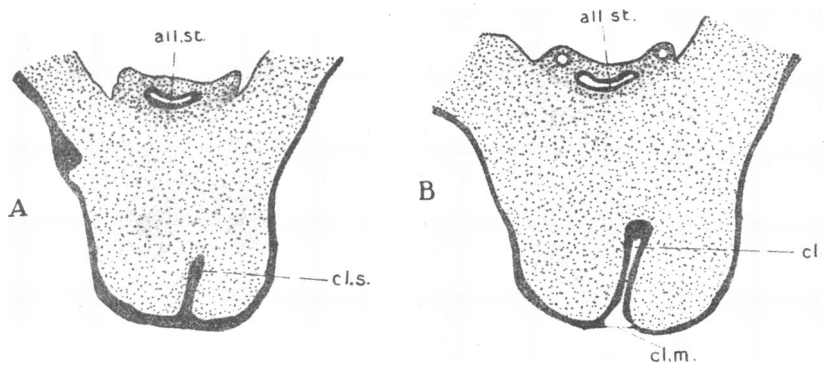
In the next embryo (Stage IX, *G.L.* 8·5 mm.) important changes have taken place. The division of the original cloaca into rectal and urogenital portions has made further progress (text-fig. 5 and plate V, fig. 23) accompanied by a further extension backwards of the urorectal coelom. The hinder portion of the gut or rectum (text-fig. 5 and plate V, fig. 23) forms a narrow tube, which opens into the dorsal side of the cloaca considerably behind the level of the openings of the Wolffian ducts (*W.D.*), and therefore much nearer to the posterior end than in Stage VIII. The urogenital portion (*u.g.s.*) has lengthened considerably; its cranial end is narrow and dorso-ventrally flattened, but more caudally it widens out into the still undivided part of the cloaca (*cl.*), posterior to the entrance of the gut (*r.*). On each side the urogenital sinus (*u.g.s.*) extends up dorsally and anteriorly into the broad Wolffian duct (*W.D.*). The bases of the latter ducts are now more markedly widened

in the sagittal direction, and have undergone lateral compression. Their hinder margins are continued into prominent lateral ridges (*l.r.*) of the sinus wall, which run as far as the opening of the rectum into the cloaca. Examination of the model (plate V, fig. 28) shows that these ridges are apparently produced by the mode of separation of the rectum from the medio-dorsal side of the original cloaca, as a result of which a small portion of the latter is left on each side, forming the ridge in question. The widened terminal end of the Wolffian duct, together with the lateral ridge of the urogenital sinus, with which the duct is connected, would appear to correspond to the so-called cloacal horn of Felix (14).



Text-fig. 5. Stage IX *b* (IV '01), *G.L.*, 8·5 mm. Reconstruction of cloacal region. *all.st.* allantoic stalk; *cl.* cloaca; *cl.m.* cloacal membrane; *g.t.* genital tubercle; *h.g.* hind-gut; *l.r.* lateral ridge; *cl.s.* cloacal septum; *r.* rectum; *ur.* ureter; *u.g.s.* urogenital sinus. *u.s.* urorectal septum; *W.D.* Wolffian duct. × 50.

The primordium of the genital tubercle (*g.t.*) is now recognisable for the first time in the form of an elevation of the abdominal wall (more prominent in Embryo IX *b* than IX *a*), on the posterior face or so-called anal slope of

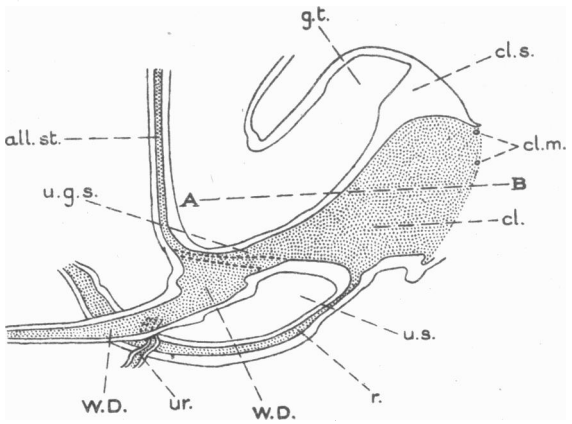


Text-fig. 6 *a*. Stage IX *b* (IV '01). *G.L.* 8·5 mm. Transverse section of genital tubercle at the level of *A*...*B* in text-fig. 5, showing the cloacal septum (*cl.s.*). *all.st.* allantoic stalk. (Sl. 15–5–2.) × 50.

Text-fig. 6 *b*. Stage IX *b* (IV '01), *G.L.* 8·5 mm. Transverse section of genital tubercle at the level of *C*...*D* in text-fig. 5, showing the laterally compressed cloaca (*cl.*) and the thin cloacal membrane (*cl.m.*). (Sl. 15–4–1.) × 50.

which the cloacal membrane (text-figs. 5 and 6 *b*, *cl.m.*) is situated. Its appearance is due to a marked increase in the amount of mesoderm surrounding the cloaca on its lateral and cranial sides, and is partly conditioned by the

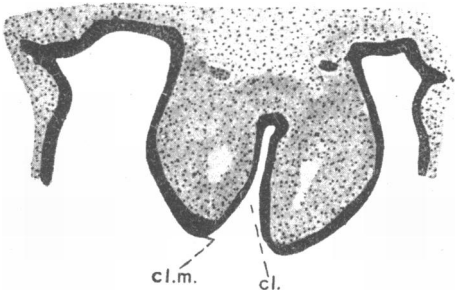
increase in extent of the ventral cloacal wall, first noted in Stage V. As the result of the formation of the tubercle, the undivided terminal portion of the cloaca has become drawn out, and now appears as an elongated laterally compressed chamber (text-figs. 5 and 6 *b*, *cl.*), lying in the mid-region of the posterior half of the tubercle. The lateral compression is more marked on the ventral side; and indeed, towards the apex of the tubercle, immediately in front of the cloacal membrane, the lateral walls have actually come into contact, and have united to form a short solid median septum (text-figs. 5 and 6 *a*, *cl.s.*), which is continuous with the ectoderm of the tubercle. This is the primordium of the urethral plate or cloacal septum. The cloacal membrane is still intact but is very thin, especially in Stage IX *b* (text-fig. 6 *b*, *cl.m.*), where its ventral portion is extremely delicate and degenerate. The tail is now much longer; the tail gut consists of a pear-shaped cavity with the broad end towards the tip of the tail, where its distal wall fuses with the surrounding mesenchyme. It is quite separated from the cloaca, only a few small isolated fragments of its proximal region remaining in the base of the tail.



Text-fig. 7. Stage XI (VI '01), *G.L.* 10 mm. Reconstruction of cloacal region. Lettering as in text-fig. 5. × 50.

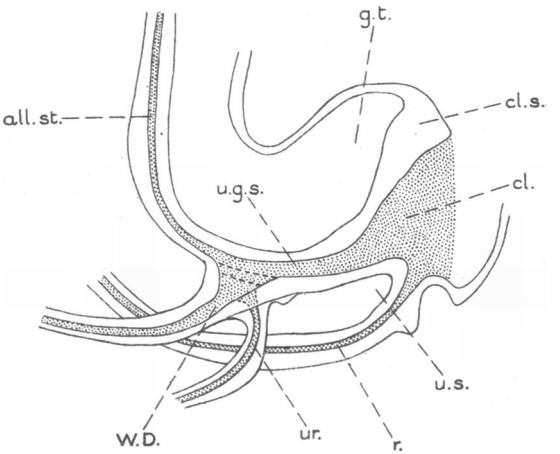
During Stages X and XI (*G.L.* 9·5 and 10 mm.), the genital tubercle becomes more prominent, and in the latter stage it already forms a well marked protuberance (text-fig. 7 and plate VI, fig. 24 *a*, *g.t.*). (It may be noted that in the embryo modelled (plate VI, fig. 24), the tubercle appears to be exceptionally well developed for this stage.) The greater part of the cloacal membrane has disappeared, only fragments of it remaining as narrow irregular strips (text-figs. 7 and 8 and plate VI, fig. 24 *b*, *cl.m.*). The cloaca (*cl.*) thus opens out to the exterior (text-fig. 7, *cl.*). It has undergone still further lateral compression, except on the anal side near the region of its opening, and, with the growth of the tubercle, has become drawn up into the latter, this extension being facilitated by the rupture of the cloacal membrane, the dorso-ventral extent of which at Stage IX is very much less than the opening of the cloaca to the exterior at Stage XI (cf. text-figs. 5 and 7). The separation of the rectum (*r.*) from the cloaca has made further progress, the rectum now forming a tube of small diameter and greatly diminished lumen The urogenital segment (*u.g.s.*) has at the same time become longer, and, in correlation with the elongation of the cloaca as a

whole, its lateral ridges, which were continuous with the Wolffian ducts at Stage IX, have now almost completely disappeared (cf. text-figs. 5 and 7 and plate V, fig. 23 and plate VI, fig. 24). Towards the tip of the tail we still find the distal portion of the tail gut, which widens at its extremity, and shows a central cavity; its posterior wall mingles with the surrounding mesoderm as in the last stage.



Text-fig. 8. Stage XI (VI '01), *G.L.* 10 mm. Transverse section of the genital tubercle at the level of *A*...*B* in text-fig. 7. *cl.* cloaca; *cl.m.* cloacal membrane. (Sl. 19–3–1.) × 50.

At Stage XII *b* (*G.L.* 11·5 mm.) (plate VI, fig. 25 *a* and *b* and text-fig. 19), the expanded base of the Wolffian duct (*W.D.*), as far forwards as the point of origin of the ureter (*ur.*), has become incorporated into the wall of the urogenital sinus (*u.g.s.*). As a result, the ureter, although really opening on the medial side of the aperture of the Wolffian duct into the sinus, practically leads directly into the cranial end of the latter. The sinus at this end expands outwards on each side, owing to the still incomplete incorporation of the bases of the Wolffian ducts (plate VI, fig. 25 *b*). In the preceding stage, the widened end of the duct curved round dorsally to pass gradually forwards into the narrower portion of the tube, the two parts lying in the same sagittal plane. Here, the persisting basal portion of the duct is much shorter, and turns forwards almost at right angles to run abruptly into the thinner part of the tube. Behind the connection of the Wolffian ducts, the urogenital sinus continues back as a long narrow dorso-ventrally flattened tube, which opens out into the entodermal cloaca.

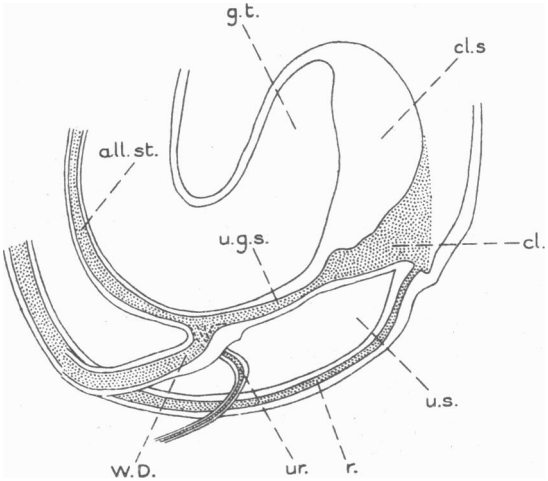


Text-fig. 9. Stage XII *a* (XXII '04), *G.L.* 11 mm. Reconstruction of cloacal region. Lettering as in text-fig. 5. × 50.

In the 11 mm. embryo (Stage XII *a*), the cloaca (text-fig. 9, *cl.*) has the form of a narrow laterally compressed cavity, which communicates with the exterior by a narrow slit-like opening, extending from the base of the tail over about two-thirds of the anal slope of the tubercle. In the 11·5 mm. embryo, however, as a result of the meeting and fusion of its lateral walls, the greater part of the

cloacal cavity has become obliterated (text-fig. 10, *cl.*). All that remains of it is a short dorsal section, forming the direct prolongation of the urogenital sinus (*u.g.s.*), and a very small terminal piece, into which both sinus and rectum open, and which is in communication with the exterior. The fused walls of the cloaca form a solid median entodermal septum (text-fig. 10, *cl.s.*), the primordium of which was already apparent in Stage IX. It now divides the anal portion of the cloacal tubercle into two halves, and extends in a vertical plane from the tip of the tubercle to the newly formed ventral wall of the sinus. This septum, in much later stages of marsupials, has been termed by van den Broek (11), after Fleischmann (16), the "Phallusleiste," and has been described under various designations in the Monodelphia[1]. We shall adopt the name cloacal septum, first proposed by Reichel (v. Born (3)).

It should be noted that, whilst by far the larger portion of the cloacal septum is formed at Stage XII *b* (text-fig. 10), it actually begins to appear at Stage IX (*G.L.* 8·5 mm.) (text-figs. 5 and 6 *a*) at the tip of the tubercle. In Stages X and XI it increases only slightly in extent, and is not finally completed until Stage XIV (*G.L.* 13·0 mm.) (text-figs. 11 and 12, *cl.s.*). From its mode of origin it is clearly entodermal.



Text-fig. 10. Stage XII *b* ('97), *G.L.* 11·5 mm. Reconstruction of hind region. Lettering as in text-fig. 5. × 50.

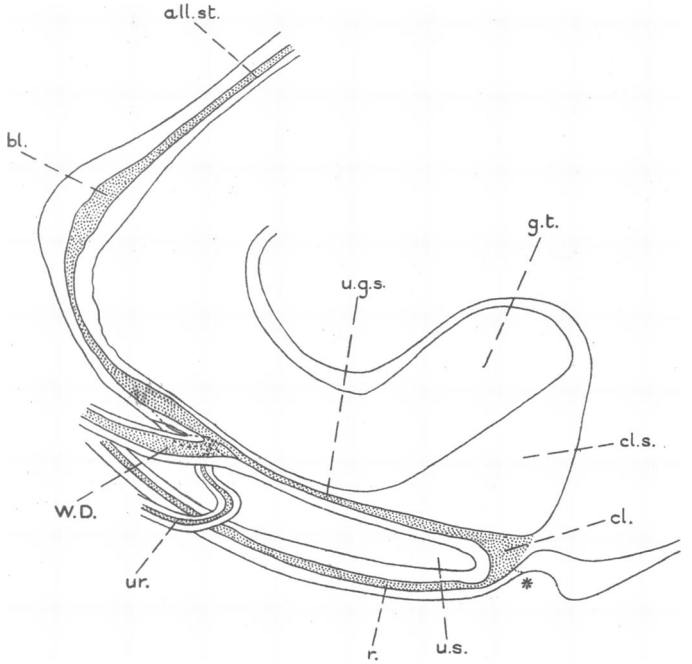
In the 11·5 mm. embryo, the hollow bulbous extremity of the tail gut is still present at the tip of the tail.

At Stage XIV *b* (*G.L.* 13·5 mm.) (text-fig. 11) only a slight advance is seen compared with Stage XII. The remnant of the entodermal cloaca is still more reduced; the part forming the hinder end of the urogenital sinus (*u.g.s.*) is much narrower, whilst the terminal piece has almost disappeared, being represented by a shallow depression (*cl.*), into which the sinus (*u.g.s.*) and rectum (*r.*) open. In other words, the urorectal fold (*u.s.*) has now advanced to within a very short distance of the original site of the cloacal membrane. Indeed, at this stage, it may be said to have practically completed its growth. The tail gut has disappeared.

In the new-born foetus (Stage XV, *G.L.* 14·5 mm.), an important advance has taken place in the formation of a definite ectodermal cloaca (text-fig. 13,

[1] Lame cloacale, uréthrale, or urogénitale, cloison cloacal, ectodermal Kloakenplatte, Ural platte, urogenital plate, urethral plate.

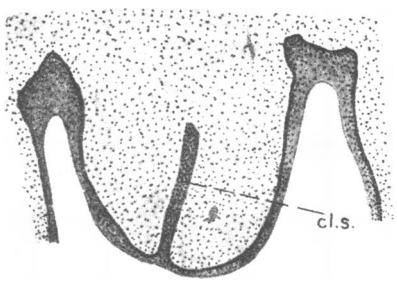
ect.cl.). The urogenital sinus (*u.g.s.*) and the rectum (*r.*) have both increased in length with the general growth of the body, and their openings are now no longer situated close to the surface, but have become secondarily displaced



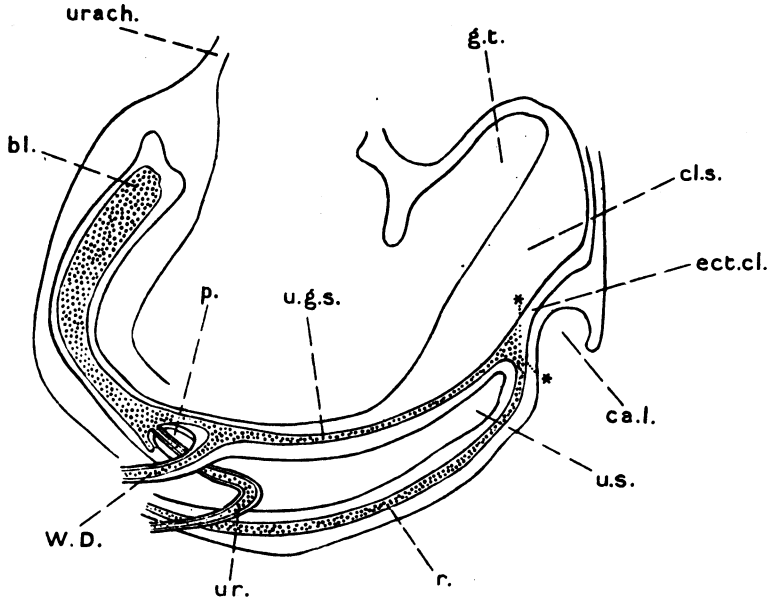
Text-fig. 11. Stage XIV *a* (XXII), *G.L.* 13 mm. Reconstruction of hind region. *bl.* bladder. Other lettering as in text-fig. 5. × 50. Dotted line at * marks the limit between ectoderm and entoderm.

inwards, and now lie at the inner end of an ectodermally lined passage, the ectodermal cloaca. This is formed, not as an invagination, but chiefly as the result of the continued enlargement and backward growth of the cloacal tubercle.

