A COMPARATIVE STUDY OF THE DESCENT OF THE TESTIS

Harry Immanuel Aaron

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A COMPARATIVE STUDY

OF

THE DESCENT OF THE TESTIS.

BY

HARRY IMMANUEL AARON

(alias) U. ZAW TUN.

A THESIS

PRESENTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OF

THE UNIVERSITY OF ST.ANDREWS.

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DECLARATION.

I hereby declare that the following thesis is based on the results of study of original work carried out by me, that the thesis is my own composition, and that it has not previously been presented for a higher degree.

The research was carried out in the department of Anatomy, Bute Medical Buildings, University of St.Andrews, in nine academic terms during the period 1955-58.

HARRY IMMANUEL AARON (a) ZAW TUN.

CERTIFICATE

I certify that HARRY I. AARON has spent nine terms at research work under my supervision and that he has fulfilled the conditions or Ordinance No.61 of the University of St.Andrews, so that he is qualified to submit the following thesis in application for the degree of Ph.D.

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iv/

INTRODUCTION.

Galen, it is said, referred to the occasional abdominal position of the testis and also recognised the descent of the testis into the scrotum from the abdomen in the apes. The ancients thought the peritoneal (processus vaginalis) sac was patent throughout life. (Weil, 1885, and Wyndham, 1943). According to Weil, closure of the sac was shown by Douglas and migration of the testes from the belly into the scrotum in man by Fabricius Hildanes. This was also known to Mott, Hirschel and others in the 17th century; but these facts were overlooked till several investigators working independently at about the same time associated the state with inguinal hernia (Haller (1750), Pott, Camper (1756), Paletta (1777), Vicq d'Azyr (1784) and John Hunter (1786)).

According to Bramman (1884) Camper was the first to study descent of the testis in man. However, it is to Haller that most authorities attribute the 'discovery' of the gubernaculum and the role it played in descent. He described the gubernaculum as a 'vagina cylindrica', a hollow cylinder which is fused at its upper end with a mucous mass and runs from the lower end of the testis to be lost in the cellular tissue of the inguinal region. He thought the testis descended through this hollow cylinder into the scrotum, the driving force being the contraction of the muscles.

Many/

Many explanations soon came forth by contemporary investigators. Paletta (1777) compared the descent with prolapse of the uterus as far as the internal ring. Hildebrandt (Weil 1885) likened it to an inversion of a glove.

John Hunter (1876) at the suggestion of his surgeon brother who had read Haller's observations on congenital hernia, made a study of the descent of the testis. Few of his observations and excellent description have needed correction since, and the term 'gubernaculum' is now primarily associated with his name. He showed that the gubernaculum was not hollow and that fibres of the internal oblique and transversus were associated with its surface. He believed that the gubernaculum, creating a pathway in its wake, guided the testis into the scrotum. In the process, part of it he imagined was evaginated to form part of the parietal wall of the tunica vaginalis. Finally, when the testis was at the bottom of the scrotum, the gubernaculum was found "shortened and compressed".

Meckel (1820) believed that the contraction of the gubernaculum brought the testis to the abdominal ring and no further. According to him, if it contracted further it would invaginate the scrotum.

Rathke (1832) noted that the distal part of the gubernaculum was mucoid and formed an olive shaped swelling. However, he believed that the proximal part of the gubernaculum sank into this swollen/

swollen part and that later there was a herniation through the external oblique with several bundles of interal oblique fibres joining it.

Till late in the 19th century authors without finding anything new, confirmed or denied existing facts and put forward many theories of their own. Towards the end of the century, however, Bramann (1884) and Weil (1885) using histological sections for study, made admirable contributions and most of their descriptions have been corroborated by recent investigators using more modern methods. However, gaps were still left in the morphological development that raised many controversial problems. Interest in the subject was revived and much investigation was again carried out. Notable amongst the investigators in this period were Lockwood (1888), Klaatch (1896) Frankl (1895, 1900), Berry Hart (1909) and Felix 1912).

Engle (1932) and Wislocki (1933) working on the hormonal control of the descent of the testes and the formation of the scrotum have shed new light on the subject but nevertheless, in spite of more work done recently on the morphological process, there remains an incomplete picture which still can raise doubts. Amongst the more recent investigators are Forsner (1928), who finds no muscle and observes progressive transformation of the gubernaculum into mucoid tissue, Moszkowicz (1935) Wells, Wyndham (1943), Pearson, Backhouse/

Backhouse and Butler 1955).

Throughout investigations in the past, work was concentrated on human specimens. There were from time to time, authors who brought in stages of development in various animals for comparison (Ratke 1832, Von Eichbaum, Bramann 1884, Klaatch 1890, Frankl 1900, Backhouse and Butler 1954-57). These were done more with an idea of corroborating their views on the developmental process in the human. However, no one has made a detailed study of the descent of the testis in any one mammalian species.

As the same biological laws govern both animals and man, it would be interesting to know the process in an animal and compare it with the various known stages in man. With this idea in mind, it was decided to make a detailed study, stage by stage, of the morphological process of the descent of the testis in one species of mammal.

For such a purpose, an animal that was easily available, convenient in size, easily mated and having a short gestation period was desirable. Such an animal was the white Norway rat and this was selected for study.

Following the study of the descent of the testis in the rat, a study was made on available human specimens for comparison.

In/

In addition pig embryo specimens of 40 mm. C.R. length, rabbit embryos of 85 mm. C.R. length and newly born cats were sectioned, and the relevant regions were studied for further comparison.

MATERIALS AND METHODS.

The oestrous cycle in rat is approximately five days. Vaginal smears of a number of female rats were examined twice daily. When one was found to be in oestrous it was mated over a period of twelve hours. Vaginal smears were then examined daily and if within six days no sign of oestrous was present, then the rat was presumed to be pregnant. At the required age, the embryos were removed from the uterus of the pregnant rat, immediately after it had been killed, and placed in a fixative.

Various fixatives were tried, and for all the stages up to the period when ossification of bones began, Bouin's fluid was found to be most satisfactory. For later stages 'Susa' was used and it gave good results. The C.R. lengths of the embryos were measured after fixation. Shrinkage due to fixation was slight and uniform, and therefore did not seriously affect the measurements.

Paraffin blocks of the various specimens were prepared and sectioned serially at a thickness of 10 u. As a rule every fourth section was mounted and stained with Ehrlich's haematoxylin and eosin. Whenever it was thought necessary, a reconstruction was made using wax plates, each of 1 mm. thickness, and magnifying the sections twenty five times.

Dissections were made under a binocular dissecting microscope of/

of the inguino-scrotal region in adult males and in embryos in the later stages of development. The method described by Dodds (1957) for the staining of striated muscle fibres in bulk (vide infra) was utilised particularly in the study of the cremasteric muscle. The early stages of descent of the testis in man was studied on series kindly lent by Professor Walmsley. The later stages in the process were investigated on specially prepared sections and dissections.

The findings in the rat and man were compared with those shown in serial sections of a 40 mm. C.R. length pig embryo, an 85 mm. C.R. length rabbit embryo and a newly born cat.

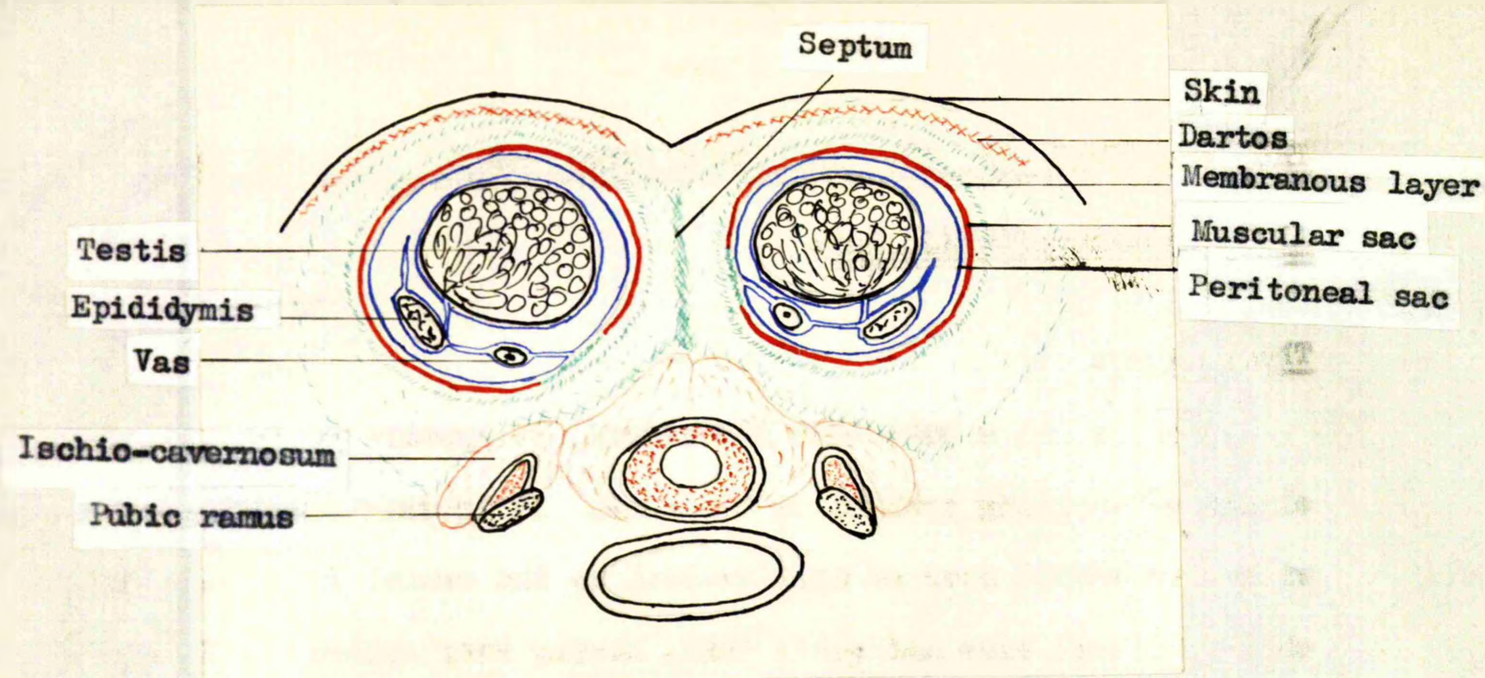

Fig. 1. Diagrammatic T.S. through the adult scrotum of a rat, showing the various layers.

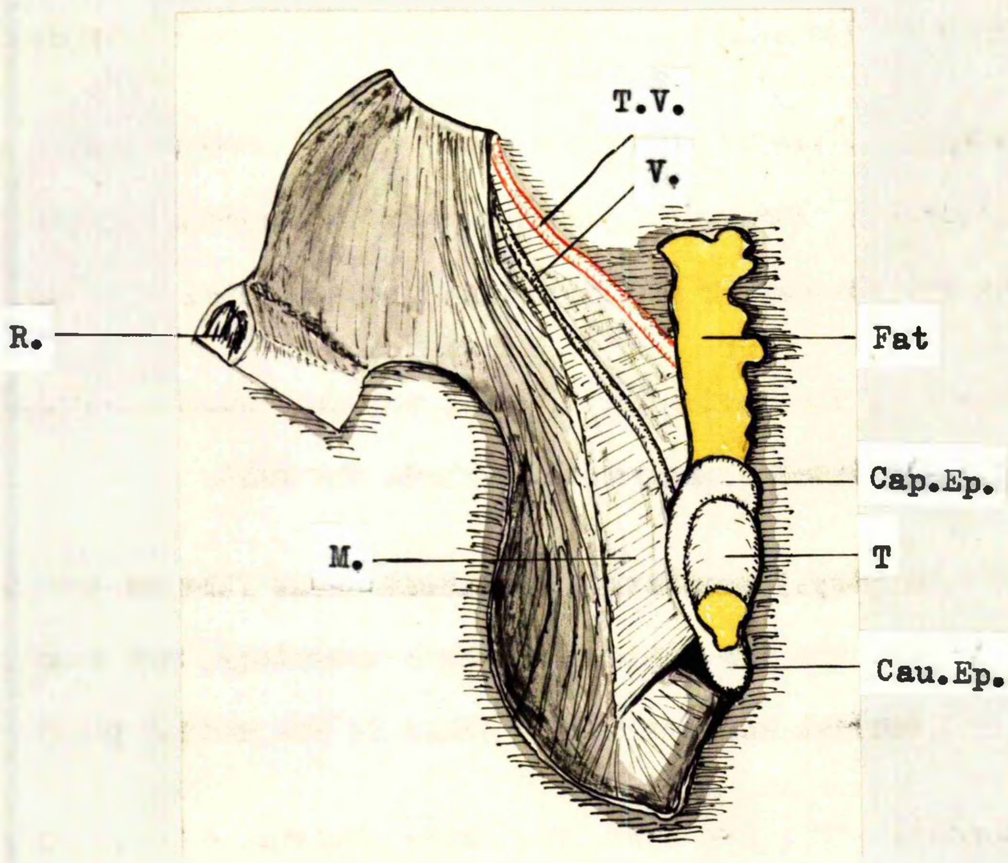

Fig. 2. The right sac opened to show the wide mesorchium attaching the testis and vas to the dorsal wall.

THE STRUCTURE OF THE INGUINO-SCROTAL REGION IN THE MALE ADULT ALBINO NORWAY RAT.

The Scrotum.

This is not a pendulous structure. It appears as a bi-lobed elongated swelling cranial to the anus. It is densely covered with hair except over an area ventral to the caudal end. Here the skin is almost bare and quite thin, having very sparse and fine hair. Hamilton (1937) terms this area, which is distinguished by its wrinkling and reddish-yellow colour, as the "sexual" skin and has shown that its differentiation and also the development and maintenance of the scrotum is controlled by the male hormone.

The skin is easily separable from the underlying testes and their coverings. There is no semblance of a scrotal ligament anchoring the testes, or its coverings, to the skin.

Strands of the Dartos may be seen running cranio-candally, in the dense subcutaneous tissue adherent to the skin.

A septum (Sep.) consisting of subcutaneous fibrous tissue extends between the two testes and their coverings, and anchors the skin to the ventral surface of the penis in the median plane. (fig. I).

The testis (T), its duct (V), blood vessels, a large mass of fat and the peritoneal sac are enclosed in a muscular sac, which in/

in turn is surrounded by a membranous layer of fascia. These three coverings of the testis and spermatic cord will be described separately from without inwards (figs. I and II).

The Membranous Sac.

A membranous layer (Mem. L.) of fibrous tissue immediately surrounds the muscular sac (fig. I). Superficial to it the fibrous tissue is loose and connects the membranous layer with the dense fibrous tissue immediately subjacent to the scotal skin. From the deep surface strands of fibrous tissue are continuous with that between the muscle fibres. Traced cranially the membranous layer is continuous with that of the abdomen superficial to the external oblique muscle. Over the external ring the fascia is adherent to the surface of the internal oblique fibres as firmly as to the margin of the ring.

The Muscular Sac.

This sac is very thin and the muscle fibres are difficult to trace even with the aid of the dissecting microscope. The following technique (Dodds 1957) was used, by means of which the muscle fibres alone were stained with benzoquinone.

1. Fix the material in 70% alcohol.

2. Thoroughly dehydrate in absolute alcohol.

3./

3. Stain for hours in an 0.05% solution of benzoguinone in absolute alcohol.

4. Rinse in absolute alcohol.

5. Place in a solution consisting of equal quantities of absolute alcohol and methyl salicylate.

6. Clear in methyl salicylate.

The muscular sac with its membranous covering and peritoneal lining, was cut out, opened up ventrally, stretched on a glass plate and then fixed in 70% alcohol. After thorough dehydration it was found that approximately 24 hours were necessary for the tissue to remain in the benzoquinone solution. This produced good results with the adult rat tissue that was used; but the time required for embryonic tissue and tissues of other species may vary considerably.

After clearing in methyl salicylate, the fibres took on a deep brown stain in contrast to the transparent matrix and could be easily followed under the dissecting microscope.

Fig. 3 is a diagram illustrating the shape of the muscular sac and the direction of the external layer of fibres of which it is composed. The investigation has shown that all the fibres of this/

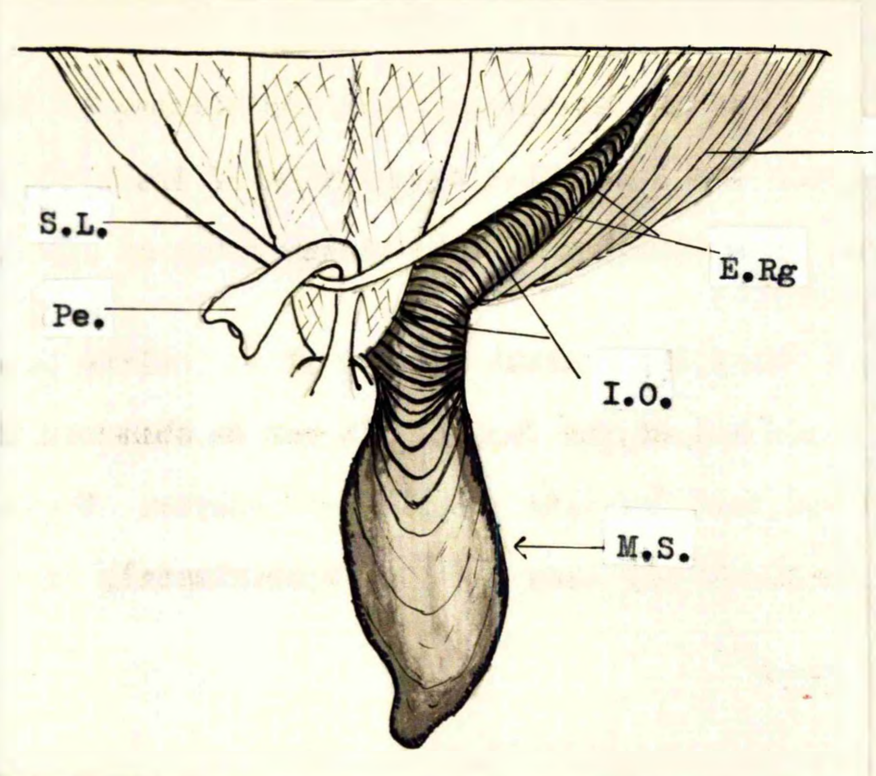

Fig. 3. Ventral view of the left muscular sac and external 'ring'.

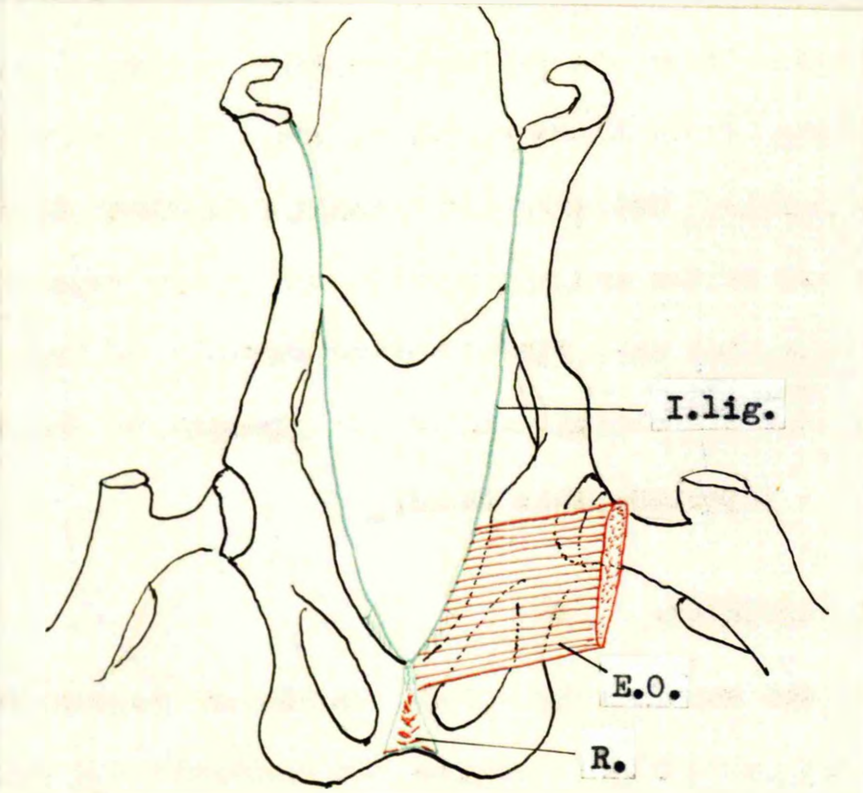

Fig. 4. Diagram of the ventral aspect of the pelvic bones with the attached upper end of the femurs, showing the inguinal ligament, caudal attachment of the external oblique and origin of the rectus abdominis.

this muscular sac are continuous with the muscles of the anterior abdominal wall and a detailed description of these requires a preliminary consideration of this latter group of muscles.

Fig. 4 shows the ventral aspects of the pelvic bones with the attached upper end of the femora. It can be observed that the pelvis is narrow and that the ilia are long and narrow. The symphysis pubis is comparatively long and lies approximately in a horizontal plane.

The inguinal ligament (I. Lig.) is shown in green. It is a very strong fibrous band which tends to be cord like in its medial part and flattened dorso-ventrally in its lateral part. It is attached laterally to the anterior-superior iliac spine and to the ventral border of the ilium as far as the lateral margin of the ilio-psoas muscle. Medially its primary attachment is to the cranial border of the symphysis pubis and to the adjacent part of the pubic bone, but some fibres extend dorsally to form a fibrous sheet attaching the medial part of the ligament to the cranial border of the superior pubic ramus.

The Rectus Abdominis.

The Rectus muscle arises from the ventral aspects of the bodies of the pubic bones close to the symphysis and extends cranially/

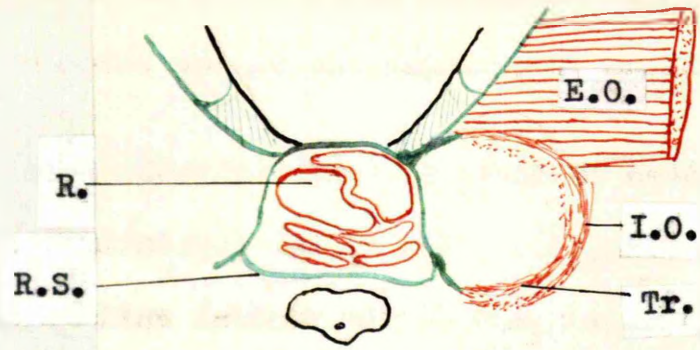

Fig. 5. Diagram showing the caudal region of the rectus surrounded by its sheath and the latter's relation to the neck of the muscular sac.

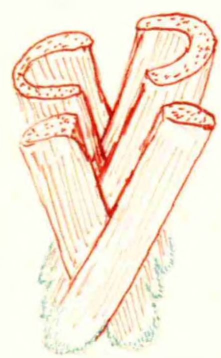

Fig. 6. Diagram showing the interdigitating slips of the rectus abdominis.

cranially in the ventral abdominal wall, lying on either side of the linea alba, to reach their insertion on the thoracic cage.

Each muscle arises by three or four digitations from the opposite pubic bone (fig. 4). The dorsal digitations are large and arise from the cranial part of the ventral surface alongside the symphysis (fig. 5). The ventral digitations tend to be smaller. Their origins are from the caudal part of the ventral surface and extend farther laterally.

From these origins the digitations extend cranially and to the opposite side of the median plane interdigitating with one another as they go. Thereafter the digitations of one side fuse together, the fusion starting at the lateral margin and extending medially (fig. 6).

At and immediately above the origin, the muscle is thick dorso-ventrally and narrow from side to side so that it presents a lateral surface of appreciable width. As the muscle proceeds cranially it becomes thinner and wider.

<u>The Rectus Sheath.</u>

This is a true sheath. Anterior to the margin of the ribs, the sheath thins out, blends with the adjacent connective tissue and is of little significance. Caudally, it attaches itself to the/

the surface of the pubic bones around the origin of the Rectus.

The lateral margin of the ventral surface of the sheath comprises the true lateral boundary and it is to this that the external oblique, internal oblique and transversus abdominis gain attachment.

The sheath is not uniform in thickness or in strength. Its ventral wall is thicker and aponeurotic. Aponeurotic fibres of the external oblique and internal oblique can be traced on to this surface beyond the actual lateral boundary throughout its length. On the other hand, the dorsal wall, besides being thinner, becomes very weak and transparent in the caudal region. Muscle fibres of the transversus extend along the wall of the sheath dorsal to the rectus muscle and far down as a little below the level of the umbilicus. Thereafter, the fibres gradually fall short and eventually do not go beyond the lateral border of the sheath.

The linea alba extends as far caudally as the point where the rectus muscles begin to decussate. Beyond that point it is absent and the rectus muscle of each side together form one interdigitating mass, within a single sheath.

<u>Aponeurotic Raphe</u>.

As the rectus muscle presents an appreciably wide lateral surface/

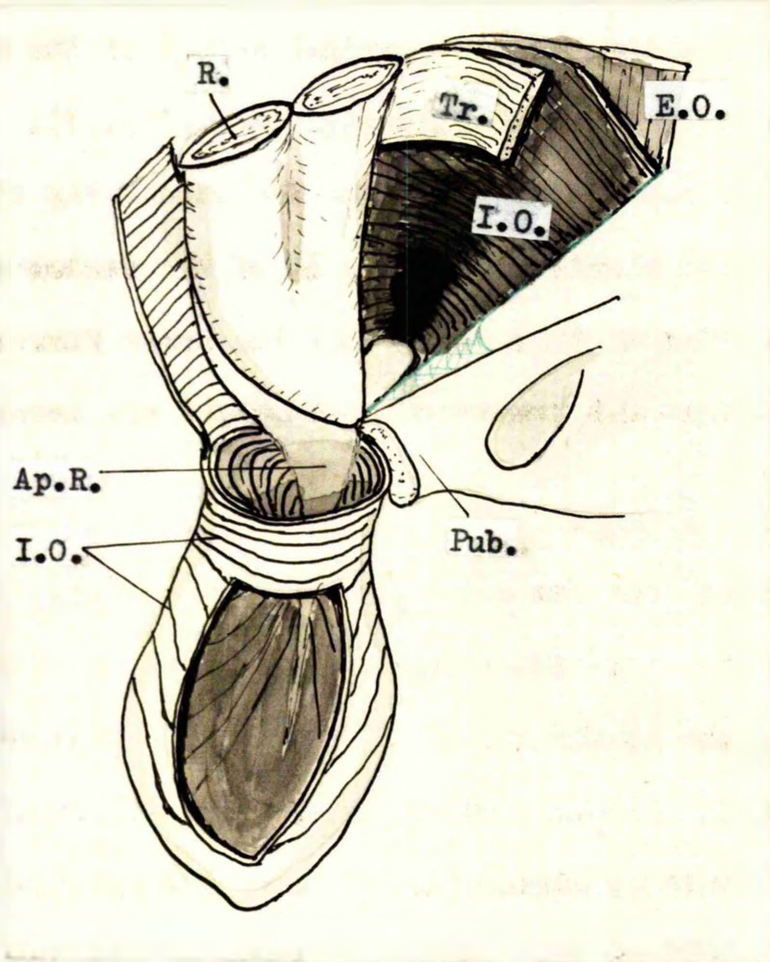

Fig. 7. Diagramatic representation of the left muscular sac, without the transversus layer, with part of the ventro-lateral wall cut away to show the aponeurotic raphe on the dorso-medial aspect continuous cranially with the lateral surface of the rectus sheath.

surface near its pubic attachment, the rectus sheath too, presents a corresponding surface over this area. Extending caudally from this surface, along the dorso-medial aspect of the muscular sac is a narrow triangular aponeurotic sheet (fig. 7). The apex of this sheet or raphe nearly reaches the caudal tip of the sac, and the narrow base blends with the side of the rectus sheath. It is into the margins of this raphe that the lower fibres of the internal oblique and transversus abdominis are inserted.

The External Oblique.

It arises from the outer surfaces of the 4th to the 12th ribs, interdigitating with the serratus magnus muscle. The dorsal margin is free and the muscle fibres of which it is composed run caudally to be inserted directly into the mid-point of the iliac crest. Thereafter there is direct insertion of the muscles' fibres into the ventral half of the crest, the whole of the inguinal ligament and the ventral surface of the pubis as shown in fig. 4. The fibres inserted into the pubis form the lateral boundary of a triangular opening in the muscle for the passage of the spermatic cord (fig. 3). The fibres forming the medial boundary of this opening becomes tendinous and after receiving some fibres from the internal oblique (vide infra), this tendon continues caudally and medially to become continuous with the tendon from the opposite side and/

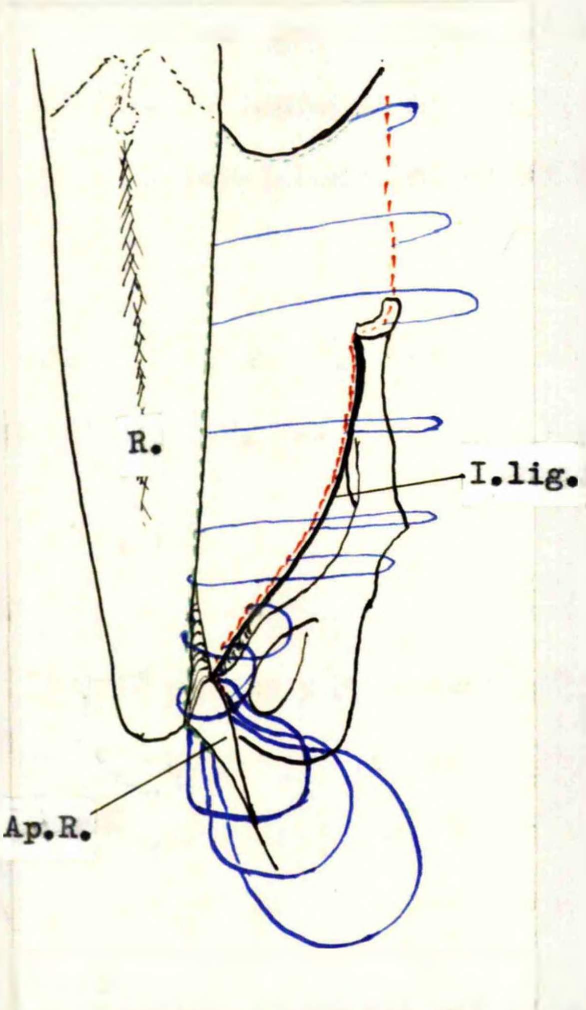

Fig.8. Diagramatic representation of the origin (red) and insertion (green) of the internal oblique (blue).

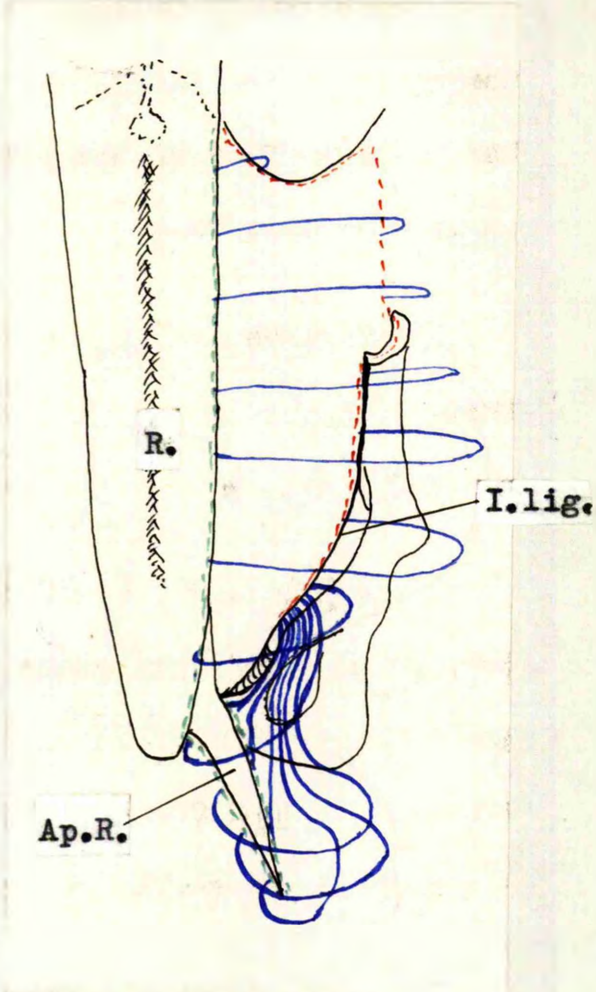

Fig.9. Diagramatic representation of the origin (red) and insertion (green) of the transversus abdominis (blue).

and forms a suspensary ligament (S.L.) for the penis (fig. 3). The most medial fibres of the muscle become aponeurotic and after crossing ventral to the rectus are inserted into the linea alba.

The Internal Oblique.