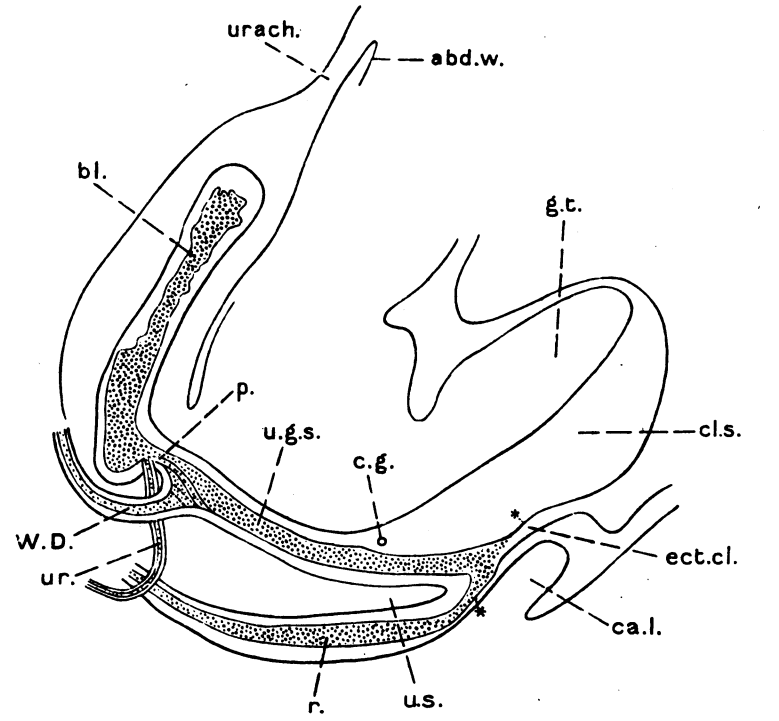
The dorsal part of the anal slope of the tubercle thus constitutes the ventral boundary of the new ectodermal cloaca. The dorsal boundary of the latter is formed by an elevation, which arises from a thickening of the mesoderm at the base of the tail. This thickening is limited behind by an ingrowth of ectoderm destined to hollow out, and in this way a distinct protuberance arises, which furnishes the dorso-caudal lip of the definite cloacal opening (text-fig. 13, *ca.l.*). The cloacal septum (*cl.s.*) stretches from the



Text-fig. 12. Stage XIV *a* (IX '01), *G.L.* 13 mm. Transverse section through the genital tubercle, showing the cloacal septum (*cl.s.*). (Sl. 29–3–4.) × 50.

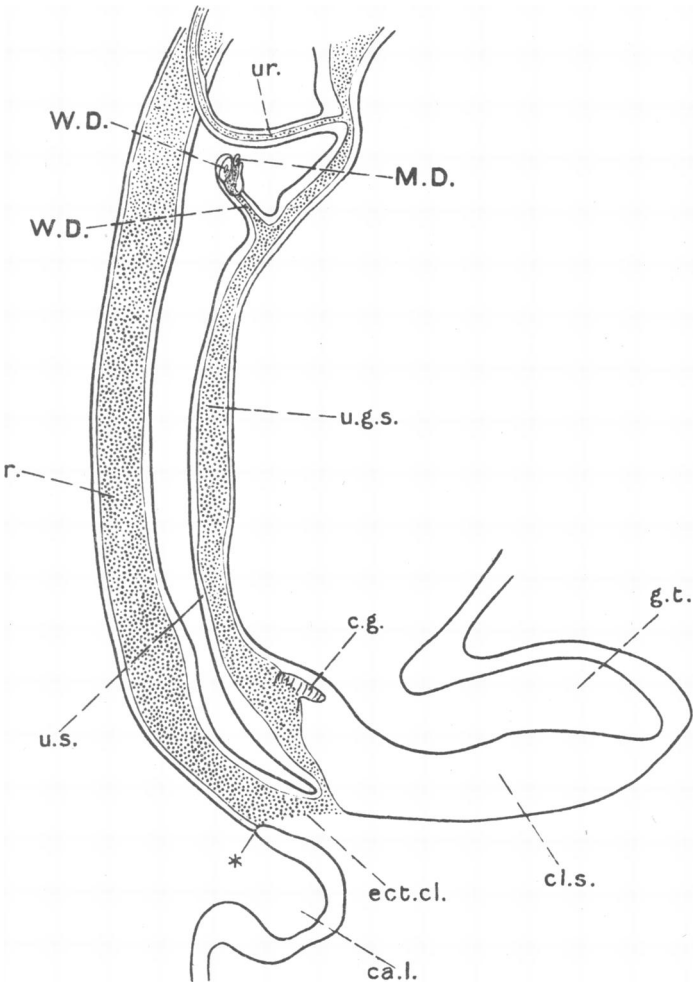


Text-fig. 13. Stage XV *b* (new-born foetus), *G.L.* 14·5 mm. Reconstruction of hind region. *bl.* bladder; *ca.l.* caudal lip of ectodermal cloaca; *ect.cl.* ectodermal cloaca; *p.* ureteric papilla; *urach.* urachus. Dotted line at * marks the limit between ectoderm and entoderm. Other lettering as in text-fig. 5. ×75 and reduced by ½.



Text-fig. 14. Stage XVII *a*, *G.L.* 17 mm. Reconstruction of hind region. *abd.w.* abdominal wall; *c.g.* level of Cowper's glands. Other lettering as in text-figs. 5 and 13. ×75 and reduced by ½.

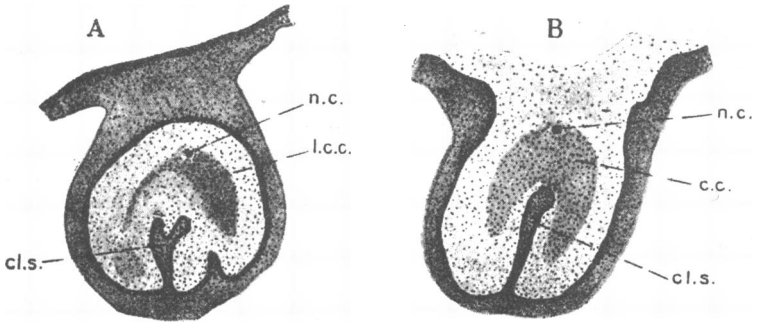
ventral wall of the sinus, with which it is continuous, up to the tip of the genital tubercle, whilst its inner free margin reaches almost to the mid-region of the latter. The ureters now open on a definite papilla (*p.*) anterior to the Wolffian ducts at the base of the bladder primordium (*bl.*).



Text-fig. 15. *G.L.* 21 mm., *H.L.* 9 mm. Reconstruction of hind region. *c.g.* Cowper's glands; *M.D.* Müllerian duct. Lettering as in text-figs. 5 and 13. ×75 and reduced by ½.

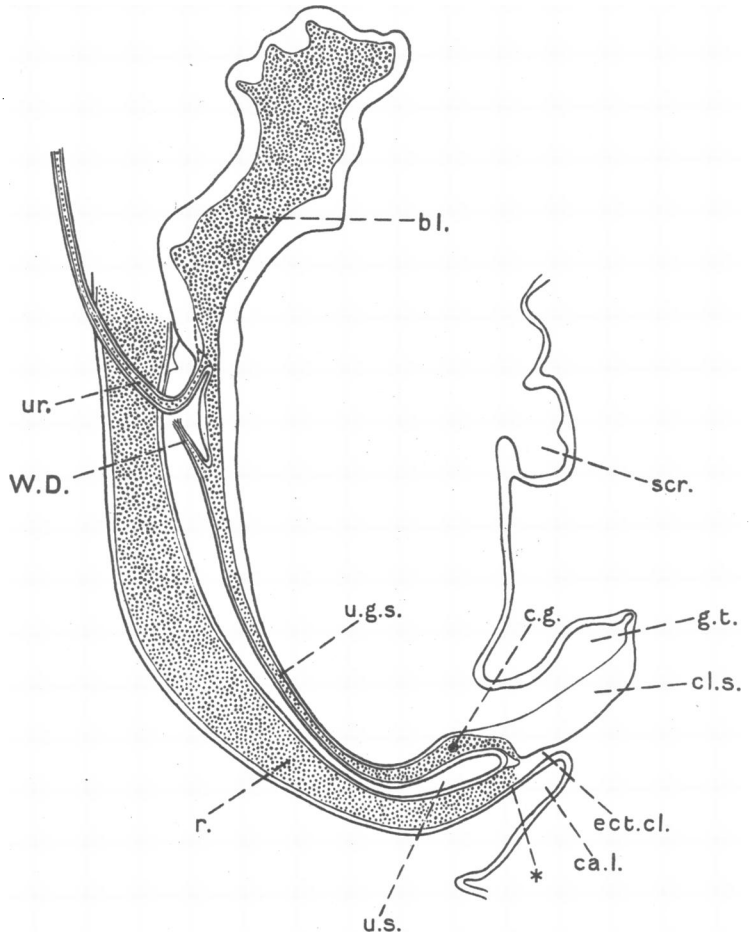
The urorectal coelom, which is well developed at birth, becomes partially obliterated in pouch-young with a head length of 7·5 mm. (*G.L.* 17 mm.), and extends only a short distance into the urorectal fold. At this stage (text-fig. 14), except for a general increase in size, no marked change has occurred in the region of the urogenital sinus and genital tubercle.

Text-fig. 15 shows the hinder region of a foetus with a head length of 9 mm. in which both urogenital sinus and rectum have increased greatly in length.



Text-fig. 16 *a*. Embryo D, *G.L.* 28 mm., *H.L.* 12 mm. Transverse section through the genital tubercle, showing the distal end of the cloacal septum (*cl.s.*) with its bilobed margin. *l.c.c.* left corpus cavernosum; *n.c.* erector penis. (Sl. XV–5–2.) ×33⅓.

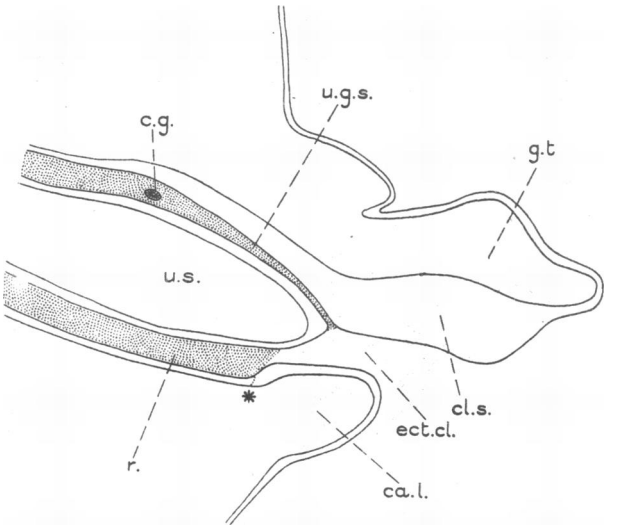
Text-fig. 16 *b*. Embryo D, *G.L.* 28 mm., *H.L.* 12 mm. Transverse section through the genital tubercle, showing the proximal end of the cloacal septum (*cl.s.*) with its bulbous margin. *c.c.* corpus cavernosum; *n.c.* erector penis. (Sl. XVI–2–8.) ×33⅓.



Text-fig. 17. *G.L.* 41 mm., *H.L.* 15 mm. Reconstruction of hind region. *scr.* scrotal sack. Other lettering as in preceding text-figs. ×12·5.

In both the male and female foetus with a head length of 11 mm. the free margin of the cloacal septum is slightly bifid at its distal end, and becomes bulbous as one passes towards the proximal end, i.e. towards the base of the tubercle. In the next (*H.L.* 12 mm.) (text-figs. 16 *a* and *b*), and later stages, the bifid margin of the septum becomes well marked.

In older pouch-young, the urogenital sinus lengthens considerably, and forms a tube of small diameter which diminishes at its hinder end, and communicates with the short ectodermal cloaca by a narrow aperture. The opening of the rectum into the cloaca, on the other hand, always remains wide (text-figs. 19 to 22). At its anterior end the urogenital sinus in cross-section has the form of a dorso-ventrally flattened tube, with the dorsal surface slightly concave and the ventral surface convex. More posteriorly it becomes narrower transversely and is gradually reduced in size, becoming almost circular in outline towards the hinder end.



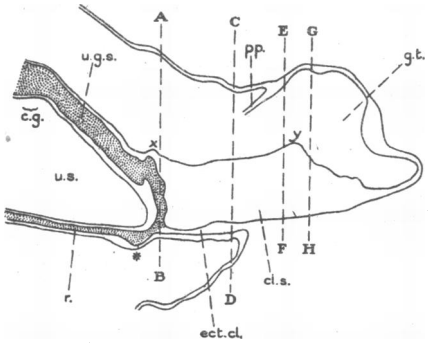
Text-fig. 18. *D.C.L.* 11 cm., *H.L.* 2·9 cm. Reconstruction of hind region. *c.g.* level of Cowper's gland; *ca.l.* caudal lip of ectodermal cloaca; *ect.cl.* ectodermal cloaca; *g.t.* genital tubercle; *cl.s.* cloacal septum; *r.* rectum; *u.g.s.* urogenital sinus; *u.s.* urorectal septum. Dotted line at * marks the limit between ectoderm and entoderm. × 12·5.

Leaving for the present the consideration of the later stages in the female, we shall now describe the later stages of development of the male alone. In a male with a head length of 15 mm. (text-fig. 17) the epithelial lining of the terminal part of the urogenital sinus has thickened, whilst the ectodermal epithelium covering the free edge of the urorectal fold (*u.s.*), which forms the dorsal margin of the sinus opening, has also markedly thickened, with the result that the latter opening is now reduced to a mere slit.

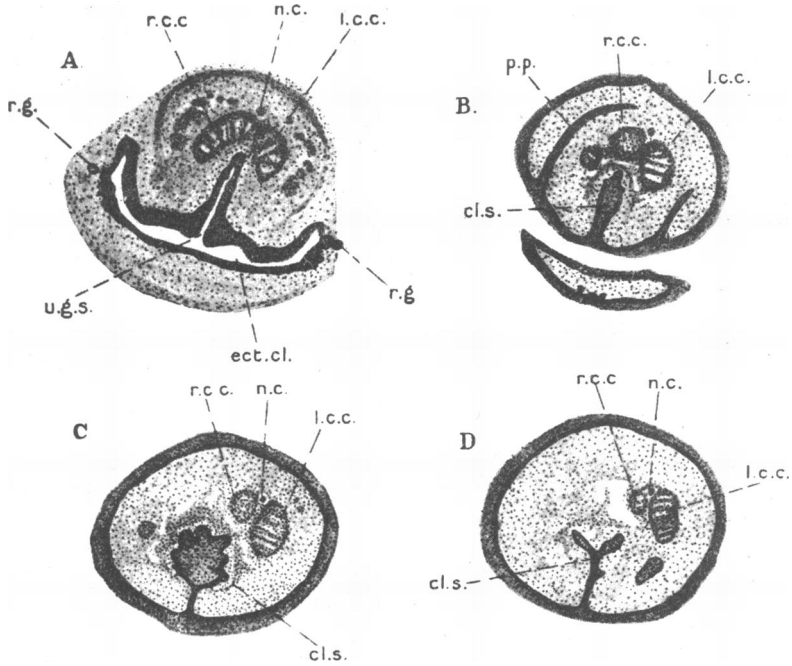
Text-fig. 18 shows a reconstruction of the hinder region of a male foetus with a head length of 2·9 cm.

In a male with a head length of 3·1 cm. (text-fig. 19), the urogenital sinus still opens into the cloaca by a narrow aperture (text-fig. 20 *a*). Sections through the cloacal septum of this stage are seen in text-figs. 20 *b*, *c*, and *d*, the last one showing the bifid character of the inner free margin of the septum towards its distal end.

In a slightly older foetus (*H.L.* 3·5 cm.), a very remarkable alteration has



Text-fig. 19. *H.L.* 3·1 cm. Reconstruction of hind region. *p.p.* preputial lamella. Other lettering as in text-fig. 18. × 12·5.



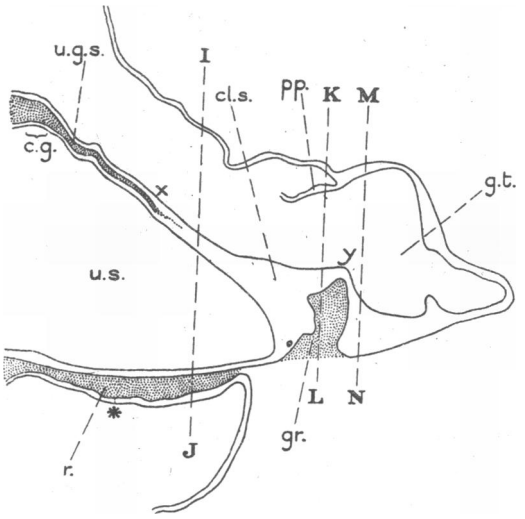
Text-fig. 20 *a*, *b*, *c* and *d*. 20 *a*. *H.L.* 3·1 cm. Transverse section through *A*...*B* in text-fig. 19, showing the slit-like aperture of the urogenital sinus (*u.g.s.*) into the ectodermal cloaca (*ect.cl.*). *r.g.* duct of rectal gland; *l.c.c.* left corpus cavernosum; *r.c.c.* right corpus cavernosum; *n.c.* erector penis. (Sl. 2–7–12.)

20 *b*. Transverse section through the genital tubercle at *C*...*D* in text-fig. 19. *cl.s.* cloacal septum; *p.p.* preputial lamella. Other lettering as in 20 *a*. (Sl. 2–3–12.)

20 *c*. Transverse section through genital tubercle at *E*...*F* in text-fig. 19, showing swollen margin of the cloacal septum (*cl.s.*). Lettering as in 20 *a*. (Sl. 1–7–10.)

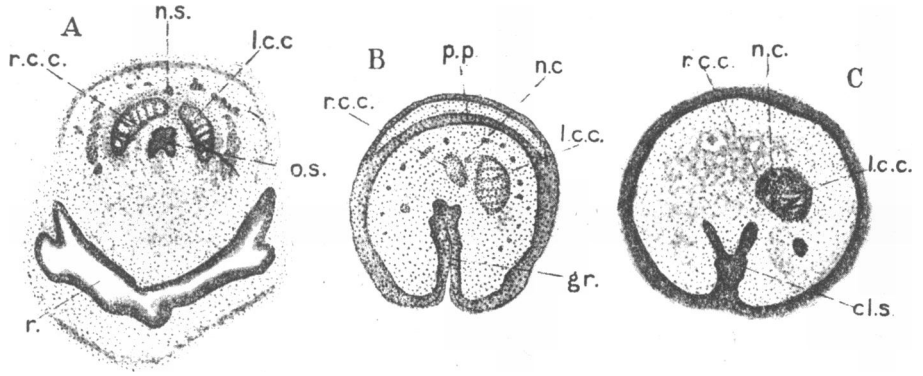
20 *d*. Transverse section through genital tubercle at *G*...*H* in text-fig. 19, showing bifid margin of the septum (*cl.s.*). Lettering as in 20 *a*. (Sl. 1–6–1.)

taken place, inasmuch as the lumen of the posterior portion of the sinus has become completely occluded (text-figs. 21 and 22 *a*), and therefore impervious to the passage of urine. That this is so is evident, not only from the examination of sections, but from the condition of the bladder. The latter was seen to be tensely swollen and of large size, having a diameter of 26 × 14 mm., the corresponding measurement in the preceding stage being 8·5 × 5·5 mm., and in the succeeding one (*H.L.* 4·1 cm.) 17 × 13 mm.



Text-fig. 21. *H.L.* 3·5 cm. Reconstruction of hind region. *gr.* groove in cloacal septum. Other lettering as in text-figs. 18 and 19. × 12·5.

It is difficult to determine exactly what has occurred between this stage and the last one, but the closure of the sinus (*u.g.s.*) is apparently the result of the continued thickening of the epithelial lining, combined with certain growth changes. Comparison of text-figs. 19 and 21 shows that considerable growth has occurred in this region. The urogenital sinus has increased in length, whilst the urorectal fold (*u.s.*) has notably thickened. As a result, the proximal portion of the septum has apparently become drawn up, and is now longer and narrower (note the distance between *x* and *y* in text-figs. 19 and 21). At this stage no trace remains of the original opening of the sinus,



Text-fig. 22 *a*, *b*, and *c*. *H.L.* 3·5 cm. 22 *a*. Transverse section through *I*...*J* in fig. 21, showing the occluded sinus (*o.s.*). Lettering as in preceding text-figs. (Sl. 4–7–8.)

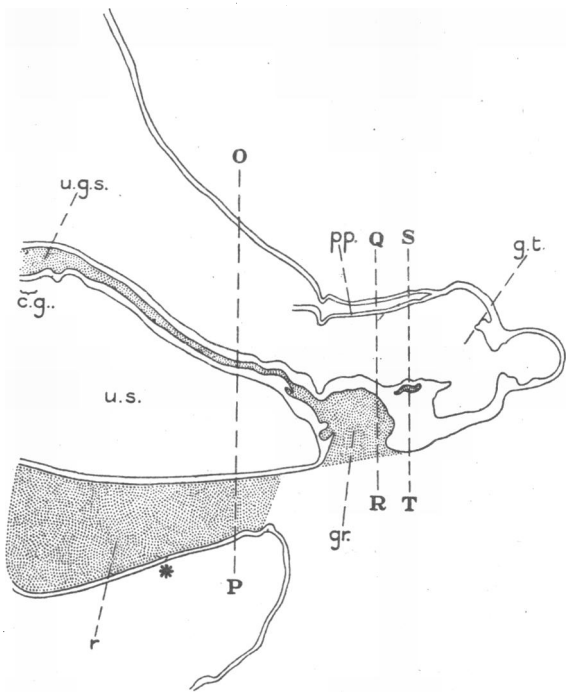
22 *b*. Transverse section through *K*...*L* in fig. 21, showing the groove (*gr.*) in the cloacal septum (*cl.s.*). (Sl. 3–5–2.)

22 *c*. Transverse section through *M*...*N* in fig. 21, showing bifid margin of cloacal septum (*cl.s.*). (Sl. 2–5–7.)

and exactly how far the obliteration of the lumen extends is difficult to ascertain, but presumably the occlusion takes place as far forwards as the point *x* in text-fig. 19.

A further change is observable in this foetus (*H.L.* 3·5 cm.), and that is in the appearance of a cleft-like space (*gr.*), which has developed independently within the cloacal septum (*cl.s.*), below and in front of the point *y* in text-fig. 21 (text-fig. 22 *b*), and which communicates with the exterior on the anal side of the tubercle. Further distally the septum is quite solid (text-figs. 21 and 22 *c*).

In our oldest male (*H.L.* 4·1 cm.) the cleft-like space referred to above has enlarged considerably and now forms a wide groove, the urethral groove, occupying rather more than the proximal half of the cloacal septum (text-figs. 23 and 24 *b*, *gr.*). Moreover, it is now directly continuous with the sinus lumen by means of a very narrow passage (text-fig. 24 *a*, *s.p.*), which has arisen in the solid portion of the septum represented in text-fig. 19 between the levels *x* and *y*. In this way the sinus has now acquired a new and direct opening to the exterior. Small vacuities are also present in the septum further distally (text-fig. 24 *c*), but the bifid distal end is still solid. It will be observed from text-fig. 23 that the ectodermal cloaca of earlier stages has temporarily disappeared, the rectum and urogenital sinus communicating quite independently with the exterior.

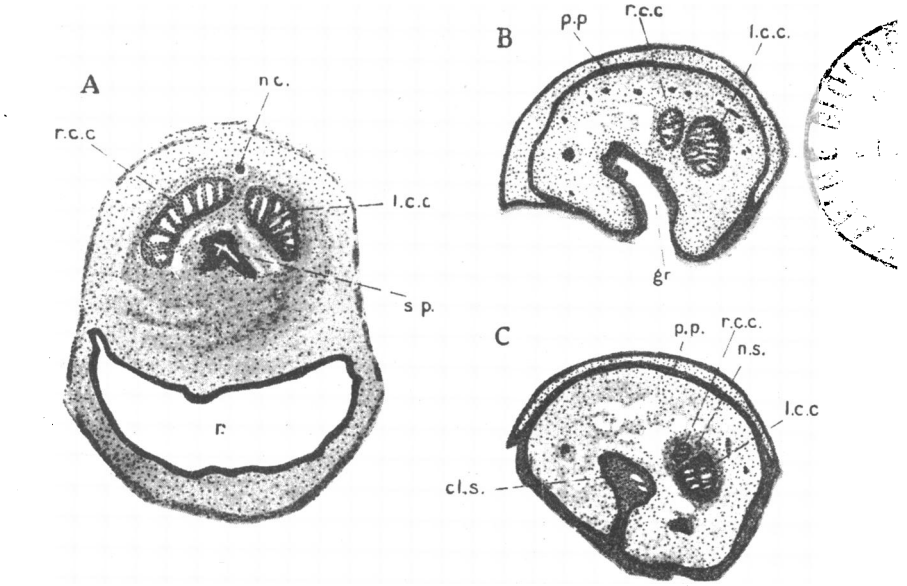


Text-fig. 23. *H.L.* 4·1 cm. Reconstruction of hind region, showing the new opening acquired by the urogenital sinus through the cloacal septum. *gr.* urethral groove. Other lettering as in text-figs. 18 and 19. × 12·5.

The newly formed passage just described, is to be regarded as arising in the site of that part of the original entodermal cloaca, which became obliterated in Stages IX to XIV by fusion of its lateral walls to form the cloacal septum. Phylogenetically, it is to be interpreted merely as a re-opening, but ontogenetically, it is an entirely new formation. In later stages, the urethral groove closes to form a canal, the future penial urethra.

The originally single primordium of the corpora cavernosa first distinctly

appears in the new-born young (*G.L.* 14·5 to 15 mm.), and takes the form of a condensed mass of mesenchyme with ill-defined boundaries, lying along the ventral side of the cloacal septum; at the proximal end of the latter it divides into the two crura which extend up antero-dorsally on each side. Dorsally to the corpora cavernosa we can distinguish the primordium of the tendon of the *erector penis* (*levator penis*, Cunningham (13*a*)), described by van den Broek (10) under the designation of "Kernzentrum" in several marsupials. It runs antero-dorsally, parallel with the edge of the septum, and widens out below the symphysis.



Text-fig. 24 *a*, *b*, and *c*. *H.L.* 4·1 cm. 24 *a*. Transverse section through *O*...*P* in text-fig. 23, showing the narrow passage (*s.p.*) newly acquired in the formerly solid septum. Lettering as in preceding text-figs. (Sl. 4–4–8.)

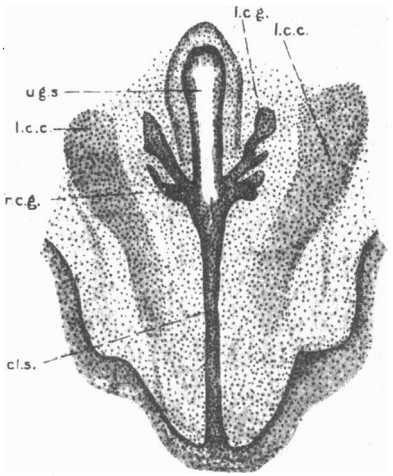
24 *b*. Transverse section through *Q*...*R* in text-fig. 23, showing the wide groove (*gr.*) in the cloacal septum. (Sl. 2–6–1.)

24 *c*. Transverse section through *S*...*T* in text-fig. 23, showing the cloacal septum further distally, containing several small vacuities. (Sl. 2–3–6.)

In a foetus with a head length of 8·5 mm., the primordium of the corpora cavernosa is well marked. It lies along the ventral side of the genital tubercle, and is seen in transverse section partially enveloping the cloacal septum in the form of a horseshoe (v. text-fig. 16 *b*, which shows a transverse section of the primordium in a foetus with a head length of 12 mm.). Towards the base of the genital tubercle, it separates into the two crura, which spread outwards on each side lateral to Cowper's glands, and then extend upwards ventro-laterally to the gut (text-fig. 25, *H.L.* 11 mm., *l.c.c.* and *r.c.c.*). The tendon of the *erector penis* is also well developed; it divides into two laterally running cords just ventrally to the symphysis.

In later stages the single primordium of the corpora cavernosa becomes completely divided into two masses of tissue running into the distal end of the tubercle. At this end, shortly after birth (*H.L.* 6·5 mm.), the corpus cavernosum of the left side is more marked than that of the right. In both sexes, this asymmetry becomes very conspicuous in older stages, the distal end of the left corpus cavernosum extending much farther into the genital tubercle (text-figs. 16 *a*, 20 *c* and *d*, 22 *b* and *c*, 24 *b* and *c*). In the adult, we find the glans penis markedly asymmetrical, that of the left side being much better developed than that of the right.

The first indication of the preputial lamella occurs in a male with a head length of 26 mm., as a short ingrowth of ectoderm, extending inwards from the oral side of the tubercle on each side, laterally to the corpora cavernosa. In our oldest foetus (*H.L.* 4·1 cm.) the preputial lamella forms a sheath surrounding the cloacal septum and corpora cavernosa along the posterior three-fifths of the tubercle (text-figs. 23, 24 *b* and *c*, *p.p.*). It is also well developed in the female.



Text-fig. 25. *H.L.* 11 mm. Transverse section through the base of the cloacal septum (*cl.s.*), showing Cowper's glands (*l.c.g.* and *r.c.g.*) and the corpora cavernosa (*l.c.c.* and *r.c.c.*). (Sl. 20–1–4.)

Van den Broek (10), finding that no preputial lamella was present in a male specimen of Trichosurus measuring 32 mm., has erroneously placed Trichosurus among those marsupials in which a praeputium is probably never developed (cf. table, p. 382).

DISCUSSION

It will be seen from the foregoing description that the early development of the cloaca in Trichosurus is essentially similar to that of the human embryo described by Felix (14). A well developed tail gut is present, which is quite shut off from the cloaca in embryos of 8·5 mm. *G.L.*, and disappears completely at Stage XIV (*G.L.* 13·5 mm.).

The rectum becomes separated off from the urogenital sinus by an ingrowth of the wall of the cloaca, the urorectal fold, which, arising between the allantoic stalk and the hind-gut, extends back towards the cloacal membrane, as in the human embryo (Felix (14) and Pohlman (29)), but which, in the marsupial, never quite reaches as far as the original site of that membrane. There is no evidence in favour of the view that the separation of the dorsal rectal segment takes place by means of two lateral folds, which meet across the middle line, a view upheld by Retterer (30) and others. At Stage IX,

whilst the first division of the cloaca is proceeding, the abdominal wall, cranial to and on a level with the cloacal membrane, rises up to form the genital tubercle, in the anal half of which lies the posterior portion of the cloaca (text-fig. 5, *g.t.* and *cl.*). After the rupture of the cloacal membrane in the next stage, and with the continued growth of the tubercle, the cavity of the cloaca becomes drawn up into the latter, its lateral walls become approximated, and finally fuse together to form the cloacal septum or urethral plate (Stage XIV, text-fig. 12). In Trichosurus, the cloacal septum is therefore an entodermal structure. There is some difference of opinion as to the origin of this septum in the Monodelphia. Fleischmann (16), in his studies of various mammals, Schwarztrauber (32) in the sheep and pig, Felix (14) in the human embryo, and van den Broek (11) (12) in marsupials, consider it to be derived from the entoderm as in Trichosurus, whilst Retterer (30) in the pig, sheep and rabbit, and Born (3) in the human embryo, regard it as arising from two ectodermal folds (genital folds, Retterer), which grow together in the median line. After careful examination, we could find no evidence in Trichosurus of any ingrowth of ectoderm into the genital tubercle, nor was there any development of ectodermal folds. Van den Broek points out that though the cloacal septum in marsupials is entodermal in character[1], the urogenital sinus, posterior to the openings of Cowper's glands, is of mixed origin, partly ectodermal and partly entodermal, the amount of ectoderm varying in different species. The ectodermal portion of the sinus, according to this author, arises as two folds on each side of the ectodermal cloaca, which join together in the median line, but we fail to follow this description. In later stages (from *H.L.* 15 mm.) of Trichosurus, the epithelial lining of the urogenital sinus, from the opening of the latter as far forwards as Cowper's glands, takes on an epidermal appearance, but we regard this change as purely secondary. An ectodermal cloaca does not develop until birth (*G.L.* 14·5 mm., text-fig. 13), and is apparently due to the enlargement of the genital tubercle on the ventral side; and on the dorsal side, to the formation of an elevation at the base of the tail, destined to form the dorso-lateral portion of the cloacal lip.

In the male foetus (*H.L.* 3·5 cm.), as a result of growth changes and a thickening of its epithelial lining, the lumen of the hinder end of the urogenital sinus becomes gradually obliterated, with the result that for a short period the sinus has no opening to the exterior (text-fig. 21). The cloacal septum then becomes hollowed out independently in the form of a groove, so that very soon the sinus acquires a new passage to the outside. A similar closure of the ostium urogenitale primitivum, during the splitting of the urethral plate, takes place in the human embryo (Herzog (18)), but it is more remarkable in the marsupial in that it occurs long after birth.

It is of interest to note that the new-born *Dasyurus viverrinus*, with respect to the cloacal region, as in all other points connected with the urogenital

[1] Van den Broek's investigations, however, begin at a late stage, the youngest specimen being a "*Phalangista*-Beuteljungen," 12 mm.

system, is much less advanced than is the corresponding stage of Trichosurus. Immediately prior to birth, the cloacal membrane is still intact, but in the newly born young (*G.L.* 5·5 mm., *H.L.* 2·5 mm.) it is ruptured, and the cloaca opens out to the exterior. The primordium of the genital tubercle at this stage forms only a slight elevation, somewhat resembling that of Stage IX *a* (*G.L.* 8·5 mm.) in Trichosurus. In a three hours pouch-young it is more prominent, and shows a condition similar to the Trichosurus embryo of 11 mm.

The final stages of the development of the penis, and the development of the clitoris in the female, are not dealt with in this paper.

BLADDER

The primordium of the bladder is first recognisable in an embryo with a length of 11 mm. (Stage XII) in the form of a slight enlargement of the intra-abdominal part of the allantoic stalk, a short distance (about ·24 mm.) behind the umbilicus. In a slightly older embryo (*G.L.* 11·5 mm.) the layer of longitudinal muscle fibres is already developing. At Stage XIV (*G.L.* 13 and 13·5 mm.), the enlarged region of the allantoic stalk is much more marked (text-fig. 11, *bl.*); its lining epithelium is here thickened, and both an outer circular and an inner longitudinal layer of muscle fibres are being laid down. At birth, the bladder is well established (text-fig. 13). Its distal end or fundus is expanded, and is attached by the urachus (*urach.*) to the body wall; posteriorly, it narrows gradually into the long neck. Into the base of the latter, just in front of the openings of the Wolffian ducts into the urogenital sinus, lies the papilla (*p.*) on which the two ureters open. In later stages, the neck of the bladder, together with the curved posterior portions of the ureters, lie enclosed with the genital ducts in the genital cord.

In a foetus with a head length of 7·5 mm. (text-fig. 14, *bl.*) the urachus (*urach.*) is much reduced, appearing as a thin vascular cord, which is still attached to the skin in the region of the closed umbilicus. In a pouch-young with a head length of 8·5 mm., the urachus has disappeared and the fundus of the bladder projects freely. A short distance behind its apex, however, and backwards along its whole length, the bladder is attached to the ventral abdominal wall by the ligamentum vesicae medium (lig. vesicale anterius). This ligament first appears soon after birth. At this time the intra-abdominal part of the allantoic stalk becomes gradually constricted off from the ventral body wall, until only a thin mesentery remains along its ventral side (text-fig. 1, *l.v.m.*). The hinder parts of the umbilical arteries would appear to persist as the vesical arteries (text-figs. 1, 26 and 31, *v.a.*). As in Macropus and Perameles, already described by Prof. Hill (19), they run up in a low ridge lying on each side of the bladder (text-figs. 26 and 31, *v.a.*).