The Internal Oblique takes origin from the lumbar fascia, the crest of the ilium, the whole of the inguinal ligament and the anterior part of the dorsal border of the aponeurotic raphe in the muscular sac.

The fibres are inserted into the inferior margin of the lower ribs, the rectus sheath and the anterior part of the ventral border of the aponeurotic raphe.

With the exception of the fibres in the inguinal region and the muscular sac, the general direction of the muscle fibres is to run cephalo-medially at right angles to the direction of the external oblique fibres. Near the inguinal region the fibres tend to run into the margin of the rectus sheath at right angles.

Figure 8 shows diagramatically the origin, insertion and general direction of the fibres.

The arrangement of the fibres in the muscular sac is shown in figures 3, 7 and 10. The fibres tend to diverge from their origin/

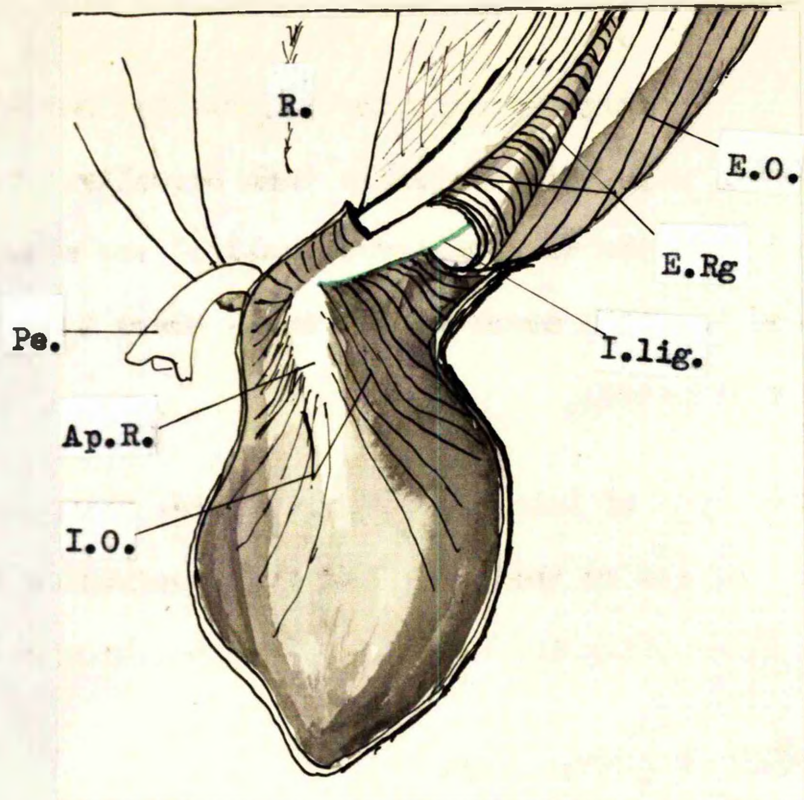

Fig. 10. The left sac, without the transversus layer, opened up ventro-laterally to show the direction of the internal oblique fibres and the aponeurotic raphe.

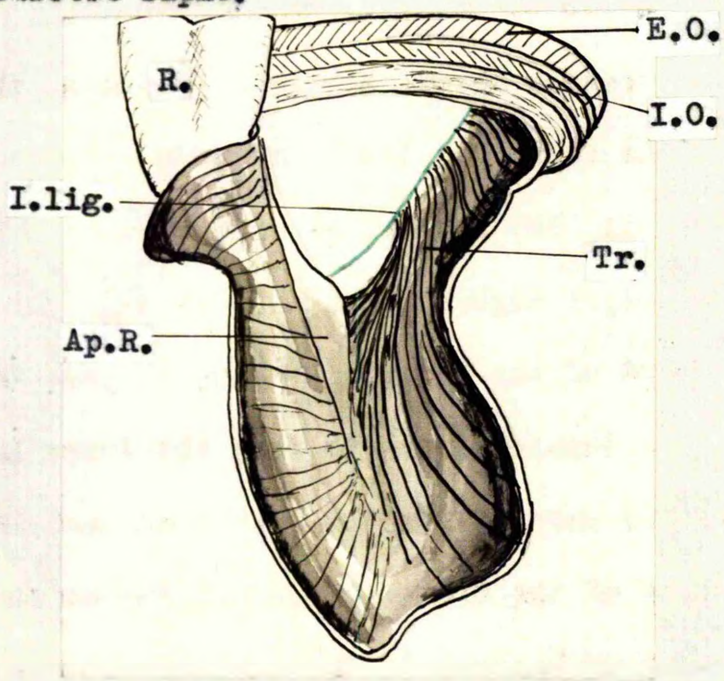

Fig. 11. The left sac, without the peritoneal layer, opened up ventro-laterally to show the direction of the transversus fibres and their insertion into the aponeurotic raphe.

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origin and converge towards their insertion so that they are more widely separated ventrally than dorsally. This is due to the fact that the ventro-lateral wall of the muscular sac is longer than the dorso-medial wall, where the aponeurotic raphe is situated.

The layer of internal oblique fibres thins out towards the apex of the sac so that on naked eye observation it is only the fibres surrounding the neck which are easily visible.

The Transversus Abdominis.

This muscle is closely adherent to the internal oblique layer and cannot be readily separated from it. Its fibres, however, run at an angle to those of the internal oblique. It arises from the inferior margin of the lower ribs, the lumbar fascia, the crest of the ilium and the lateral two thirds of the inguinal ligament. All but the caudal fibres run medially and meet the rectus at right angles. The most caudal fibres arising from the middle third of the inguinal ligament, pass through the superficial inguinal ring to form the inner layer of the muscular sac surrounding the testis and are inserted into both the borders of the fibrous raphe on its dorso-medial wall. The fibres arising highest on the inguinal ligament sweep across the ventral aspect of the sac to reach the ventral border of the raphe,/

Fig. 12. View of a fresh specimen of the muscular sac opened along its long axis through the ventral wall showing the appearance of the transversus muscle fibres as seen through the peritoneum.

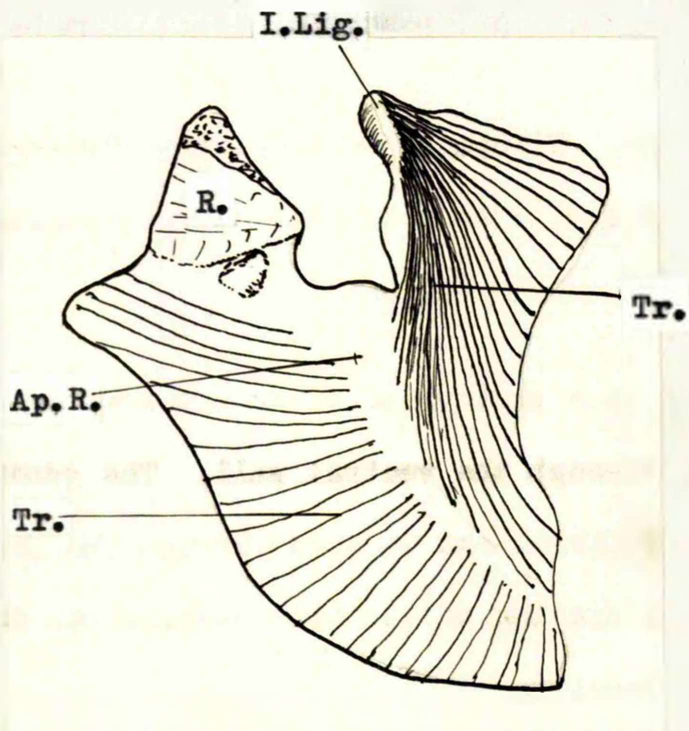

Fig. 12A. A drawing of the above specimen showing the direction of the transversus muscle fibres and the aponeurotic raphe.(Ap.R.)

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raphe, whereas the lower fibres of origin run caudally across the lateral and dorsal aspect of the sac to reach the dorsal margin of the raphe as shown in figure 9.

In comparing the lengths of origin and insertion of these fibres of transversus abdominis that form the inner layer of the muscular sac, one is struck by the difference. The length of origin from the inguinal ligament is less than a quarter of the length of the combined ventral and dorsal borders of the raphe. Moreover, the origin lies in a dorsal plane to the sac and most of the fibres destined for the dorsal border and the caudal half of the ventral border of the raphe have to run along the dorsal wall of the neck before radiating to their insertion. This gives the appearance of the fibres running parallel with the raphe on its lateral side. Figure 11 is a drawing showing the inner surface of the muscular sac, with a diagramatic representation of the direction of the muscle fibres.

Figure 12 is a photograph of an actual fresh specimen of a sac opened up through the ventral wall. The general direction of the transversus fibres can be seen through the peritoneal layer. Figure 12A is a drawing of the same specimen as seen under a dissecting microscope.

Figure/

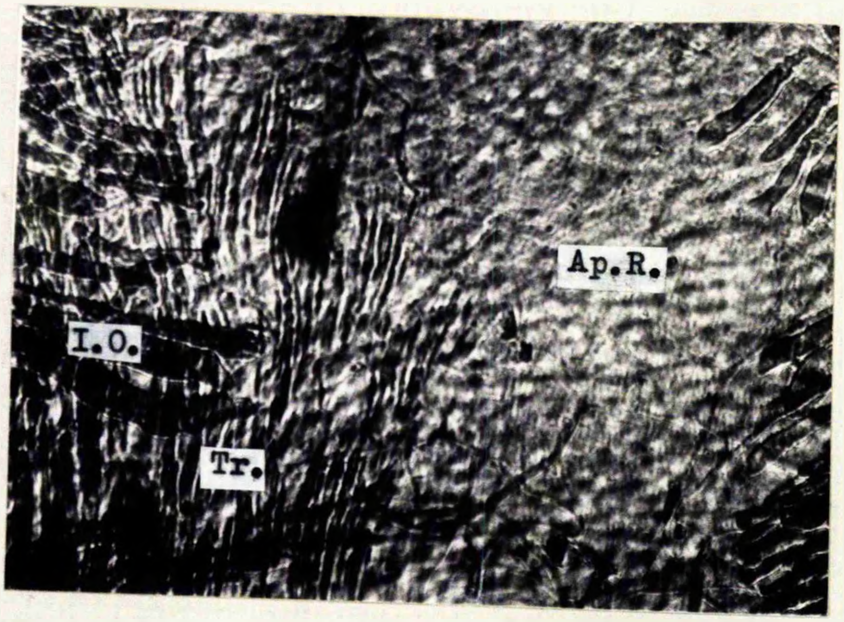

Fig. 13. A benzoquinone stained cleared specimen of the muscular sac showing the two layers of muscle fibres and the aponeurotic raphe.

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Figure 13 is a high powered view of a cleared specimen, stained with benzoquinone, showing clearly the existence of two layers of muscle in the sac wall. Also shown in the same figure is the fibrous raphe. On the surface of the raphe is seen the root of the mesorchium (M.).

<u>The Peritoneal Sac.</u>

Similar in shape to the muscular sac whose inner surface it lines, the peritoneal sac is continuous with the peritoneum of the abdominal cavity through the wide neck of the muscular sac.

From its dorso-medial aspect, a fold of peritoneum stretches ventrally to invest the vas deferens, epididymis, testis, blood vessels and the mass of fat. The root of this fold overlies the aponeurotic raphe and caudally is co-extensive with that structure.

Figure 2 is a drawing of a dissected specimen with the testis drawn medially to display the peritoneal fold (M) connecting the vas to the dorsal wall of the sac.

It is to be noted that the mesorchium (M.) is a very wide fold and permits the testis a great deal of mobility. Without disturbing the muscular sac the testis can be passively raised as far as the neck of the sac. Contraction of the muscular sac without actually producing any invagination, would be sufficient to allow the testis to enter the abdominal cavity.

TERMS OF DESCRIPTION.

Due to the extreme caudal curvature and the degree of variation at different ages, the conventional terms Cephalic, Caudal, Ventral and Dorsal cannot always be used without causing ambiguity. For the sake of clarity, all descriptions will refer to the specimens orientated so that the C.R. axis is vertical, as depicted in the figure below.

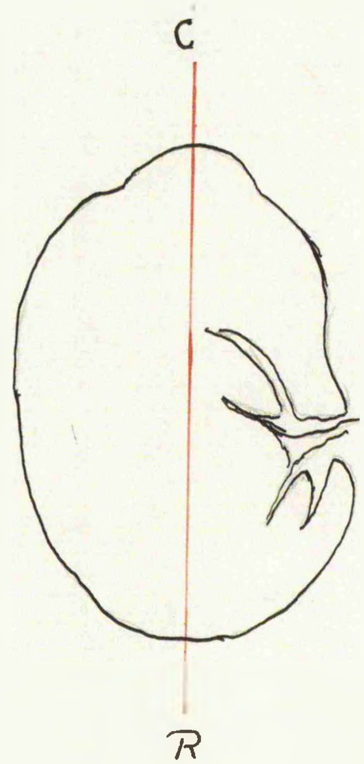

FIG. 14.

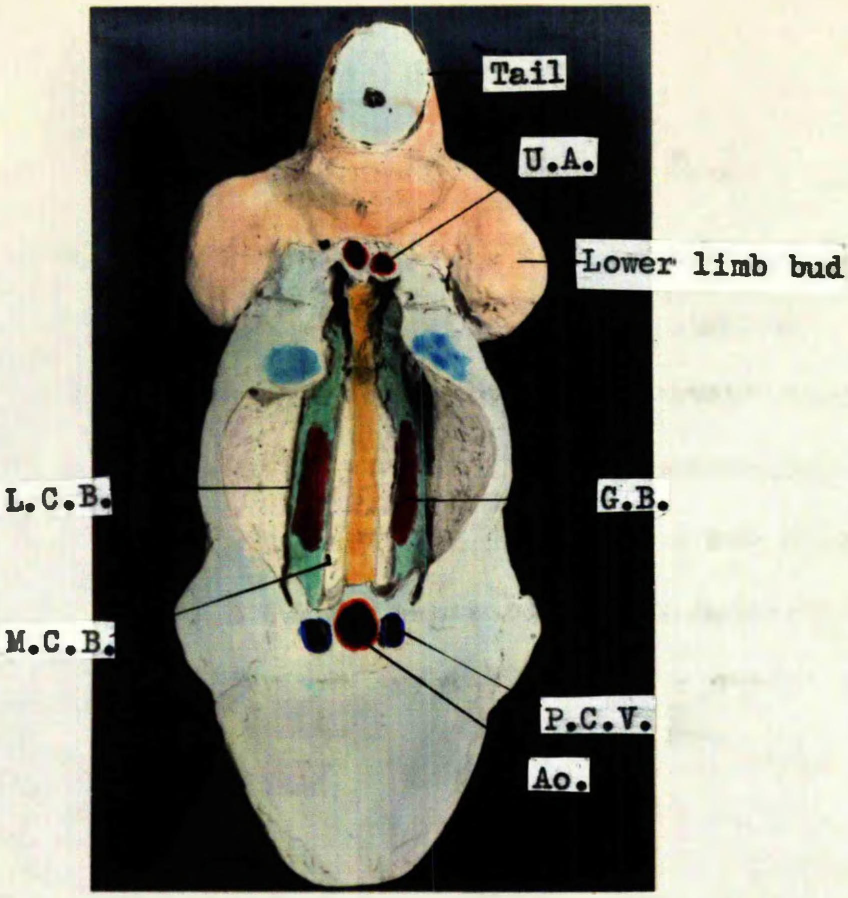

Fig. 15. RAT EMBRYO. C.R.L. 7.3 mm. A wax plate reconstruction model of the caudal region. Viewed from the cephalic aspect. X 15.

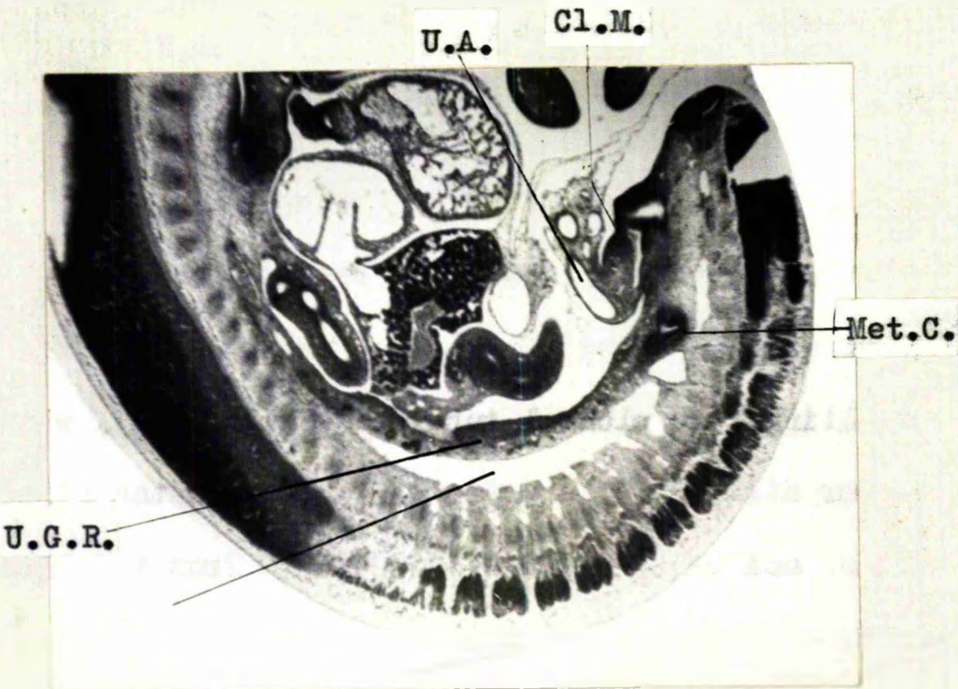

Fig. 16. RAT EMBRYO. C.R.L. 7.3 mm. Median sagittal section through the caudal region. X 20.

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9½ DAY EMBRYO OF A WHITE NORWAY RAT.

A number of embryos were obtained from the uterus of a rat 9½ days after mating. Their average C.R. length was 7.3 mm. From these specimens, serial sections at 10u were prepared in sagittal, coronal and transverse planes and every fourth section of the relevant region was mounted and stained with H. & E. A wax plate reconstruction from transverse sections, of the caudal region of an embryo was made, showing the urogenital ridges and the cloacal tubercle.

Cloacal Tubercle.

The form of the ventral surface of the embryo in the region bounded by the umbilicus and the coccygeal tubercle was studied in the reconstruction in figure 15 and in sagittal sections such as that shown in figure 16.

The cephalic margin of the area is marked by the two umbilical arteries lying against one another on either side of the median plane. Caudal to these vessels the surface is in the form of a single swelling, the cloacal tubercle (Felix 1912), which extends on either side of the middle line as far as the attachments of the limb buds, and which is limited caudally from the coccygeal tubercle by a faint groove. In the rat the swelling faces in a dorsal direction owing to the curvature of the caudal end of the/

the embryo. In the middle of the swelling and nearer to the caudal margin than the cephalic margin is a linear depression, the floor of which is the external surface of the cloacal membrane.

At this stage as Wyburn (1937) has noted, there is no interval between the cloacal tubercle and the umbilicus, and consequently there is no infra umbilical abdominal wall.

It will be demonstrated in subsequent sections that in later stages of development, the infra umbilical portion of the abdominal wall, the muscular coat of the bladder, the symphysis pubis and part of the external genitalia are derived from the mesoderm underlying the surface of the cephalic part of the cloacal tubercle.

The development of the infra-umbilical portion of the abdominal wall produces a separation of the tubercle from the umbilicus and from that stage onwards it would therefore seem convenient to refer to the swelling as the genital tubercle, the terminology used by Spaulding (1921).

<u>The Urogenital Ridge or Fold.</u>

Figure 17 is a sagittal section of one of the 7.3 mm. embryos, a little to the right of the median plane. When the embryo/

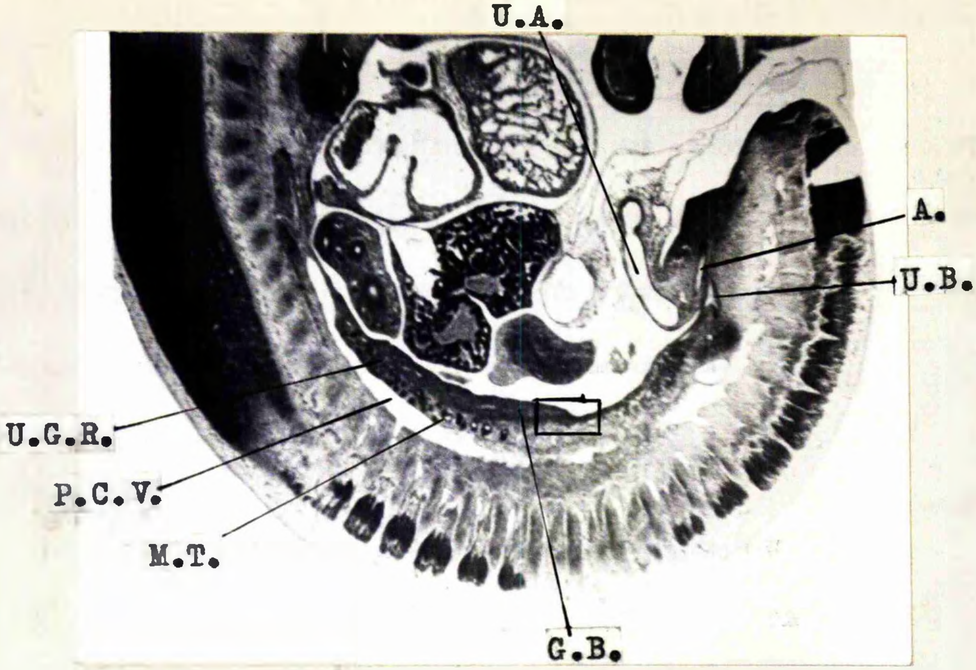

Fig. 17. RAT EMBRYO. C.R.L. 7.5 mm. Paramedian sagittal section passing through the urogenital ridge on the right side. X 20.

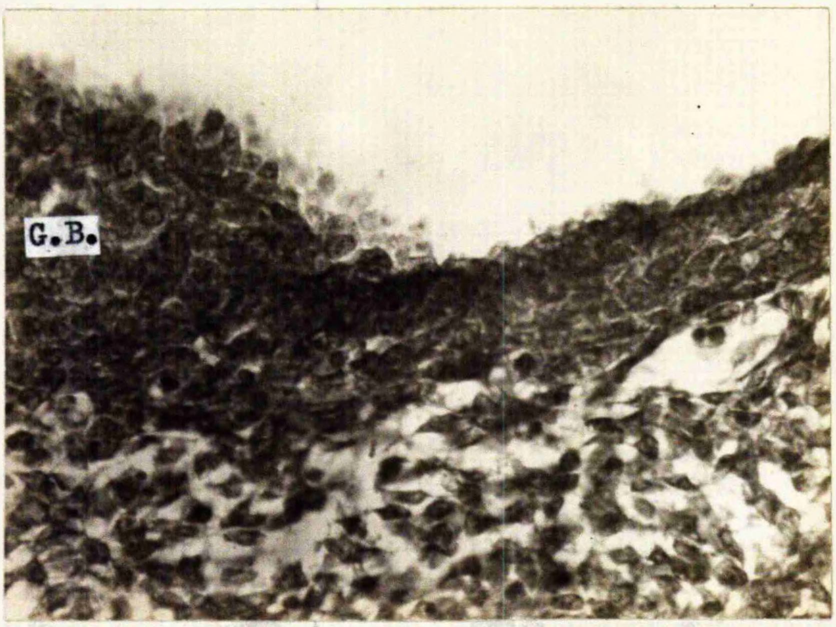

Fig. 18. Same as above. High powered view of the area marked by a square. X 300.

embryo is orientated, as in this figure, so that the C.R. axis is vertical, the urogenital ridge (Ur.R.) lies in the caudal curvature of the coelomic cavity and the cephalic and cloacal ends of the ridge are at the same horizontal level. Throughout its length the ridge overlies the right posterior cardinal vein (p.c.v.).

The right umbilical artery (U.A.) is cut transversely between the caudal (cloacal) end of the urogenital ridge and the posterior cardinal vein. Beyond this point the artery passes ventrally across the lateral side of the ridge towards the umbilicus. The urogenital ridge is separated from the body wall on the lateral side and the mesentery on the medial side by two deep bays or gutters (figures 15 and 19). These have been termed the lateral (L.C.B.) and medial (M.C.B.) bays respectively by Felix (1912).

Within the cranial half of the urogenital ridge may be seen fully developed mesonephric vesicles and tubules (M.T.). (figure 17.)

A well developed mesonephric (Wolffian) duct (W.) runs deep to the lateral surface of the ridge (figure 19). With the ridge it inclines gradually towards the median plane, following the curvature of the body. Soon after the ridge has crossed the umbilical/

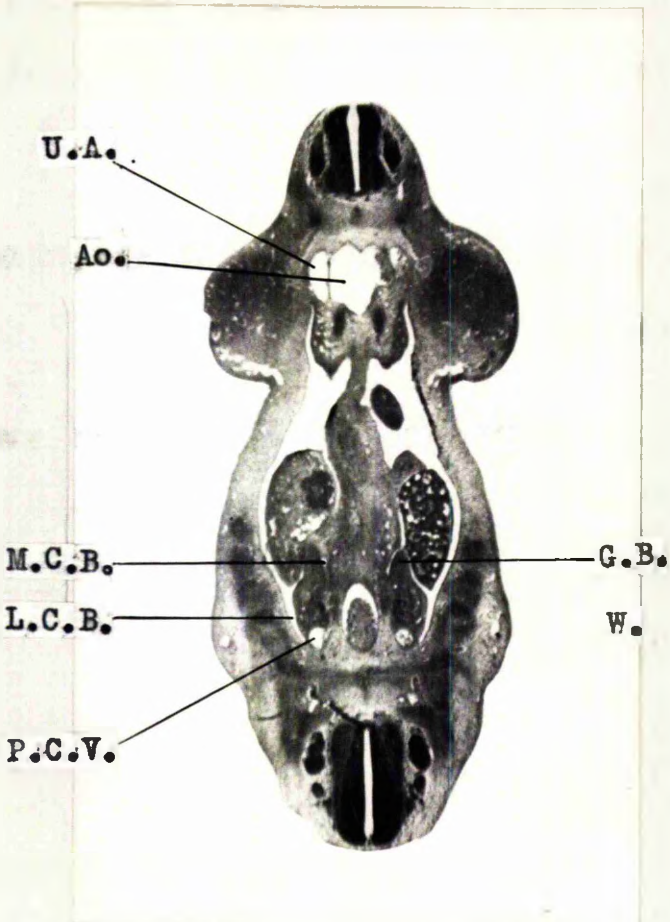

Fig. 19. RAT EMBRYO. C.R.L. 7.5mm. T.S. at the level of the origin of the umbilical arteries, passing through the cephalic and caudal portions of the urogenital ridge. X 20.

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umbilical artery, the duct gives off the ureteric bud (Ur.B.), from its dorsal aspect and then enters the side of the cloaca obliquely, near the base of the allantoic diverticulum (A). (figure 17).

No paramesonephric (Müllerian) duct is recognisable at this stage of development.

In figure 17, a condensation, part of the metanephric cap (Met. C.) is seen just cephalic to the ureteric bud, in the mesoderm at the caudal end of the ridge. Similarly, in figure 16 which is a section 40u medial to that of fig. 17, the cephalic end of the ureteric bud is seen surrounded by the metanephric cap (Met. C.).

Gonadal Blastema.

The central part of the mesonephric ridge bulges more prominently into the coelomic cavity than do the cranial and caudal parts. The prominence is due to a mass of round mesodermal cells which is termed the gonadal blastema (G.B.) (figure 17) and is situated on the medial part of the ventral aspect of the urogenital ridge (G.B.) (figures 15 and 19). It is approximately 1 mm. in length, 0.2 mm. in width and 0.1 mm. in depth. Figure 18 shows a high power view of the junction between the gonadal blastema and the more caudal part of the urogenital ridge. In the caudal part there is a distinct mesothelium of darkly staining/

staining cells. At its junction with the gonadal blastema the mesothelium disappears as a distinct layer and beyond this point the proliferating mass of mesodermal cells show no distinct surface layer.

There are two views concerning the origin of primordial germ cells that give rise to the gonadal blastema. One states that primordial germ cells originate from the yolk sac and migrate from there along the gut mesentery to the region of the gonadal blastema. (Fuss, Felix, 1912; Hamlett, 1935), The other considers that the primordial germ cells are solely derived from the mesothelium (Stieve, 1927; Simpkins, 1928; Swezy & Evans, 1930). This dispute has yet to be solved.

It is a fact, however, that the primary sex cords are developed from a definitive gonadal blastema that overlies the urogenital ridge. Here again, there is a dispute as to whether the cords are derived solely from the surface mesothelium or the underlying mesoderm. Simpkins (1928) states that the primary sex cords were invaginations from the surface mesothelium into the underlying mesoderm. Fischel (1930), according to Gruenwald (1942), was of the opposite opinion, believing the sex cords were solely differentiated in the underlying mesenchyme. Van Vloten (1927) and Higuchi (1932) considered the gonadal blastema "as a mingling/

mingling of epethelial and mesenchymal cells" (Gruenwald 1942). This according to Gruenwald, "is misleading, because it creates the impression that there is a mixture of cells of different properties and potencies". He has shown that the mesothelium and the underlying mesenchyme in the gonadal blastema, cannot be distinguished at the time when differentiation of the primary cords begins. The early condition of the coelomic wall, when the surface cells are no different morphologically from the deeper mesenchymal cells, is re-established for a limited period of time. The basement membrane breaks down and the surface cells together with the underlying mesoderm now form a uniform blastemal mass, within which differentiation of sex cords takes place. He does not believe that sex cords are "distinctly bounded epithelial buds and cords growing into the subjacent mesenchyme."

From observations in the rat, my histological findings conform with that of Gruenwald. As shown in figure 18, there is no distinct basement membrane separating the surface layer from the underlying mesoderm, in the gonadal blastema. The cells of the gonadal blastema are in contrast to those underlying the blastema; but within itself the cells all appear alike.

From subsequent stages of development, it will be seen that the sex cords develop in situ within the gonadal blastema, and not as invaginations from the surface.

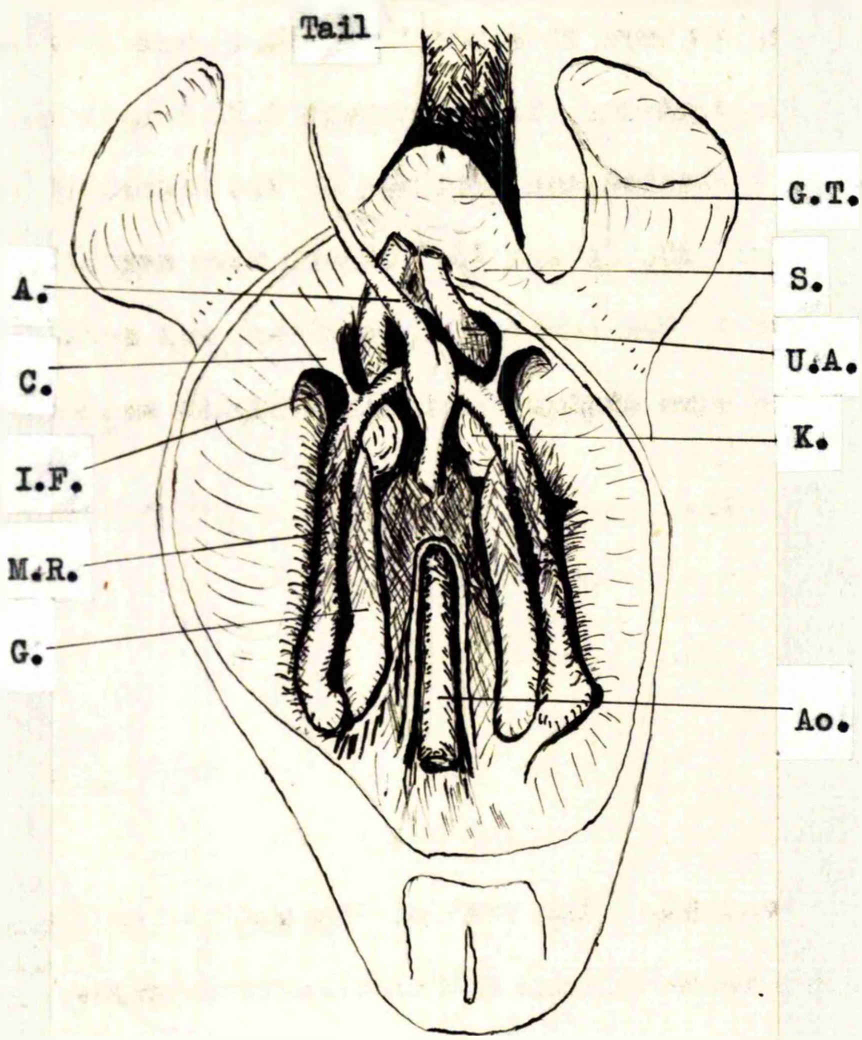

Fig. 20. 10.6 MM. RAT EMBRYO. View into the caudal curvature of the coelomic cavity from the cephalic aspect. The gut and liver have been removed.