The main portion of the bladder in Trichosurus thus arises from the hinder part of the intra-abdominal allantoic stalk. From our account (p. 57)

of the incorporation of the bases of the Wolffian ducts into the wall of the urogenital sinus, and the consequent opening of the ureters into the latter, medial to the Wolffian ducts, it is evident that the lower part of the neck of the bladder must be formed from the bases of the Wolffian ducts, together with that portion of the entodermal lining of the cloaca lying between them. The lining of the bladder is thus of twofold origin; the larger part of it is derived from the allantoic entoderm, a small part from that of the cloaca, and yet another small part from the mesodermal epithelium of the Wolffian ducts. A similar mode of origin has been observed in the Chelonia (Felix (15)).

Katz (22) and van den Broek (12) have put forward suggestions as to the origin of the bladder in marsupials, but they lacked the material necessary for the decision of this question. Katz thought that the bladder was probably derived from the whole allantois, which he imagined was taken into the body during development. From his investigations on many pouch-young, in which the allantois had completely disappeared, van den Broek ((12) p. 440) comes to the conclusion that the bladder develops almost entirely from the entodermal cloaca, to which is added a small mesodermal section arising from the Wolffian ducts. In the foetal Macropodinae this author (10) found a thick mass of connective tissue, uniting the apex of the bladder to the abdominal wall, but in all other marsupials which he examined, the distal end of the bladder lay free in the body cavity between the coils of the intestine, whilst all traces of the umbilical arteries and the lig. vesico-umbilicale had disappeared. As we have seen in Trichosurus, however, the urachus, whose condition in pouch-young with a length of 14·5 mm. very much resembles that of *Macropus ruficollis*, figured by van den Broek ((10) p. 360), degenerates soon after birth, whilst the hinder portions of the umbilical arteries apparently persist as the vesicle arteries.

With van den Broek (5, 9 and 10) we believe the early formation and rapid growth of the bladder is to be correlated with the very early intestinal (milk) nutrition, which is characteristic of marsupials.

WOLFFIAN DUCT

From the material at our disposal, it was impossible to observe the origin of the Wolffian duct. In our earliest embryo (*G.L.* 5 mm.), the duct is already completely developed. Its anterior end lies on a level with the third and fourth spinal ganglia, and behind this, it is discontinuous and evidently degenerating (plate I, fig. 2, *W.D.*). The urogenital fold at this stage is well marked. Within it, lie the ventral part of the cardinal vein on the lateral side, and the anterior end of the Wolffian duct and the rudimentary excretory tubules ventro-medially. The Wolffian duct may, as in Stage I *a*, lie in a secondary outbulging of the fold ventro-medially to the cardinal vein. Anterior to the Wolffian duct, the ventral portion of the vein alone fills the fold, which gradually flattens out cranially until it disappears altogether. Posteriorly, in the region of the mesonephric tubules, both duct

and tubules lie ventrally to the cardinal vein, and occupy almost the whole of the fold. The Wolffian duct, which is hollow throughout its length, passes back on the lateral side of the fold, and bends medially shortly behind the last mesonephric tubules, to become attached on each side by a solid connection to the ventral part of the lateral wall of the cloaca (plate III, fig. 15, *W.D.*), some distance behind the primordium of the allantois (text-fig. 2, *W.D.*).

At Stage II the urogenital fold is not present anterior to the Wolffian duct. By Stage III (*G.L.* 7 mm.) its posterior portion is larger, and has grown down farther into the coelom, almost the whole of the cardinal vein, as well as the excretory organ, lying within it. The Wolffian duct on the right side has just acquired an opening into the cloaca, whilst on the left the junction is still solid. The primordium of the ureteric bud (v. p. 46) (plate II, fig. 9, *u.b.*) makes its appearance at this time, just in front of the ventro-medial bend of each Wolffian duct. By the next stage (*G.L.* 7·5 mm.), both ducts communicate with the cloaca by a narrow opening on each side, posterior to the entrance of the gut and allantois.

In the embryo with a length of 7 mm. (Stage III), just posterior to the anterior end of the Wolffian duct, the coelomic epithelium at the tip of the urogenital fold is thickened. At Stage IV (*G.L.* 7·5 mm.), this thickening extends from the anterior end of the duct down to the eighth spinal ganglion, where distinct mesonephric tubules with developing glomeruli are found. By the following stage (*G.L.* 6 mm.) it is still more marked, and here, the pointed margin of the fold at the cranial end approaches nearer to the lateral wall of the coelom. In the next stage (Stage VI, *G.L.* 7·75 mm.), the anterior end of the fold lies some distance behind the end of the Wolffian duct, and its margin is now united with the lateral wall of the coelom, so that there is now present a coelomic bay or pocket between the fold and the dorso-lateral wall of the body cavity. Immediately behind this union, where the margin of the fold is again free, we find a slight groove, which is the first indication of the ostium abdominale of the Müllerian duct (v. p. 74). At Stage VII (*G.L.* 7·25 mm.), the coelomic pocket or cul-de-sac is better developed, having an antero-posterior length of ·12 mm. on the right side. In later stages, however, it gradually disappears, until, in embryo with a length of 11·5 mm., it is no longer present, the ostium abdominale opening out at the anterior free end of the urogenital fold. Plate IV, fig. 19 *a* (*c.p.*), shows the coelomic pocket in an embryo of 8·5 mm. (Stage IX *b*).

In embryos with a length of 7·25 mm. (Stages VII and VIII), the hinder ends of the two Wolffian ducts have widened considerably in a sagittal direction (cf. text-figs. 3 and 4, *W.D.*), and now open on a level with the allantois, ventral to and in front of the hind-gut, which has already begun to separate off from the rest of the cloaca. At Stage IX (*G.L.* 8·5 mm., text-fig. 5 and plate V, fig. 23), the sagittal widening is still more marked, and the ducts have undergone lateral compression, the hinder margins of their

openings being continuous with the lateral ridges of the sinus wall (v. p. 55). By Stage XII *b* (*G.L.* 11·5 mm., text-fig. 10 and plate VI, fig. 25) the enlarged base of the duct, up to the point of origin of the ureter, has become incorporated in the wall of the sinus, so that the duct now enters the latter as a narrow tube, whilst the ureter acquires an independent opening into the same. It may be noted that after Stage X (*G.L.* 9·5 mm.) the Wolffian duct runs continuously back from its anterior end, which is situated at the cranial end of the mesonephros.

In the new-born foetus (*G.L.* 14·5 and 15 mm.) the Wolffian duct, now provided with a large lumen, runs back along the lateral side of the urogenital fold, medially to the Müllerian duct. Behind the latter (text-fig. 1) it gradually moves medially, and, towards the posterior end of the mesonephros, extends along the ventro-medial border of the excretory organ, finally passing ventrally to enter the dorsal side of the anterior end of the urogenital sinus on each side (text-fig. 13).

In female pouch-young with a head length of 8·5 mm. and succeeding stages, behind the mesonephros, the posterior end of each urogenital fold, containing both Wolffian and Müllerian ducts, passes inwards to unite with its fellow of the opposite side in the middle line, thus forming the genital cord (v. p. 75). The Müllerian duct runs back close to the medial side of the Wolffian duct, and, in pouch-young of both sexes with a head length of 11 mm., unites with the Wolffian duct shortly before the entrance of the latter into the sinus (text-fig. 29, *M.D.*). In the male, the genital cord is always short and never extends much beyond the neck of the bladder, the posterior ends of the urogenital folds joining with the dorso-lateral side of the tissue surrounding the bladder, near the level of the entrance of the ureters into the latter.

At this time (*H.L.* 11 mm. and following stages), the anterior end of the Wolffian duct bends round medially in the presexual portion of the mesonephros, and remains connected with the degenerate excretory tubules destined to develop into the epididymis and epoophoron.

In a female with a head length of 16 mm., the Wolffian duct is much reduced in size and is already smaller than the Müllerian duct. In an older foetus (*H.L.* 19 mm.), it appears to be disconnected both from the epoophoron and paroophoron, and runs from below the former as a fine solid cord as far as the union of the Müllerian duct with it, behind which point it increases considerably in diameter (v. text-fig. 30, *H.L.* 22 mm.). In a foetus with a head length of 22 mm., however, it enlarges at the anterior end and can still be followed into the canals of the epoophoron. In an older pouch-young (*H.L.* 24 mm.) the Wolffian duct is absent for a short distance anterior to the junction with the Müllerian duct, and in one with a head length of 38 mm., except perhaps for a few isolated pieces, it has completely disappeared in front of this point. Its persistence as the hinder end of the lateral vagina in Trichosurus is described in our section on the Müllerian duct.

In the male, the anterior end of the Wolffian duct always remains connected with the canals of the epididymis (v. Part II of this paper). In a foetus with a head length of 15 mm. the ventral part of the postsexual portion of the mesonephros, which is now very degenerate, together with the Wolffian and narrow Müllerian ducts, is beginning to descend into the saccus vaginalis. The description of the consequent elongation and characteristic bending of the vas deferens does not come within the scope of this paper.

MÜLLERIAN DUCT

The primordium of the Müllerian duct is first recognisable at Stage VI (*G.L.* 7·75 mm., XX '04). In this embryo, directly behind the coelomic pocket, which is described in our section on the Wolffian duct (p. 72), there is a marked thickening of cells over the now free margin of the urogenital fold. Within this thickening, on the ventro-lateral side of the fold, between the level of the eighth and ninth spinal ganglia, is a groove. This is the first indication of the ostium abdominale tubae of the Müllerian duct. It is, however, not very distinct at this stage, and is better seen on the left side of the body. At Stage VII (*G.L.* 7·25 mm.) the groove is well marked, now lying between the tenth and eleventh spinal ganglia, ventro-laterally to the Wolffian duct, in the greatly thickened margin of the fold. In this embryo it is best developed on the right side. Plate IV, fig. 17 shows a longitudinal section through the thickened coelomic epithelium at Stage VIII *a* (*G.L.* 7·25 mm.).

At Stage IX (*G.L.* 8·5 mm.) the ostium abdominale is well defined. As a result of the degeneration of the cranial portion of the urogenital fold, together with the anterior tubules of the mesonephros, it now lies between the fifteenth and sixteenth spinal ganglia, and forms an ingrowth on the ventral side of the urogenital fold immediately behind the coelomic pocket, and slightly behind and lateral to the anterior end of the Wolffian duct. It consists of an anterior (plate IV, fig. 19 *a*) and a posterior (plate IV, fig. 19 *b*) bay, in each of which are two smaller ingrowths. The posterior bay extends back for a short distance as a canal (plate IV, fig. 18), the Müllerian duct. The ostium is lined by thickened coelomic epithelium, which stretches medially towards the suprarenal body as a low indentation of the urogenital fold; this medial indentation is the anterior termination of the rete ridge (v. Part II of this paper).

In succeeding stages (X to XII), the ostium abdominale comes to consist of a single large ingrowth, the subsidiary bays having become obliterated.

In the new-born foetus (*G.L.* 14·5 mm., Stage XV) the ostium lies just cranially to the mesonephros at the extreme anterior free edge of the urogenital fold. It runs in from the medial side, the Müllerian duct extending laterally across the anterior margin of the fold, where it turns posteriorly, and passes back on the lateral side of the Wolffian duct. It may be solid for

a few sections at its posterior end (i.e. for ·06 mm. on the left side in one foetus), or it may remain tubular throughout. The margin of the fold, in which lies the Müllerian duct, represents the tubal portion of the latter. It forms a slight salient, only marked off from the major portion of the fold by the denser mesenchyme which surrounds the Müllerian duct. Towards the hinder end of the latter, which, in one of our examples, terminates after a length of ·78 mm., the tubal portion contains both genital and excretory ducts, and a slight groove now separates it from the rest of the fold on the ventral side. Not far from the posterior end of the mesonephros, this tubal portion, in which only the Wolffian duct is now present, becomes more distinctly marked off, and behind the excretory gland it becomes attached to the lateral wall of the coelom, the Wolffian duct running back posteriorly and ventrally into the dorso-lateral side of the urogenital sinus.

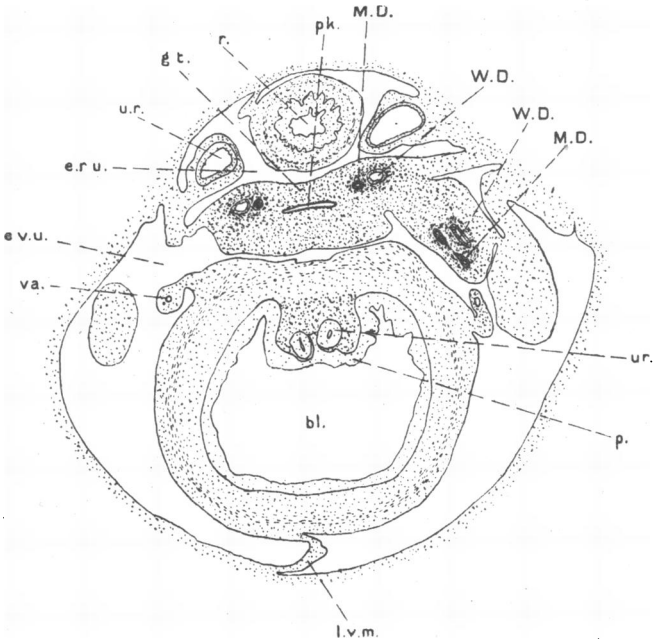
In pouch-young with head lengths of 6·75 to 7·5 mm., the Müllerian duct has an average length of ·9 mm., its posterior end lying a short distance behind the mesonephros.

In a foetus with a head length of 8·5 mm., the duct has more than doubled its length compared with the last stage, now measuring 2·2 mm. The ostium abdominale has the form of a narrow aperture running in from the ventro-medial side of the anterior end of the urogenital fold, in front of the meso-nephros. The Müllerian duct extends back immediately laterally to the Wolffian duct, gradually passing more ventrally until at the hinder end of the mesonephros it bends medially, and ends on the ventral side of and closely adjacent to the Wolffian duct (plate IV, fig. 20 *a* and *b*); its course in relation to the latter is thus a loose spiral.

Behind the excretory gland, the posterior portion of the urogenital fold, which represents the backward continuation of its tubal portion, and which at this stage as yet contains only the Wolffian duct, passes back, and unites on each side with the tissue surrounding the bladder at the level of the entrance of the ureters into the latter. As a result, the Wolffian and Müllerian ducts, as well as the neck of the bladder, come to be enclosed in a common sheath of mesodermal tissue. The entire formation constitutes the primordium of the genital cord. In the male, the genital cord always remains very short, never extending forwards very far beyond the neck of the bladder, whilst in the female, it undergoes progressive development from this region forwards, and becomes a conspicuous structure. In the female of this stage (*H.L.* 8·5 mm.) it has a length of ·49 mm., and is already better developed than in the male, where it measures only ·18 mm. in length. Comparison of the two sexes shows that, in the female, the genital cord has already extended in front of the bladder neck, as the result of the progressive postero-anterior fusion of the urogenital folds. This region in section takes the form of a transverse band of tissue, which has an antero-posterior length of ·04 mm., and in which the Wolffian ducts alone are situated, the tissue immediately around each duct being dense and darkly staining. Posteriorly, it becomes

continuous with the tissue on the dorsal side of the bladder neck, and shortly behind this level, the ureters enter the genital cord, and, running through the latter medially to the Wolffian ducts, curve forwards to open on a papilla at the base of the bladder.

In pouch-young with a head length of 11 mm., the anterior end of the urogenital fold has become bent round ventrally to the presexual portion of the mesonephros, the Müllerian duct curving round dorsally and laterally, and passing back laterally to the mesonephros. In the female, the postero-anterior fusion of the hinder portions of the urogenital folds has made further

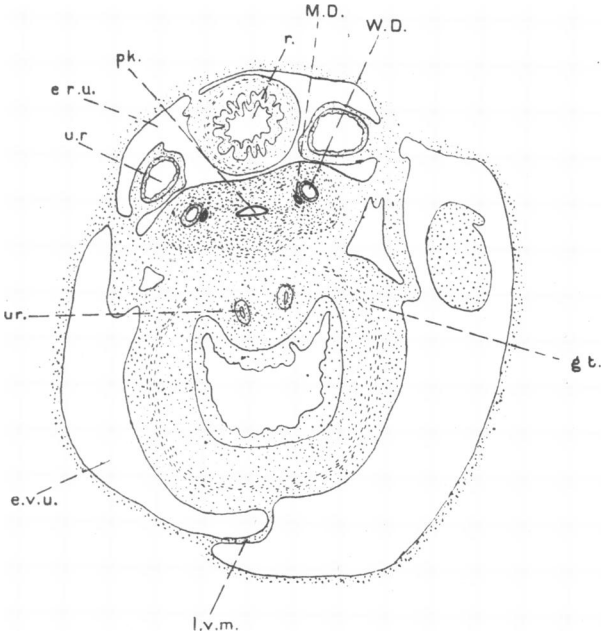


Text-fig. 26. *H.L.* 11 mm. ♀. Transverse section, showing the anterior end of the genital cord (*g.t.*) in which lie the Wolffian (*W.D.*) and Müllerian (*M.D.*) ducts. The papilla (*p.*) on which the ureters (*ur.*) open is seen projecting into the base of the bladder (*bl.*). *e.r.u.* excavatio recto-uterina; *e.v.u.* excavatio vesico-uterina; *pk.* peritoneal pocket extending into anterior end of genital cord; *l.v.m.* ligamentum vesicae medium; *r.* rectum; *v.a.* vesicle artery. (Sl. 13–4–4.)

progress, and they now meet across the middle line to form the anterior portion of the genital cord (text-fig. 26, *g.t.*), which, after a length of ·075 mm., becomes continuous with the connective tissue surrounding the bladder (text-fig. 27). In both sexes the posterior part of the Müllerian duct now runs back medially to the Wolffian duct on each side dorso-laterally to the bladder and urethra (text-figs. 27 and 28), and, a short distance in front of the opening of the Wolffian duct, it enlarges slightly and unites with the medial side of the latter (text-fig. 29, *M.D.*, *W.D.*). In pouch-young (♂ and ♀) with a head length of 12 mm., the cavities of the two ducts appear to be actually continuous (plate V, fig. 21 *a* and *b*). In the female at this stage the genital

cord has lengthened considerably, now measuring about 1·2 mm. Its anterior portion, measuring ·43 mm. in length, and situated in front of the union with the neck of the bladder, divides the coelom into two portions, viz. an excavatio recto-uterina (text-figs. 26 to 29, *e.r.u.*), dorsal to the genital cord and partially surrounding the gut, and an excavatio vesico-uterina (text-figs. 26 to 29, *e.v.u.*), ventral to the genital cord and round the bladder. Plate V, fig. 22 shows the passage of the ureters (*ur.*) through the hinder portion of the genital cord, and their openings on the papilla (*p.*) at the neck of the bladder.

In the male with a head length of 15 mm., the ostium abdominale is closed, and the anterior end of the Müllerian duct is attached to the coelomic

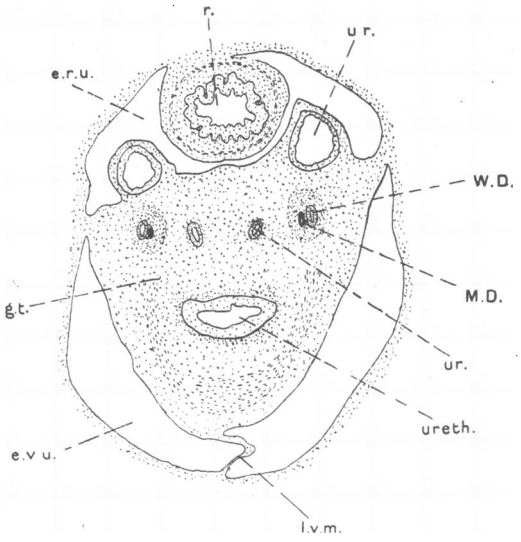


Text-fig. 27. *H.L.* 11 mm. ♀. Transverse section, showing the genital cord (*g.t.*) posterior to fig. 25, in which are enclosed the Wolffian (*W.D.*) and Müllerian (*M.D.*) ducts, together with the neck of the bladder (*bl.*). Lettering as in text-fig. 26. (Sl. 14–1–3.)

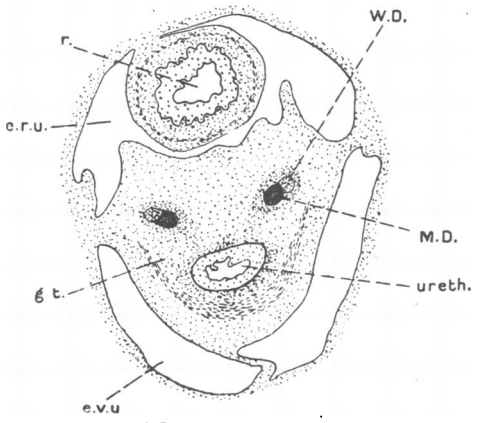
epithelium by a solid cord of cells. Further back, the duct has decreased in diameter. In an older male (*H.L.* 20 mm.), where the testis lies in the saccus vaginalis on the ventro-lateral side of the body, the cranial end of the Müllerian duct forms a closed tube, lying posterior to the epididymis on a level with the rete. As it passes posteriorly, it soon becomes a solid cord of small diameter, which follows the course of the Wolffian duct, and, along the ascending limb of the latter, is only present as a fine strand, which was not definitely traced through all the sections. Just in front of the entrance of the Wolffian duct into the urogenital sinus, it widens suddenly into a tube, and enters the medial side of the Wolffian duct. At this point, the cavity of the latter appears to be almost obliterated, but behind the union, it is dis-

tinctly enlarged. In our oldest male (*H.L.* 26 mm.) the anterior portion of the Müllerian duct is still present, but the duct has completely disappeared along the ascending loop of the vas deferens. The widened terminal portion, however, is still seen running into the latter as in the preceding stage.

In the female with a head length of 16 mm., the ostium abdominale is much enlarged. It opens on the medial side of the urogenital fold, just dorsal to the ovary and medial to the epoophoron. The Müllerian duct runs in directly laterally as a well-marked tube, which then makes a definite bend, curving forwards and then backwards to run ventro-laterally to the ovary, at first medial to and then below the Wolffian duct. Behind the ovary, the Müllerian duct runs back with the Wolffian duct in the posterior part of the urogenital fold; as the two folds unite to form the genital cord, the Müllerian ducts pass inwards to the medial side of the Wolffian ducts, and continue back close to the latter. They bend slightly outwards as the ureters pass medially to them through the tissue of the genital cord to enter the bladder, and, finally, each unites with the corresponding Wolffian duct, shortly before the latter enters the dorsal side of the urogenital sinus. The Wolffian duct is now greatly reduced, and much smaller than the Müllerian duct. In a foetus with a head length of 19 mm., it is reduced to a fine strand, which, posterior to the union with the Müllerian duct, however, widens considerably. In this foetus, the ostium abdominale, on the right side, lies medial to the anterior end of the ovary, and on the left, it is

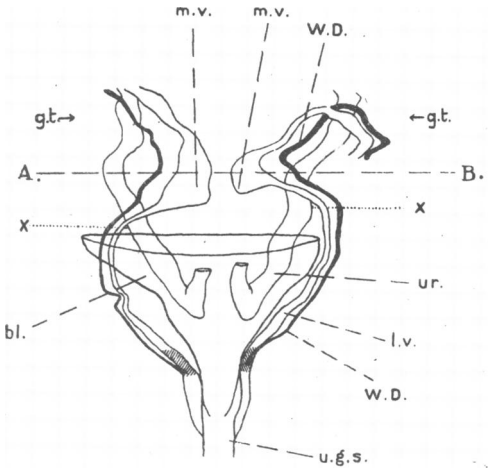


Text-fig. 28. *H.L.* 11 mm. ♀. Transverse section through the genital cord (*g.t.*) posterior to text-fig. 26, showing the passage of the ureters (*ur.*) through the cord. *ureth.* urethra. Other lettering as in text-fig. 26. (Sl. 14–2–7.)

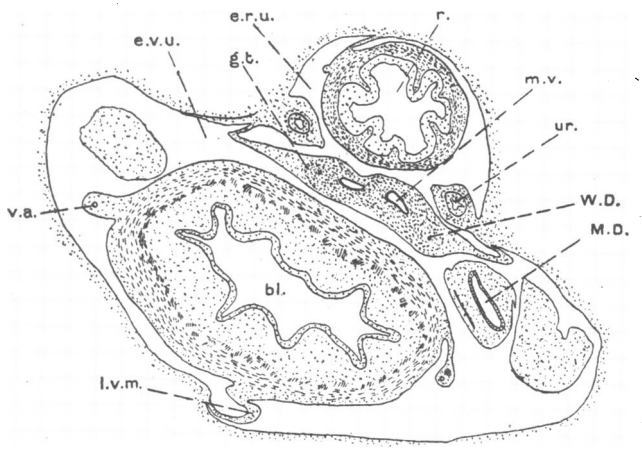


Text-fig. 29. *H.L.* 11 mm. ♀. Transverse section through the genital cord (*g.t.*) showing the union of the Müllerian (*M.D.*) with the Wolffian (*W.D.*) duct. *ureth.* urethra. Other lettering as in text-fig. 26. (Sl. 14–3–6.)

placed further back, behind the rete and on the ventro-lateral side of the ovary. The genital cord has now a length of about 2 mm., of which the anterior half (·84 mm.) forms a band of tissue simply uniting the genital ducts of the two sides.



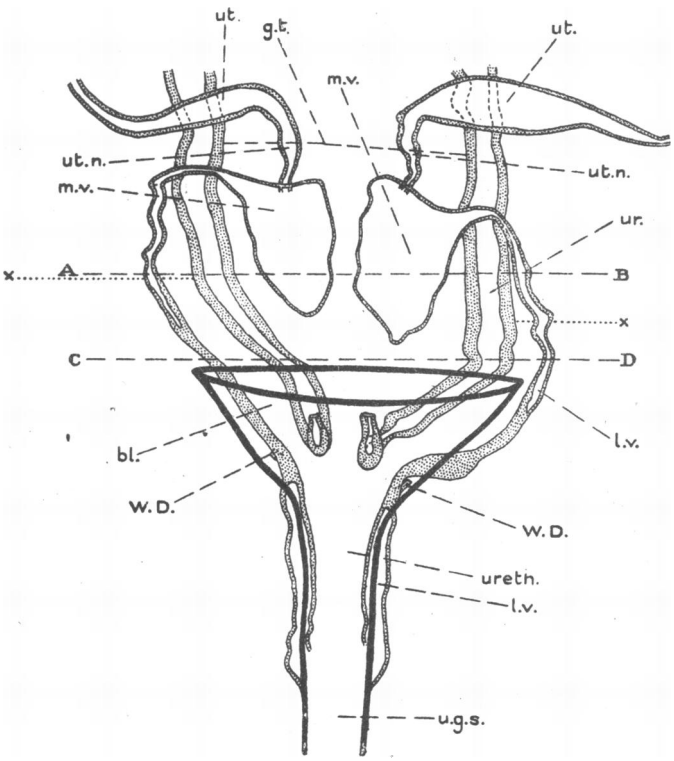
Text-fig. 30. *H.L.* 22 mm. ♀. Reconstruction of the hinder ends of the Müllerian (*l.v.*) and Wolffian (*W.D.*) ducts, showing the primordium of the median vaginae (*m.v.*). The ureters (*ur.*) are seen to pass medially to the Müllerian ducts into the bladder (*bl.*). At the level of the dotted lines x the ureters enter the genital cord. The solid portion of the Müllerian duct (*l.v.*), near its union with the Wolffian duct, is shaded. (*g.t.*) shows the level of the anterior end of the genital cord. Ventral view. ×20.



Text-fig. 31. *H.L.* 22 mm. Embryo F. Transverse section through the anterior end of the genital cord (*g.t.*) in the region shown by the broken line *A*...*B* in text-fig. 30, showing the primordia of the median vaginae (*m.v.*). Other lettering as in text-fig. 26. (Sl. 3–3–5.)

In the next stage (*H.L.* 22 mm.) the Müllerian ducts, shortly after entering the genital cord, enlarge considerably, their walls becoming thinner and their cavities wider. These enlargements are destined to produce the median

vagina (text-figs. 30 and 31, *m.v.*). The duct of each side then bends outwards, and continues back as the gently curved lateral vaginal canal (text-fig. 30, *l.v.*), situated on the dorso-lateral side of the urethra. It eventually becomes solid (text-fig. 30, *l.v.*, shaded portion), and joins the Wolffian duct shortly before the entrance of the latter into the sinus, the Wolffian duct enlarging considerably behind the point of union (text-fig. 30, *W.D.*).

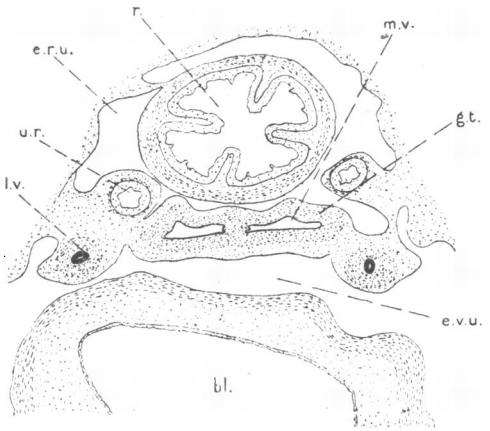


Text-fig. 32. *H.L.* 38 mm. Reconstruction of hinder ends of Müllerian ducts, showing the primordia of the uteri (*ut.*) and uterine necks (*ut.n.*), and the now conspicuous median vaginae (*m.v.*). The posterior portions of the lateral vaginae (*l.v.*) are practically solid for some distance, the lumen again appearing shortly behind the last remnant of the Wolffian duct (*W.D.*). The broken line (*g.t.*) marks the anterior end of the genital cord. At the level of the dotted lines x, the ureters enter the genital cord. Ventral view. ×20. *ureth.* urethra. Other lettering as in text-fig. 30.

In a foetus with a head length of 38 mm., just in front of the genital cord, the Müllerian duct, especially that of the left side, enlarges slightly to form the first indication of the future uterus (text-fig. 32, *ut.*). The ducts then decrease markedly to form the uterine necks (*ut.n.*), which communicate by two definitely projecting orae with the median vaginae (*m.v.*).

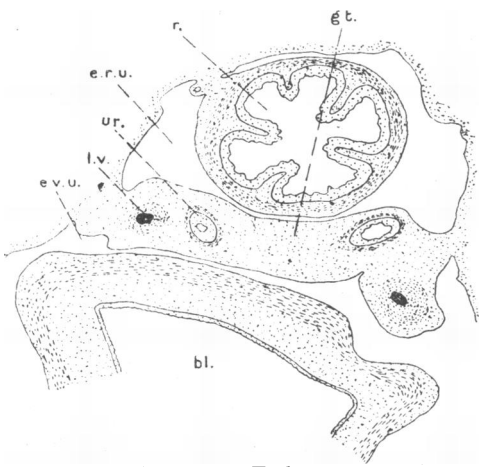
These are now definitely formed, and appear as two thin-walled dorso-ventrally flattened sacks which project backwards between the lateral vaginae on either side of the middle line (text-fig. 33). From the antero-

lateral corners of the median vaginae, the ducts bend outwards and then backwards as the lateral vaginae (text-figs. 32 and 33, *l.v.*), the walls of which are now thickened. Posterior to the entrance of the ureters into the bladder—in text-fig. 34, the ureters (*ur.*) have just entered the genital cord (*g.t.*), and have not yet reached the bladder (*bl.*)—the two vaginae approach each other, and, at the same time, they become reduced in diameter and their epithelial lining becomes folded (text-fig. 35 *a*). A small blind cord, evidently the last remnant of the Wolffian duct, unites with the Müllerian duct at this point (text-figs. 32, 35 *b* and *c*, *W.D.*). Posterior to this junction, the lateral vagina (*l.v.*) increases considerably in size (text-figs. 32 and 35 *d*), and runs into the dorso-lateral wall of the urogenital sinus (*u.g.s.*).



Text-fig. 33. *H.L.* 38 mm. Embryo H. Transverse section through the anterior end of the genital cord (*g.t.*), showing the median vaginae (*m.v.*) and lateral vaginae (*l.v.*) at the level of the broken line *A*...*B* in text-fig. 32. Other lettering as in text-fig. 26. (Sl. 3–5–3.)

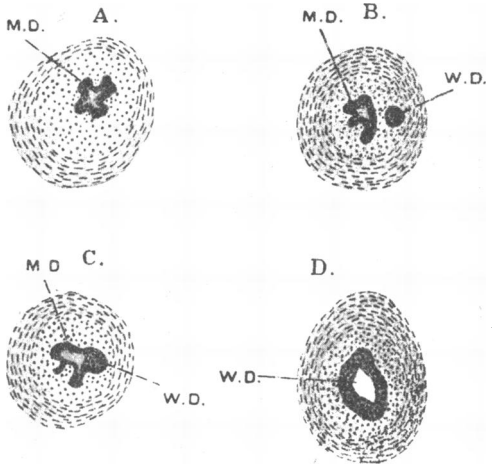
In a foetus with a head length of 18·5 cm., the primordia of the uteri are well marked. The median vaginae now consist of two very large culs-de-sac, separated by a narrow median partition, and lined by a thin layer of epithelium with closely packed elongated nuclei. They extend back and terminate a short distance in front of the passage of the ureters into the bladder. The lateral vagina of each side, towards its posterior end, shows a constriction, which doubtless marks the former point of union of the Müllerian with the Wolffian duct.



Text-fig. 34. *H.L.* 38 mm. Embryo H. Transverse section through the region shown by the broken line *C*...*D* in text-fig. 32, showing the passage of the ureters (*ur.*) through the genital cord (*g.t.*). Other lettering as in text-fig. 26. (Sl. 4–3–5.)

If the interior of the lateral vagina be examined in the adult, it will be seen that the lumen shows a localised constriction towards the lower third of the duct, and, in the virgin female, may be completely occluded in this region. Posteriorly it again widens. Further,

the upper two-thirds of the tube, which is derived from the Müllerian duct, has a reticulated lining, outside which is a layer of very loose connective tissue. Posterior to the constricted region, in that portion of the tube which is derived from the Wolffian duct, the lining is markedly different in character; it is thrown into longitudinal ridges, about four in number, whilst the layer of connective tissue, immediately outside it, is much denser than



Text-figs. 35 *a*, *b*, *c* and *d*. *H.L.* 38 mm. Embryo H. Transverse section through the lateral vagina at the union of Müllerian (*M.D.*) and Wolffian (*W.D.*) ducts.

35 *a*. Lateral vagina a short distance before union with the Wolffian duct.

35 *b*. The two ducts immediately in front of union.

35 *c*. Point of union of the two ducts.

35 *d*. Posterior to union, showing enlarged Wolffian duct.

the corresponding layer of the upper portion of the tube. Throughout its length, the lateral vagina is enveloped by an inner layer of longitudinal and an outer layer of circular muscle fibres, but around the constricted portion there is a second layer of circular fibres within the longitudinal layer, and surrounding the compact connective tissue. Unfortunately, owing to the lack of suitable sections, we were not able to deal with the corresponding region of the vas deferens.