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14½ DAYS EMBRYO OF (WHITE NORWAY) RAT.

Eight embryos were obtained from the uterus of a rat 14½ days after fertilization. Their average C.R. length was 10.6 mm. Two were dissected and drawings of the dissected specimens are shown in figs. 20, 21 and 22. Others were serially sectioned at 10u in the transverse, sagittal and coronal planes and the sections were stained with Haematoxylin and Eosin.

Here, as in the previous stage, and in all future stages, the description will be of the embryos so orientated that their C.R. axis is vertical.

In fig. 20 the embryo has been cut transversely at the level of the umbilicus. The mesentery has been cut at its root and the gut and liver removed. The rest of the structures are untouched and viewed from above through a binocular microscope.

In fig. 21 the tail has been cut transversely at its base, in line with the caudal slope of the cloacal tubercle, so as to show the tubercle's caudal surface.

Further Development of the Cloacal Tubercle.

Figs. 21 and 22 are views of the superior and anterior aspects of the cloacal tubercle.

The/

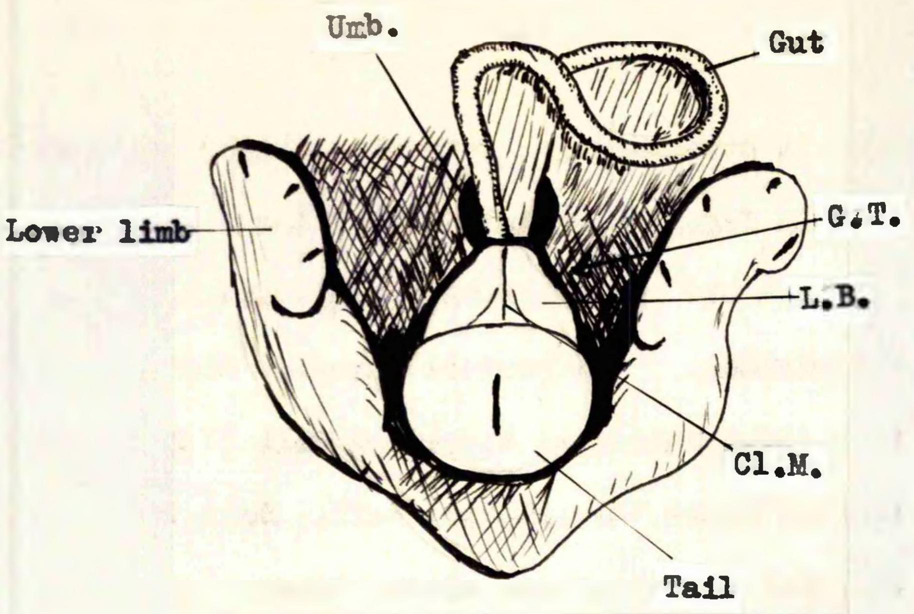

Fig. 21. 10.6 MM. RAT EMBRYO. View of the caudal aspect of the genital tubercle.

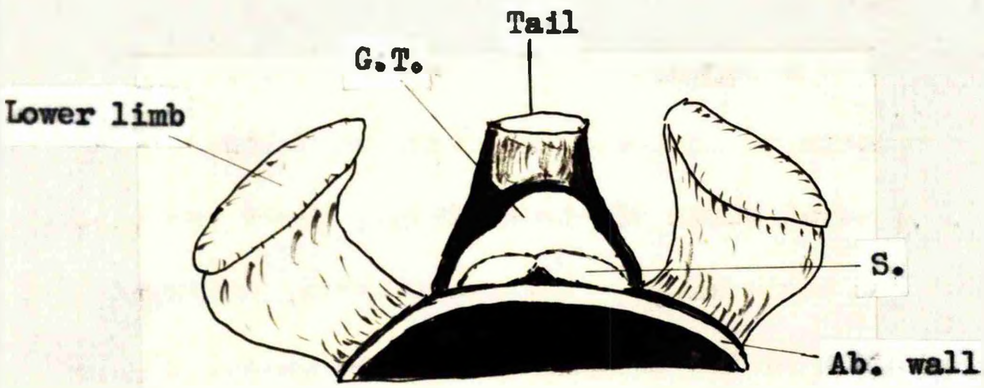

Fig. 22. 10.6 MM. RAT EMBRYO. View of the cephalic aspect of the genital tubercle.

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The tubercle is directed upwards with a slight inclination forwards, as seen in fig. 23, a sagittal section passing approximately through the median plane of the tubercle. Its tip is blunt and cylindrical. The postero-superior surface faces the newly formed thin infra-umbilical abdominal wall (I.U.A.W.), while the anterior surface faces the upturned tail. Both these surfaces are somewhat flattened and appear triangular when viewed as in figs. 21 and 22. The lateral margins of these two surfaces are connected by and continuous with the two lateral surfaces of the tubercle. These lateral surfaces are rounded, before backwards, and slope obliquely downwards and laterally towards the base of the tubercle. They give the appearance of overlying an oblique swelling on each side of the tubercle, a little below the apex. Fig. 24 is a coronal section through the cloacal tubercle. It appears triangular in shape with the two oblique lateral slopes forming the lateral sides of the triangle. Note the slight swelling on the right slope. Spaulding (1921) referred to these lateral swellings as the 'lateral buttresses' (L.B.) of the genital tubercle. Later development shows that the corpora cavernosa and ischio-cavernosa develop within these swellings.

A gentle swelling is present on each side on the postero-superior surface of the tubercle, near its base (s) (fig. 22). That/

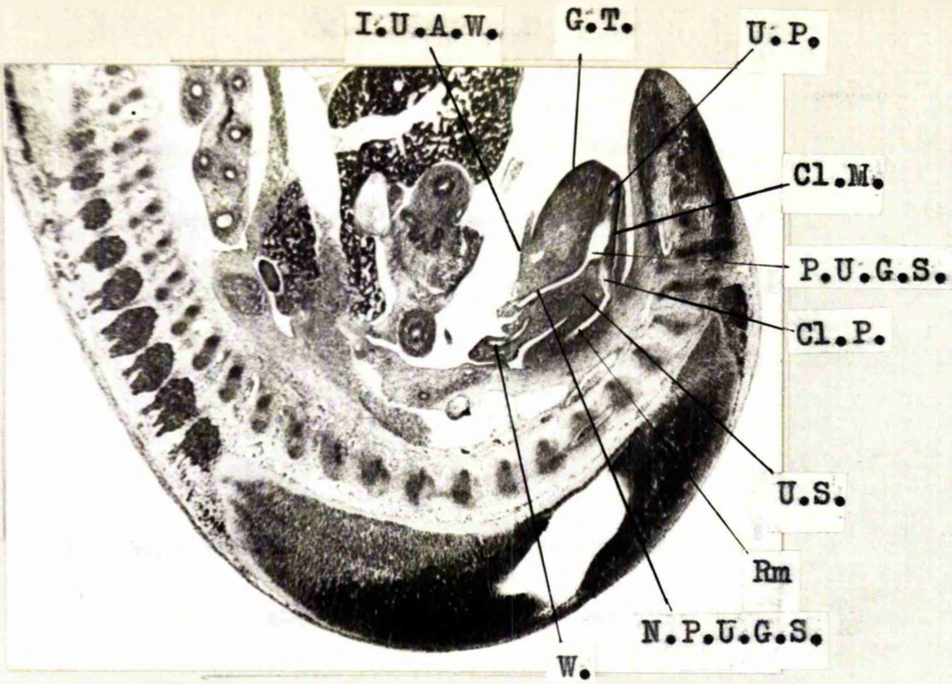

Fig. 23. RAT EMBRYO. C.R.L. 10.6 mm. Median Sagittal section through the caudal region. X 26.

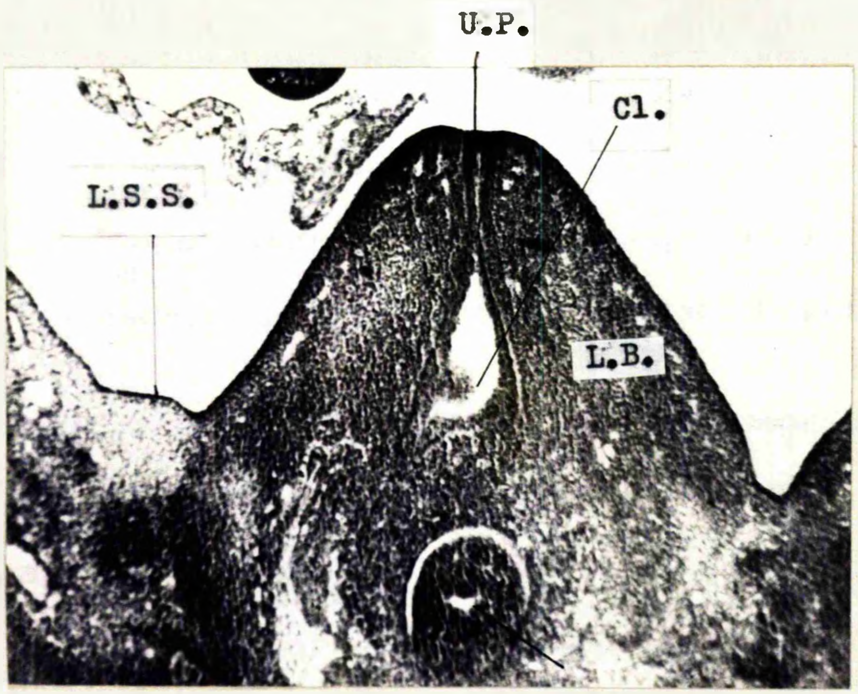

Fig. 24. RAT EMBRYO. C.R.L. 10.6. mm. Coronal section through the genital tubercle. X 70.

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That this swelling is not imaginary or an artefact is seen from paramedian sagittal sections, such as shown in fig. 25. A definite swelling (s) is seen here, between the anterior abdominal wall and the lateral buttress (L.B.) of the tubercle.

The caudal surface of the cloacal tubercle (fig. 21) presents a shallow linear median groove extending from the apex to the base. This is the external surface of the very narrow cloacal membrane. Fig. 23 shows the membrane (Cl.M.) cut in the median plane.

The position of the cloacal membrane is in contrast to that seen in the previous stage. In that stage, the membrane was found facing backwards (fig. 16); here it is facing forwards. The cause of this is found in the fact that the mesoderm between the cloacal membrane and the umbilicus has proliferated so rapidly that it has caused a 'push over' rotation of the cloacal membrane so that it now faces the opposite direction.

At this stage the urorectal septum (U.S.) has almost completely divided the cloaca into a rectal and a urogenital part (fig. 23). Immediately above the septum is the narrow non-phallic part of the urogenital sinus (N.P.U.G.S.). This leads forwards into the wider phallic part (P.U.G.S.) of the sinus which is directed upwards towards the tip of the tubercle. Beyond/

Beyond the wide portion of the sinus the urethral plate (U.P.) extends to the tip of the tubercle. The inferior or proximal part of the urethral plate has been partly broken down by the extension of the phallic portion of the urogenital sinus towards the tip. The urethral plate is a plate of cells lying in the median sagittal plane extending from the base of the tubercle to its tip. Throughout its length it is in contact with the caudal surface of the tubercle, and keeps pace with the growth of the phallus. The phallic part of the urogenital sinus extends along this plate breaking it down to form the penile urethra. The urogenital membrane does not break down in the male rat. Connecting the rectum with the phallic portion of the sinus is the very narrow cloacal passage (Cl.P.) fig. 23.

Behind the base of the tubercle is the thin infra-umbilical abdominal wall (I.U.A.W.). Immediately below this near the median plane, as in fig. 23, there is a wedge of mesoderm, bounded posteriorly by the coelomic cavity and antero-inferiorly by the non-phallic part of the urogenital sinus, which will develop into the symphysis pubis and the muscular coat of the bladder.

The Umbilical Arteries.

The two umbilical arteries (U.A.) which in the previous stage was in contact with the cloacal tubercle, are now separated from the latter by the newly forming infra-umbilical portion of the/

the abdominal wall (fig. 20). Arising from the abdominal aorta they run laterally for a short distance, beneath the floor of the coelomic cavity, before curving upwards and medially towards the umbilicus. In the dissected specimen, (fig. 20) they are seen in this latter part of their course to bulge backwards into the coelomic cavity from the ventral abdominal wall. At the lower margin of the umbilicus they meet and together exit through the umbilical opening.

At the point where the arteries make their sharp bend upwards and medially they give off a large limb bud artery (L.B.A.). In fig. 27, the section passes through this junction and may be seen below and behind the genital tubercle. Immediately behind the junction may be seen a section of the kidney. (K)

<u>The Urogenital Ridge or Fold.</u>

This ridge now appears to be cleft into a medial developing genital fold and a lateral "degenerating" mesonephric ridge. The term "degenerating" is not quite accurate. True, mesonephric tubules within the ridge are degenerating, but on the whole the ridge is re-moulding and developing itself towards its ultimate form (fig. 20).

The/

The cephalic portion of the mesonephric ridge has completely degenerated and remains as the plica diaphragmatica (P.D.). The mesonephric tubules (M.T.) too, have mostly disappeared. What few remain are situated at the cephalic end of the mesonephric ridge in its medial portion, adjacent to the genital fold (G.F.) (fig. 29).

The mesonephric ridge is attached to the floor of the coelomic cavity by a short thick fold, commonly termed a mesentery or better still, a mesogenitale. Due to differential growth the caudal portion of the ridge, accompanied by its mesogenitale makes a fairly sharp bend antero-medially towards the urogenital sinus.

The Wolffian duct traverses the whole length of the ridge. In its cephalic part it lies beneath the lateral surface of the ridge. It runs downwards, forwards and medially, following the curve (fig. 30) of the ridge till it reaches the side of the base of the allantoic diverticulum. Here, (fig. 23) it makes a sharp turn forwards and runs with a curve upwards to enter the non-phallic part of the urogenital sinus (N.P.U.G.S.).

The anterior half of the gonad lies horizontally in the floor of the coelomic cavity and extends as far forwards as the/

the bend of the mesonephric ridge. The posterior half of the gonad follows the curvature of the coelomic cavity and is directed backwards and upwards as far as the cephalic end of the mesonephric ridge. This latter part of the gonad eventually degenerates, considerably in the male, less so in the female.

Sex differentiation is not yet clearly defined at this stage. There are signs of sex cord formation and some of them show a parallel arrangement of cells. However, in the majority of specimens, there is no constant regularity in the arrangement of the cells. In no specimen was there any sign of a developing tunica albuginea. There was also no appearance suggesting ingrowing cords of cells from the surface mesothelium. The cells were uniformly scattered throughout the substance of the gonad. The nearest approach to sexual differentiation at this stage is shown in fig. 32 a high powered view of a gonad cut in the sagittal plane. Here, the cells are arranged in parallel rows, throughout the substance of the gland. This regularity suggests that this gonad might be a testis. However, there is no appearance of a tunica albuginea.

Kidney.

Differential growth is causing the caudal portion of the embryo to 'open-out', like the unrolling of a carpet, in the sagittal/

sagittal plane. Structures relatively fixed in the dorsal wall of the coelomic cavity thus appear to be at a higher level than previously. This factor in addition to the active growth of the kidney backwards, has shifted the latter far from where it was seen in the previous stage of development. It is now seen as a swelling (K) in the floor of the coelomic cavity in the angle between the anterior end of the gonad and the bend of the mesonephric ridge (fig. 20). In fig. 29 a sagittal section passing just medial to the bend of the mesonephric ridge, the kidney (K) is seen raising a swelling in the floor of the coelomic cavity between the caudal end of the gonad and the transversely cut mesonephric ridge containing the Wolffian duct (W).

The Inguinal Fold and Cone.

In the dissected specimen (fig. 20) a ridge of tissue runs ventro-laterally from the mesonephric ridge, at the region where it bends medially, to a gentle swelling (C) on the ventral wall of the coelomic cavity, lateral to the ascending portion of the unbilical artery (U.A.). This is the inguinal fold and the swelling on the ventral abdominal wall will be referred to as the inguinal cone because of its appearance, particularly in the later stages of development.

A study of these structures was made from serial sections in/

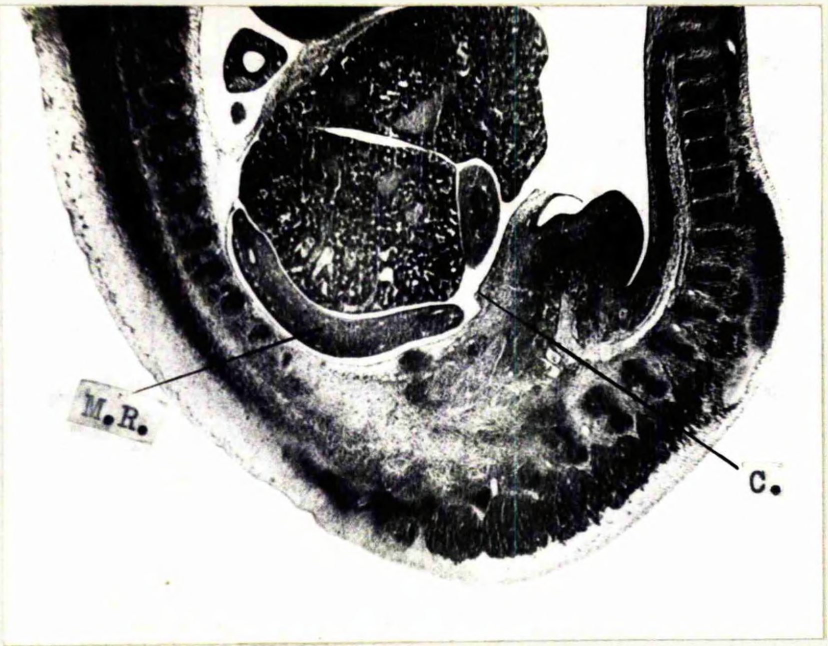

Fig. 25. RAT EMBRYO. C.R.L. 10.6 mm. Sagittal section passing through the right inguinal cone. X 20.

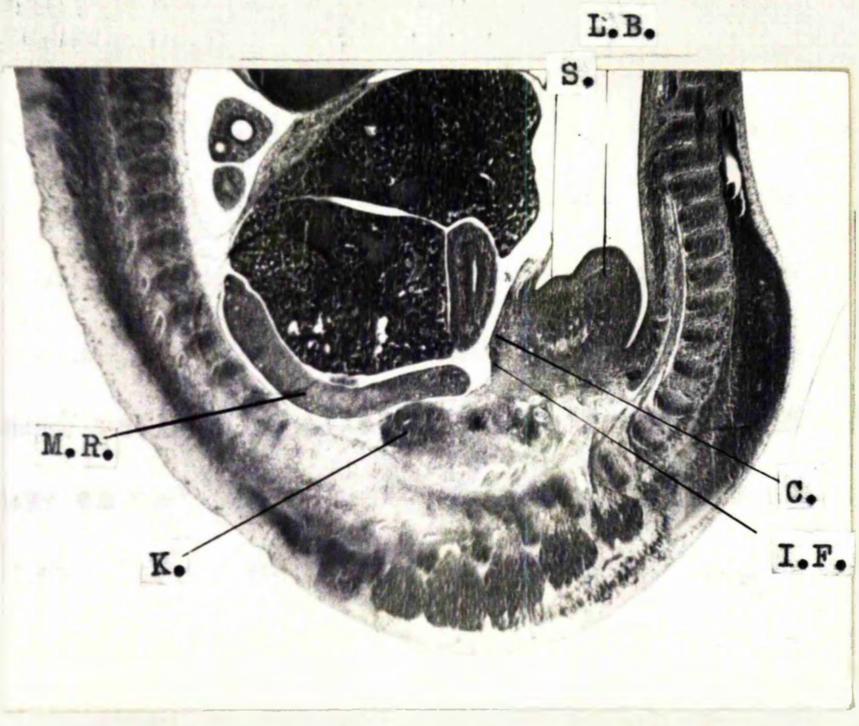

Fig. 26. RAT EMBRYO. C.R.L. 10.6. mm. Sagittal section 40u medial to the section shown in fig. 25. X 20.

in the sagittal, transverse and coronal planes. Figs. 25 to 29 are photographs of sagittal sections taken serially from lateral to medial to show the shape, structure and relations of the cone and fold.

In fig. 25 we see a conical projection(C) from the ventral abdominal wall. This is the cone of the right side and the section passes through the lateral part of it. The surface layer is more deeply stained while the underlying mesenchyme is lighter stained and looser in texture. No part of the inguinal fold can be seen in this section as that structure approaches the cone from below and medially.

Fig. 26 passes through the middle of the cone (C). In addition, however, it passes through the lateral part of the distal end of the inguinal fold (I.F.) which is continuous with the lower half of the cone and so makes the swelling appear less prominent. The cells beneath the coelomic surface of the cone in this region are closely packed and darker in colour, in contrast to the adjacent cells. Cells of similar appearance extend through the inguinal fold reaching as far as the mesonephric ridge. In a high powered view of this region (fig. 26A), the actual condensation of cells in the cone (C.C.) and fold are seen with loose mesenchym beneath them.

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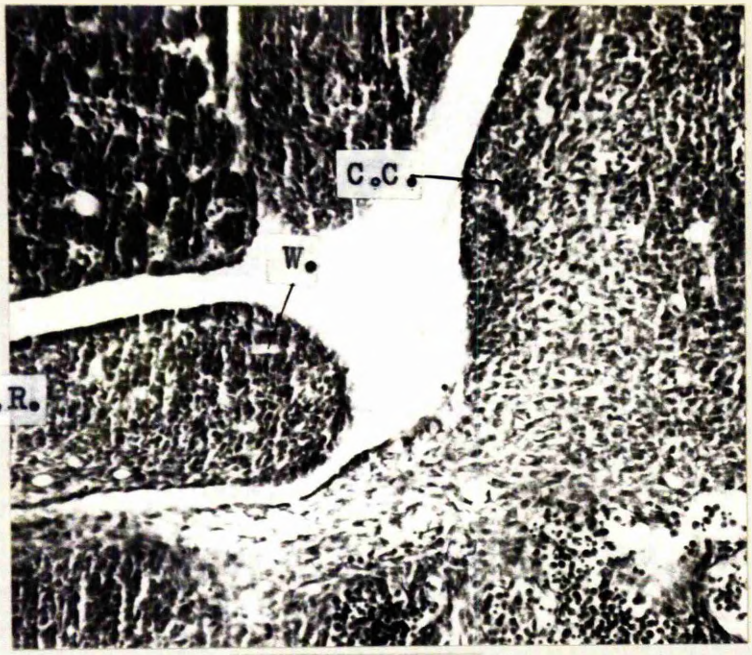

Fig. 26 A. same as fig. 26. High powered view of the inguinal cone (c) X 20.

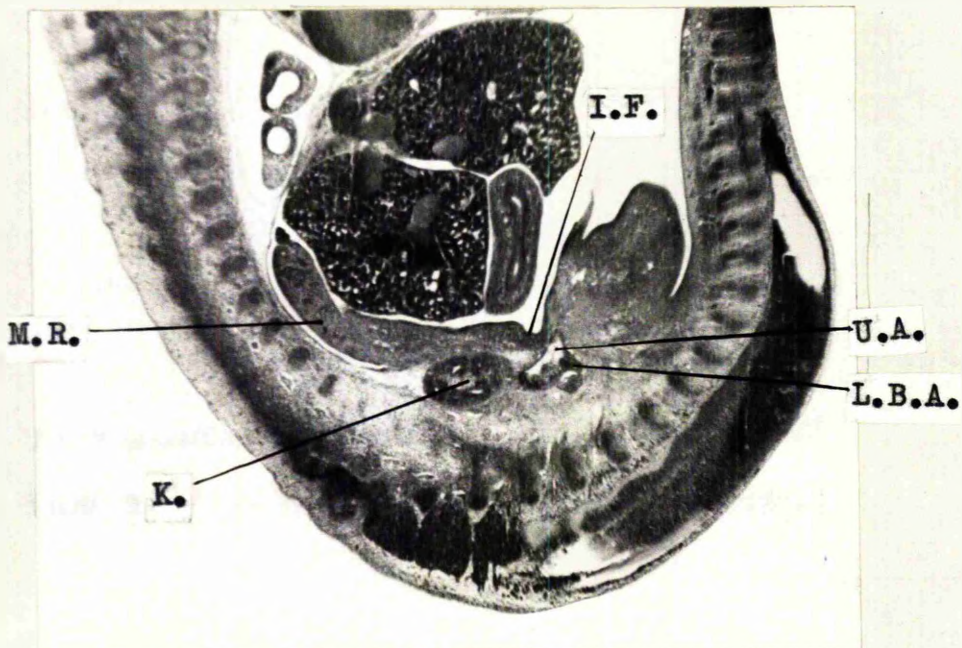

Fig. 27. RAT EMBRYO. C.R.L. 10.6.mm. Sagittal section 40u medial to the section shown in fig. 26. X 20.

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In fig. 27 the inguinal fold is seen actually connecting the ventral abdominal wall to the mesonephric ridge. The section does not pass along the long axis of the fold but obliquely from the lateral edge of the cephalic end to the medial edge of the caudal end. Notice the coloration of the cells in the fold (I.F.). They are darkly stained like the cells within the cone. A high powered view of the fold (fig. 27A) shows the cells (C.C.) to be more closely packed in comparison to the cells in the mesodem below the fold. Their appearance is like that of the cells in the condensation beneath the surface of the cone in fig. 26A. It also is similar to that of the cells in the mesonephric ridge where the fold is attached. It would therefore be logical to assume that the cells being alike, could be a stream of cells from the mesonephric ridge into the cone.

In the same figure, immediately below the fold there is seen the umbilical artery (U.A.) giving off the limb bud artery (L.B.A.) (the future external iliac) and turning upwards and medially. Cephalic to the artery is a section of the right kidney (K).

Fig. 28 is a section passing through the vertical limb of the/

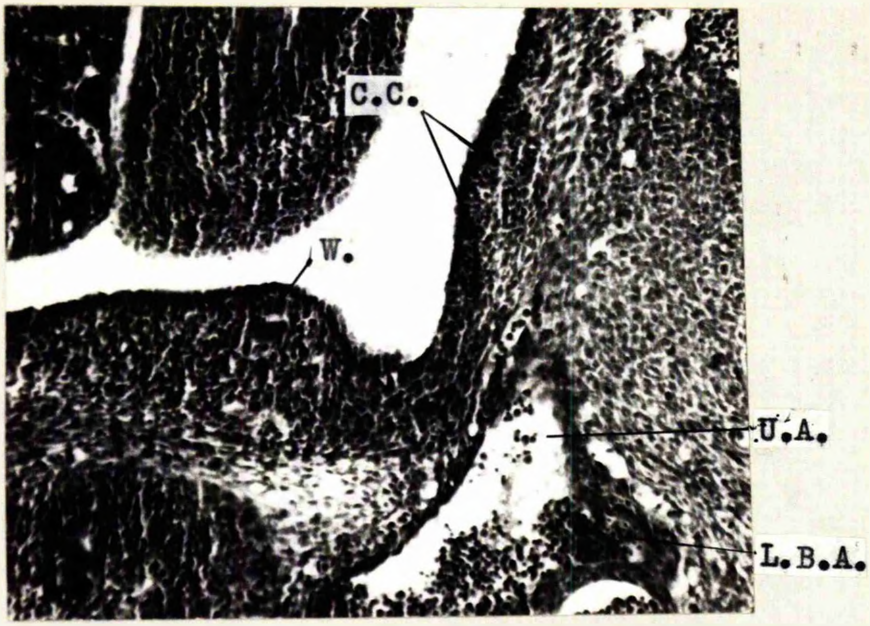

Fig. 27A. Same as in fig. 27. High powerdd view of the inguinal fold. (I.F.) X 20.

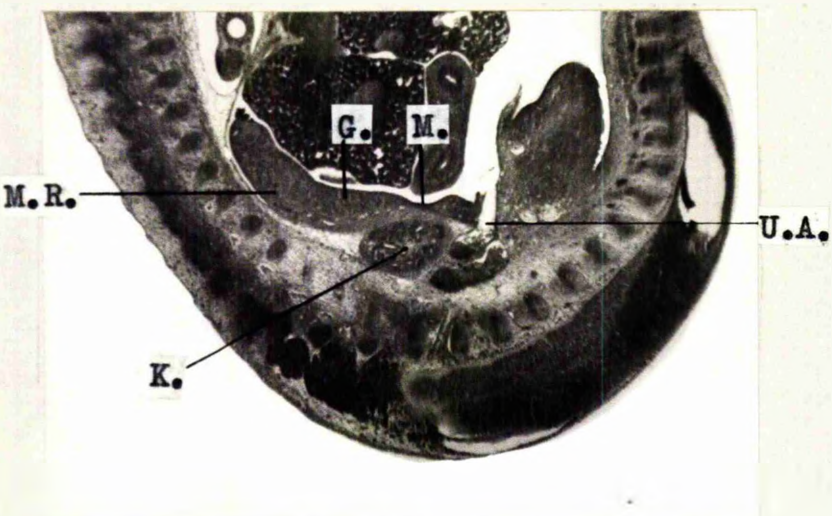

Fig. 28. RAT EMBRYO. C.R.L. 10.6.mm. Sagittal section 40u medial to the section shown in fig. 27. X 20.

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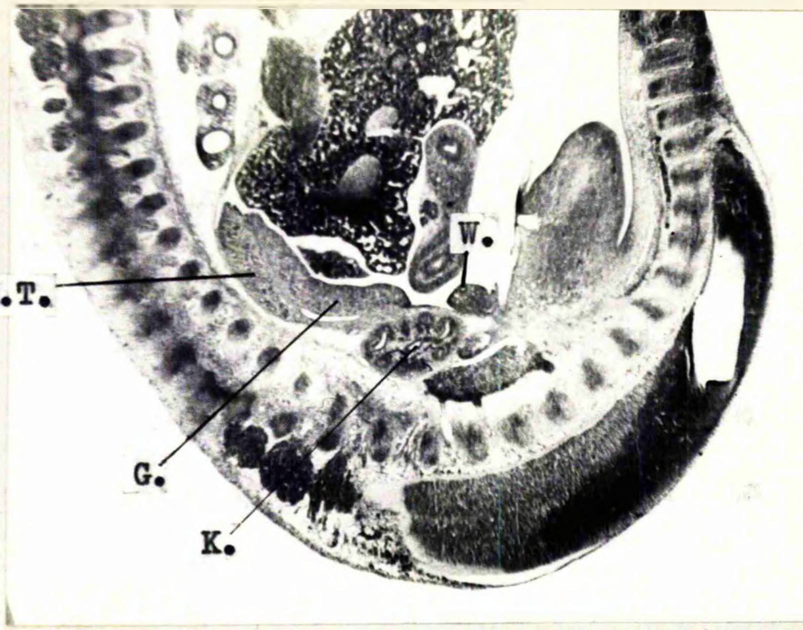

Fig. 29. RAT EMBRYO. C.R.L. 10.6.mm. Sagittal section 40u medial to the section shown in fig. 28. X 20.

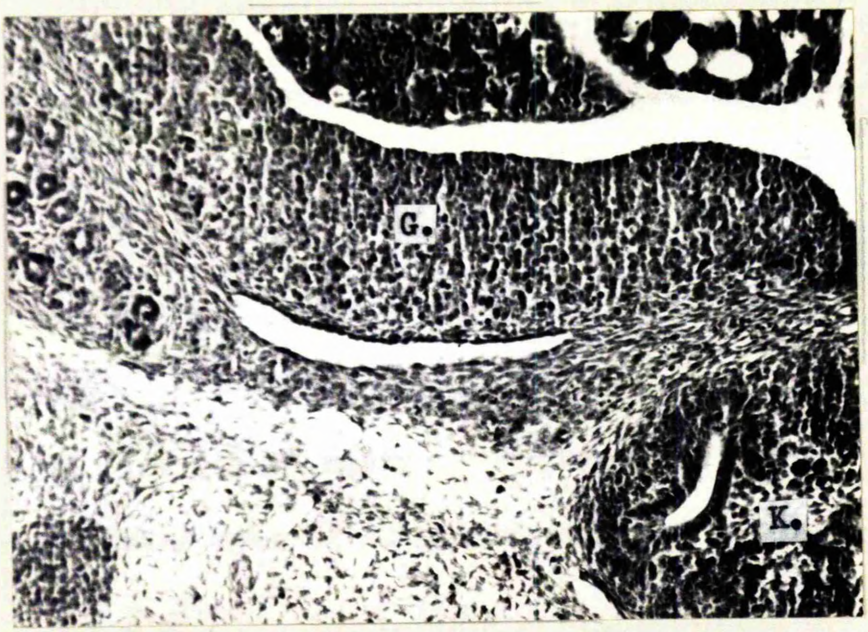

Fig. 29A. Same as above. High powered view of the gonad. X 20.

the umbilical artery (U.A.). Between the lower part of the artery and the mesonephric ridge is seen the medial portion of the proximal part of the fold. A portion of the mesorchium (M.) appears as a dark band of tissue connecting the anterior (caudal) end of the gonad (G.) with the posterior surface of the mesonephric ridge (M.R.). Directly below is the kidney (K.).

In the last section (fig. 29) the inguinal fold has almost disappeared and there is no mesorchium between the caudal end of the gonad (G.) and the mesonephric ridge (M.R.). Instead, in that region, the kidney raises a swelling in the floor of the coelomic cavity, between the two structures.

Fig. 30 is an oblique transverse section passing through the left side at a higher level. The left cone (C.) has been cut through its middle and shows a prominent cellular condensation. On the right side, the cone has been cut at a slightly lower level and therefore does not appear so prominent as the left one. The mesonephric ridge (M.R.) with the Wolffian duct (W.) coursing within it is seen within the abdominal cavity, behind the right cone. There is no inguinal fold visible; but in the fig. 31, a section 40u lower down, it is seen connecting the right mesonephric ridge (M.R.) with the/

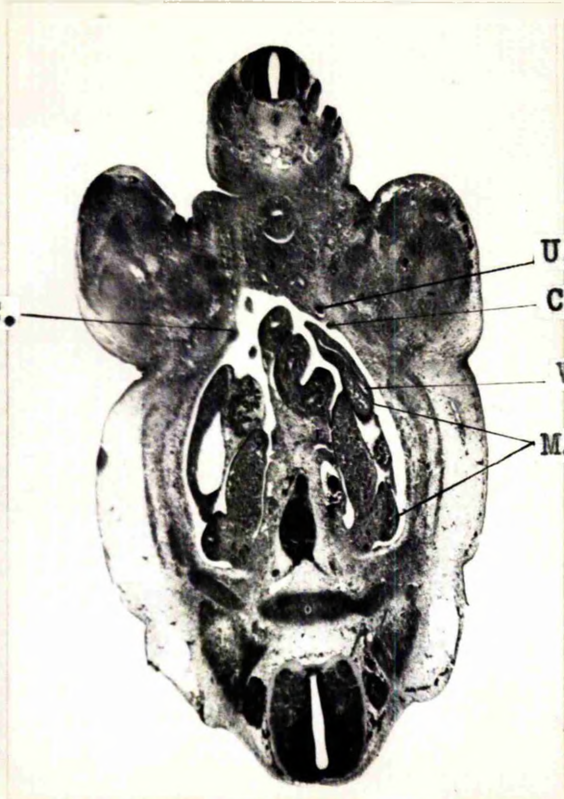

Fig. 30. RAT EMBRYO. C.R.L. 10.6.mm. T.S. at the level of the [illegible] inguinal cone. X 20.

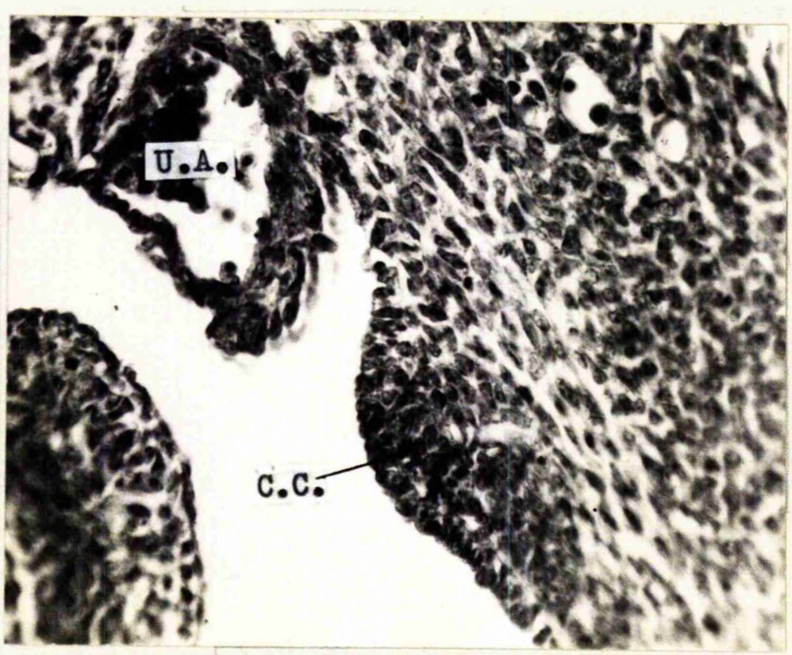

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Fig. 30A. RAT EMBRYO. C.R.L. 10.6.mm. High powered view of T.S. through the right inguinal cone. (c). X 300.

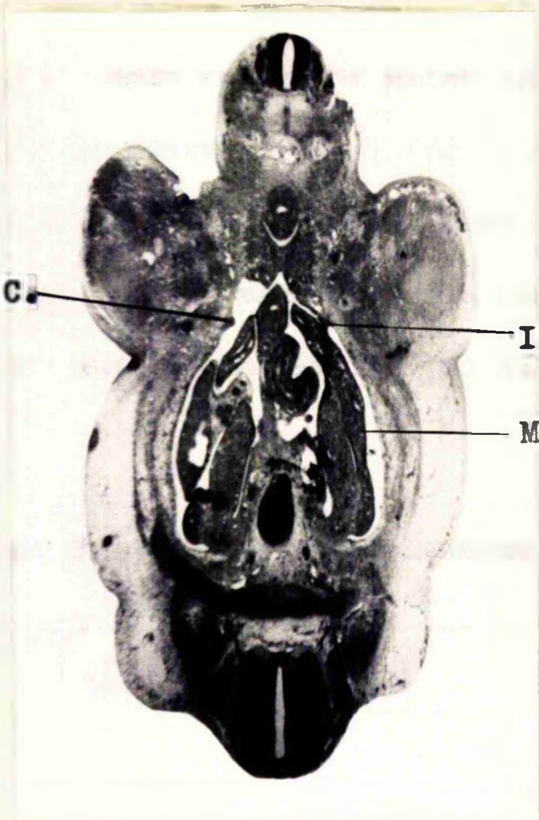

Fig. 31. RAT EMBRYO. C.R.L. 10.6mm. T.S. 40u below the level of the section shown in fig 30., passing through right inguinal fold. (I.F.) X 20.

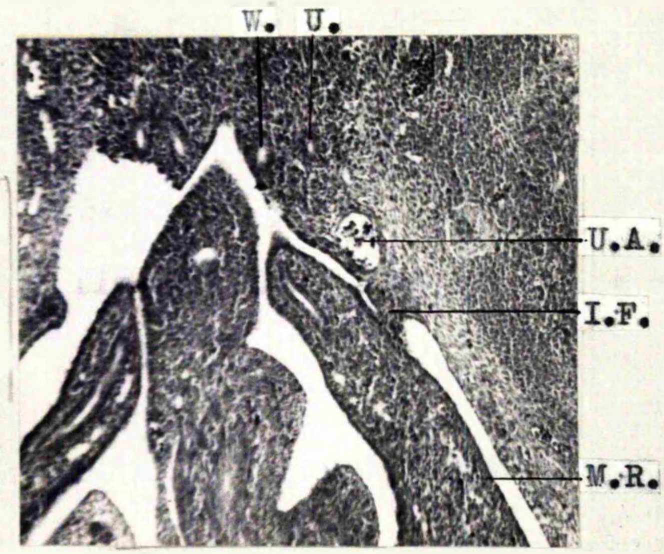

Fig. 31A. Same as above. High powered view of right inguinal fold. (I.F.). X 70.

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the ventral abdominal wall below the right cone. Fig. 31A is a higher powered view of the right mesonephric ridge (M.R.) and inguinal fold (I.F.) seen in fig. 31. The cellular appearance in the fold and ridge are similar in contrast to the lighter coloured cells beyond the fold in the ventral abdominal wall.

Fig. 30A is a high powered view of the right cone and umbilical artery 80u above the section shown in fig. 30. Note the condensation of cells (C.C.) beneath the surface of the cone and contrast it with the rest of the cells lying in the neighbourhood.

From this study, it is concluded that the inguinal fold contains a band of cells, in appearance like the mesodermal cells in the mesonephric ridge, which are continuous into the ventral abdominal wall to a region underlying the surface of a conical swelling, which at this stage is at a higher level than the free margin of the inguinal fold. This conical projection marks the site where the processus vaginalis will eventually develop.

Ventral Abdominal Musculature.

Condensations of the lateral abdominal wall musculature are recognisable at this stage. In fig. 30 the three parallel condensation/

condensation of the External oblique, Internal oblique, and Transversus abdominis can be seen. They are separated by wide layers of loose mesenchyme. The Transversus appears to be continuous internally with the lining of the coelomic cavity. Ventrally, all three muscles fall short of, by a considerable distance, the region where the conical swelling is present. Nowhere in the mesoderm of the ventral abdominal wall between the anterior ends of these muscle condensations and the middle line, at the level of the cone, is there any indication of the rectus abdominis.

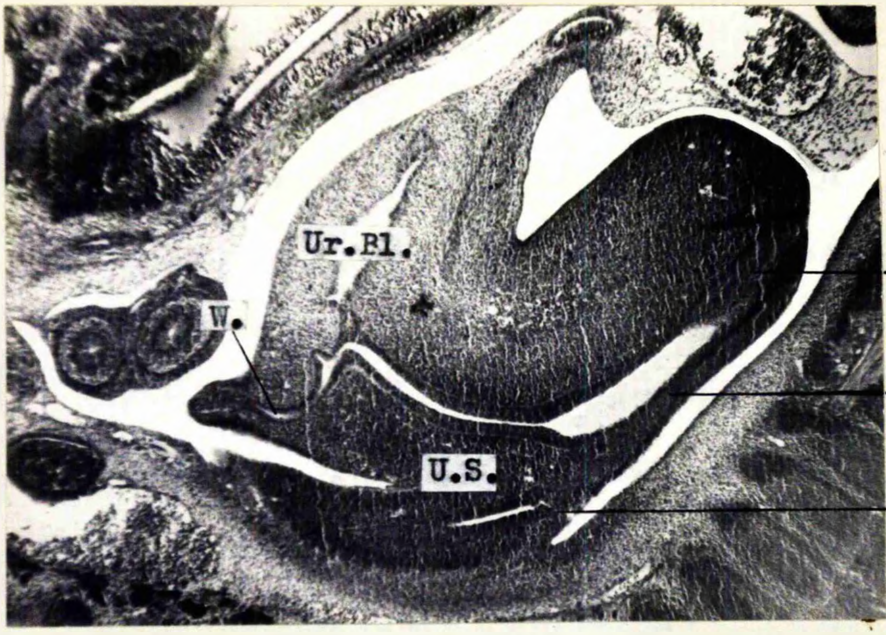

Fig. 32. RAT EMBRYO. C.R.L. 11.4mm. Median sagittal section through the developing bladder and phallus. X 40.

Fig. 33. RAT EMBRYO
C.R.L. 11.4mm.
A wax plate reconstruction model of the caudal region between the median sagittal plane and the lateral edge of the mesonephric ridge (M.R.) Viewed from the cephalic aspect, showing the inguinal cone (c) on the surface of the ventral wall of the coelomic cavity.

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$15\frac{1}{2}$ D. RAT EMBRYOS.

Two sets of embryos of approximately the same age were obtained from rats' uteri fifteen and a half days after mating. One, numbering thirteen embryos, had an average C.R. length of 11.0 mm. The other, numbering twelve embryos, had an average C.R. length of 11.4 mm. As before, transverse, coronal and sagittal sections were prepared, and stained with Haematoxylin and Eosin. The region between the median plane and the middle of the right lower limb in the caudal curvature of the embryo was re-constructed from sagittal sections magnified 25 times, using wax plates.

The Genital Tubercle.

The external appearance of the tubercle presents no striking change from that in the previous stage. A median sagittal section, however, fig. 32, shows that there has been some advancement of the underlying structures. The urorectal septum (U.S.) has completely separated the rectum from the urogenital sinus and therefore the cloacal membrane now is divided into an anal membrane (A.M.) and a urogenital membrane (U.G.M.). The anal membrane is very thin and is at the point of breaking down. The urogenital membrane, on the other hand, is still fairly wide and shows no sign of dissolution.

The/

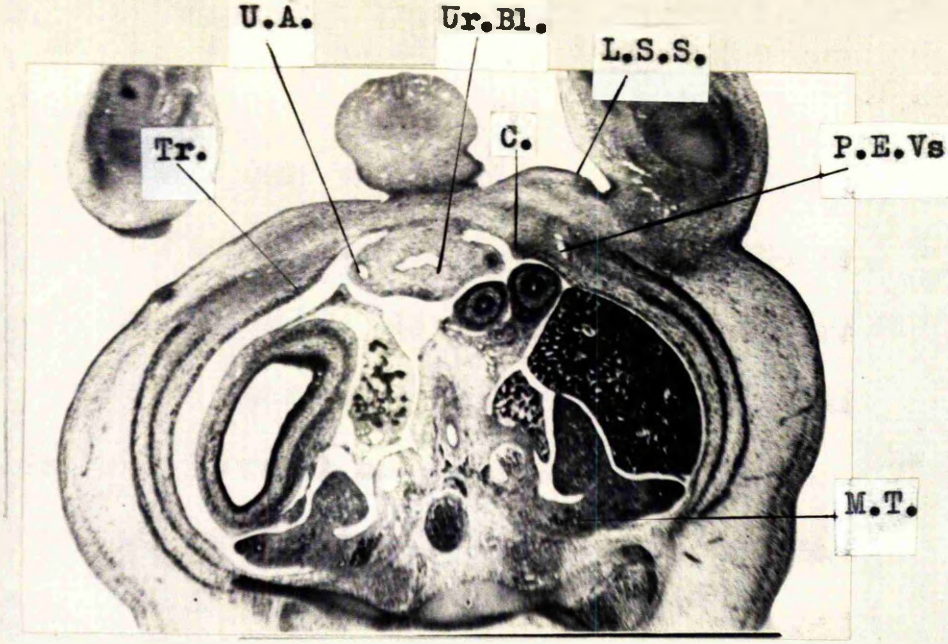

Fig. 34. RAT EMBRYO. C.R.L. 11.4mm. A slightly oblique T.S., higher on the left side, passing through the level of the right inguinal cone. (c).X 20

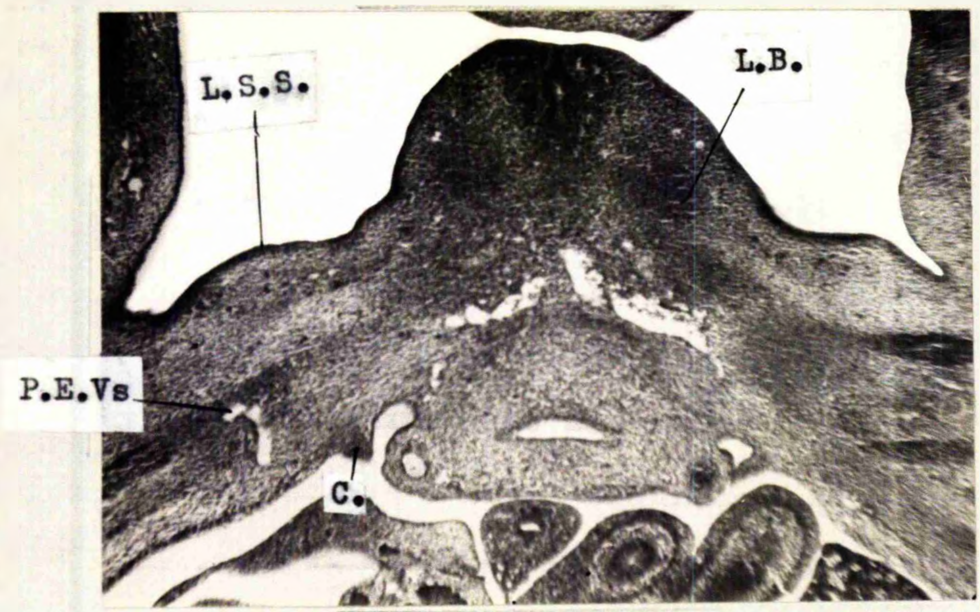

Fig. 35. RAT EMBRYO. C.R.L. 11.4mm. T.S. 160u below the level of the section shown in fig. 34. X 45.

40

The phallus has grown in length and the urethral plate has kept pace with it. The phallic part of the urethra is extending along the plate towards the tip of the phallus. Dorsal to the urethral plate there is a large blood channel. This is the result of the union of two internal pudendal vessels at the base of the tubercle between the phallic part of the urethra in front and the anterior wall of the bladder behind (fig. 35).

The Umbilical Arteries.

Due to the 'opening out' of the caudal curvature, the origins of the arteries are migrating cranialwards. This has tended to straighten the first part of each artery which was directed laterally, so that it now runs in a more forward direction towards the ventral abdominal wall. Its course is shown in fig. 33. The red line represents the line of its direction on the right side. The artery runs in an antero-lateral direction and after crossing below the mesonephric ridge it appears on the ventral abdominal wall medial to the inguinal fold. It then travels upwards on the lateral surface of the developing bladder (fig. 34) to meet the left artery at the umbilicus.

Mesonephric Ridge./

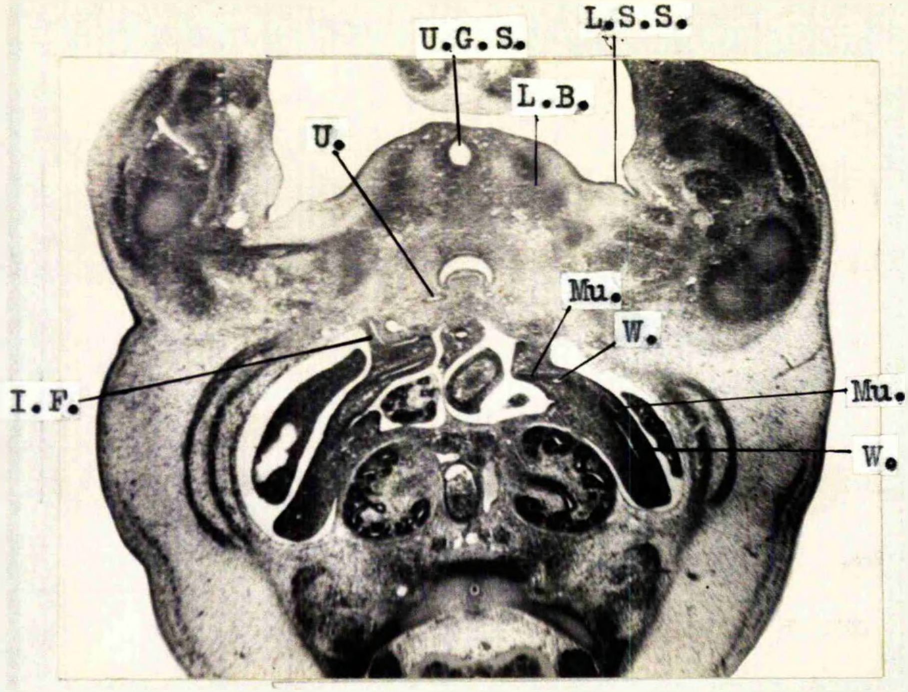

Fig. 56. RAT EMBRYO. C.R.L. 11.4mm. T.S. 120u below the level of the section shown in fig. 35. X20.

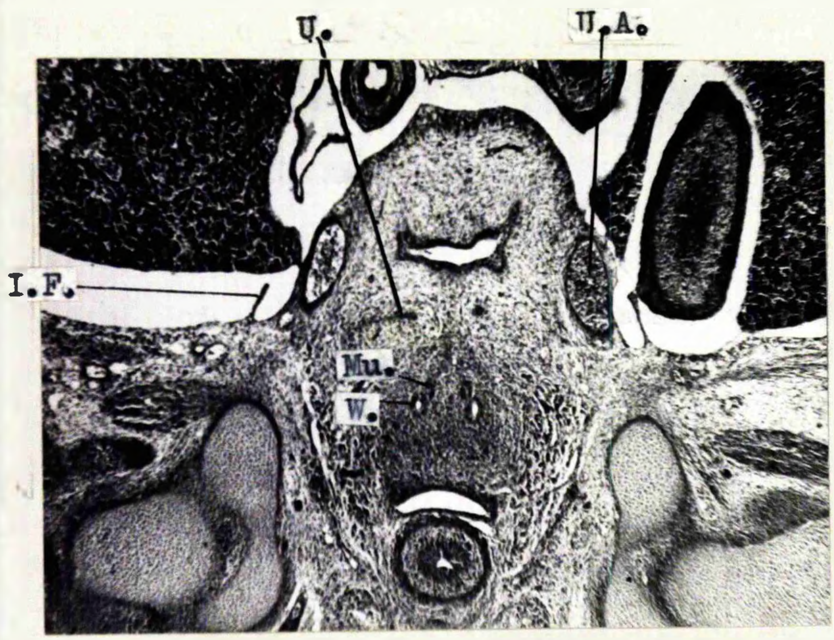

Fig. 37. RAT EMBRYO. C.R.L. 11.4mm. A Coronal section passing through the posterior wall of the bladder showing the openings of the various ducts, and the very thin inguinal folds (I.F.) X 40.

Mesonephric Ridge.

Partly due to the rapid growth of the suprarenal gland and the kidney in the interval between the mesenteries of the gut and the mesonephric ridge, the cephalic half of the latter is carried further away from the median plane. Since the caudal end of the ridge is anchored in the pelvic mesoderm near the median plane, the ridge now makes a pronounced curve inwards (fig. 36).

The cephalic part of the ridge tends to fall over laterally partly because of its deepening and thinning mesentery, partly due to the growing gonad on its medial side and partly due to the pressure of the rapidly growing intra-abdominal viscera. In fig. 34 we see the ridges directed laterally and related ventrally to the stomach on the left side and to the liver on the right. In the same figure Mesonephric tubule remnants (M.T.) are just recognisable, but under higher magnification as in fig. 38, they are clearly visible.

The Müllerian duct (Mü) has by this stage developed sufficiently to be easily recognisable in the cephalic part of the ridge (fig. 38). It lies just beneath the lateral surface in this region and is separated from the Wolffian duct (W.) by a considerable interval of mesenchymal tissue. The/

The cephalic end of the duct opens at the cephalic extremity of the undegenerated mesonephric ridge, into the coelomic cavity. From there it runs caudally, lateral to the Wolffian duct, gradually approaching the latter. At about the level of the caudal pole of the gonad where the mesonephric ridge takes a sharper turn medially, the Müllerian duct begins to cross the Wolffian duct obliquely on its ventral aspect. It reaches the medial side of the Wolffian duct where the mesonephric ridge is about to merge with the pelvic mesoderm. In the transverse section shown in fig. 36 the Müllerian duct can be seen in the anterior part of the right ridge, as a dark condensation running in a transverse plane. Dorsal to the right extremity of this dark streak is the lumen of the sectioned Wolffian duct (W.). The mullerian duct here has just crossed the Wolffian duct. Excepting at its cephalic region, the lumen of the duct is very poorly developed. However, it can faintly be recognised and traced in serial sections. Having reached the medial aspect of the Wolffian duct, whose lumen is widely patent, the Müllerian duct runs along with it and blends with its termination. Fig. 37 is a coronal section near the dorsal surface of the bladder. The Müllerian ducts (Mü) can be seen between the two Wolffian ducts (W.).

The/

The course of the Wolffian duct is similar to that in the previous stage, except that it is more deeply placed in the mesonephric ridge, with the Müllerian duct separating it from the lateral surface.

Following the curve of the mesonephric ridge the Wolffian duct runs into the pelvic mesoderm surrounding the urogenital sinus. Here it abruptly changes direction and turning forwards it runs with a curve upwards to reach and open into the upper part of the urogenital sinus, just below the developing bladder. This terminal part of its course is seen in fig. 32, a slightly oblique sagittal section which passes through the median plane ventrally and slightly to the right of it dorsally.

As has been mentioned, the mesentery of the mesonephric ridge has lengthened and narrowed considerably to permit a certain amount of mobility. At the level where mesonephric tubules are still present, this mesentery is wide. Caudal to that region it rapidly narrows and past the caudal pole of the testis it is very thin. Near the termination of the ridge, this mesentery shortens and disappears rapidly, merging with the pelvic mesoderm.

The Gonads./

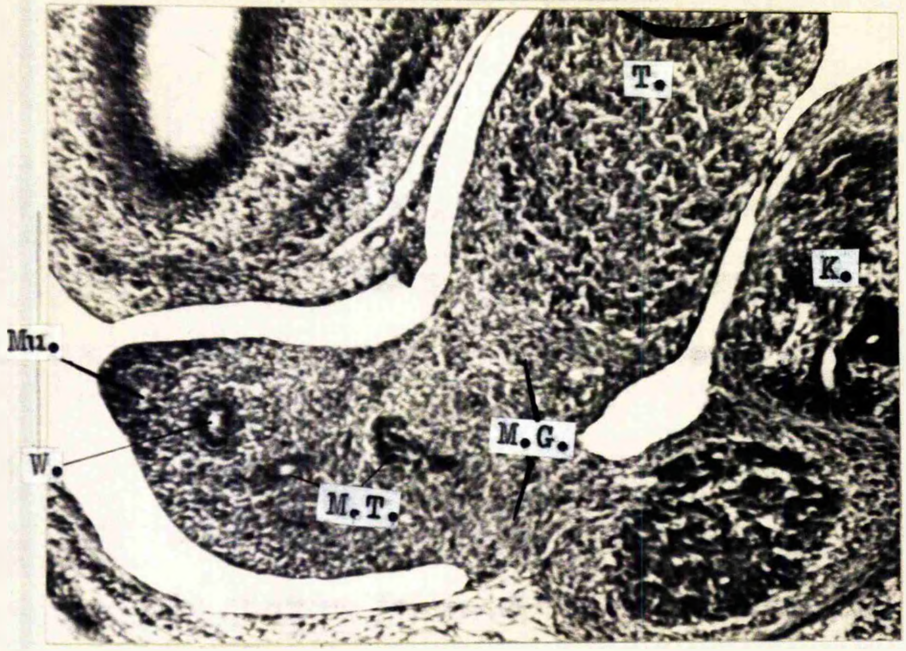

Fig. 38. RAT EMBRYO. C.R.L. 11.4mm. T.S. left mesonephric ridge and testis. X 95.

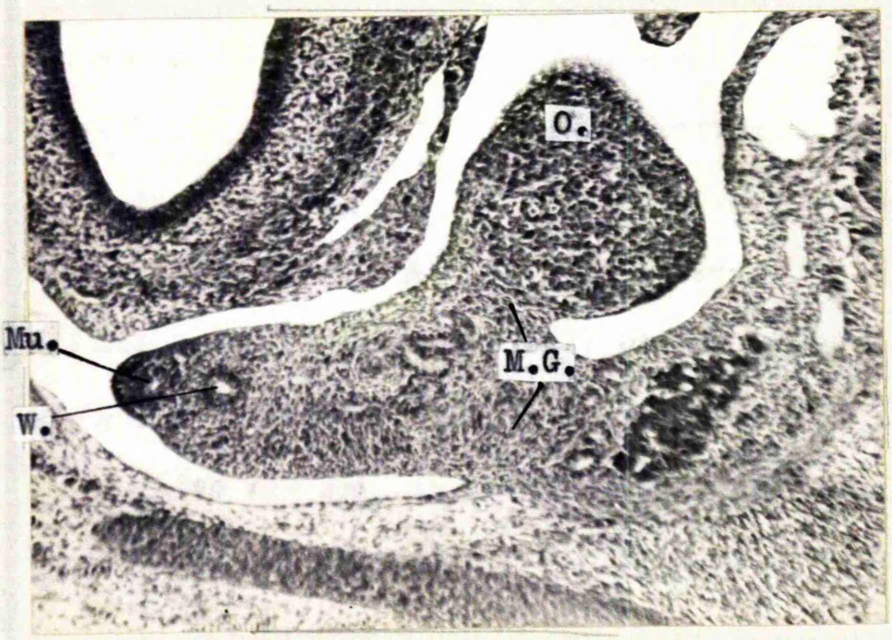

Fig. 39. RAT EMBRYO. C.R.L.11.4mm. T.S. left mesonephric ridge and ovary. X 95.

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The Gonads.

These are situated in the lateral part of the floor of the abdominal cavity. Their long axis is set so that the caudal end is directed slightly downwards and medially.

Each is like an elongated cylinder (T.) (fig. 40) with rounded ends. A mesongonad (M.G.) along its dorso-lateral aspect attaches the gonad to the medial surface of the mesonephric ridge (figs. 38 and 39).

Sex differentiation is now apparent. The cordlike arrangement of the cells (S.C.) the tunica albuginea (Tu.A.) and the generally large size distinguish the testis. In comparison the ovary does not show a similar regularity in the arrangement of its cells, has no tunica albuginea and is smaller in appearance. Compare figures 38 and 39. Each is an equally magnified view of an ovary and testis respectively, attached to the adjacent mesonephric ridge. The sections shown in the figures have been cut approximately in the same transverse plane through the cephalic region of each gonad. The testis is larger, and its cells are arranged in definite cellular cords which are seen here cut in both transverse and oblique planes. Figs. 41 and 42 are more/

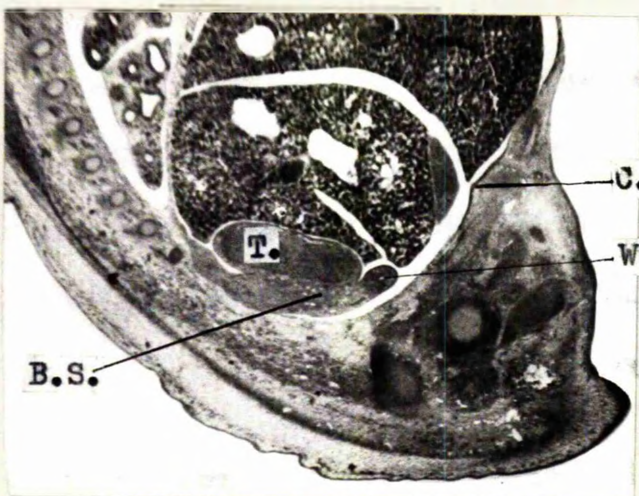

Fig. 40. RAT EMBRYO. C.R.L. 11.4mm. Sagittal section ofthe caudal region passing through the right testis (T) and the inguinal cone (c). X20.

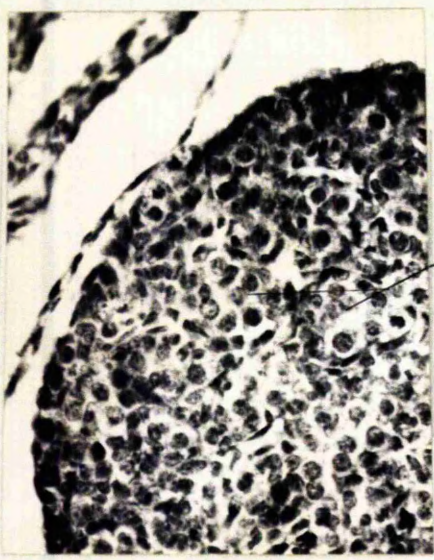

Fig. 41. RAT EMBRYO C.R.L. 11.4mm. Internal structure of the ovary. X300.

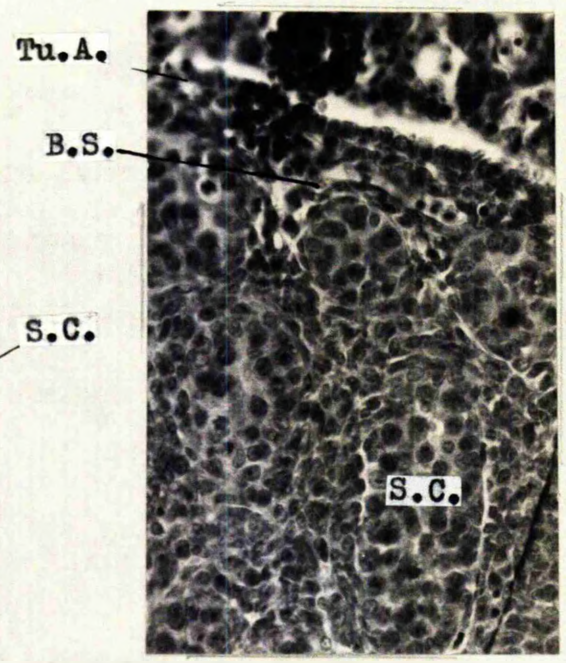

Fig. 42. RAT EMBRYO. C.R.L. 11.4mm. Internal structure of the testis. X 300.

more highly magnified views of the testis and ovary. The difference in the appearance of the sex cords (S.C.) is very striking. The male sex cords are clearly delimited from each other, but not so in the ovary. The tunica albuginea (Tu.A.) is clearly defined in the testis and blood channels (B.S.) may be seen beneath it.