SUMMARY AND DISCUSSION

The Müllerian duct arises as an ingrowth of the thickened coelomic epithelium towards the anterior end of the urogenital fold. It is first apparent in an embryo with a length of 7·75 mm. (Stage VI) between the eighth and ninth spinal ganglia, a short distance behind the anterior end of the Wolffian duct. The ostium abdominale tubae at first (embryo of 8·5 mm. *G.L.*) consists of several subsidiary ingrowths (fig. 19 *a* and *b*), which apparently merge into one large opening (*G.L.* 9·5 and 10 mm.). Whatever may have been its phylogenetic origin it has no ontogenetic connection whatever with

pronephric nephrostomes in Trichosurus, as has been suggested for other mammals by von Winiwarter (38), Wichmann (37) and others. The thickened coelomic epithelium, from which it arises, lies much nearer the lateral side of the urogenital fold than these nephrostomes, or than any other degenerate excretory tubules, which may occur at the same level: From the ostium, the Müllerian duct grows back as a tube, situated close to the margin of the fold, and immediately laterally to the Wolffian duct; in the newly born young, it has reached a length of ·78 mm. In pouch-young with a head length of 11 mm., it unites at its hinder end with the medial side of the Wolffian duct (text-fig. 29). The Müllerian duct always lies close to the Wolffian duct; in its anterior region, it is situated on the lateral, and more posteriorly, on the ventral side of the latter; in the posterior part of the urogenital fold, it passes up medially to the Wolffian duct, and in that position runs backwards with the latter in the genital cord.

In both sexes, the tubal portion of the urogenital fold contains both the Müllerian and the Wolffian ducts. In the human embryo according to Felix (14), there is a distinction between the male and the female in this respect; whereas, in the former, both ducts lie within the tubal portion, in the latter, only the Müllerian duct is found within it. Along the region of the mesonephros it is seen as a well-marked projection, separated from the rest of the fold by a groove. Behind the mesonephros, it forms the posterior continuation of the urogenital fold.

In the male, the hinder ends of these folds unite directly with the tissue on the dorsal side of the neck of the bladder to form the very short genital cord[1]. In the female, the two folds not only become enclosed with the neck of the bladder in a common sheath, but actually join together in the middle line in front of this region. Thus there is formed the elongated genital cord of the female, in which both Wolffian and Müllerian ducts lie embedded in a common mesodermal sheath (text-fig. 26). The genital cord, once established in the female, undergoes a progressive increase in length, as the result, partly of the lengthening of the neck region of the bladder and the formation of the urethra, partly of the progressive union of the urogenital folds from behind forwards, and partly of general interstitial growth. Sections through the mid-region of the cord (text-fig. 34 and plate V, fig. 22) show the two lateral vaginae situated dorso-laterally to the urethra, whilst the curved hinder portions of the ureters may be seen to penetrate the tissue of the genital cord, between the lateral and median vaginae, to reach the bladder.

The primordia of the median vaginae first appear in the female with a head length of 22 mm. (text-fig. 30) as two enlargements of the Müllerian

[1] Our use of the term genital cord is in accordance with the definition of Thiersch (*Illustr. med. Zeitschr.* 1852) and Mihalkovics (27). The latter states (p. 326): "Dieses Bindegewebe am Harnblasengrund ist die erste Anlage des Geschlechtsstranges" and "Der Geschlechtsstrang weiter nichts ist als das dichter gewordene Bindegewebe um den distalen Teil der Wolff'schen Gänge, das sich in der Medianlinie mit jenem der anderen Seite zu einer gemeinsamen Platte vereinigt hat."

ducts, just where they bend outwards in the tissue of the genital cord to form the lateral vaginae. These enlargements grow rapidly backwards, and soon form two large thin-walled dorso-ventrally flattened culs-de-sac, lying between the lateral vaginae, and separated from each other by a narrow median partition. In a foetus with a head length of 38 mm., we find the first indication of the uteri and uterine necks (text-fig. 32, *ut.* and *ut.n.*), and in an older female with a greatest length of 18·5 cm., both median vaginae and uteri are well established.

In Trichosurus, therefore, there is a genital cord of exactly the same character as that described and figured by Professor Hill (19) in Peramelidae and in foetal Macropodidae. Van den Broek (10) is under a complete misapprehension when he denies the occurrence of a continuous genital cord in marsupials (p. 369). According to this author, the tissue round the genital ducts only unites across the middle line at the level of the future uteri and the passage of the latter into the vaginae, whilst the ureters bend round the lower edge of the cross connection thus formed. On the contrary, our observations demonstrate that the lateral vaginae are embedded in the genital cord throughout their length, and that the ureters actually pass through the cord to reach the bladder (plate V, fig. 22).

In the male Trichosurus, the ostium abdominale tubae is already closed in a pouch-young with a head length of 15 mm. The Müllerian duct is reduced in diameter, and in the oldest male (*H.L.* 26 mm.) which we examined, where the testis lies in the saccus vaginalis on the ventro-lateral side of the body, it has altogether disappeared along the ascending loop of the vas deferens.

The union of the Müllerian with the hinder end of the Wolffian duct in marsupials was first recorded in a female pouch-young of *Macropus rufus* by Lister and Fletcher (26). In more recent times, van den Broek (10) has described this condition in a female of *Macropus ruficollis*, measuring 34 mm. *D.C.L.*, and in a male of *Macropus thetidis* (*D.C.L.* 2·8 cm.). The figures of this author ((12) p. 445), showing the connection of the two ducts in *Macropus thetidis*, almost exactly resemble those from the same region in Trichosurus (cf. text-fig. 35 *b*, *c*, *d*). Van den Broek compares these figures with cross sections of the segmental duct in Elasmobranchs, where, as is well known, the Müllerian and Wolffian ducts arise by the longitudinal splitting of the pronephric duct. Van den Broek supposes that if the Müllerian duct develops in the same manner in the Macropodinae, then the cleavage is never completed, but he also suggests that the conditions here may point to secondary union of two primitively separate ducts. We have seen that in Trichosurus, and indeed in all the Didelphia which we have investigated, the Müllerian duct does not arise by cleavage from the Wolffian duct, but quite independently of the same; moreover, the Müllerian duct in Trichosurus, and evidently also in the Macropodinae, never reaches the urogenital sinus, but joins with the caudal end of the Wolffian duct. This condition, however, as the study of other marsupials demonstrates, is not primary, but purely secondary.

In early pouch-young of Dasyurus (*H.L.* 8 to 10·5 mm., Stages H to I), the Müllerian duct opens independently into the urogenital sinus, ventrally and just cranially to the Wolffian duct. In the next stages, the two ducts appear to run into each other at their extreme posterior ends, so that they have a common opening into the sinus. The oldest foetus, which we have examined, was a female with a head length of 2·8 cm.; here, the point of union of the two ducts occurred about ·37 mm. in front of the connection with the sinus, whilst the single duct thus formed appears to be practically solid throughout.

In pouch-young of Didelphys (*H.L.* 8 to 10·5 mm.), the Müllerian duct joins the wall of the urogenital sinus, just medially to the Wolffian duct, and the connection is apparently solid. In a male foetus with a head length of 12 mm., the posterior end of the Müllerian duct is no longer present, but the Wolffian duct, posterior to the passage of the ureters through the genital cord, and shortly before its entrance into the sinus, becomes reduced in diameter, and its lumen almost obliterated for a short distance. On the other hand, in an older male (*H.L.* 18 mm.), the small solid end of the Müllerian duct is still embedded in the wall of the sinus, medially to the Wolffian duct. In a female of the same age (*H.L.* 18 mm.), posterior to the passage of the ureters through the genital cord, the two lateral vaginae become solid. Shortly behind this point, in the region of the hinder third of the vagina on each side, a change takes place in the character of the lining of the duct. Whereas the epithelium of the upper part of the latter stains deeply with eosin, here it is hardly affected by the counter-stain, the change first becoming apparent on the lateral side of the tube, whilst a small central cavity again makes its appearance. Very soon, however, the duct becomes once more solid, and continues so almost up to its opening into the urogenital sinus. This differential staining in the wall of the lateral vagina seems to point to a difference in origin of the two portions of the tube, but the material at our disposal is not sufficient for the settlement of this question.

In Perameles, for want of the requisite stages, we had no opportunity of observing whether or not the Müllerian duct opens independently into the sinus. In a female pouch-young with a head length of 12·5 mm., the Müllerian duct, shortly in front of the passage of the ureters into the bladder, passes round from the medial to the ventral side of the Wolffian duct, and, not far from the entrance of the latter into the urogenital sinus, it unites with the ventral side of the Wolffian duct. In a female with a head length of 16 mm., the Müllerian duct lies medially to the Wolffian duct in the genital cord, and joins the dorso-medial side of the duct a considerable distance farther from the sinus than in the last stage.

Van den Broek (10) (12) also observed that, in both Dasyurus and Didelphys, the Müllerian and Wolffian ducts have separate openings into the urogenital sinus, but, not being in possession of the earlier stages, he was unable to determine whether this condition was primitive or not. It is

undoubtedly primitive. In later stages, the two ducts apparently join together at their posterior ends, but we have found it impossible to decide whether the terminal portions of the genital ducts, in these two forms, are constituted by the Wolffian duct alone or by the united Müllerian and Wolffian ducts, or whether, indeed, they may not represent constricted off parts of the dorso-lateral walls of the sinus itself.

In later stages of Trichosurus, after the disappearance of the whole of the more anterior part of the Wolffian duct in the female, the former point of union of the Müllerian with the Wolffian duct is marked by a constriction in the lateral vagina. In the adult, this constriction lies about one-third from the posterior end of the latter, whilst there is also a marked difference in the appearance of the epithelial lining of the anterior and posterior portions of the tube, and in the structure of their walls. In the adult Macropodinae, van den Broek (10) observed a gradual decrease and sudden widening of the lumen towards the caudal end of the lateral vagina, and rightly suggests that this point may coincide with the opening of the Müllerian into the Wolffian ducts, the posterior portion of the vagina being therefore derived from the Wolffian duct. The same constriction has been previously noted by Lister and Fletcher (26) in Macropus and Petrogale. Van den Broek ((10) p. 372) also refers to the marked convolution in the posterior region of the lateral vagina in adult Didelphys, and he thinks it may have some connection with the point of union of the two ducts. We have examined the lateral vagina in the adult of both *Marmosa murina* and *Didelphys aurita*, in which genera the above mentioned convolution is developed. We find that immediately posterior to the curved region, the epithelial lining of the tube is greatly thickened, whilst the lumen becomes gradually much reduced. It is therefore most probable that van den Broek's interpretation is correct, and that, as pointed out to us by Professor Hill, the bending is due to unequal growth, the portion of the Müllerian duct above the point of junction growing more markedly than the part of the tube derived from the Wolffian duct. In Trichosurus, the Macropodidae, and presumably also in Dasyurus and Didelphys, the major portion (about two-thirds in Trichosurus) of the lateral vagina is derived from the Müllerian duct, whilst the lower terminal portion (about one-third in Trichosurus) may arise, on the one hand, from the Wolffian duct alone (Trichosurus and Macropus), or, on the other, from the united Müllerian and Wolffian ducts, or from an outgrowth of the urogenital sinus itself (Dasyurus and Didelphys).

In higher mammals, several observers have concluded that the hinder end of the vagina is not derived entirely from the Müllerian duct. In 1884, Tourneux and Legay (35) pointed out that, in many mammals, the posterior portion of the genital canal is formed by the fusion of the Wolffian and Müllerian ducts, and that this hinder portion is primitively solid. In 1901, Hart (17) put forward the view that the vagina was Müllerian only in its upper two-thirds, the lower third being formed by the coalescence of the "Wolffian

bulbs" with the urogenital sinus. From his investigations in the calf, Bergschicker (2) comes to the conclusion that the hindermost part of the vagina is a product of both the Müllerian duct and the urogenital sinus. According to this author, the Müllerian duct lacks an open communication with the sinus, but "es bildet sich vielmehr eine ganz eigentümliche Verklebung der Übergangsstelle der Vagina in das Vestibulum aus, die sich erst kurz vor oder nach der Geburt löst."

SUPRARENAL BODY

No attempt has been made to work out the development of the suprarenal bodies in detail, the material not being suitable for such a study.

In our earliest stages the accurate determination of the primordium of the suprarenal body is a matter of some difficulty. At Stage I (*G.L.* 5 mm.), it appears on each side as a slight projection into the coelom, lying medially to the anterior end of the urogenital fold, from which it is separated by a slight groove. In the region of this groove and over the projection itself, the coelomic epithelium is thickened, whilst within the projection the cells are slightly denser. The primordium extends back just beyond the beginning of the Wolffian duct, and has roughly a length of ·18 mm., but it is impossible to determine with certainty its anterior and posterior limits.

By Stage III (*G.L.* 7 mm.), the body is better developed and quite definite. At its anterior end it forms a low ridge, which increases in size posteriorly, where the denser mass of cells are no longer confined to the outgrowth itself, but extend up dorsally between the aorta and the cardinal vein. At this stage, vascular connective tissue has begun to penetrate into the gland. Plate II, fig. 8 (*u.b.*), shows a section through the suprarenal body of the right side at Stage V.

In succeeding embryos the body undergoes degeneration at the anterior end, whilst it gradually extends further caudally. Intimate connection is established with the sympathetic ganglia, and the ingrowth of sympathochromaffin cells has definitely begun at Stage VII (*G.L.* 7·25 mm., III '01), and becomes very marked in following stages.

In the newly born young (*G.L.* 14·5 to 15 mm.) the suprarenal body lies opposite the twentieth spinal ganglion, not far from the anterior end of the urogenital fold, and extends back to the cranial end of the metanephros. The right gland produces an indentation on the dorso-medial side of the liver in which it lies, whilst the left, which is situated more caudally, forms an outgrowth into the coelom dorsally to the anterior end of the stomach. The ingrowth of sympatho-chromaffin cells seems to be usually completed at birth, and these cells now form irregular masses of varying size, scattered throughout the gland.

In older pouch-young we find the suprarenal body lying medially to the kidney on each side. In our oldest foetus (*H.L.* 15 mm.) in which the organ

was examined, the sympatho-chromaffin cells are distributed in small clumps in the centre of the gland, although some lie not far from the periphery. The primordia of the cortex and of the medullary nucleus are present, but are not yet definitely established.

CONCLUSIONS

1. A definite pronephros is present, which, though rudimentary, is as well developed as in any other mammal, including Echidna. It consists of a number of tubules (approximately 14 to 16), many hollow but some solid, which are usually connected with the Wolffian duct, and may be joined to the coelomic epithelium, where a distinct but rudimentary nephrostome is sometimes developed. A vestigial external glomerulus may also be present. There is no metamerism, several primordia occurring in one segment. The pronephros in Trichosurus apparently extends from between the third and fourth spinal ganglia (Stage I) as far back as the sixth, or possibly the ninth spinal ganglion, but for want of early stages and owing to the similar degeneration of the anterior tubules of the mesonephros, it was not possible to definitely determine its posterior limit. By Stage VII (*G.L.* 7·25 mm.) the organ has completely disappeared.

2. The mesonephros is a large and well-developed gland, which functions for a considerable period both before and after birth. The most anterior tubules are incompletely formed, with rudimentary glomeruli, and undergo a progressive degeneration, which begins before the hindermost tubules have completed their development, and continues up to Stage XI (*G.L.* 10 mm.). Mesonephric nephrostomes, usually in the form of solid cellular cords uniting the tubule with the coelomic epithelium, are often found in connection with the more rudimentary primordia. The mesonephric tubules develop much as in other mammals, and have a similar histological differentiation. Glomeruli first appear at Stage III (*G.L.* 7 mm.), and the gland attains its maximum development from Stages XI (*G.L.* 10 mm.) to XVII (*H.L.* 7·5 mm.). During this period, the average number of glomeruli is about forty-six, and these may attain a transverse diameter of ·20 mm.

3. The ureter arises in Stage III (*G.L.* 7 mm.) as a small rounded outgrowth from the dorso-medial side of the Wolffian duct shortly in front of the opening of the latter into the cloaca. The distal end of the outgrowth becomes enlarged to form the pelvis, whilst the proximal portion develops into the ureter. With the gradual incorporation of the posterior ends of the Wolffian ducts into the urogenital sinus, the ureters come to open directly into the latter (*G.L.* 11·5 mm.). The ureteric openings, and with them the characteristically curved posterior portions of the ducts, are finally carried forwards, and come to be situated on well-marked papillae projecting into the bladder, some distance in front of the openings of the Wolffian ducts into the urogenital sinus. The ureters permanently retain their medial

positions in relation to the latter, piercing the genital cord between the Wolffian as well as the Müllerian ducts.

The metanephric blastema arises from the nephrogenic tissue, which is the caudal continuation of the blastema out of which the mesonephric tubules develop. It surrounds the primordium of the pelvis, and moves with the latter as it grows forward dorsally to the mesonephros. The development of the tubules is similar to that of other mammals. At Stage IX (*G.L.* 8·5 mm.) the pelvis is expanded, and we find the first indication of primary collecting tubules. At Stage XIII (*G.L.* 12 mm.), the blastema is becoming transformed into metanephric vesicles, and at birth (*G.L.* 14·5 mm.), these are united with the collecting ducts of the pelvis. Definite Malpighian corpuscles are established at Stage XVII (*H.L.* 7·5 mm.), and the gland soon becomes functional. As the mesonephros still appears functional in the pouch-foetus with a head length of 12·5 mm., the two excretory glands evidently work together for a considerable period.

4. There is a well-developed entodermal cloaca, into which the allantois and hind-gut open. The cloacal membrane is well marked and consists of fused ectoderm and entoderm.

A conspicuous tail gut is present, whose bulbous extremity is connected for some time with the persisting remnant of the tail bud, in which the notochord and the medullary tube also terminate. It is separated off from the cloaca by Stage IX (*G.L.* 8·5 mm.), and disappears entirely at Stage XIV (*G.L.* 13·5 mm.).