Minute blood sinuses containing immature blood cells are present within the substance of the gonads. No large regular channels are seen here. In the testis, however, there are larger sinuses present immediately subjacent to the tunica albuginea. (Tu.A.) These sinuses are connected to a rich network of sinuses that is present in the mesogonad, particularly in its caudal portion (fig. 40). It is noted that there is a fairly large channel constantly present beneath the tunica in the region furthest from the mesogonad running parallel with the long axis of the testis. Many parallel channels of smaller dimensions are seen scattered in the same plane.

Kidney.

The kidney has grown considerably in size. It is now bigger than the testis and is situated in the mesoderm immediately/

immediately lateral to the aorta and its accompanying large veins. Its growth has pushed the mesonephric ridge and testis laterally. The ureters, emerging from its medial aspect course caudally just beneath the mesothelial surface of the abdominal cavity. Following the caudal curvature of the embryo in the sagittal plane, it passes ventral to the common iliac vessels and dorsal to the mesonephric ridge. Reaching the pelvic mesoderm it curves upwards till it reaches the level of its termination. Then it makes first a sharp turn medially and, on reaching the terminal portion of the Wolffian duct, a second turn forwards to enter the Wolffian duct. In fig. 37 a coronal section through the posterior wall of the bladder, from above downwards, just lateral to the median plane, can be seen the ureter (U.) Müllerian (Mü) and Wolffian (W.) ducts. On the left side the Ureter (U.) can be seen like a comet with its tail, running transversely across. This is the portion that makes a sharp medial turn. It is high above the Wolffian ducts (W.) in this region. This same portion of the ureter can be seen in fig. 36. It appears as an oblique lumen (U.) just behind the left horn of the crescentic space that represents the urogenital sinus just below the bladder. The actual termination is clearly shown in fig. 32, where the Ureter (U.) enters the Wolffian duct (W,) from behind, near the latter's termination into the upper/

upper end of the urogenital sinus.

Inguinal Fold.

From the wax plate reconstruction (fig. 33) and a study of serial sections the inguinal fold was found to be longer, deeper and thinner than that in the previous stage. Furthermore, owing to differential growth its obliquity has altered somewhat so that now it is nearer the sagittal plane and parallel with the ascending part of the umbilical artery. On either side of the fold the recesses separating it from the umbilical artery on the medial side and the abdominal wall on the lateral side, have deepened. The inguinal cone (C.) to which the fold is attached ventrally is situated well above the level of the mesonephric ridge as is seen in fig. 40. Transverse sections through the cone, e.g. figs. 34 and 35 do not therefore show a fold connecting the ventral abdominal wall with the mesonephric ridge. Only sections near the base of the fold such as that shown in fig. 36 will show such a connection because it is close to the attached margin of the fold (I.F.). Near its attached margin the inguinal fold has considerable width but its walls rapidly approach one another as they are traced towards the free margin and the greater part of the fold is thin and tenuous (fig. 37). Furthermore, the darkly staining cells noted/

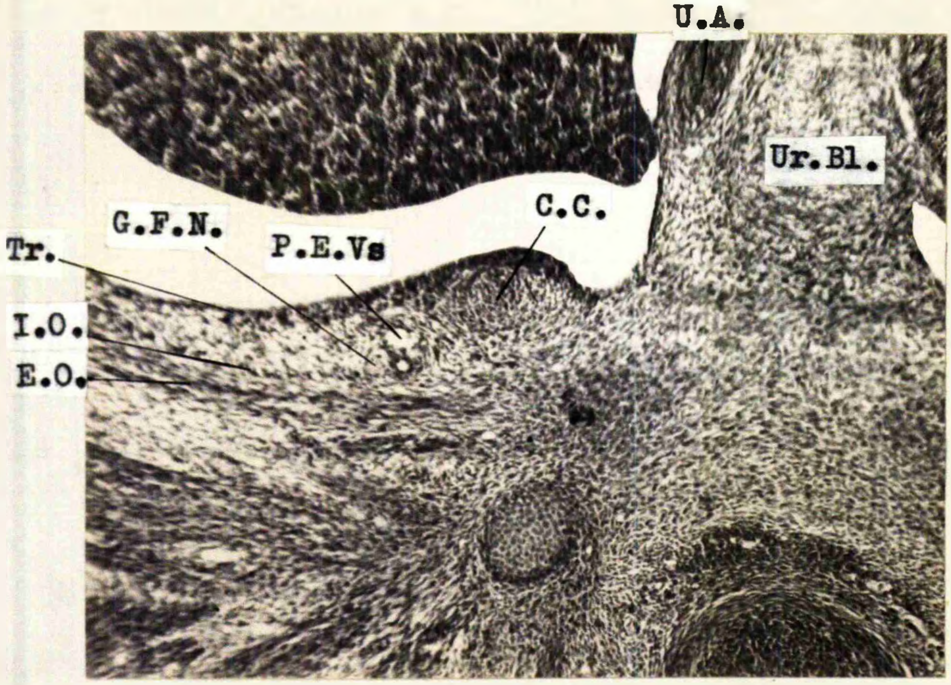

Fig. 43. RAT EMBRYO. C.R.L. 11.4mm. A Coronal section through the left inguinal cone near its base showing the cellular condensation(c.c.) and its relation to the oblique muscles. X 80.

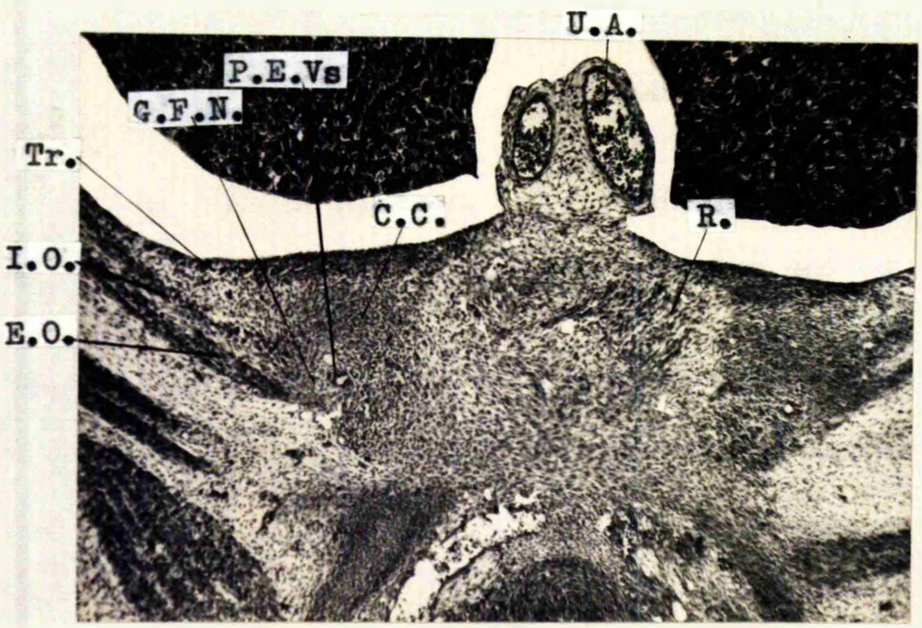

Fig. 44. RAT EMBRYO.C.R.L. 11.4.mm. A Coronal section 200u ventral to that shown in fig. 43. X 40.

noted in the fold in the previous stage have now almost disappeared.

The Inguinal Cone.

This conical elevation already noted in the previous stage lies on the deep aspect of the ventral abdominal wall, just lateral to the umbilical artery and opposite the 12th thoracic vertebra. Its apex is directed dorsally with slight medial and caudal inclinations and the ventral extremity of the inguinal fold merges with its inferior aspect.

The cone consists of a mass of closely packed, darkly staining rounded cells (C.C.). From the base of the cone a process of similar cells extends ventro-laterally into the abdominal wall and ends at the level of the external oblique (figs. 40, 43 and 44).

The cone has important relationships to various structures in the anterior abdominal wall.

(a) The muscles of the anterior abdominal wall. The condensation representing rectus abdominis can now be clearly seen except in its most caudal part. In the upper part of the abdominal wall and at the umbilicus the two muscles are widely separated. Figure 45, a transverse section/

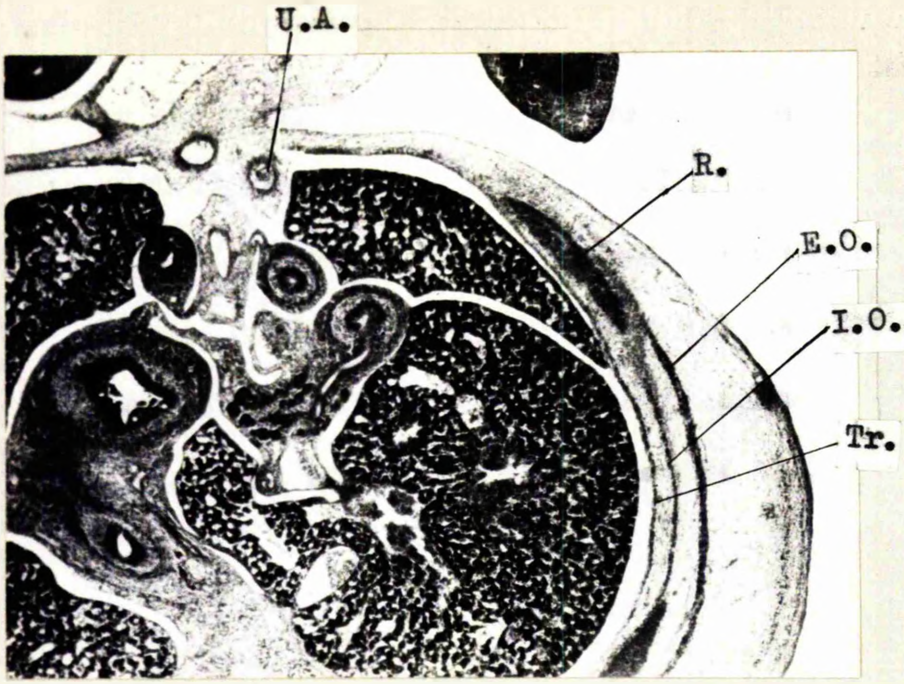

Fig. 45. RAT EMBRYO. C.R.L. 11.4mm. T.S. at the level of the umbilicus showing the condensation of the rectus abdominis (R). X 20.

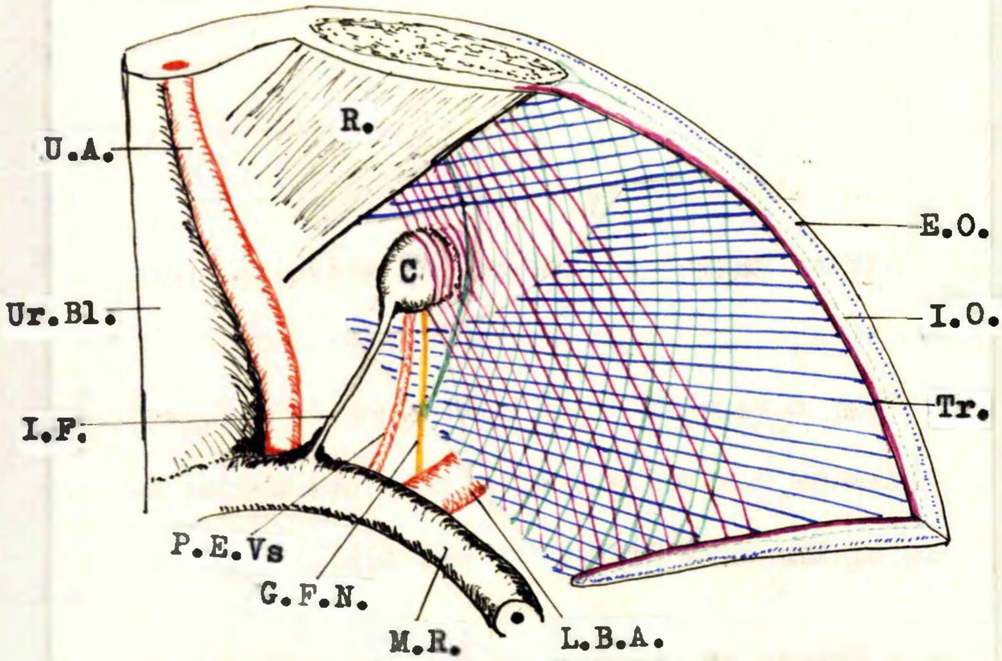

Fig. 46. A diagramatic representation of the cone and its relations. Note particularly the caudal margins of the transversus (purple) and internal oblique (green) which arch over the cranio-lateral aspect of the cone. The external oblique (blue) shows a definite 'opening'.

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section at the level of the umbilicus shows the rectus condensation (R) well developed and a considerable distance away from the mid line. Traced below the umbilicus however, the muscles rapidly approach the midline so that their lateral borders run medially as well as caudally. The caudal part of the muscle lies above and medial to the inguinal cone (figs. 46, 47 and 44).

The layer representing the transversus abdominis (Tr.) is now well defined and is still continuous with the mesothelial lining of the coelomic cavity. Traced ventrally its lowest fibres extend obliquely across the coelomic aspect of the cone, covering its lateral and cephalic aspects (figs. 46 and 47). The fibres of the internal oblique (I.O.) run cranially and medially and the ventral margin of this layer reaches the lateral margin of the rectus abdominis (R) (figs. 46 and 45). With a curve the caudal margin of the muscle skirts the lateral surface of the process of cells (C.C.) that extends ventro-laterally into the abdominal wall from the base of the cone (figs. 46 and 44).

The lower fibres of the external oblique (E.O.) extend caudally and medially towards the precartilaginous condensations representing the pubis, but over the base of the/

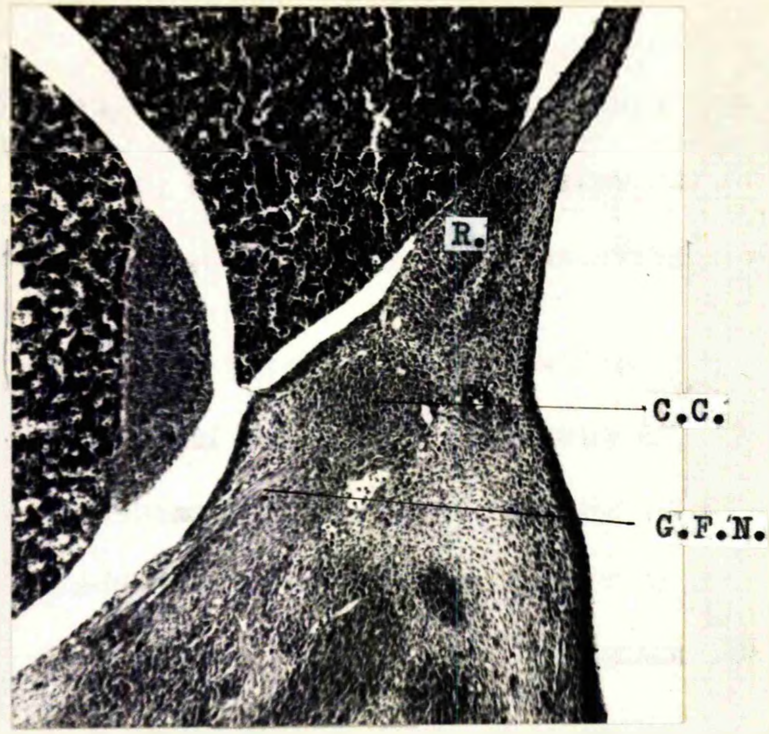

Fig. 47. RAT EMBRYO. C.R.L. 11.4mm. Same as fig. 40, showing a high powered view of the cone cellular condensation (CC) and genito-femoral nerve. X 65.

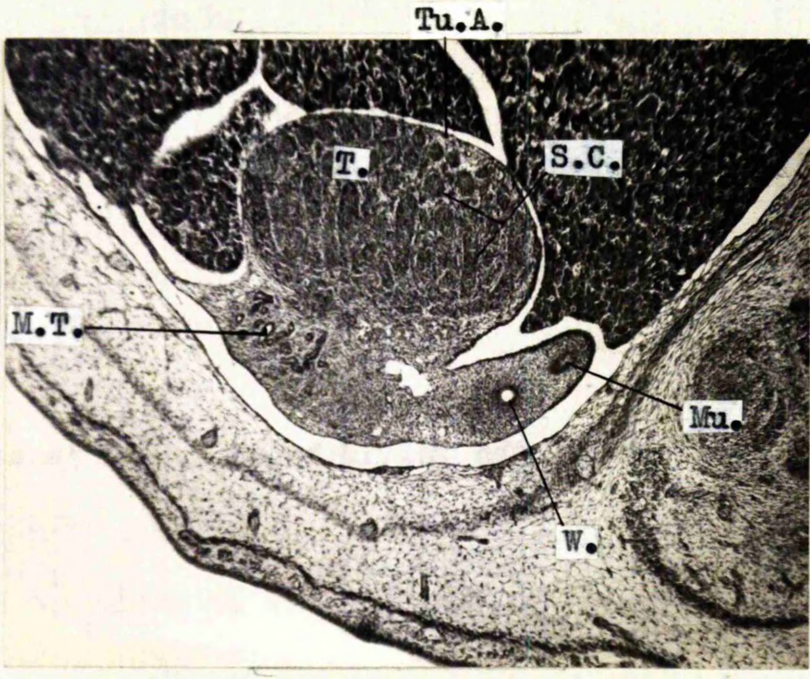

Fig. 48. RAT EMBRYO. C.R.L. 13.8mm. Sagittal section of the caudal region showing the testis in the floor of the cavity. X 40.

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the cone the layer presents a triangular deficiency in which lies the ventral extremity of the cellular extension of the cone previously noted (figs. 46, 43 and 44).

(b) The pudic-epigastric artery (P.E.A.) arises at the junction of the umbilical (U.A.) and limb bud arteries (L.B.A.) dorsal to the inguinal cone (C). It passes ventrally and comes to lie dorsi-lateral to the base of the inguinal cone, deep to the caudal margin of the internal oblique (I.O.) and caudal to the lowest fibres of the transversus (Tr.). There the vessel breaks up into a plexus which can be traced ventrally and medially across the caudal margin of the cone until it ramifies into the substance of the rectus abdominis (figs. 46, 43 and 44).

(c) The genito femoral nerve (G.F.N.) follows the pudic epigastric artery on its lateral side and reaching a position caudal to the inguinal cone divides into medial and lateral branches (figs. 47 and 43).

(d) The outline of the pelvic bones appear in a pre-cartilaginous condition as and seen in figure 40. The plane of the condensation is directed upwards forwards and medially and the anterior end of the pubic ramus is situated caudal to the inguinal cone (C.) (figs. 43 and 44).

16½ D. RAT EMBRYOS.

Eleven embryos were obtained from a rat's uterus 16½ days after fertilization. Their average C.R. length was 13.8 mm. Two were dissected and six of the rest were prepared, sectioned and stained in the usual manner.

Testis and its Mesonephric Ridge.

In spite of the gradual "unrolling" of the caudal curvature, instead of being carried cranially, the testis (T.) as in the previous stages, is found situated in the floor of the abdominal cavity. It has grown considerably in size and taken an oval shape (fig. 48). The tunica albuginea (Tu.A.) is well established and the cords (S.C.) are beginning to appear discrete. Mesenchymal cells have begun to invade ? the intervals between the sex cords, which are directed towards the hilus; the region where the mesorchium is attached.

The mesorchium (M.) of the testis is a thick sheet that anchors the testis to the cephalic (posterior) end of the mesonephric ridge and the floor of the cavity. Its medial surface is continuous with the peritoneum covering the kidney, while its lateral surface is continuous with the surface of the mesonephric ridge.

Blood/

Blood vessels (B.Vs.) coming from the medial side form a rich network between the hilus of the testis and the cephalic end of the mesonephric ridge.

The rapidly growing liver and visceral organs have pushed the testis downwards and laterally so that it overlaps the mesonephric ridge laterally. The adjacent part of the ridge naturally conforms to the curvature of both the testis and the floor of the cavity.

With the increasing growth of the bladder, the caudal ends of the Wolffian ducts are carried backwards and so the mesonephric ridge (M.R.) caudal to the anterior pole of the testis tends to get more and more horizontal so that by the next stage, as shown in figure 51, they run in a coronal plane.

Excepting at its cephalic end, where mesonephric tubules still persist, the ridge now appears like a thick cord and is attached to the floor by means of a thin sheet of mesentery. Where the testis is adjacent to it this mesentery is continuous with the mesorchium.

The course of the Wolffian and Müllerian ducts is much the same, but the lumen of the former is well developed while that/

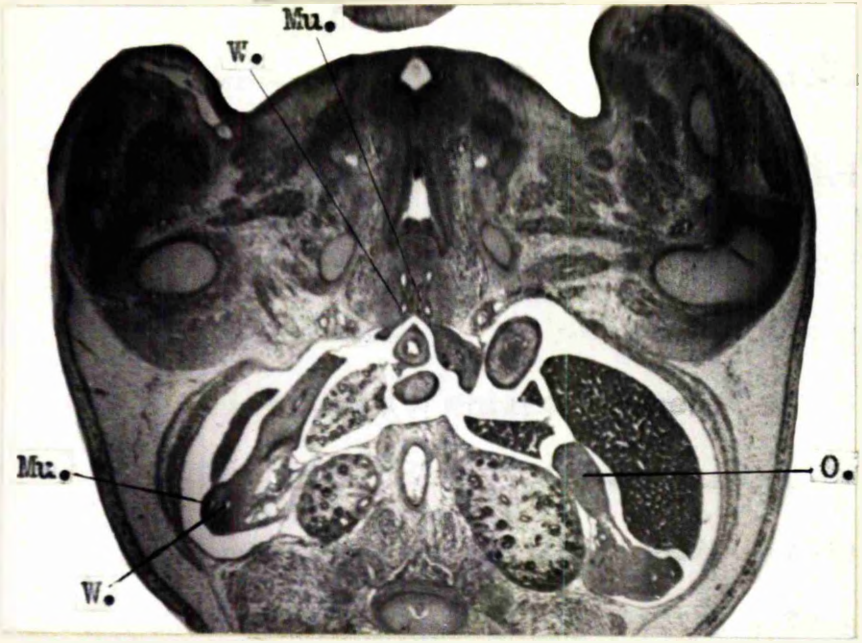

Fig. 49. FEMALE RAT EMBRYO. C.R.L. 13.8mm. T.S. of the caudal region passing through the left mesonephric ridge X 20.

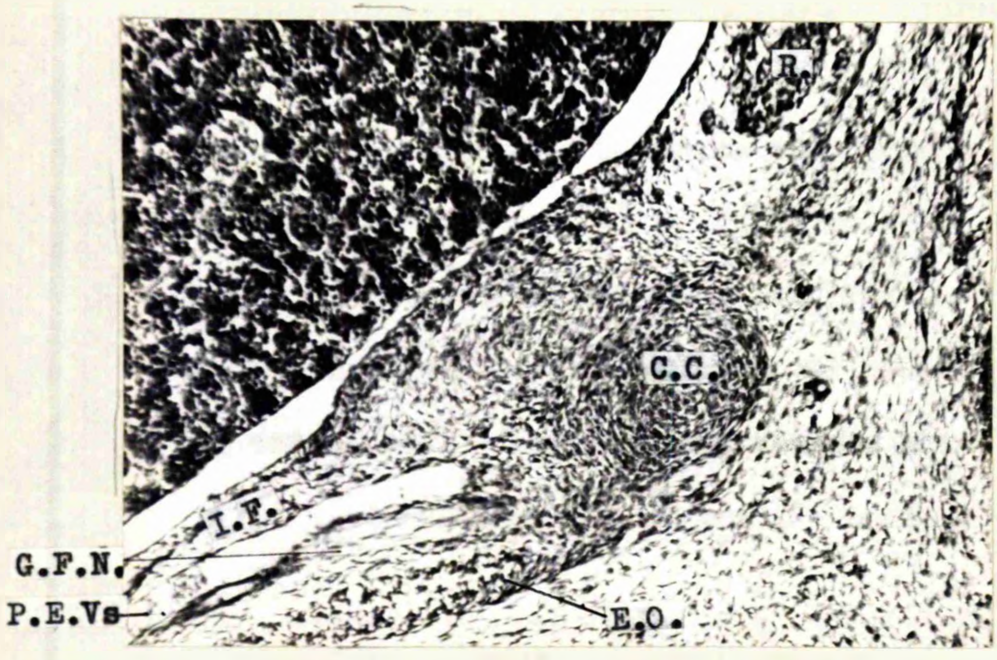

Fig. 50. RAT EMBRYO. C.R.L. 13.8mm. Sagittal section through the right inguinal cone showing concentric whorl of cells (c.c.) and its relations. X 120.

that of the Müllerian duct is hardly recognisable.

<u>The Ovary and its Mesonephric Ridge.</u>

The ovary itself does not present any noteworthy change beyond being more easily distinguishable. The mesonephric ridge however displays a remarkable contrast. It is directed forwards medially and slightly downwards in an almost straight line, towards the lower end of the bladder (fig. 49), giving an impression of the shape of things to come. In the adult rat, the uterus is bi-cornuate and long. The horns of the uterus are directed upwards, backwards and laterally from the pelvis.

The Müllerian duct has advanced in its development and its lumen, though narrow, is easily recognisable. In figure 49, parts of its course are recognised. In the cephalic part of the ridge it is seen as a lumen (Mu.) lateral to the wider one of the Wolffian duct. After the ducts have crossed the Wolffian ducts, they lie between them and run forwards towards the urethra. This latter portion of the course can also be seen in the same figure.

The ovary (O.) is on a level with the kidney. It is quarter the size of either the kidney or the testis and whereas the latter reaches way below the caudal pole of the kidney/

kidney, the ovary reaches only half way. This can be appreciated in figure 49, where the left ovary has already disappeared at a level a good distance above the caudal pole of the kidney.

The Inguinal fold and Cone.

The inguinal fold (I.F.) extends as before from the mesonephric ridge to the caudal aspect of the inguinal cone. It is now very thin and very little trace of the darkly staining cells which it originally contained can now be seen (figure 50).

The inguinal cone is still in the same position and still bears the same relationship to the musculature of the abdominal wall. The condensation of cells (C.C.) of which the cone consists is still present and still extends ventro-laterally as a solid process as far as the plane of the external oblique (E.O.). However, it can be observed that the cells at the apex of the cone are now less closely packed and lighter in colour so that the main condensation occupies the base of the cone and the process ventral to it. Thus from the fourteenth to the seventeenth day there has been a gradual migration of this condensation of cells from the inguinal fold, into the inguinal cone and beyond it into the process extending as far as the external oblique.

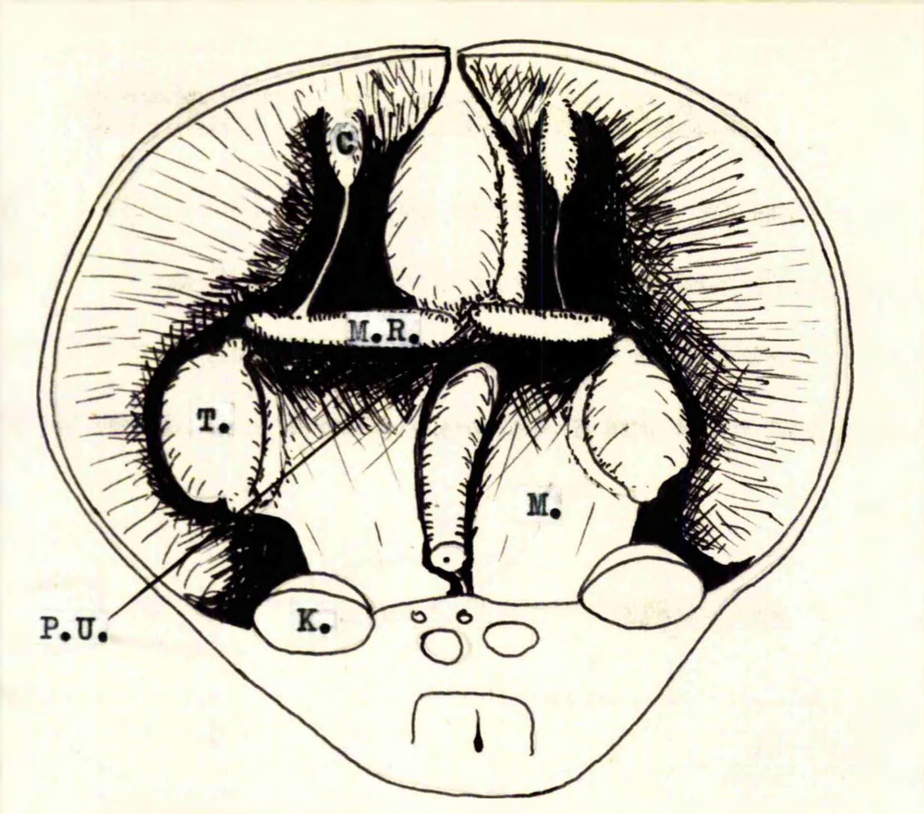

Fig. 51. 17.0 MM. MALE RAT EMBRYO. View of the cephalic aspect of the structures in the floor of the coelomic cavity.

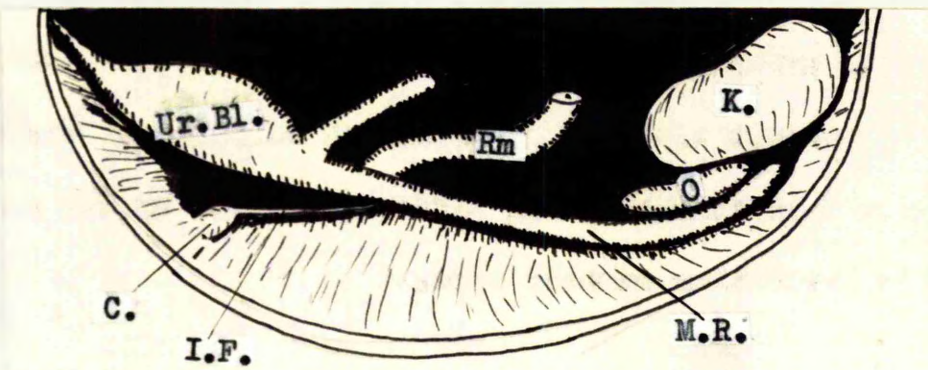

Fig. 52. 17.0 MM. FEMALE RAT EMBRYO. A lateral view of the left ovary, mesonephric ridge and inguinal cone, the abdominal wall on the left side having been removed.

17 - 20 MM. STAGE IN RAT EMBRYOS.

A series of embryos were obtained at 18 and 19 days after the fertilization varying in C.R. length from 17 - 20 mm. Some were sectioned serially and others dissected. Drawings of dissected male and female specimens are shown in figs. 51 and 52.

The Testis and Mesonephric Ridge.

Figure 51 is a view of the floor of the coelomic cavity in a male specimen, with the irrelevant viscera removed and figure 53 is a transverse section of this area cutting through the testes and inguinal cones.

The testes (T.) large and prominent, lie in the bottom of the cavity. They are situated below the level of the kidneys (K.) well lateral to the middle line. Their mesorchia (M.) have lengthened considerably and the testes overlap the cephalic parts of the mesonephric ridges so that the latter are in contact with the dorso-medial surfaces of the testes. This part of each ridge is hidden from view by the medial surface of the mesorchium on each side.

The ridge has moulded itself along the dorsal curvature

of/

of the testicular surface and its cephalic extremity is seen like a little knob, free of mesenteric attachment, sitting on the upper and medial end of the testis. At the caudal pole of the testis, the ridge, having curved upwards forwards and laterally from the dorso-medial surface of the testis, now makes a sharp bend medially. It then runs horizontally as far as the middle line meeting its opposite fellow behind the bladder. A shallow groove separates the bladder from the two united ridges behind. This horizontal ridge dorsal to the bladder has been termed the 'plica urogenitalis' (P.U.) in the human embryo.

Within the testis the solid sex cords (S.C.) have been pushed away from the centre of the gland towards its periphery. The cords are arranged in parallel rows (figure 48) beneath the tunica albuginea, following the contour of the gland towards the mediastinum testis dorso medially. Inter cordal mesodermal cells which were previously noted have now multiplied profusely and much of this tissue occupies the centre of the gland and is loosely arranged there. They are more closely knit nearer the periphery.

Scattered within the intercordal mesenchyme there are recognisable a different type of cell, usually found grouped in clumps. These cells are approximately the size of the cells/

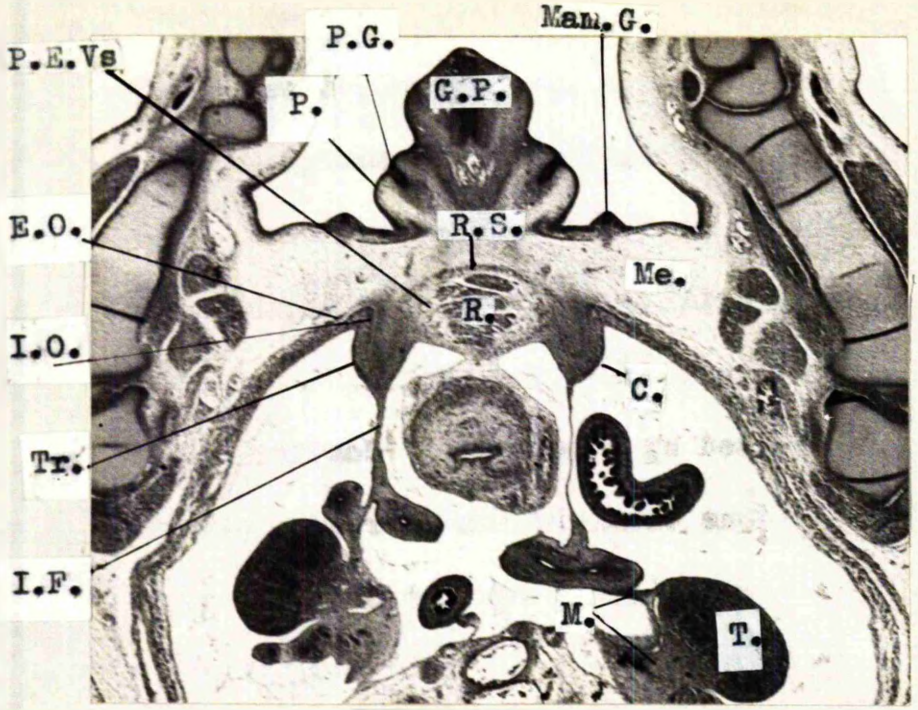

Fig. 53. RAT EMBRYO. C.R.L. 20.1 mm. T.S. of the caudal region, passing through both the inguinal cones. X 15.

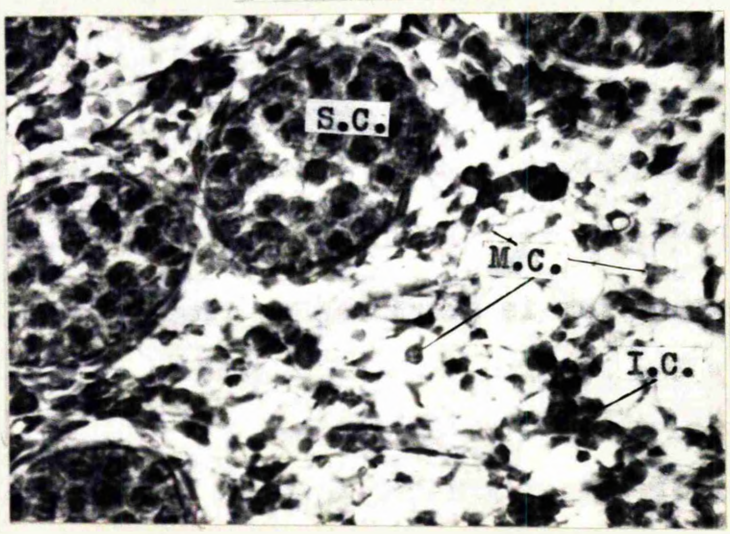

Fig . 54. RAT EMBRYO. C.R.L. 20.1 mm. T.S. showing internal structure of testis. X 300.

57

cells within the sex cords and have round and lighter staining nuclei. The rest of the mesenchymal cells are irregular in shape, small, have smaller deeply staining nuclei and show fibrillar connections. Figure 54 is a high powered view of a section of the testis. The cords (S.C.) are clearly delimited by a membrane. Scattered between the cords is loose tissue within which is recognised the larger cells in clumps (I.C.) and the ordinary mesenchymal cells (M.C.). The former are the interstial cells of Leydig.

The Ovary and its Mesonephric Ridge.

Figure 52 is a drawing of a female specimen with the left side of the abdominal wall cut away and the intestines removed.

The ovary (O.), a quarter the size of the testis, is situated on a level with the lower half of the kidney (K.) thus lagging behind the testis in the process of abdominal descent.. Its mesovarium is short and is attached to the medial aspect of the mesonephric ridge (M.R.).

The mesonephric ridge itself, in contrast to that seen in the male, is much shorter and runs almost in a straight line forwards and medially along the floor of the coelomic cavity, to unite with its opposite fellow behind the neck of/

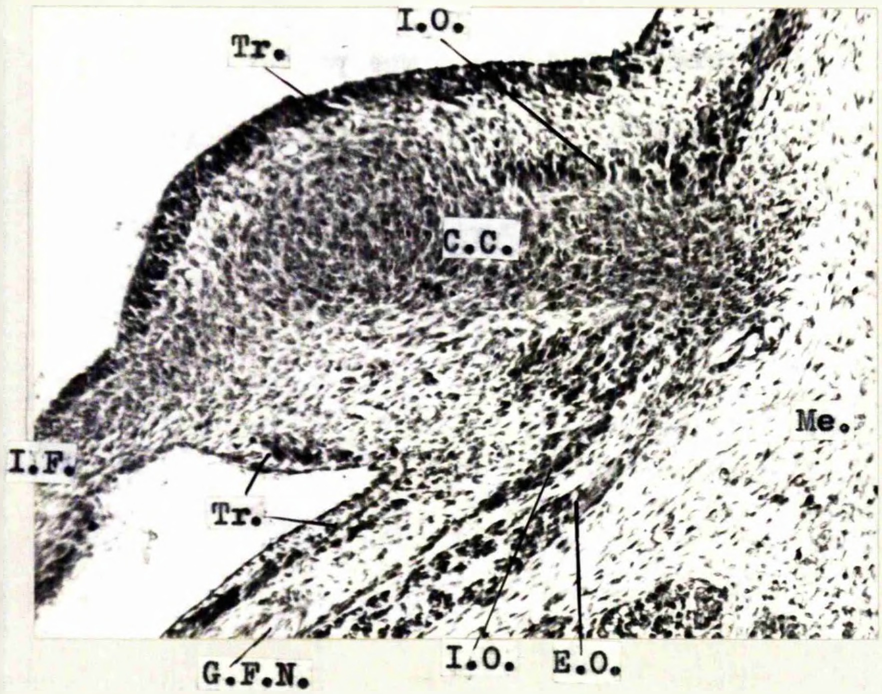

Fig. 55. RAT EMBRYO. C.R.L. 17.0 mm. Sagittal section through the left inguinal cone showing the new position of the ball of cells and the relations of the muscles to the cellular condensation.

58

of the urinary bladder.

The Inguinal Fold and Cone.

As before the inguinal fold (I.F.) extends from the genu of the mesonephric ridge (M.R.) to the caudal aspect of the inguinal cone. However, the posterior attachment extends below the ridge on to the surface of its elongated mesentery and is thus indirectly continuous with the mesorchium (M.). This may be seen in figure 53, on the left side.

The inguinal cone (C.) itself has grown in all dimensions and presents a typical conical appearance. Its base is directed forwards (figure 51) and the medial side of the base blends with the side of the rectus sheath (R.S.) (figure 53).

The deeply staining condensation of cells (C.C.) which was noted at the base of the cone in the previous stage (figure 50) now lies within the cone near its apex (figure 55). This condensation appears like a ball of cells (C.C.) which is recognised as early as in the 15½ day embryo (figure 43). From this ball a cylindrical column of cells extends forwards, to reach the base of the inguinal cone (figure 55). The caudal margin of the internal oblique (I.O.) which previously was related to the lateral surface of the cell condensation at/

at the base of the inguinal cone, now appears invaginated within the cone. Thus the caudal most fibres of the internal oblique (I.O.) are related to the lateral surface of the cylindrical column of cells that extends forwards from the ball of cells in the apex of the cone into the ventral abdominal wall (figures 53 and 55).

The transversus layer (Tr.) is similarly carried inwards by the growth of the cone and extends not only on the lateral surface of the cone as its apex, but also on the cranial and caudal surfaces (figures 53 and 55).

The deficiency noted in the external oblique layer (E.O.) is more marked and the margins are well defined (figures 53 and 55).

External to the opening in the external oblique, the mesenchyme (Me.) is markedly loose in texture and continuous with the cells that stream out from within the cone.

In the female, the cone and its fold are conspicuously smaller though well developed (figure 52).

Rectus Abdominis and its sheath.

The interdigitating slips of the caudal portion of the rectus/

rectus abdominis (R.) are now clearly recognisable as shown in figure 53. Note that the dorso-ventral depth of the muscle in this region is roughly equal to its transverse diameter.

As mentioned before, the mesoderm lateral to the rectus is continuous with the medial side of the base of the inguinal cone. Within this mesoderm, in figure 53, are seen branches of the pudic-epigastric vessels (P.E.Vs) which have coursed upwards and medially from the caudal surface of the inguinal cone.

Ventral to the rectus, the rectus sheath condensation (R.S.) is plainly visible, but laterally it is vague. Later, however, it will develop between the inguinal cone and the muscle and will include the blood vessels within the sheath.

The Genital Tubercle.

The swellings (S.) present on the dorsal aspect of the genital tubercle in the 10.6 mm. stage (figure 22), now appear as two prominent swellings (P.) on each side of the tubercle (figure 53). They are connected with each other across the dorsal surface of the phallus and also extend on to the ventral surface without actually meeting. This continuous swelling is the developing prepuce and the part of/

of the tubercle distal to it, seen in figure 53, will develop into the glans penis (G.P.). From the left and right surfaces of the preputial swelling, there grow inwards a condensation of epithelial cells (P.G.) which eventually give rise to the preputial glands of the rat.

Mammary glands (Mam. G.).

These have begun appearing along the milk line as early as the $15\frac{1}{2}$ day stage. One pair, however, constantly appears to lie at the same horizontal level as the base of the inguinal cone. These are seen in figure 53 as conical swellings on the surface of the skin between the preputial swellings (P.) and the lower limbs.

Umbilical arteries.

The left artery has almost completely degenerated while the right is very well developed. In figure 53 the right artery (U.A.) is seen lying against the right side of the urinary bladder.

20 - 40 MM STAGE IN THE RAT EMBRYO.

Between the 20th day of intra-uterine life and full term at the 22nd day the C.R. length of the rat embryo increases from 20 mm to 40 mm. The specimens obtained in this age group are shown in the table below.

No. of Embryos.	Age	C.R. length	No. dissected.	No. sectioned and stained c H. & E.
7	Unknown	27.6 mm.	-	5
12	"	30.3 mm.	4	6
7	"	34.8 mm.	-	6
13	"	36.0 mm.	4	4
7	"	39. mm.	-	4

Drawings of two views of a dissected specimen were made and are shown in figures 56 and 57.

Two wax plate reconstructions of the relevant caudal region were also made of specimens of C.R. length 27.6 mm. and 34.8 mm.

External appearance of an almost full term embryo.

The spinal curvature, except at the caudal end, has practically disappeared. The head is held so that its snout is at right angles to the C.R. axis.

More/

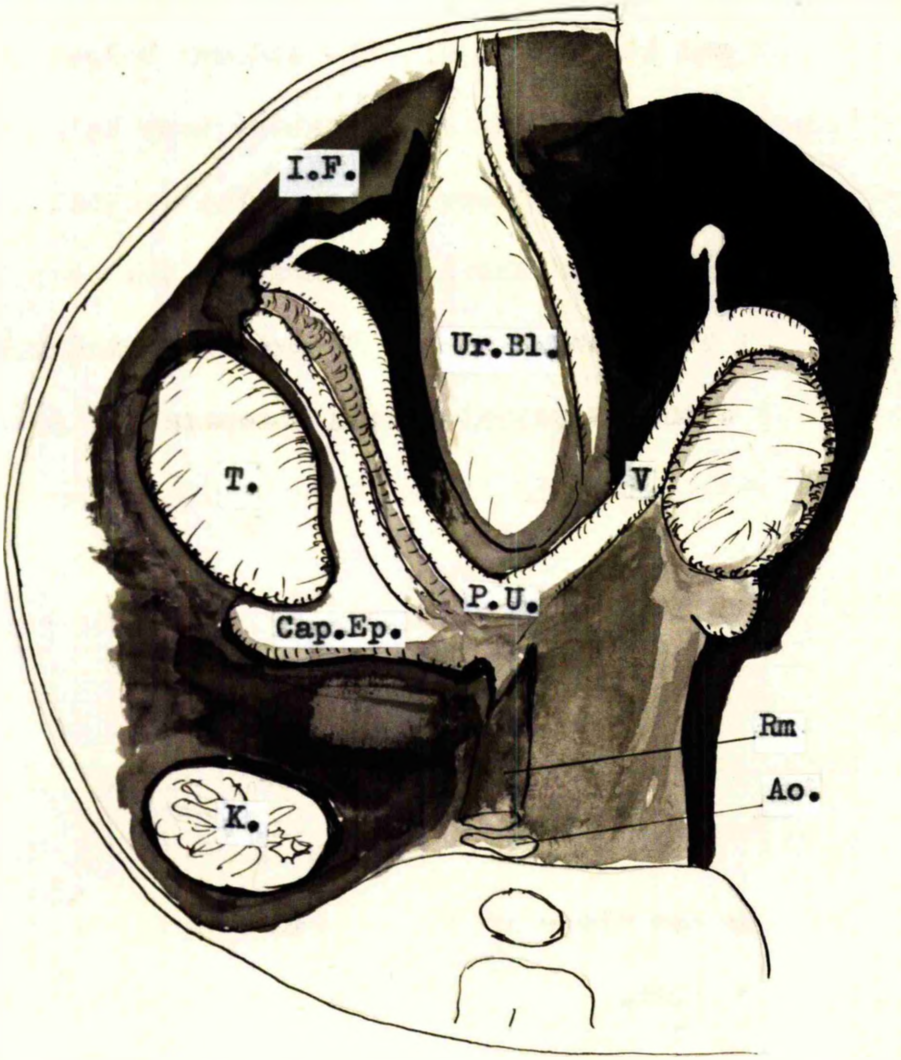

Fig. 56. 30.3 MM. MALE RAT EMBRYO. View of the cephalic aspect of the urogenital organs and associated structures in the floor of the coelomic cavity. The right half of the abdominal wall has been cut away.

63

More than half the bulk of the embryo now consists of the abdomen and its contents. The abdomen bulges in all directions, more so at the sides, where they bulge beyond the lateral surface of the lower limbs. The ventral surface presents a well formed umbilical cord and the abdominal wall caudal to it is somewhat flat. Between the hind limbs the glans penis with its prepuce still unseparated, forms a prominent swelling which is directed horizontally forward.

The Gross Appearance of the Relevant Structures in the Floor of the Coelomic Cavity.

After sectioning a 30.3 mm. specimen transversely and removing the intestines the impression of the latter on the structures in the floor of the coelomic cavity was clearly visible for a time.

Figure 56 is a view from above. The left half of the abdominal wall is intact but the right half of the abdominal wall has been cut away to enable a clear view of the structures from the side as shown in figure 57.

The testis (T.), and mesonephric ridge, occupy the middle of the floor a little distance away from the middle line. In the middle line, from before backwards are the urinary bladder (Ur.Bl.) plica urogenitalis (P.U.), rectum (Rm.) and aorta(Ao.).

The/

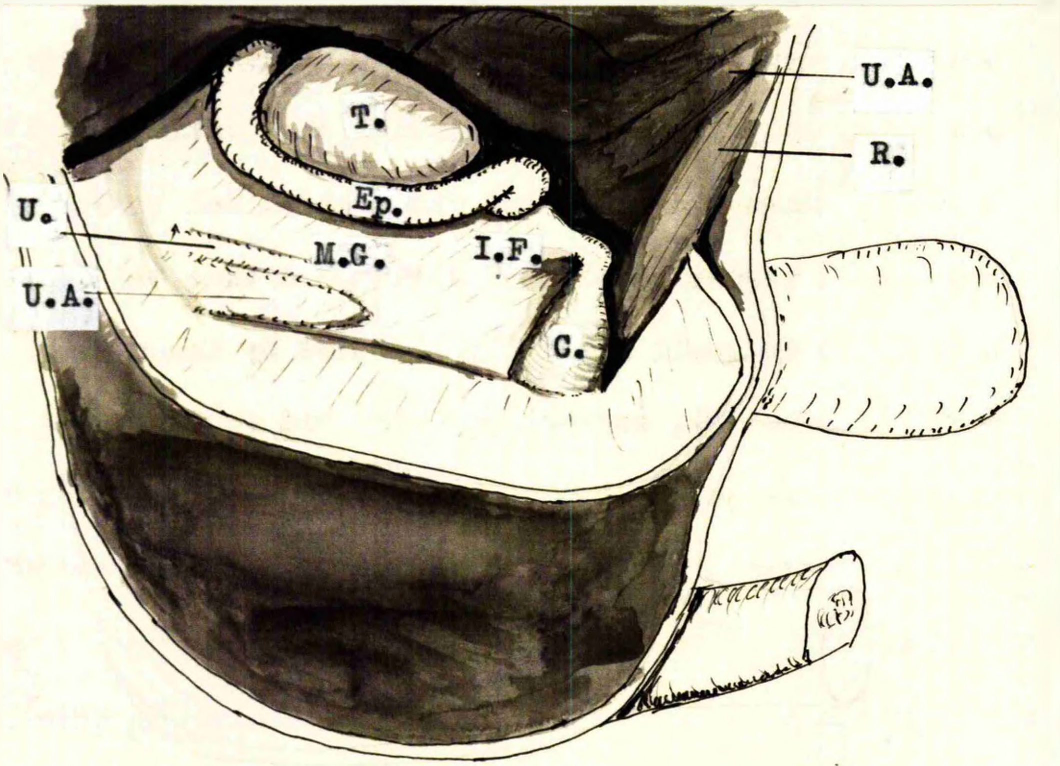

Fig. 57. Same specimen as Fig. 56, viewed from the right side. The testis (T.) has been pulled upwards to display the epididymis (Ep.) and stretch the mesogenitale (M.G.). Note the vertical inguinal cone (C.) and the shortened free margin of the inguinal fold (I.F.)

6A

The cephalic portion of the mesogenitale has progressively disappeared while the caudal portion has simultaneously lengthened, allowing the testis to slide forwards through gravity and intra-abdominal pressure to the lowest region possible. Although a true pelvic cavity exists, it is small and fully occupied by the neck of the bladder, prostate, seminal vesicles and rectum. The testis is far too large to possibly slip into the potential slit that is present on the ventral and lateral surfaces of the rectum. There is no fold raised by the umbilical artery that might be a cause for preventing the testis from entering the pelvic cavity. In fact, the left umbilical artery has started degenerating at an early stage and has by this stage almost completely disappeared.

In figure 57 the testis (T.) has been pulled upwards and medially to display the mesogenitale (M.G.) and the inguinal fold (I.F.). The testis is seen situated midway between the anterior and posterior walls of the coelomic cavity. Closely hugging its dorso-medial aspect is that part of the mesonephric ridge which develops into the epididymis (Ep.). At the caudal end of the testis the ridge makes a hairpin bend backwards and medially and continues between the testis and bladder to reach the plica urogenitalis (P.U.) (figure 56).

Due/

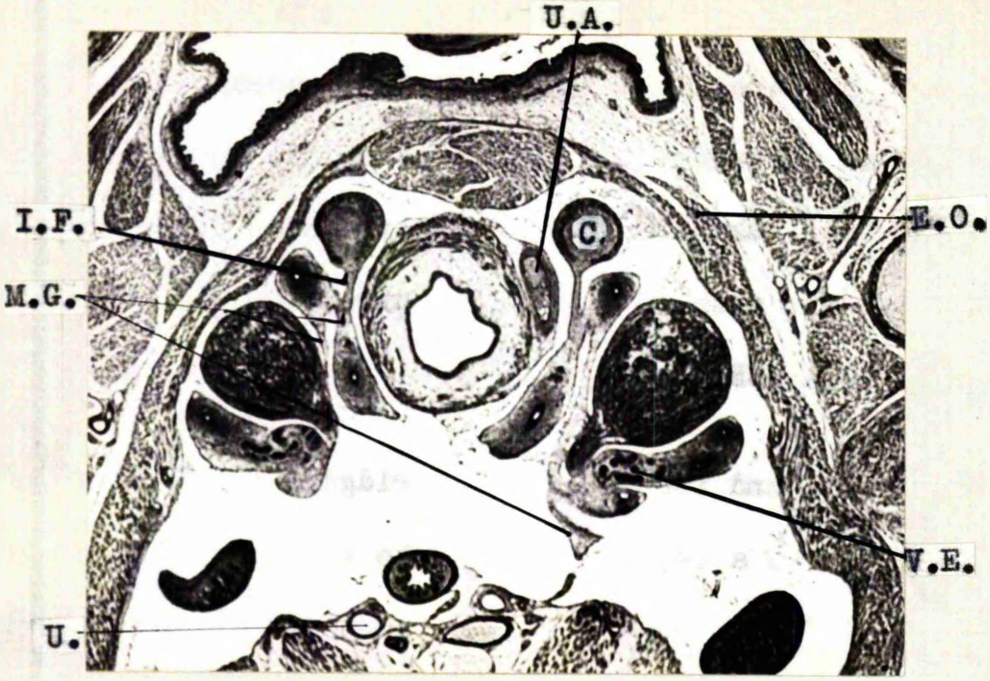

Fig. 58. RAT EMBRYO. C.R.L.27.6mm. T.S. near the floor of the coelomic cavity showing the situation of the testes and the inguinal cones. (c). X 15.

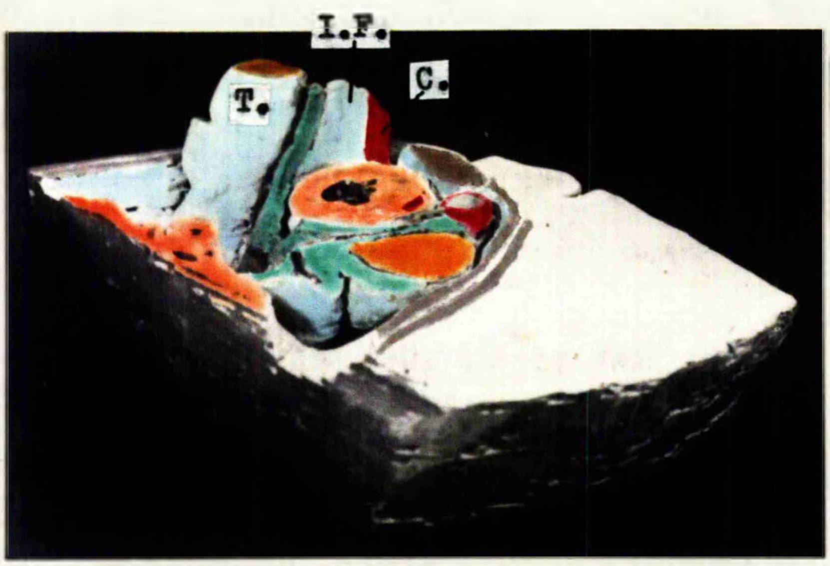

Fig. 58.A. RAT EMBRYO. C.R.L.27.6 mm. A wax plate reconstruction of the caudal part of the embryo showing the coelomic cavity and its contents. Viewed from above, behind and right side. X 10.

Due to differential growth and the gradual 'unrolling' of the caudal curvature the long axis of the inguinal cone (C.) has rotated through 90° so that its base is directed caudally and its apex in a cephalic direction (See also figures 58A and 58B).

The testis and the mesonephric ridge are now very close to the cone. As a result it would be expected that the long inguinal fold (I.F.) present in the previous stage (figures 51 and 53) should be thrown into loose folds, particularly at the free margin. This, however, is not so. The fold appears to have been taken in at the free margin and is shorter and thicker in that region. Through this region the apex of the cone is attached to the hairpin bend of the mesonephric ridge. Below the level of the ridge, the inguinal fold (I.F.) is continuous with the mesogenitale (M.G.) (figures 57 and 58).

Medial to the root of the mesogenitale runs the right umbilical artery (U.A.) with the right ureter (U.) just above it. The artery curves upwards and medially (figure 56 and 57) on the surface of the urinary bladder.

<u>The testis.</u>

The sex cords present much the same appearance. No lumen/

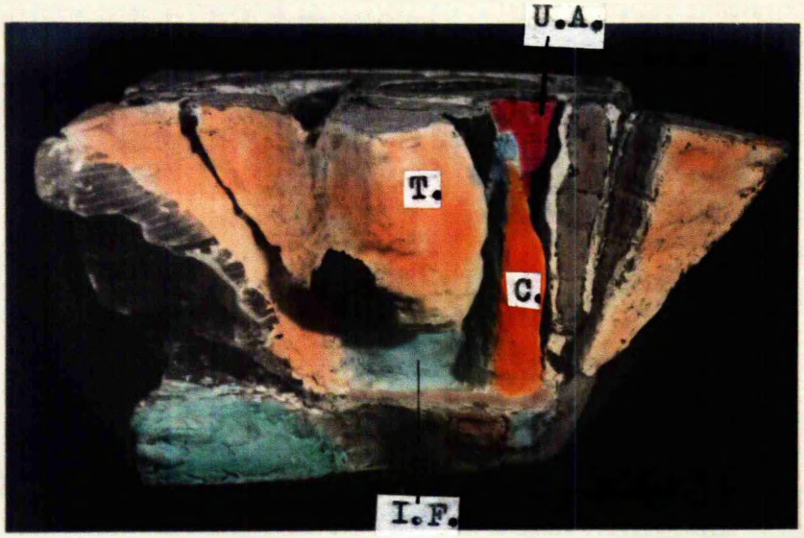

Fig. 58. B. RAT EMBRYO. C.R.L.34.8 mm. A wax plate reconstruction similar to the one above, with the abdominal wall removed from the lateral side, to show the vertical cone and its relations. Viewed from the right side. X 15

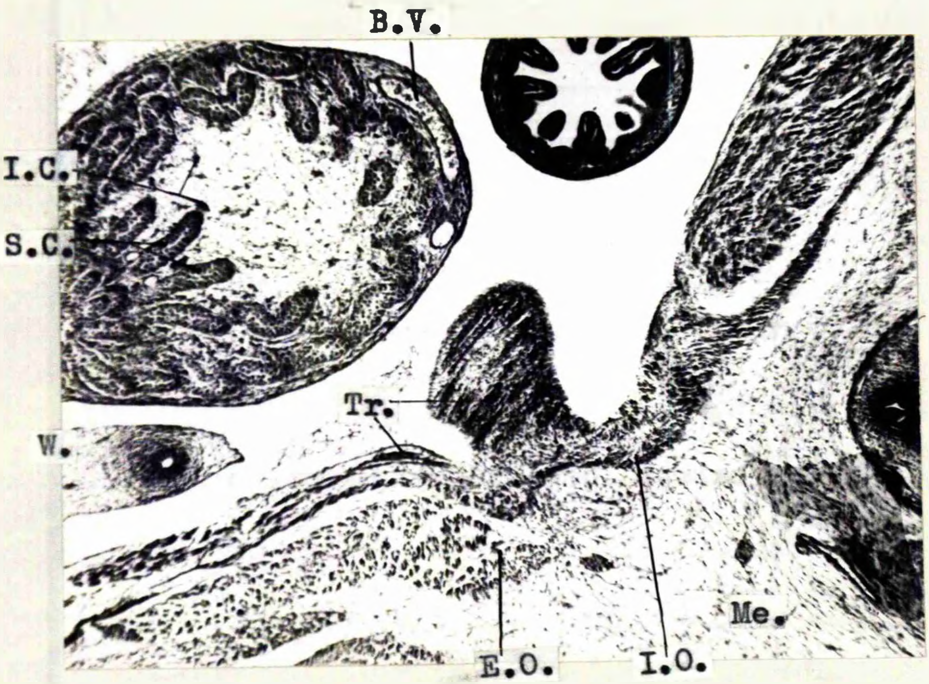

Fig. 59. RAT EMBRYO. C.R.L.30.3 mm. Sagittal section passing through the lateral surface of the right inguinal cone, showing depression of abdominal wall towards the external ring. X 45.

No lumen is present within the cords. The loose mesenchyme (Me.) in the centre of the testis, however, has increased remarkably, and so also have the interstitial cells of Leydig (I.C.) (figure 59).

Blood vessels (B.V.) arising from a rich plexus in the mesorchium near the hilus of the testis run between the tunica albuginea and the cords. They ramify beneath the tunica and send branches into the substance of the gland.

A tortuous testicular artery is seen developing at this stage. It can be traced from the aorta, running downwards and laterally within the mesogenitale to reach the plexus in the meorchium.

The Mesonephric ridge.

In the male, at this stage, it can be clearly demarcated into the part that will develop into the epididymis (Ep.) and the part that will become the vas deferens (V.). (figures 56 and 57). The hairpin bend of the mesonephric ridge marks the junction of the empididymis with the vas deferens, the cephalic portion developing into the epididymis.

Remains of the mesonephric tubules which were present in the cephalic part of the ridge have modified themselves into ducts/

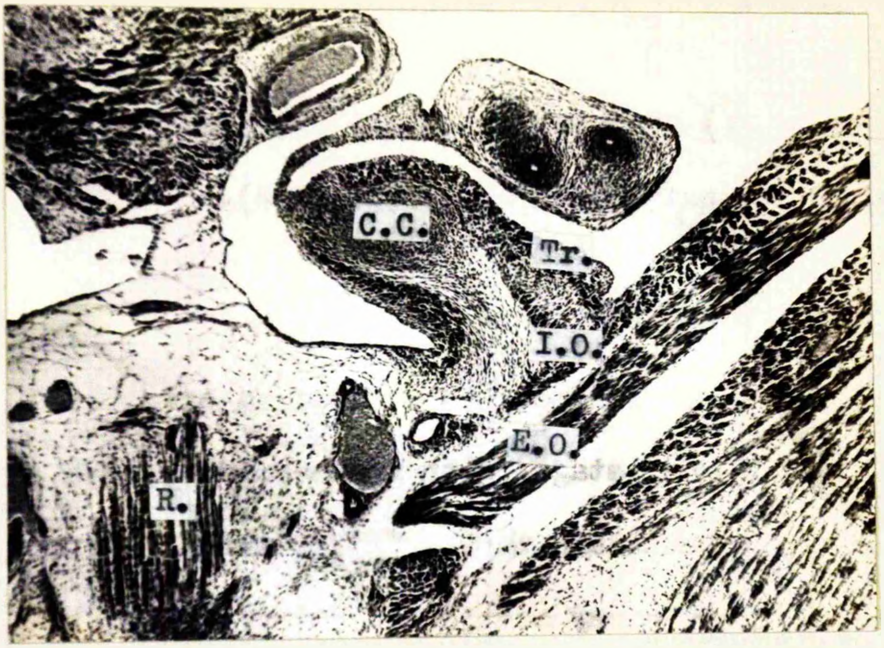

Fig. 60. RAT EMBRYO. C.R.L. 39.0 mm. Coronal section through the right cone. X 45.

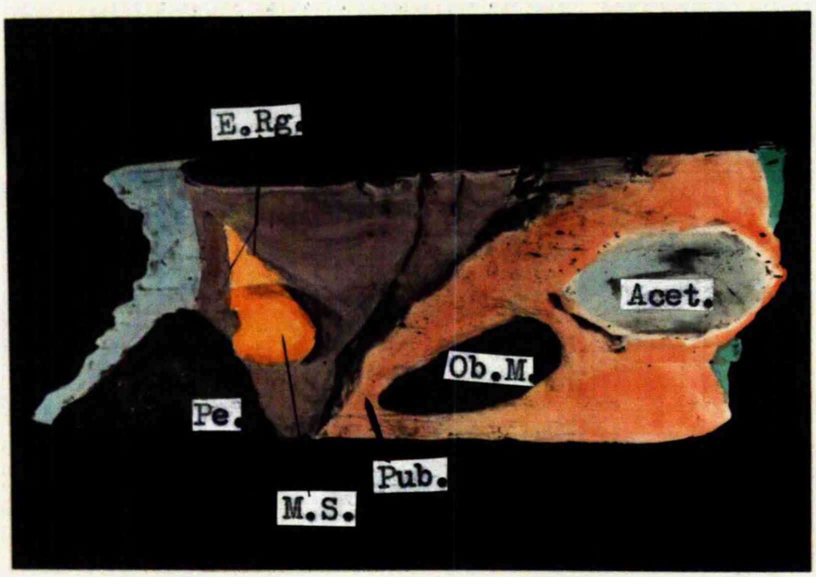

Fig. 61. NEWBORN RAT. 48 Hours. C.R.L. 44.2 mm. Lateral view of a wax plate reconstruction showing the inguinal region without the skin and subcutaneous tissue. The processus vaginalis invested by the muscular sac is seen protruding through the external ring. X 10.

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ducts (V.E.) that will eventually connect the rete testis with the caput epididymis (figure 58).