The cloaca becomes divided into a dorsal and a ventral segment, forming the rectum and urogenital sinus respectively. This division is effected by means of a mesodermal ingrowth, the urorectal fold, which originates between the openings of the hind-gut and allantois into the cloaca, and gradually extends posteriorly, but which never quite reaches the original site of the cloacal membrane. The ingrowth at first contains a prolongation of the coelom. The separation into dorsal or rectal, and ventral or urogenital segments, begins at about Stage VII (*G.L.* 7·25 mm.), and is completed at Stage XIV *b*.

At Stage IX (*G.L.* 8·5 mm.) the abdominal wall, cranial to and on a level with the cloacal membrane, rises up to form the primordium of the genital tubercle. The cloacal membrane is ruptured in the next stage, and, with the continued growth of the tubercle, the cavity of the cloaca becomes drawn up into the latter, its lateral walls become approximated, and finally fuse together to form the cloacal septum, which is not quite completed until Stage XIV (*G.L.* 13·5 mm.). This septum is thus an entodermal structure.

An ectodermal cloaca develops at birth, apparently as the result of the outgrowth of the tubercle on the ventral side, and the formation of an elevation at the base of the tail, destined to form the dorso-lateral cloacal lip.

In the later male foetus, as a result of growth changes and thickening of its walls, the hinder end of the urogenital sinus becomes temporarily occluded;

after a short period, the cloacal septum becomes hollowed out independently, and the sinus again acquires an opening.

5. The main portion of the bladder arises from the hinder part of the intra-abdominal allantoic stalk, whilst the lower portion of the neck is formed from the bases of the Wolffian ducts, together with that part of the urogenital sinus lying between them. The lining of the bladder is thus mainly of entodermal origin, whilst a small portion is derived from the mesoderm.

6. The anterior end of the Wolffian duct in our earliest stages is already discontinuous, and in process of degeneration; this portion finally disappears altogether. Its posterior end is attached by a solid connection to the wall of the cloaca, but by Stage III, it has acquired an opening into the latter. At Stages VII and VIII (*G.L.* 7·25 mm.) the terminal portions of the two ducts, just in front of their openings into the urogenital sinus, widen in a sagittal direction and undergo lateral compression. In succeeding embryos, the widened bases of the ducts, as far as the point of origin of the ureters, become incorporated into the sinus wall, with the result that the ureters acquire an independent opening into the latter (*G.L.* 13 and 13·5 mm.).

The Wolffian ducts run back along the ventro-lateral sides of the mesonephroi. Behind the latter in later stages (*H.L.* 8·5 mm.), the posterior ends of the urogenital folds, containing the Wolffian and later the Müllerian ducts, pass medially and unite across the middle line to form the genital cord, in which the ducts continue back, and finally enter the urogenital sinus.

The anterior end of the duct remains connected with the degenerate excretory tubules of the mesonephros, destined to develop into the epididymis and epoophoron. In the male, this condition is permanent. In the female, the whole duct gradually becomes much reduced; it becomes detached from the epoophoron (*H.L.* 19 mm.), and, in front of the junction with the Müllerian duct, altogether disappears. The posterior end persists as the hinder portion of the lateral vagina.

7. The Müllerian duct arises as an ingrowth of thickened coelomic epithelium towards the anterior end of the urogenital fold at Stage VI (*G.L.* 7·75 mm.). From the ostium abdominale the duct grows back as a tube along the margin of the fold, laterally to the Wolffian duct. Towards the hinder end of the mesonephros, it passes to the ventral side of the Wolffian duct, and, behind the excretory gland, in the posterior end of the fold, it extends up medially to the excretory duct, to run back in the genital cord. It finally unites with the medial side of the latter duct (*H.L.* 11 mm.).

The primordium of the genital cord first appears in pouch-young with a head length of 8·5 mm. It arises as the result of the union of the posterior ends of the urogenital folds, behind the mesonephros, with the tissue on the dorsal side of the bladder, so that the neck of the latter, together with the Wolffian ducts, becomes enclosed in a common connective tissue sheath. In the male, the genital cord always remains short, never extending very far beyond the neck of the bladder. In the female, the genital cord, which

contains both Wolffian and Müllerian ducts, increases in length in a postero-anterior direction, as the result of the lengthening of the neck region of the bladder, of the progressive union of the urogenital folds from behind forwards, and of general interstitial growth.

The median vaginae develop as two enlargements of the Müllerian ducts, just where they bend outwards in the genital cord to form the lateral vaginae (*H.L.* 22 mm.). These enlargements grow downwards between the lateral vaginae as two large culs-de-sac, which eventually come to possess a common median partition wall. The uteri appear as two widenings of the Müllerian ducts, shortly in front of the median vaginae.

The point of union of the Müllerian with the Wolffian duct is marked in older stages and in the adult by a constriction situated towards the hinder end of the lateral vagina, whilst there is a noticeable difference in the appearance of the epithelium lining the portions of the tube in front of and behind the constriction. The anterior portion (about two-thirds) of the lateral vagina is derived from the Müllerian duct, whilst the terminal portion (about one-third) arises from the Wolffian duct.

In the male the ostium abdominale is closed in a foetus with a head length of 15 mm. The Müllerian duct is very reduced, and has partially disappeared in our oldest male (*H.L.* 26 mm.).

The suprarenal body is first recognisable as a low inconspicuous ridge of thickened coelomic epithelium, projecting into the coelom medially to the anterior end of the urogenital fold (Stage I, *G.L.* 5 mm.). In succeeding stages the body increases in size, and gradually extends further posteriorly, whilst the anterior end undergoes degeneration. It early becomes vascular (Stage III), and later, intimate connection is established with the sympathetic ganglia. The immigration of sympatho-chromaffin cells begins at Stage VII, and is usually completed at birth.

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REFERENCE LETTERS

all.st. allantoic stalk; *aort.* dorsal aorta; *b.c.* Bowman's capsule; *bl.* bladder; *c.e.* coelomic epithelium; *c.f.* coelomic funnel; *cl.* entodermal cloaca; *cl.m.* cloacal membrane; *cl.s.* cloacal septum; *c.t.* collecting tubule; *c.v.* cardinal vein; *e.gl.* external glomerulus; *gl.* primordium of glomerulus; *g.t.* genital tubercle; *h.g.* hind-gut; *m.bl.* metanephric blastema; *M.D.* Müllerian duct; *m.t.* medullary tube; *ost.abd.* ostium abdominale; *p.* ureteric papilla; *p.t.* pronephric tubule; *r.* rectum; *s.b.* primordium of suprarenal body; *u.f.* urogenital fold; *ur.* ureter; *u.b.* ureteric bud; *u.g.s.* urogenital sinus; *W.D.* Wolffian duct.

DESCRIPTION OF PLATES

Fig. 1. Stage I *a*. (α '97), *G.L.* 5 mm. Transverse section, showing the second pronephric tubule (*p.t.*) united with the Wolffian duct (*W.D.*). Left side. (Sl. 6–4–9.) ×215 and reduced by $\frac{1}{3}$.

Fig. 2. Stage I *b* (I A '01), *G.L.* 5 mm. Longitudinal section, showing the anterior end of the left Wolffian duct (*W.D.*). (Sl. 5–1–15.) ×70 and reduced by $\frac{1}{3}$.

Fig. 3. Stage II *a*. (β '98), *G.L.* 4·5 mm. Longitudinal section through the anterior end of the left Wolffian duct (*W.D.*), showing a pronephric coelomic funnel (*c.f.*). (Sl. 6–5–3 and 4.) ×215 and reduced by $\frac{1}{3}$.

Fig. 4. Stage II *b* (α '99), *G.L.* 4·5 mm. Transverse section, showing the first pronephric rudiment of the left side, with its nephrostome (*c.f.*). (Sl. 3–3–7, 8 and 9.) ×200 and reduced by $\frac{1}{3}$.

Fig. 6. Stage IV (XIX '04), *G.L.* 7·5 mm. Transverse section, showing a developing mesonephric tubule. Left side. It is seen to be differentiated into a narrow collecting duct (*c.d.*), a larger median portion (*m.p.*) and a thinner segment (*t.s.*), joined to the Malpighian corpuscle with its glomerulus (*gl.*). (Sl 9–2–5, 6, 7 and 8.) ×200 and reduced by $\frac{1}{3}$.

Fig. 7. Stage V (II '01), *G.L.* 6 mm. Transverse section, showing the rudimentary external glomerulus (*e.gl.*), and the second pronephric tubule (*p.t.*), which is connected both with the Wolffian duct (*W.D.*) and the coelomic epithelium (*c.e.*). (Sl. 6–2–1.) ×215 and reduced by $\frac{1}{3}$.

Fig. 8. Stage V (II '01). *G.L.* 6 mm. Transverse section, showing the third excretory tubule of the right side with its rudimentary nephrostome (*c.f.*). The latter is situated on the lateral side of and close to the primordium of the suprarenal body (*s.b.*). (Sl. 6–3–11.) ×215 and reduced by $\frac{1}{3}$.

Fig. 9. Stage III (a '97), *G.L.* 7 mm. Transverse section, showing the ureteric bud (*u.b.*) arising from the left Wolffian duct (*W.D.*), and encircled by the metanephric blastema (*m.bl.*). The metanephric blastema of the right side and the right Wolffian duct, anterior to the origin of the ureteric bud, are also seen. (Sl. 9–1–4.) × 75 and reduced by ½.

Fig. 10. Stage V (II '01), *G.L.* 6 mm. Transverse section, showing the right ureteric bud (*u.b.*) arising from the Wolffian duct (*W.D.*), and encircled by the metanephric blastema (*m.bl.*). The left Wolffian duct (*l.W.D.*) is seen immediately anterior to its opening into the cloaca (*cl.*). The level of the section is shown by the dotted line *A*...*B* in text-fig. 3. (Sl. 5–2–1.) × 75 and reduced by ½.

Fig. 11. Stage VIII *a* (XII A '02), *G.L.* 7·25 mm. Longitudinal section, showing the right ureteric bud (*u.b.*) arising from the Wolffian duct (*W.D.*). The metanephric blastema (*m.bl.*) surrounds the distal end of the bud and is continuous with the hindermost mesonephric tubule (*h.mes.t.*). *mes.t.* mesonephric tubule. (Sl. 8–2–8 to 8–3–4.) × 100 and reduced by ½.

Fig. 12. Stage XIV *b* (XXIV), *G.L.* 13·5 mm. Longitudinal section, showing a primary collecting tubule (*p.c.t.*) which has divided into two secondary collecting tubules (*c.t.*). The metanephric blastema (*m.bl.*) forms a thin investing layer, the free edge of which is thickened and becoming transformed into metanephric vesicles (*m.v.*). (Sl. 4–3–3.) × 215 and reduced by ½.

Fig. 13. Stage XV *b* (new-born), *G.L.* 14·5 mm. Longitudinal section (composite), showing a developing metanephric tubule. The metanephric vesicle has united with the collecting duct (*c.t.*) of the pelvis, and is becoming curved and elongated to form a secretory tubule (*s.t.*), whilst Bowman's capsule (*b.c.*) is developing. × 215 and reduced by ½.

Fig. 14. Stage XV *b* (new-born), *G.L.* 14·5 mm. Longitudinal section, showing a more advanced tubule in the same foetus. The secretory portion (*s.t.*) is larger, is becoming tubular, whilst Bowman's capsule is still further developed, and has the primordium of a glomerulus (*gl.*). × 215 and reduced by ½.

Fig. 15. Stage I *c* (I '01), *G.L.* 5 mm. Transverse section through the cloaca (*cl.*), showing the junction of the Wolffian ducts (*W.D.*) with the cloacal wall. Ectoderm and entoderm are not fused together in this embryo in the region of the cloacal membrane (*cl.m.*). *n.* notochord. (Sl. 2–5–5.) × 75 and reduced by ½.

Fig. 16. Stage V (II '01), *G.L.* 6 mm. Transverse section, showing the fusion of ectoderm and entoderm in the cloacal membrane (*cl.m.*). (Sl. 5–1–5.) × 215 and reduced by ½.

Fig. 17. Stage VIII *a* (XII A '02), *G.L.* 7·25 mm. Longitudinal section, showing the thickened coelomic epithelium (*ost.abd.*) in the region of the primordium of the ostium abdominale of the Müllerian duct. The anterior end of the Wolffian duct (*W.D.*) is also seen. Left side. (Sl. 7–2–7.) × 215 and reduced by ⅓.

Fig. 18. Stage IX *a* (5 '97), *G.L.* 8·5 mm. Longitudinal section, showing the ostium abdominale tubae (*ost.abd.*). The anterior end of the Wolffian duct is again seen. Right side. (Sl. 5–1–10 to 5–2–2.) × 215 and reduced by ⅓.

Fig. 19 *a*. Stage IX *b* (IV '01), *G.L.* 8·5 mm. Transverse section, showing the anterior bay of the ostium abdominale (*ost.abd.*). The margin of urogenital fold (*u.f.*) is united with the lateral wall of the coelom, forming a coelomic pocket (*c.p.*). (Sl. 12–4–3.) × 130 and reduced by ½.

Fig. 19 *b*. Stage IX *b* (IV '02), *G.L.* 8·5 mm. Showing the posterior bay of the ostium abdominale (*ost.abd.*), ·03 mm. behind fig. 19 *a*. The margin of the urogenital fold (*u.f.*) is here free. *r.* shallow medial bay, in the epithelium of which the anterior end of the rete terminates. *n.* = (Sl. 12–4–6.) × 130 and reduced by ½.

Fig. 20 *a* and *b*. *G.L.* 23 mm., *H.L.* 9 mm. Transverse section, showing the blind end of the Müllerian duct (*M.D.*) at the level of the medial bend of the Wolffian duct (*W.D.*). Fig. 20 *b* lies ·024 mm. posterior to fig. 20 *a*. *a* = (Sl. 31–3–7); *b* = (Sl. 32–1–1). × 100 and reduced by ⅓.

Fig. 21 *a* and *b*. Embryo D. *G.L.* 28 mm., *H.L.* 12 mm. Transverse section, showing the junction of the Müllerian (*M.D.*) with the medial side of the Wolffian (*W.D.*) duct. *a* = (Sl. X–2–2); *b* = (Sl. X–2–4). × 215 and reduced by ⅓.

Fig. 22. Embryo D. *G.L.* 28 mm., *H.L.* 12 mm. Transverse section, showing the passage of the ureters (*ur.*) through the genital cord (*g.t.*), and their openings at the base of the bladder (*bl.*) on the urinary papilla (*p.*). The Müllerian ducts (*M.D.*) lie close to the medial side of the Wolffian ducts (*W.D.*) within the cord, laterally to the ureters. (Sl. IX–4–9.) × 70.

Fig. 23. Stage IX *b* (IV '01), *G.L.* 8·5 mm. Drawing of wax-plate model of cloacal region and genital tubercle (*g.t.*). Right side. (Cf. text-fig. 5.) The anterior portion of the original cloaca is already divided into urogenital sinus (*u.g.s.*) and rectum (*r.*). The bases of the Wolffian ducts (*W.D.*) are widened sagitally and their hinder margins continuous with the lateral ridges (*l.r.*) of the sinus wall. *l.r.* lateral ridge. × 150 and reduced by ⅓.

Fig. 24 *a*. Stage XI (VI '01), *G.L.* 10 mm. Drawing of wax-plate model of cloacal region and tubercle. Right side. (Cf. text-fig. 7.) The genital tubercle (*g.t.*), in the anal side of which lies the entodermal cloaca (*cl.*), is exceptionally well developed in this embryo, and forms a well-marked prominence on the ventral abdominal wall. The division of the cloaca into urogenital sinus (*u.g.s.*) and rectum (*r.*) has made further progress, and the lateral ridges of the sinus wall (Stage IX, fig. 23, *l.r.*) have now almost disappeared. × 150 and reduced by ⅓.

Fig. 24 *b*. Stage XI (VI '01), *G.L.* 10 mm. Ventro-lateral view, showing the opening of the cloaca to the exterior. Only fragments of the cloacal membrane (*cl.m.*) remain. × 150 and reduced by ⅓.

Fig. 25 *a*. Stage XII *b*, *G.L.* 11·5 mm. Drawing of wax-plate model of cloacal region and genital tubercle. Right side. (Cf. text-fig. 9.) The division of the cloaca into urogenital sinus (*u.g.s.*) and rectum (*r.*) is almost completed, whilst the greater part of the cloacal cavity has become obliterated by fusion of its lateral walls to form the cloacal septum (*cl.s.*). The expanded bases of the Wolffian ducts (*W.D.*), as far as the point of origin of ureters (*ur.*), have become incorporated into the urogenital sinus, so that the ureters open almost directly into the latter. × 150 and reduced by ⅓.

Fig. 25 *b*. Stage XII *b*, *G.L.* 11·5 mm. Ventral view, showing the ureters (*ur.*) opening into the sinus at the extreme bases of the Wolffian ducts (*W.D.*). The incorporation of the Wolffian ducts is not quite complete, so that the urogenital sinus (*u.g.s.*) at this level expands outwards on each side. × 150 and reduced by ⅓.