The Inguinal Cone.

Although the cone has assumed an erect vertical position by the 27.6 mm. stage, with further development it does not necessarily maintain this erect position. By the time of birth, the cone may assume odd shapes due to the pressure of viscera and also due partly to the early phases of degeneration. A coronal section through the cone in such a stage is shown in figure 60. The cone here is tilted medially. The feature to which attention is drawn is the persisting presence of the ball of deeply staining cells (C.C.) near the apex of the cone. Through the apex of the cone, the cells of the inguinal fold are continuous with those within the cone. No muscle layer intervenes.

The cylinder of cells leading externally from the ball of deeply stained cells, which themselves were deeply stained in the previous stage (figure 55) have either disappeared or have lost their staining character. In their place, cells of an almost mesenchymous nature are present and these insiduously merge with the subcutaneous mesenchyme (figure 60).

The/

The caudal fibres of the internal oblique (I.O.) which were related to the lateral surface of the cylinder of cells, not only maintains that relationship but also extends on to the ventral and dorsal surfaces till finally it almost completely invests the cylinder in the caudal part of the cone, leaving only the dorso-medial aspect free (figures 60 and 58).

Nearer the surface of the cone, beneath the peritoneal layer, the transversus (Tr.) also gradually extends over the dorsal and ventral surfaces till it finally covers the whole cone except at the apex and dorso-medially along the attachment of the inguinal fold (figures 58, 59 and 60).

The abdominal wall adjacent to the ventro-lateral aspect of the cone begins to show signs of herniating through the external abdominal ring. A suggestion of this is seen in the early depression that is present in a sagittal section shown in figure 59. In this figure the cone of the right side is cut tangentially on its right surface and fibres of the transversus are seen. Surrounding the base of the cone is a depression of the internal oblique and transversus layers lying opposite the external ring. The external oblique (E.O.) with its free margin is seen below the cone. The depression is the beginning of a hermiation that will carry the internal oblique and transversus through the external ring.

Twenty six newly born rats of ages varying between 5 hours and 28 days were prepared and serially sectioned. The details are tabulated below.

Age of specimen	C.R. length in mm.	No. sectioned and stained c̄ H & E.
5 hrs.	38.8	2
9 hrs.	42.0	3
13 hrs.	39.9	2
18 hrs.	44.0	2
18 hrs.	42.5	2
25 hrs.	40.9	2
31 hrs.	43.4	2
37 hrs.	38.6	2
48 hrs.	44.2	2
61 hrs.	48.15	2
85 hrs.	47.8	1
?	55.4	1
12 days	61.0	1
13 days	67.0	1
28 days	?	1

Two wax plate reconstructions were made of a 48 hour old specimen to study the exact shape of the early processus vaginalis/

vaginalis and its muscular covering and their relation to the surrounding structures.

At birth the processus vaginalis is very small and is not much deeper than the depression shown in figure 59. By the second day, however, there appears a clear indication of how it develops and how the testis begins to descend into its cavity.

Figure 61 is a lateral view of a wax plate reconstruction showing the inguinal region. To the right, part of the pelvis including the obturator membrane (Ob.M.) and acetabulum (acet.) is seen. Ventral or anterior to the pubis (Pub.), the processus vaginalis with its muscular covering (M.S.) is seen bulging through the external ring (E.Rg.). This herniating mass lies embedded within the wide layer of loose subcutaneous mesenchyme, and therefore cannot be located on the surface of the young rat of this age.

A reconstruction of larger dimensions of the same sac (M.S.), seen in figure 61, was made and figure 62 is an anterior view of this model with the anterior wall cut away to show the interior.

The cone (C.) is seen occupying the floor and dorso-medial wall/

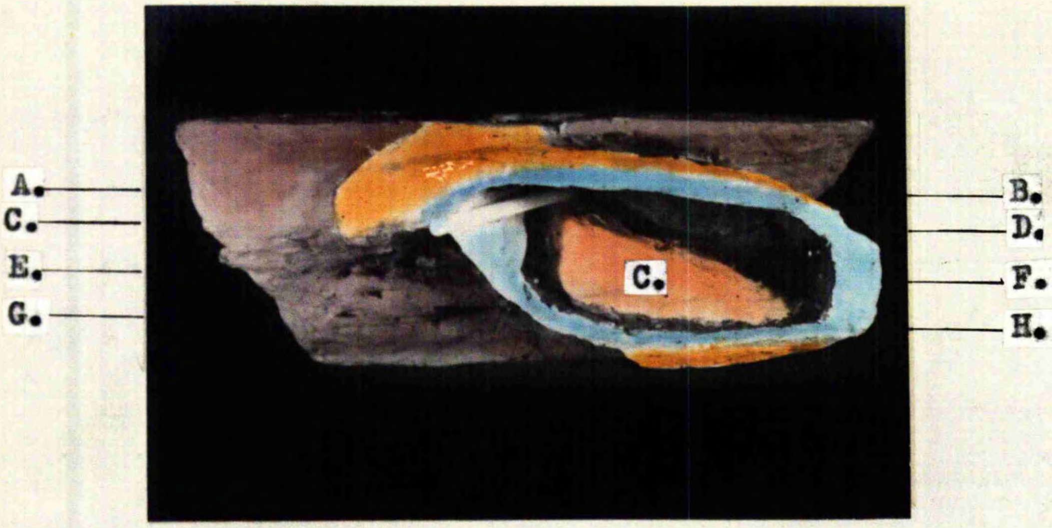

Fig. 62. Same specimen as shown in fig. 61. An enlarged wax plate reconstrustion of the herniating sac with its ventral wall cut away to show the inguinal cone. Viewed from the front. X 35.

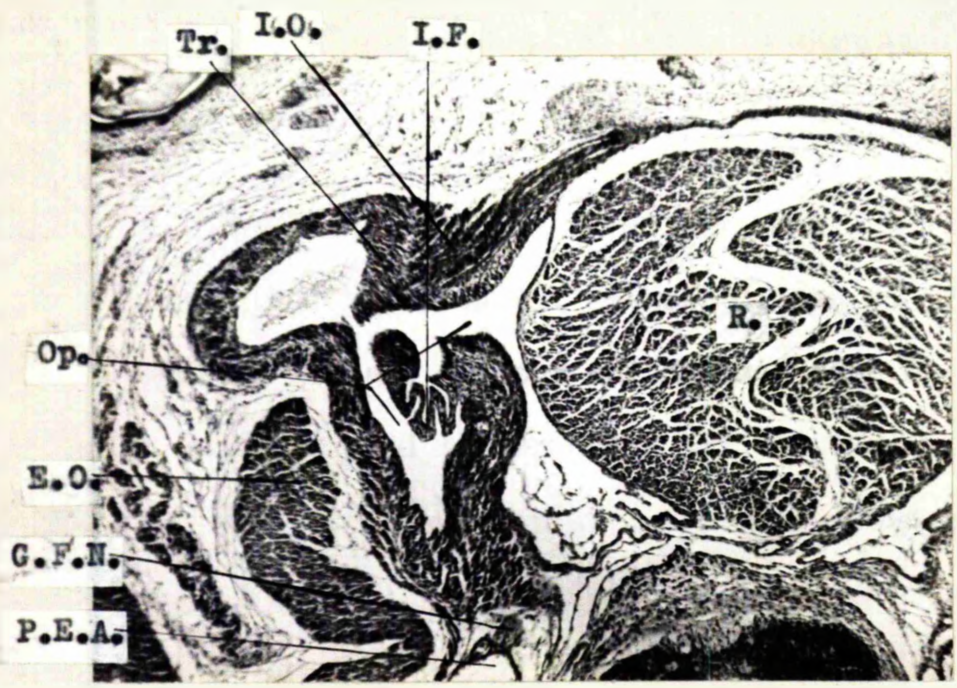

Fig. 63. T.S. of sac shown above at the level A-B. showing opening into the sac from the coelomic cavity.
X 45.

wall of the sac. The shape of the cone has altered considerably. Its ventro lateral surface has lengthened to about three times the dorso-medial surface with the result that the base has stretched enormously and is directed downwards, backwards and medially. The apex, however, still retains its upward, backward and medial direction towards the opening of the sac.

The widest part of the cavity within the sac lies ventro-laterally to the cone and so also does the widest part of the sac wall. The sac communicates with the abdominal cavity through a fairly wide neck. In this specimen the apex of the cone almost fills the neck and the pointer indicates the situation of the opening which is directed downwards, forwards and laterally from the coelomic cavity into the sac.

Figure 63 is a transverse section at level A-B of figure 62. It passes through the level of the opening (Op.) medially and just beneath the roof of the sac laterally. The opening into the sac being placed in a slightly oblique plane, the section passes through the medial wall (M.W.) just above the bottom of its curve and through the lateral wall (L.W.) just below the peak of its arch. The ventro-lateral wall/

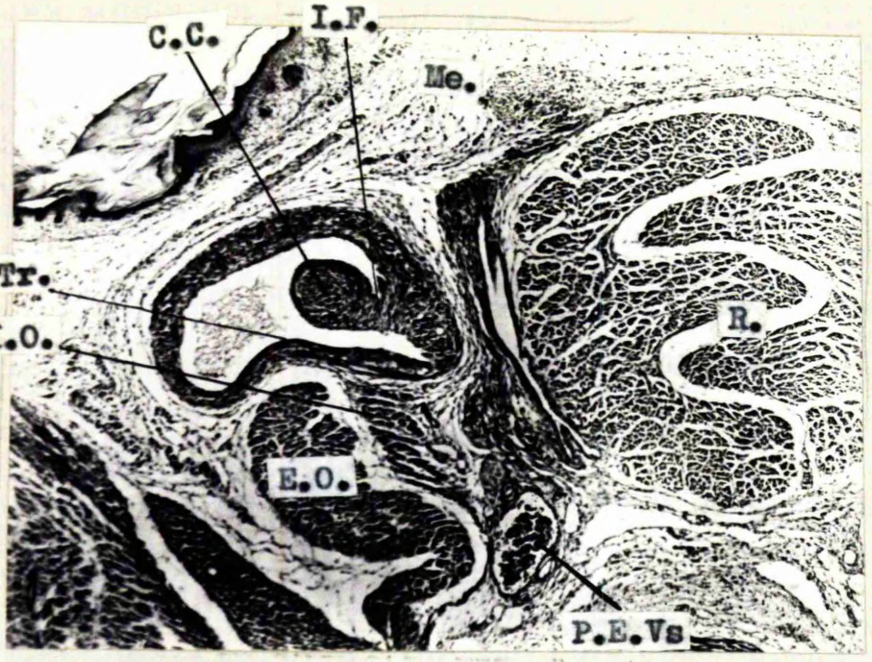

Fig. 64. T.S. of same sac (fig.62.) at the level C-D passing through the apical region of the cone. X 45.

Fig. 65. T.S. of same sac (fig.62.) at the level E-F passing through the base and ventro-lateral surface of the cone. X 45.

wall of the sac is seen protruding beyond the margin of the external ring, and the margin of the infero-lateral crus of the external oblique (E.O.) is clearly seen just behind the protrusion. There is no layer extending from the margin of the external oblique across the surface of the muscular sac. Therefore the external spermatic fascia cannot be said to be derived from the external oblique muscle.

The inguinal fold (I.F.) is seen attached to the medial wall of the neck of the sac and is thrown into folds. The free margin is fairly wide. This free margin has shortened and widened relatively and its attachment to the cone is difficult to demarcate because of this.

Figure 64 is a transverse section at the level C - D in figure 62. It passes through the apical region of the cone and the concentric cluster of deeply stained cells (C.C.) is still noticeable within it. The inguinal fold (I.F.) attaching the cone to the medial wall of the sac is much shorter than that seen in figure 63, and it lies opposite the non-muscular raphe of the sac. As the inguinal fold approaches the base of the cone, its distance between the cone and dorso-medial wall decreases while its thickness increases till it merges with the junction between the dorso-medial/

dorso-medial wall and the base of the cone.

Loose mesenchyme is seen separating the medial wall of the sac from the rectus sheath. Except for a small narrow raphe on the medial wall, the sac is completely invested by two layers of muscle which are continuous with the internal oblique (I.O.) and Transversus (Tr.). In fact in the dorsal wall of the sac in this figure, the internal oblique (I.O.) is shown to be continuous with the outer layer.

The large pudic-epigastric artery (P.E.A.) with its accompanying veins and the genito-femoral nerve (G.F.N.) can be seen lying a little distance away dorso-medially to the sac, deep to the internal oblique.

Figure 65 is a transverse section at the level E - F in figure 62, and it passes through the upper and medial part of the cone's base. The internal oblique and transversus layers of the sac wall are seen reflected on to the surface of the cone beneath its peritoneum. The cells within the cone are seen emerging through the open base to blend with the loose mesenchyme external to the sac.

Medial to the sac, next to the rectus abdominis (R), lie/

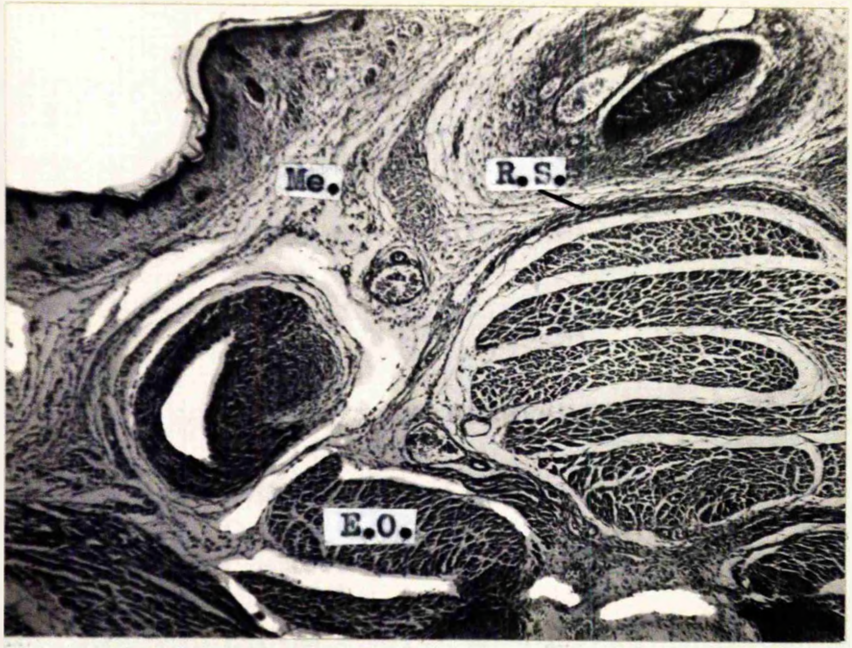

Fig. 66. T.S. of same sac. (fig. 62.) at the level G-H passing through the caudal extremity of the sac. X 45.

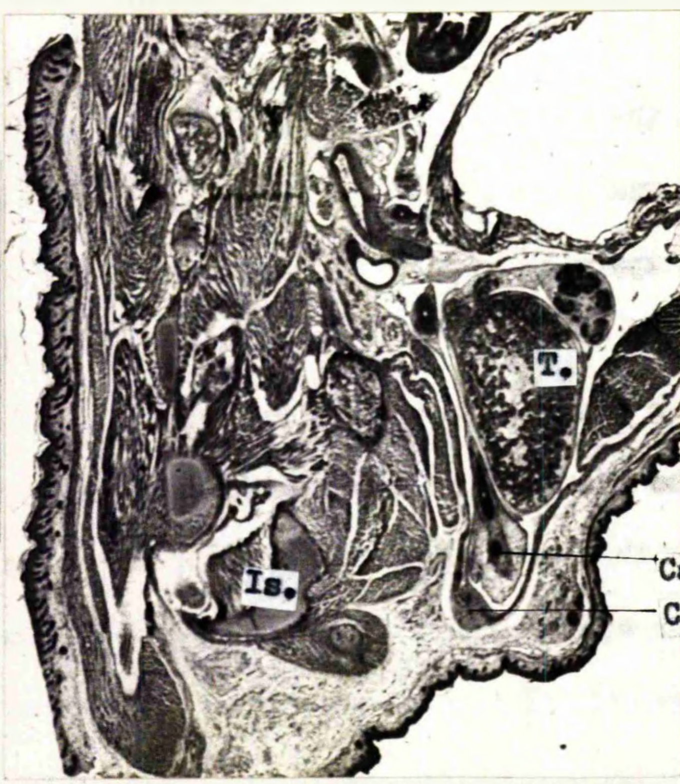

Fig. 67. NEWBORN RAT. 48 Hours. C.R.L.59.0 mm. Sagittal section through the sac on the right side showing cauda epididymis within it and the shape of the testis which is about to enter the sac. X 10.

lie branches of the pudic epigastric vessels, while dorsal to the sac, separating it from the margin of the external oblique (E.O.) is the femoral branch of the genito-femoral nerve (F.B. G.F.N.).

Figure 66 is a transverse section at the level G - H of figure 62 and it passes through the lower end of the sac. The medial wall, formed mainly by the lateral surface of the cone, is about to meet the other walls at a slightly lower level. Completely surrounded by loose mesenchyme, the sac is superficial to the external ring, the margin of the external oblique (E.O.) being seen dorso medially.

From the study of the reconstructions and serial sections it appears that the whole thickness of the abdominal wall deep to the external ring, herniates through the ring in a manner like an expanding rubber balloon with the exception that the expansion does not take place evenly, the dorso-medial wall being restricted by the presence of the cone and its attachment to the side of the rectus abdominis. Hence it is found that the ventro-lateral wall is far more extensive and the space within the sac is greatest on the ventro-lateral aspect of the cone. Clearly the sac is not a pure evagination of the cone, i.e. like the eversion of a/

a finger stall, nor is the cone instrumental in dragging out the abdominal wall through the ring. In fact, it rather appears that the ventro-lateral margin of the base of the cone was subject to tension with resultant stretching, caused by the rapidly bulging ventro-lateral wall of the sac.

The entrance of the testis into the sac begins round about this age. Although this specimen which was reconstructed did not show signs of it, the other specimen from the same litter did. Figure 67 is a sagittal section through the right sac of the latter specimen. The bottom of the sac has reached the level of the ischium (Is.). The neck is wide and the caudal pole of the testis (T.) is seen sitting at the mouth of this opening. Already within the sac is the cauda epididymis (Cau. Ep.) embedded in a mass of mesenchyme close to the degenerating cone (C.). The testis (T.) presents a shape which suggests its mode of entry into the sac. It has taken on an egg shape, the narrower end being no wider than the bulky cauda epididymis. The centre of the testis is filled with a large quantity of loose mesenchyme which can, and probably does, act as a fluid buffer allowing the testis to squeeze past the neck. It is common knowledge that a real egg with its shell softened in vinegar can be easily pushed/

Fig. 68. 15 Days Old Rat with skin reflected to show the newly formed processus vaginalis on each side of the penis. X 4.

Fig. 69. 28 Days Old Rat with the skin reflected to show the thin-walled sacs fully developed and having the testes within them. X 3.

pushed into a narrow necked glass bottle without bursting the egg. Why could not then the testis do the same? The neck in this instance, is yielding and elastic and so is the testis with its tough tunica and internal 'fluid buffer'.

By the second week the testis occupies the elongated wide neck and the upper part of the sac, while the cauda epididymis fills its lower part. The skin was reflected off the lower abdominal wall of a 13 day rat and figure 68 is a view of that region. The glans penis (G.P.) has been reflected cranial wards to enable a clearer view of the two sacs. These are well defined and thin walled. The coils of the epididymis (Cau. Ep.) can be made out through the ventral wall of the sac. The neck (N.S.) is funnel shaped because of the testis within it. The tip of the sac is curved but because it faces backwards and medially is not visible.

About the 4th week the sac has attained its adult shape and structure on a miniature scale. Figure 69 is a ventral view of the sacs of a 28 day rat. The testis (T.) and all other contents found in the normal mature muscular sac/

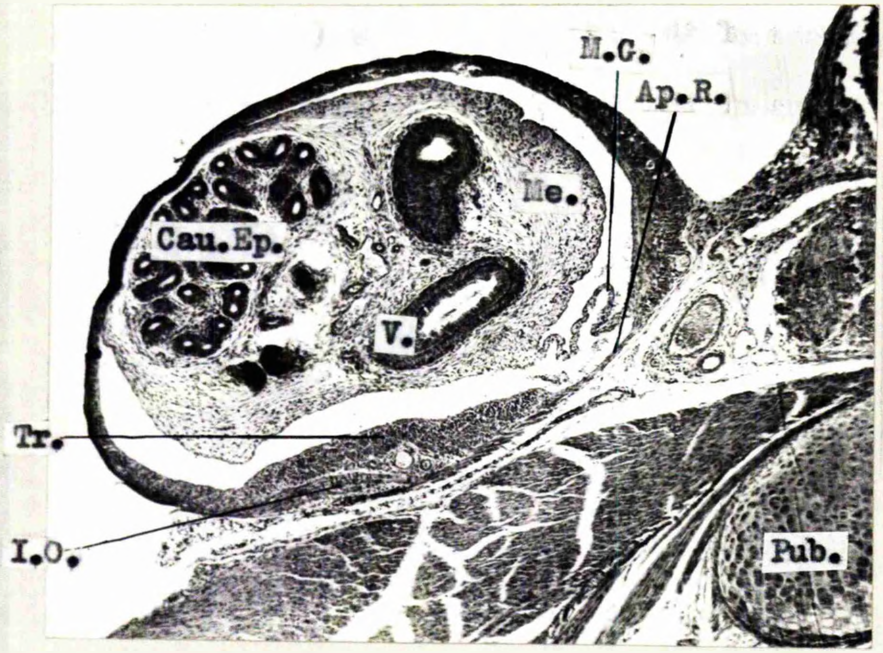

Fig. 70. T.S. of the left sac of the 15 days old rat shown in fig. 68. X 45.

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sac of the adult rat are now present within each of these muscular sacs. The curved tip of the right sac can be seen in this figure. The neck of the sac (N.S.) though widely patent, is narrower than the body of the sac.

A transverse section through approximately the middle of the left sac of the 13 day specimen (figure 70) shows the disposition of the two muscle layers and the direction of their fibres. The two layers are no longer separated by a distinct layer of mesenchyme. They are in contact, and as mentioned, almost inseparable in the adult. On the dorso-medial aspect the non-muscular aponeurotic raphe (A.R.) is distinct. From its dorsal margin the fibres of the outer layer of muscle (I.O.) are seen running transversely. Traced around the wall of the sac, this outer layer shows a change in direction of its fibres, till those nearest the ventral margin of the raphe are cut nearly transversely. The reverse is true for the inner layer of fibres (Tr.). Note how thick the layer is adjacent to the dorsal margin of the raphe, quite the reverse of the outer layer which is thickest on the medial surface of the sac adjacent to the ventral margin. Between these thickened areas the two layers together are very thin on the ventro-lateral aspect of the sac,/

sac, and this is far more noticeable in the adult rat. Diagrams showing the direction of the fibres in the adult muscular sac have been shown in figures 10 and 11.

The root of the thin mesogenitale (M.G.) is attached to the deep surface of the raphe. Within the sac is the genu of the mesonephric ridge. The lateral limb of the genu has developed into the elongated thin coils of the cauda epididymis (Cau. Ep.) while the medial limb has developed into the thick walled vas deferens (V.) with its large lumen. These two structures are still embedded in a common mass of loose mesenchyme (Me.), which in time will gradually disappear and leave a compact mass of cauda epididymis from the dorso-medial aspect of which will arise the vas deferens (figure 2).

Rectus Abdominis.

The wide dorso ventral diameter and the nature of the inter-digitating slips of the caudal region of this muscle, previously described in the adult rat, are admirably seen here in figures 63 to 66.

Scrotal skin.

Throughout the development of the muscular sacs, there was/

was no specialised outpouching of the skin that could be specifically denoted as the labio-scrotal swellings. However, the skin over the caudal portion of the 'lateral buttresses' cover the sacs. This is because the herniating sacs are directed caudally alongside the penis within the loose mesenchyme towards the side of the bulb, and therefore have to pass ventral to the ischio-cavernosa within the caudal part of the 'lateral buttresses'.

During the early stages of development the lateral buttresses of the genital tubercle contained no loose mesenchyme. From the 18th day (17.0 mm. stage) onwards, however, there began a rapid proliferation of loose mesenchymous tissue in the inguinal region extending caudally alongside the penis as far as the bulb. This proliferation naturally extended beneath the caudal part of the lateral buttresses. It was as if a path was being created for the easy passage of the herniating muscular and peritoneal sacs.

A similar condition will be shown to exist in the human, where cephalic to the level of the penis on each side the subcutaneous tissue proliferates rapidly and produces swellings, termed the labio-scrotal swellings. The/

The tissue within each is continuous with the loose tissue alongside the corpora and bulb and is of the same jellylike consistency. It is along this path that the processus vaginalis (P.V.) develops (vide infra) (figures 75, 76 and 77). This mesenchymous proliferation is particularly marked at the region just preceding the tip of the peritoneal and muscular sacs, and, in the pig, has been referred to as the infravaginal portion of the gubernacular mass (Backhouse and Butler 1957).

On examination of the skin surface in the 13 day rat specimen, there was found to be a bare hairless area overlying the caudal portion of the muscular sac. This has been referred to as the sexual skin (Hamilton, 1936). A thick layer of jelly like tissue underlies this area. With further development this layer gradually dissolves, till in the adult there is a very thin layer of connective tissue between the sexual skin and the muscular sac.

40 MM. C.R. LENGTH PIG EMBRYO.

A number of preserved specimens of the same litter were obtained. Their average C.R. length was 40 mm. Four were dissected and three prepared, sectioned and stained.

After the removal of the very large liver, it was found that there was an unusually bulky mesonephros present on each side. These occupied most of the dorsal wall and floor of the coelomic cavity, completely overlapping and hiding from view, the metanephros. The mesonephric duct was on the ventro-medial surface of the mesonephric ridge.

Attached by means of a slender sheet of mesorchium to the medial surface of the upper half of the mesonephros was the gonad. From the cephalic pole of the gonad there extended cranially the plica diaphragmatica. A thickened band ran caudally from the caudal pole of the testis to the mesonephric duct which had a very wide lumen.

A slender inguinal fold was found running downwards and forwards across the umbilical artery towards a conical swelling on the ventral abdominal wall. The ventral end of this fold was attached to the floor of a crescentic depression in the abdominal wall.

Serial/

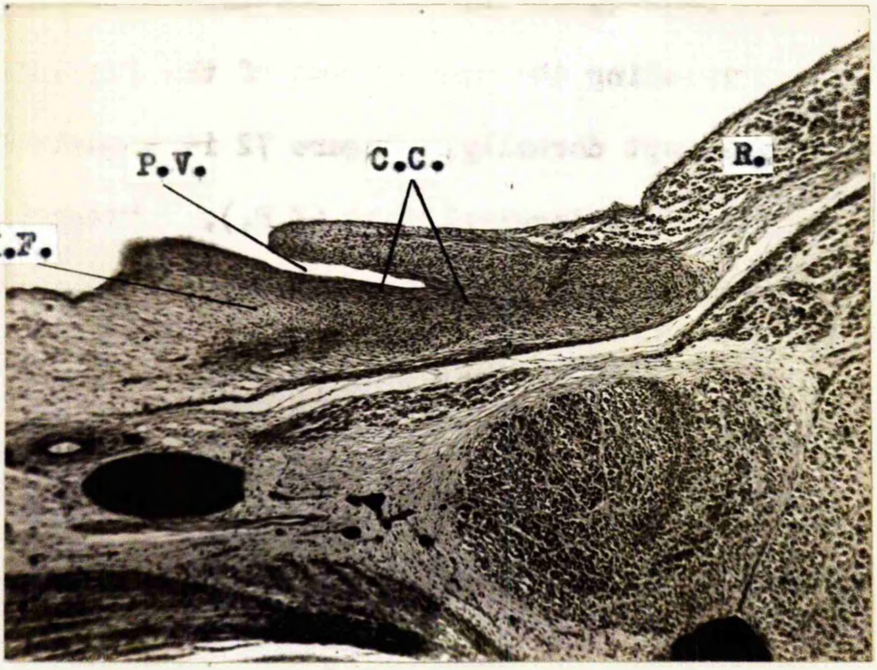

Fig. 71. PIG EMBRYO. C.R.L. 40 mm. A sagittal section along the inguinal fold. X 45.

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Serial sections showed that the inguinal fold was similar in appearance to that found in man and rat. The crescentic depression was the early processus vaginalis (P.V.) surrounding the ventral end of the inguinal fold on all sides except dorsally. Figure 72 is a sagittal section through the right inguinal fold (I.F.). Extending from the mesoderm surrounding the mesonephric duct along the fold to the region of the external ring is a condensation of deeply staining cells (C.C.). These are seen in the figure, immediately subjacent to the free margin of the inguinal fold and in the ventral abdominal wall below the rectus abdominis muscle (R.).

The internal oblique and transversus muscles bear the same relationship to this condensation of cells as they do in the rat (figure 46). The cellular condensation passes between the lateral margin of the rectus and the arched caudal margins of the internal oblique and transversus abdominis. As in the rat there is a definite deficiency in the external oblique layer.

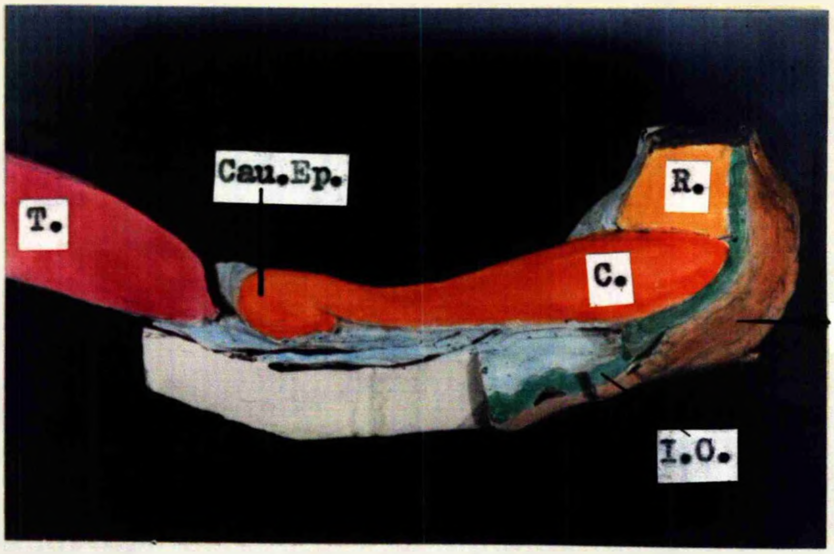

Fig. 72. RABBIT EMBRYO. C.R.L. 85 mm. A lateral view of a wax plate reconstruction of the inguinal cone and its relations.

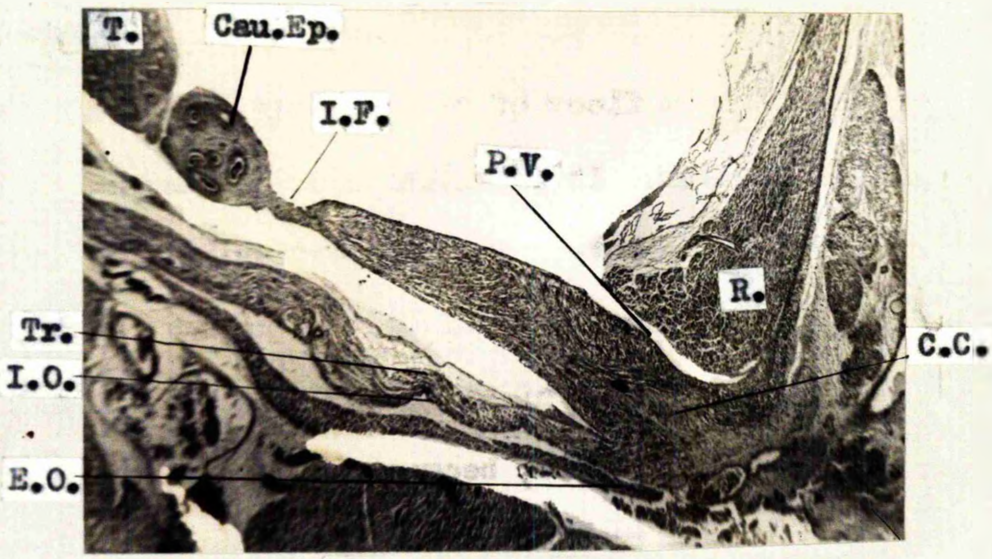

Fig. 72A. RABBIT EMBRYO. C.R.L. 85 mm. A sagittal section approximately through the middle of the right inguinal cone X 20.

RABBIT EMBRYO OF 85 MM. C.R. LENGTH.