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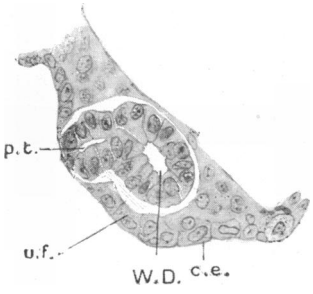


FIG. 1

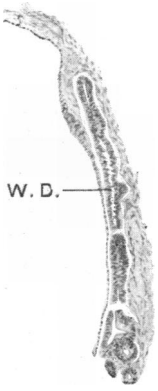


FIG. 2

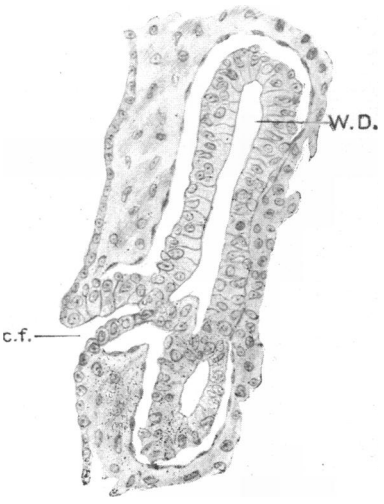


FIG. 3

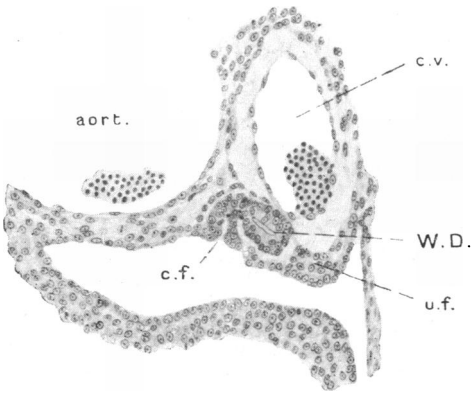


FIG. 4

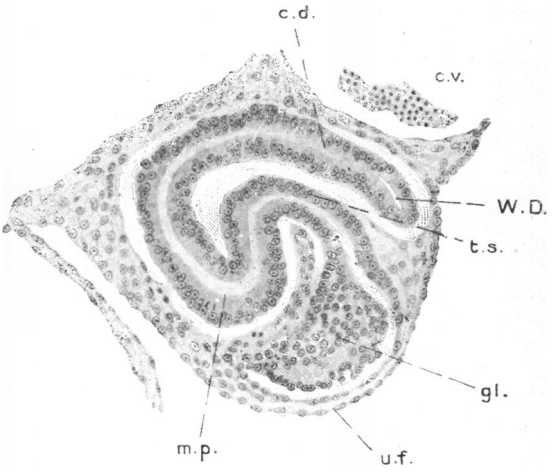


FIG. 6

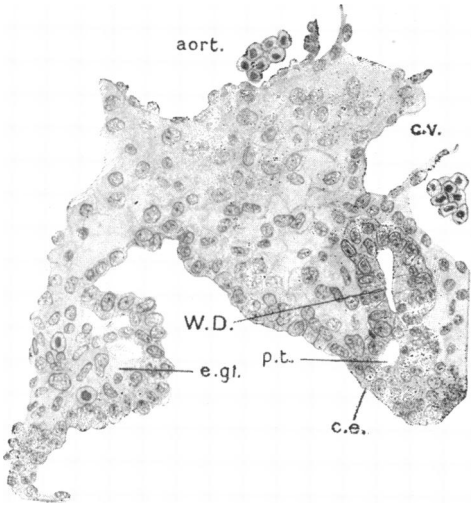


FIG. 7

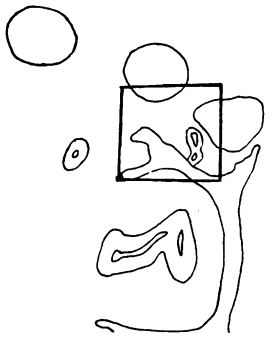


FIG. 7*a*

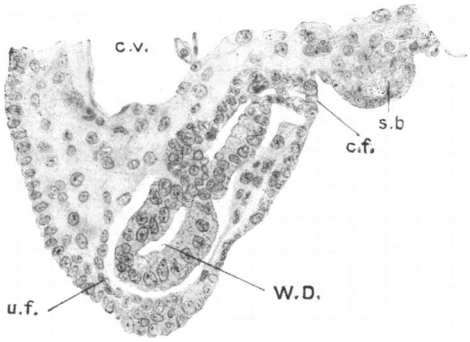


FIG. 8

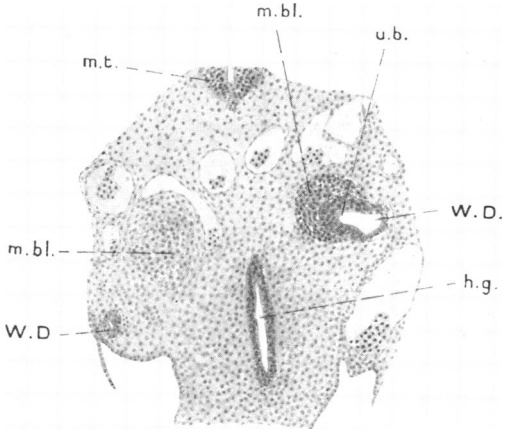


FIG. 9

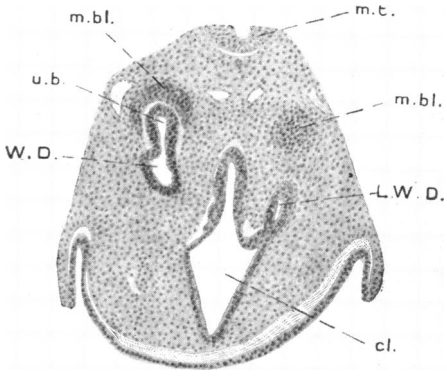


FIG. 10

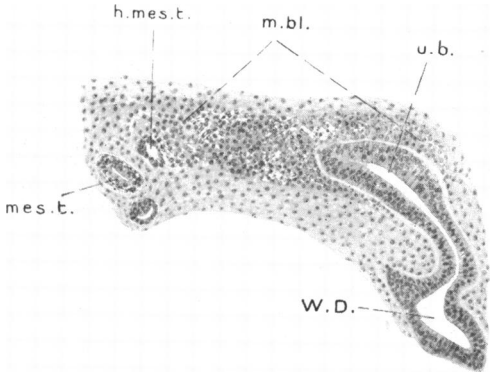


FIG. 11

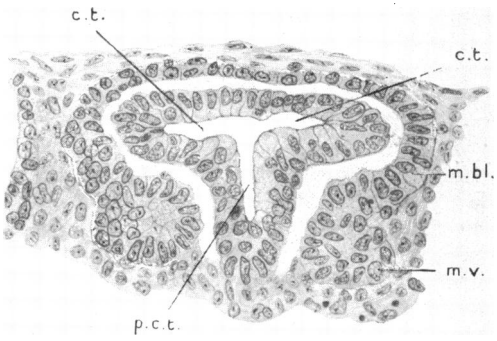


FIG. 12

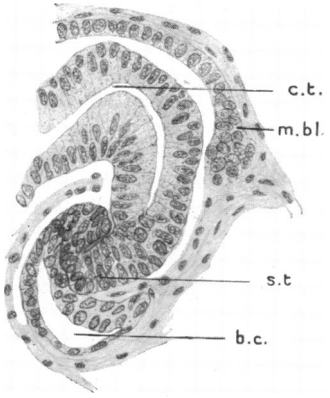


FIG. 13

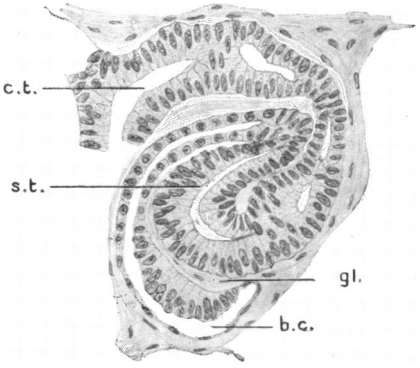


FIG. 14

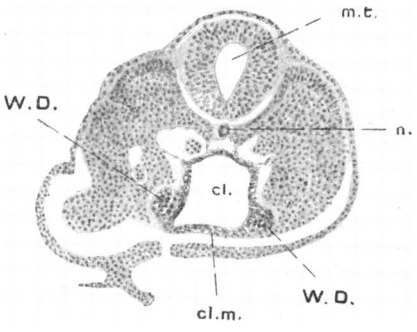


FIG. 15

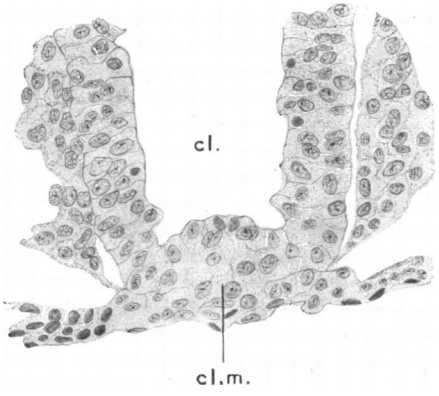


FIG. 16

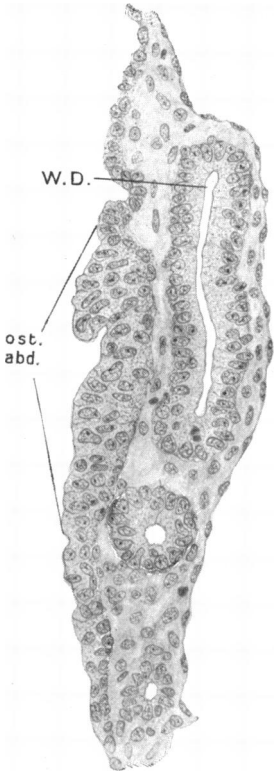


FIG. 17

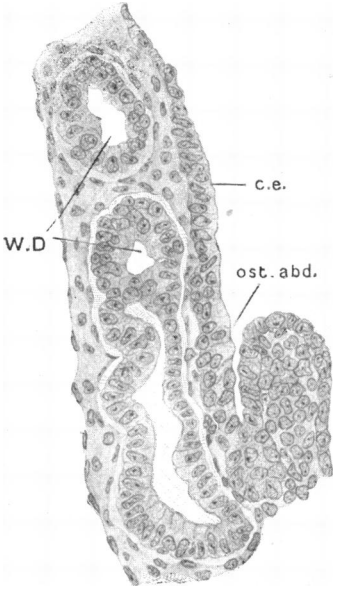
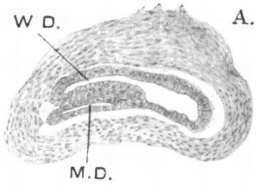


FIG. 18



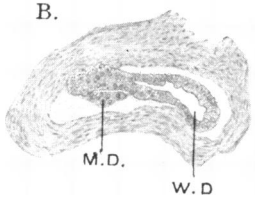
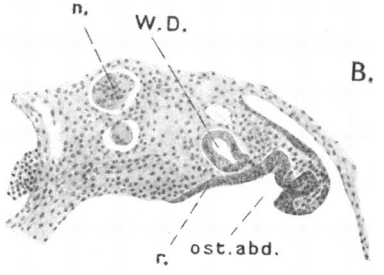


FIG. 20



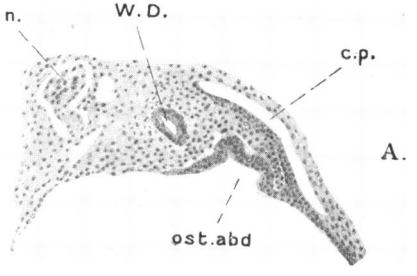


FIG. 19

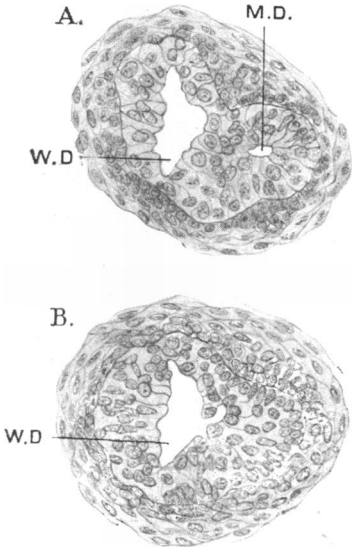


FIG. 21

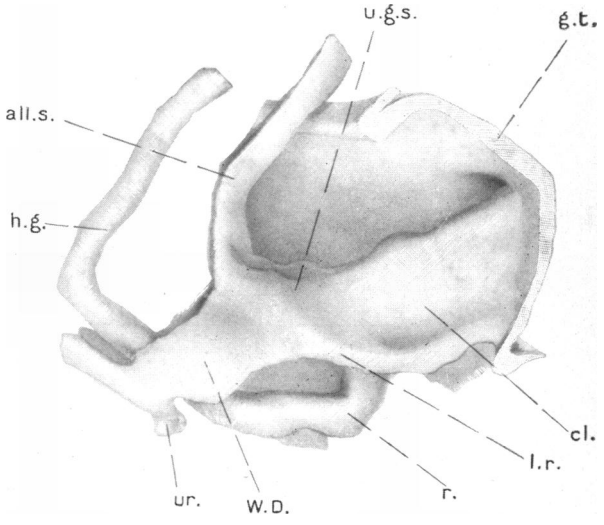


FIG. 23

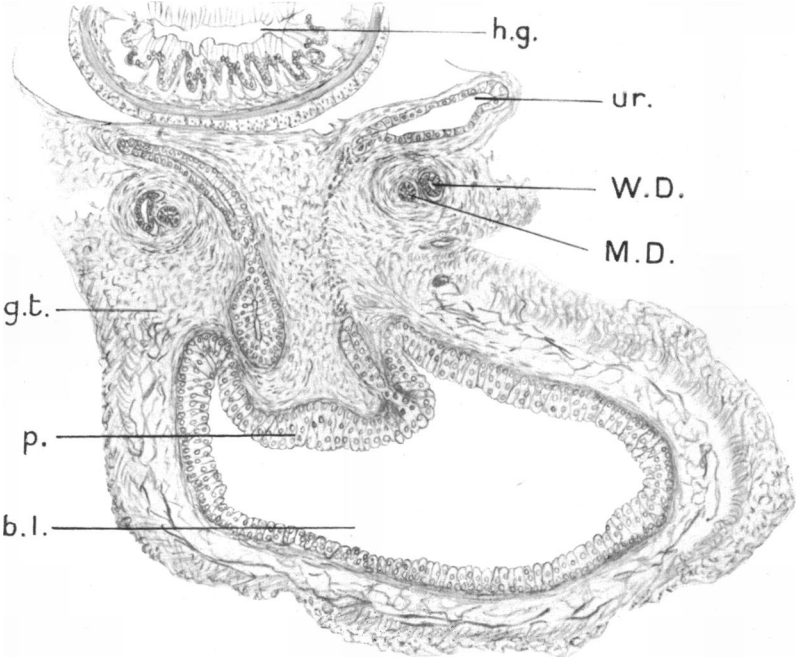
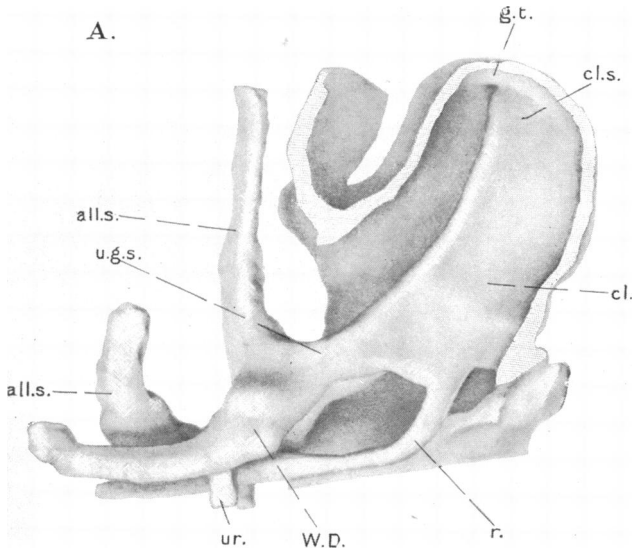


FIG. 22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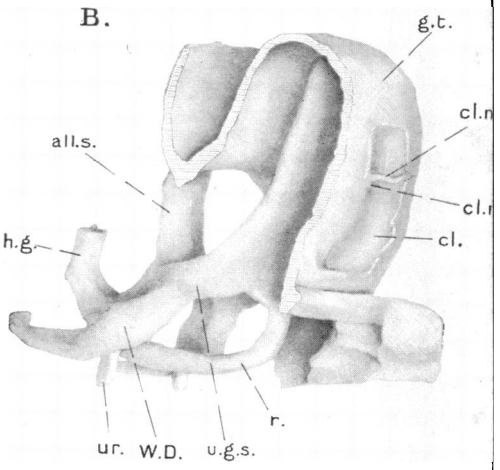
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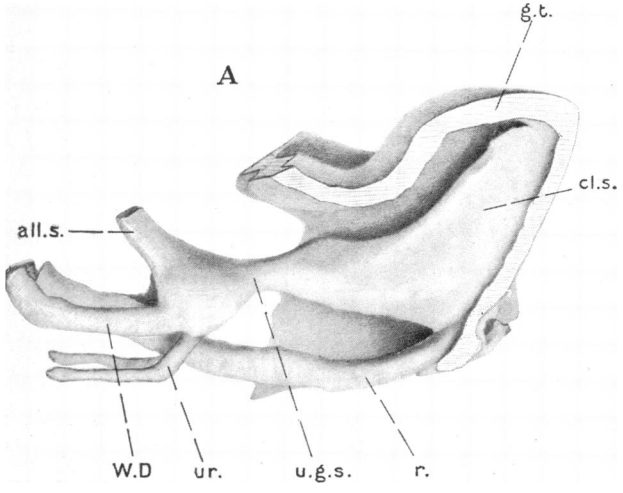
W.D.
u.g.s.

FIG. 24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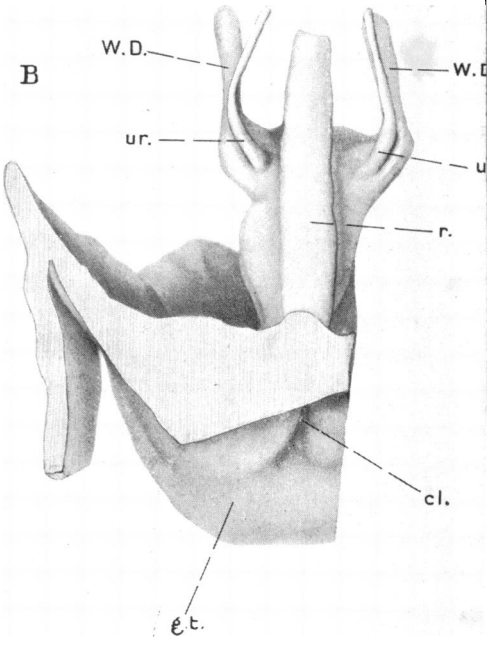
B
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FIG. 25