Three embryos were obtained from the uterus of a pregnant rabbit. One was a female and the other two were male. Their average C.R. length was 85 mm. The caudal region of the males were stripped of their skin and fixed in Bouin's fluid. They were then prepared, serially sectioned and stained. A wax plate reconstruction was made of the cauda epididymis (Cau. Ep.) and the inguinal cone (C). Fig. 72 is a lateral view of the reconstruction and Fig. 72A is a sagittal section approximately through the middle of the cone along its long axis.

The inguinal cone is a prominent conical structure situated in the floor of the coelomic cavity lateral to the urinary bladder. It is rather more slender and elongated than the cone of the rat. At this particular stage the free margin of the inguinal fold that extends from the apex of the cone to the junction of the cauda epididymis and Wolffian duct is unrecognizable, because it has shortened and is taken in by the proliferating mesenchyme which invests the cauda epididymis. The remainder of the inguinal fold, which is very thin between the 'free margin' and the dorsal attachment to the floor of the cavity, is thrown into loose folds/

folds. Fig. 72 shows the wide attachment between the apex of the cone (C.) and the cauda epididymis (Cau. Ep.) whereas a little more medially, as in fig. 72A, the inguinal fold (I.F.) dorsal to the free margin, is recognised as a narrow isthmus between the two regions.

Unlike the rat, the central loose mesenchymal core is surrounded by a comparatively thick layer of muscle except for a very narrow strip dorso-medially. The thickness of the muscle layer is seen in fig. 72A. The core of mesenchyme (C.C.) is not seen as a continuous strip because of the slightly tortuous disposition of the cone, but parts of it are seen both near the apex and at the base. The presence of the core was confirmed from the reconstruction. This core of mesenchyme is continuous with the loose subcutaneous mesenchyme through the opening in the external oblique, the free edge of the lateral margin of the opening being clearly seen below the base of the inguinal cone.

The caudal portion of the inguinal cone lies in a depression lateral to the rectus abdominis which gives the appearance of an early processus vaginalis (P.V.) before the stage of actual herniation through the external ring (Fig. 72A).

Having made a detailed morphological study of the process of testicular descent in the rat, a study of human specimens was undertaken. In addition to those personally prepared and sectioned, stained serial sections of human embryos of various ages were examined.

The following were the C.R. lengths of the specimens personally prepared and stained.

Specimen	C.R. length	Age in lunar months.
1	42 mm.	2½
2	63 mm.	3
3	85 mm.	3½
4	110 mm.	4
5	170 mm.	5 - 6
6	255 mm.	8

7.0 MM. C.R. LENGTH HUMAN EMBRYO.

This stage presents much the same picture as that seen in the 7.0 mm. rat embryo.

The urogenital ridge is in the caudal curvature of the embryo and ventrally it merges with the mesoderm of the cloacal tubercle. There is no ventral abdominal wall at this stage and/

and therefore no inguinal fold.

The gonadal blastema (G.B.), similar to that noted in the rat, is a mass of deeply stained spherical cells on the ventro-medial aspect of the urogenital ridge, uniform in character and not separated from the surface layer by any limiting membrane. Its more deeply stained appearance, however, contrasts it from the underlying mesoderm within the ridge.

One noticeable difference from the rat embryo is the greater caudal extent of the mesonephric vesicles and their very large size.

15 MM. C.R. LENGTH HUMAN EMBRYO.

The stage of development seen here is similar to that found in between the 10.6 and 11.4 mm. C.R. length rat embryos.

With the embryo orientated so that its C.R. axis is vertical, the human testis is seen to lie in the floor of the coelomic cavity below the level of the inguinal cone, which is situated in the infra-umbilical ventral abdominal wall (cf. fig. 40). The testis is recognised firstly by its more clearly defined sex cords which tend to be arranged in parallel/

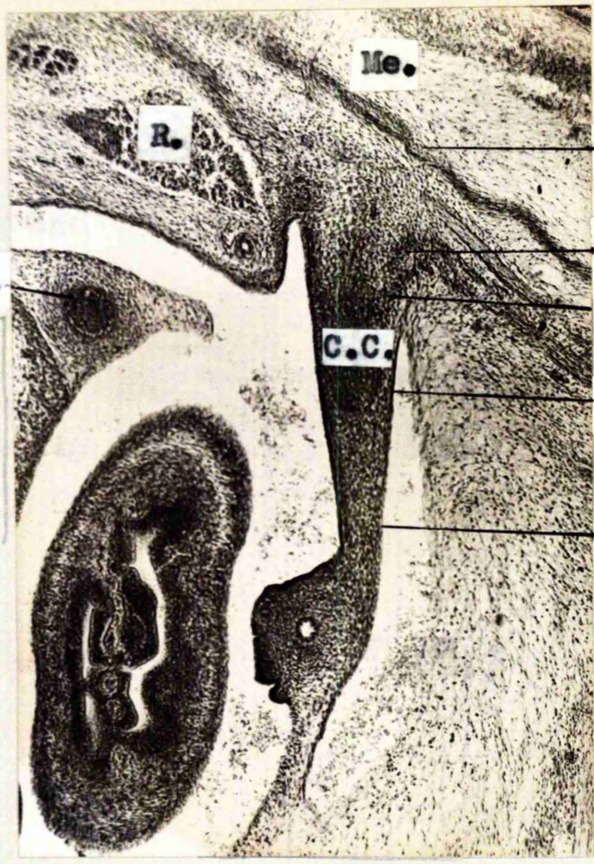

Fig. 73. HUMAN EMBRYO. C.R.L. 35 mm. T.S. through the right gubernaculum (inguinal fold + cone). X 45.

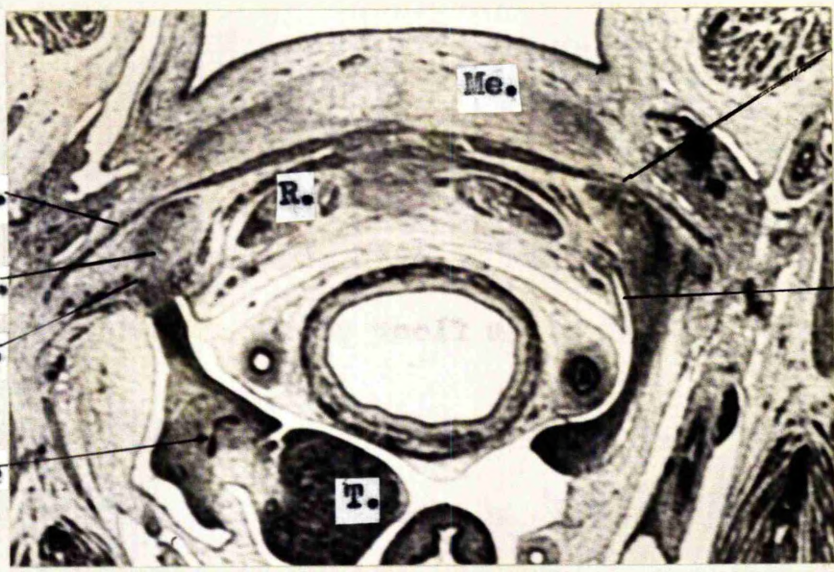

Fig. 74. HUMAN EMBRYO. C.R.L. 42 mm. An oblique T.S. at a slightly higher level on the left side. X 25.

The relationship of the internal oblique and transversus muscles to the cellular condensation is exactly like that shown in figure 46. The caudal fibres of both muscles arch over the lateral and upper surfaces of the condensation to reach the lateral margin of the rectus sheath.

35 MM. TO 42.0 MM. C.R.L. STAGE IN THE HUMAN EMBRYO.

A study of serial transverse sections of a 35 mm. and a 42.0 mm. C.R. length human embryos has shown that the inguinal fold and cone have undergone considerable modification. Figure 73 is a transverse section through right inguinal fold (I.F.) and cone (C.) of a 35 mm. embryo.

The factors involved in producing these changes appear to be:-

1. The persistence of a thick short inguinal fold with a firm attachment to the floor of the coelomic cavity.

2. Wide and firm attachments of the fold, cranially to the mesonephric ridge and caudally to the apex and dorsal aspect of the inguinal cone.

3./

3. The unrolling of the caudal curvature carrying the base of the cone to a lower level.

4. The rapid lateral expansion of the false pelvis.

5. Intra-abdominal visceral pressure.

With the unrolling of the caudal curvature of the embryo, the base of the cone is carried caudally and, as in the rat, its long axis should rotate so that its apex points upwards (figures 51 and 58B). This however, is prevented by the short and stout inguinal fold, aided perhaps by visceral pressure from above.

The lateral expansion of the pelvic floor carries the inguinal fold further away from the middle line and this, coupled with the relative fixity of the cone base, causes the inguinal fold and cone to appear in one plane as an oblique stout band extending from the mesohephric ridge to the ventral abdominal wall (figure 73).

A study of figure 73 and comparison with corresponding structures in the rat embryo will show that basically, the developmental process has not altered.

The/

The cephalic attachment of the inguinal fold to the mesonephric ridge marks the junction of the future cauda epididymis and vas deferens. It was seen in the rat, that the mesenchyme surrounding this junction proliferated and that the cauda epididymis developed within it. Already, in this 35 mm. stage of the human embryo, this proliferation has begun and the cephalic end of the inguinal fold is beginning to look wider. In later stages (figure 74) this wide attachment is so striking that it has been described as conical, with its apex directed towards the inguinal ring (Bramman 1884).

The caudal limit of the inguinal fold and the beginning of the apex of the cone cannot be exactly demarcated. However, a glance at figure 55 and a comparison here with figure 73 will show a dense condensation of cells (C.C.) about the middle of the 'stout band' or gubernaculum, as it is commonly referred to. From this dense condensation a stream of cells extends ventrally past the internal oblique (I.O.) and transversus (Tr.) layers towards the external oblique (E.O.). The dense condensation may be taken as situated near the apex of the cone, which cannot now be identified merely by its shape. At the base of the cone, on the lateral side, the internal oblique (I.O.) and transversus (Tr.) muscles are reflected into/

into the cone, bearing the same relationship to the central cellular condensation as they do in the rat (cf. figure 53). Careful observation shows that the transversus (Tr.) reaches the level of the apical margin of the dense condensation while the internal oblique (I.O.) extends only a little way in from the base.

The processus vaginalis appears as a crescentic depression surrounding the base of the cone on all sides except dorsally where the cone is attached to the floor.

By the 42.0 mm. stage a new development is initiated that distorts the relationship of the internal oblique and transversus to the gubernaculum and processus vaginalis. The mesenchymal cells ventral to the base of the 'gubernaculum' begin proliferating so rapidly that they form a globular mass between the base and the external ring. This expanding mass of cells extends beyond the margins of the base of the gubernaculum and begins to invade the interval between the internal and external oblique muscles. The latter evidently being more resistant does not yield so easily as do the internal oblique and transversus, and consequently, these two muscles bulge inwards over the surface of the gubernaculum.

Thus/

Thus in figure 74 on the left side the proliferating globular mass of cells (G.M.C.) can be seen widely separating the external oblique (E.O.) from the internal oblique (I.O.) and in addition causing the latter to bulge inwards to overlie part of the processus vaginalis (P.V.).

The conical appearance of the cephalic attachment of the gubernaculum to the mesonephric ridge is well seen on the left side in figure 74. The dense cellular condensation within the gubernaculum is seen to be continuous cranially, with similar cells beneath the surface of the mesonephric ridge and ventrally as far as the external ring (E. Rg.).

On the right side the ventral portion of the gubernaculum presents a conical outline reminiscent of its origin from the inguinal cone. The attachment of the gubernaculum to the floor of the coelomic cavity is very short and practically non-existent.

Labio-Scrotal Swellings.

The subcutaneous mesenchyme in the lower abdominal region is very loose. Traced caudally between the limbs the layer is wide and equally loose in texture. However, the lower limbs in the human approach each other far more than/

than they do in the rat and the interval between them narrows considerably in the pudendal region. As early as in the 15 mm. C.R. length human embryo, this fact is noticeable and the subcutaneous area between the limbs and the lateral buttresses of the genital tubercle with their natural proliferation are forced to appear as a swelling on each side, termed the labio-scrotal swellings. By the 42 mm. stage, these swellings are very prominent and contain very loose mesenchymal tissue which is continuous above with the subcutaneous mesenchyme (Me.) seen in figure 74.

63.0 - 110 MM. C.R. LENGTH STAGE IN THE HUMAN EMBRYO.

From the 42.0 mm. stage onwards the direction of the gubernaculum begins to alter as a result of differential growth. The dorsal end is carried further outwards to reach a plane immediately above the acetabulum of the pelvis, while the ventral end is carried downwards with the unrolling of the caudal curvature. Thus, the gubernaculum is directed forwards with an increasingly medial and downward inclination from about the middle of the inguinal region towards the scrotal swellings.

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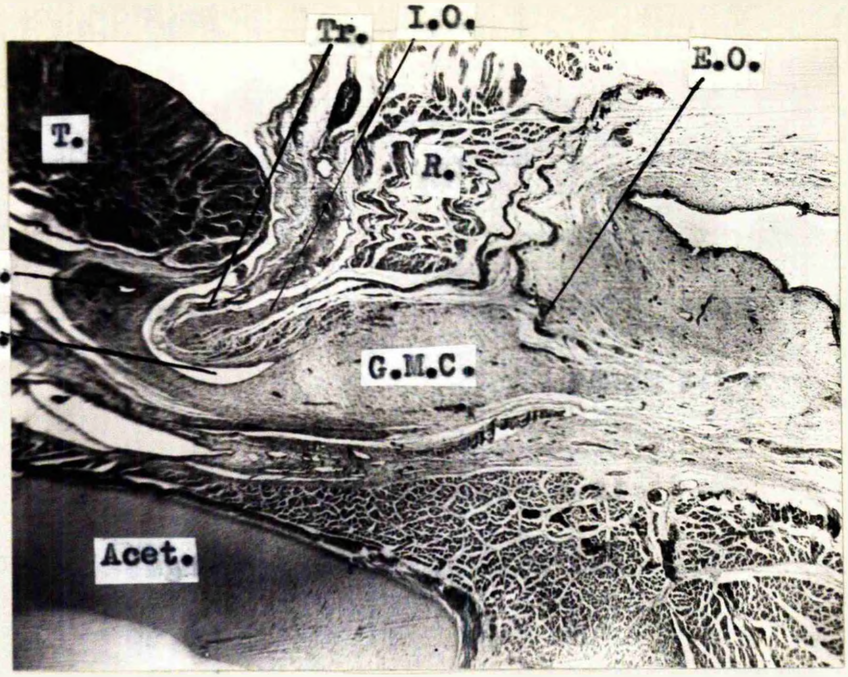

Fig. 75. HUMAN EMBRYO. C.R.L. 85 mm. A sagittal section passing through the gubernaculum on the left side. X 20.

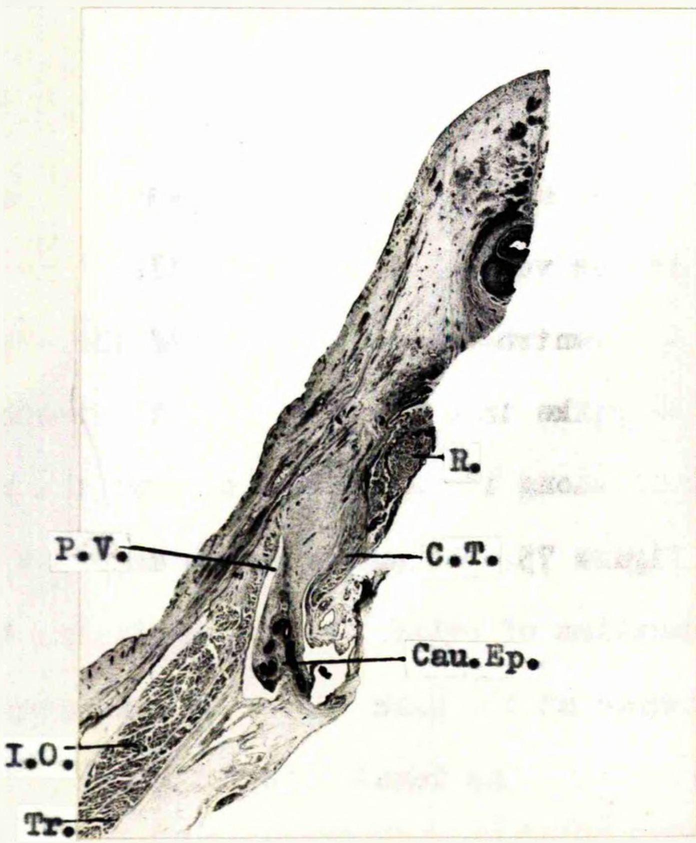

Fig. 76. HUMAN EMBRYO. C.R.L. 110 mm. A transverse section passing through the gubernaculum and scrotum (scr.) on the left side. X 6.

A 63.0 mm., 85.0 mm. and 110 mm. C.R. length human embryos were sectioned along the long axis of the left gubernaculum, the former two in a vertical and the latter in a transverse plane.

Figure 75 is a representative section of the 85.00 mm. embryo. The testis is narrow and elongated and is situated in the floor of the pelvic cavity close to the internal ring. At its caudal pole it is attached to the cauda epididymis which in turn is embedded in a bulky mass of loose mesenchyme continuous with the gubernaculum.

To the naked eye, the gubernaculum appears as a thick white cord extending obliquely downwards, forwards and medially from the caudal pole of the testis into a crescentic depression in the ventral abdominal wall. This depression is deepest on the ventro-lateral aspect of the cord. The cord itself is jellylike in consistency and is attached to the adjacent floor along its dorso-medial aspect. Histologically, as seen in figure 75, the gubernaculum does not show any dense condensation of cells. On the contrary, the proliferating loose mesenchyme at the base of the gubernaculum, seen in the previous stage, has invaded the whole length of the gubernaculum/

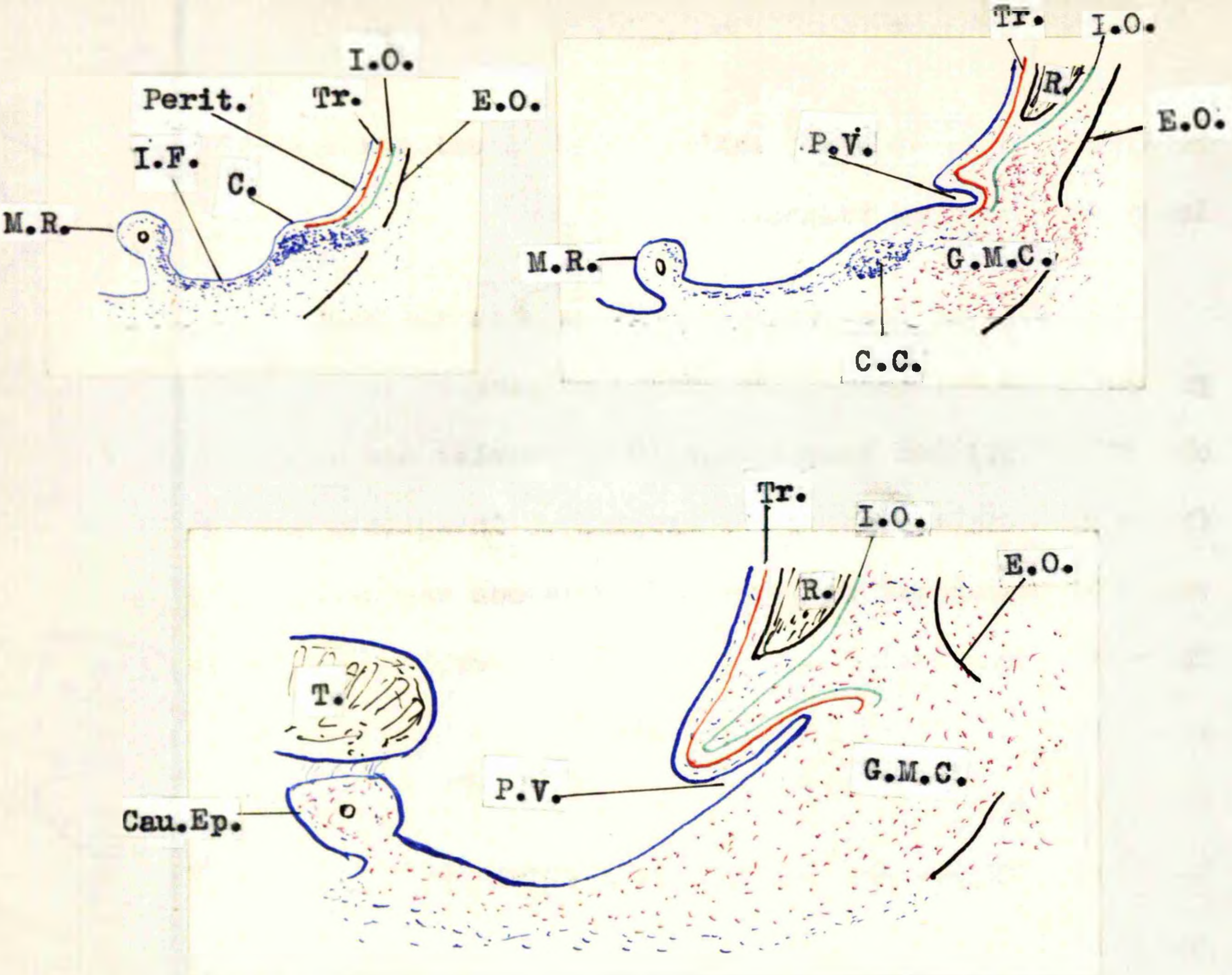

Fig. 77. A diagramatic representation of the developing gubernaculum.

(A) (29 mm. stage) Relation of caudal margins of Internal oblique (I.O.) and transversus (Tr.) to cellular condensation (C.C.) within cone (C.)

(B) (42.0 mm. stage) Proliferating globular mass of cells (G.M.C.) at base of gubernaculum separating external (E.O.) and internal oblique (I.O.). Early processus vaginalis (P.V.).

(C) (85 mm. stage) Invagination (X.) of internal oblique (I.O.) and transversus (Tr.) over processus vaginalis (P.V.). Note caudal margins (Y.) still reflected into substance of gubernaculum.

gubernaculum so that the latter is now a uniform mass of loose mesenchymous tissue.

The bulge of the proliferating mass at the base of the gubernaculum has raised the caudal margins of the internal oblique (I.O.) and transversus (Tr.) muscles and caused their fibres immediately above the margins to invaginate over the ventral and lateral walls of the processus vaginalis (P.V.). The caudal marginal fibres of these two muscles themselves are fixed to the gubernaculum, being reflected into its substance on its ventral and lateral aspects in the region that formerly was the base of the inguinal cone (figures 75 and 77).

Figure 77 is a diagramatic representation of the relationship of the internal oblique and transversus muscles to the developing gubernaculum and processus vaginalis (P.V.). In figure 77A, representing the 29.0 mm. stage, the inguinal fold (I.F.) and cone (C.) are shown containing the dense cellular condensation (C.C.). The internal oblique (I.O.) and transversus (Tr.) are shown being reflected into the cone. In figure 77B, as at the 42.0 mm. stage, the proliferation of cells at the base of the gubernaculum is shown separating the

the/

internal and external oblique muscles causing an invagination of the internal oblique (I.O.) and transversus (Tr.) above (and lateral) to the processus vaginalis (P.V.). Figure 77C shows the invagination well advanced, similar to that seen in the 85.0 mm. stage (cf. figure 75).

Figure 76 is a transverse section in the long axis of the gubernaculum in the 110 mm. embryo including the left half of the scrotum. It was stained by Masson's Haematoxylin - Acid Fuchsin - Auiline blue method to see the development of collagen fibres.

Histologically, the gubernaculum (Gub.) appears similar to that seen in the 85.0 mm. stage. On either side of it the processus vaginalis (P.V.) is seen to be fairly well advanced and has a wide internal opening. This peritoneal pouch or sac is deepest on the ventro-lateral aspect of the gubernaculum (cf. rat). The cauda epididymis (Cau. Ep.), embedded in loose mesenchyme at the dorsal end of the gubernaculum, is situated at the internal opening of the sac.

Lateral to the peritoneal sac the internal oblique (I.O.) and transversus (Tr.) muscles are seen to be cut across obliquely. These muscle layers extend ventrally beyond the processus/

processus vaginalis and a few fibres are seen to be reflected into the bulbous part of the gubernaculum (Gub.). The fibres of the internal oblique and transversus which will eventually develop into the conjoined tendon (C.T.) are at present seen medial to the processus and ventral part of the gubernaculum. Ventro-medial to these fibres is the rectus abdominis (R.).

The processus vaginalis and the gubernaculum are directed in an almost straight line towards the lateral side of the penis (Pe.) where the subcutaneous tissue is loose, abundant and richly supplied with blood. The skin overlying this area caudal to the penis, is continuous with that of the opposite side and presents a single scrotal swelling (Scr.). In the median sagittal plane stretching from the caudal surface of the penis to the skin of this scrotal swelling there now appears a fibrous partition which eventually will become the scrotal septum (Sep.). Although a scrotum is now definitely formed there is no fibrous or muscular connection between it and the gubernaculum, though, from the special stain used, collagen fibres have appeared in other areas, notably the scrotal septum and the tissue surrounding the penis.

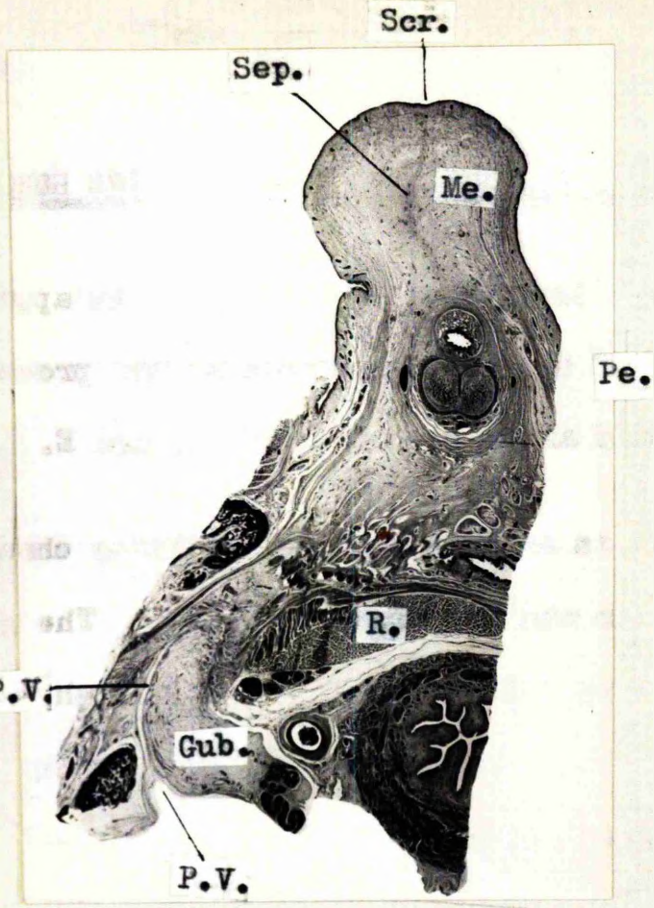

Fig. 78. HUMAN EMBRYO. C.R.L. 170 mm. A transverse section passing through the left gubernaculum (Gub.) and the scrotum (scr) X 4.

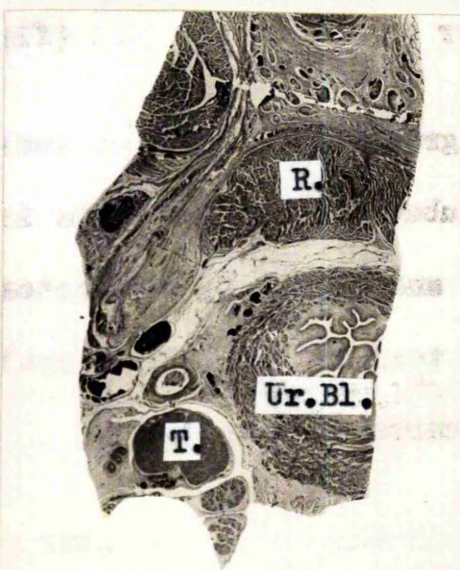

Fig. 79. HUMAN EMBRYO. C.R.E. 170 mm. T.S. through the maximum width of the left testis in the same specimen as that shown in fig. 78. X 4.

98

170 MM. C.R. LENGTH STAGE IN THE HUMAN EMBRYO.

Serial transverse sections made in approximately the long axis of the left gubernaculum and processus vaginalis were studied after staining with H. and E.

There is an important and striking change in the gubernaculum and processus vaginalis. The gubernaculum (Gub.) is now a very bulky mass of loose mesenchymous tissue which extends from the cauda epididymis ventrally along the course of the processus vaginalis (fig. 78). The cavity of the processus that was seen extending alongside the cord-like gubernaculum in the earlier stages (figs. 75 and 76) now appears to be obliterated by the expansion of the latter. The width of this expanded gubernacular mass (Gub.) is greater than the maximum transverse diameter of the testis (T.) (figs. 78 and 79).

Differential growth has caused an increase in the obliquity of the gubernaculum. From the internal ring, it is directed ventrally and medially, but instead of running in a straight line down towards the scrotal swelling, it does so with a pronounced curve downwards.

It will be noted that in the earlier stages the gubernaculum, (as the inguinal fold and cone), was curved upwards, ventrally and/

and laterally. Even in the early stages of the processus vaginalis, the curvature was slightly upwards (figs. 75 and 77). Now the direction has changed; there has been a rotation of the long axis of the gubernaculum, marked in the distal half. This will naturally stretch the ventro-lateral wall of the processus far more than it will the dorso-medial wall. Furthermore, the gubernaculum being attached to the dorso-medial wall will further restrict growth on that aspect. There is thus a great similarity to the process taking place in the rat.

The rapid lateral growth of the false pelvis and the resultant flattening of the ventral abdominal wall in the inguinal region, in contrast to the bulging abdominal wall in the rat, tend to obliterate the cavity of the developing processus vaginalis. The swelling of the gubernaculum appears to be a modification that would prevent the fibrous and muscular tissue that is rapidly developing in the neighbourhood, from compressing and obliterating the track along which the testis is to descend. The tissue within the gubernaculum is of a loose texture and non-fibrous. Both Mallory's and Masson's methods of staining for collagen fibres, using light green and aniline blue respectively, failed to demonstrate within the gubernacular mass, any fibrous tissue.

As/

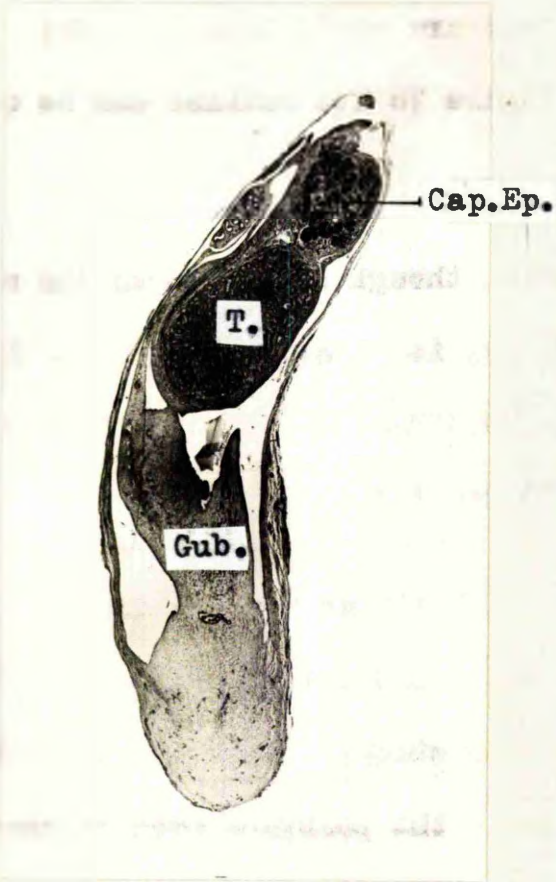

Fig. 80. CAT. 5 days old. A sagittal section through the middle of the left sac. x 7.

100

As the gubernaculum swells, it would necessarily bring into contact its own surface with that of the inner surface of the processus vaginalis. The mesothelia of these surfaces still being immature would tend to fuse; but this fusion is weak and in figure 78 its outline can be quite clearly visible.

The testis, though separated by the mesorchium from the cauda epididymis, is in contact with the latter, which, on the other hand is embedded in the cephalic end of the gubernacular mass (figs. 78 and 79).

Developmental stages between this and the 255 mm. C.R. length stage where the testis was found to have descended, was not available for study. However, the stage found in a new born cat suggests the possible mode of descent.

In a 5 day old kitten, the processus vaginalis was found to have reached the level of the base of the penis, and the testis was halfway down. The lft sac of such a specimen was cut out prepared and serially sectioned in the sagittal plane. Figure 80 is a section approximately through the middle of the testis. The sac besides curving downwards, is directed medially, hence, in this section although the caput epididymis (Cap. Ep.) and testis (T.) are cut through, the cauda epididymis is not. Below/

Below the testis is the wide gubernacular mass (Gub.). Embedded in its upper half in a more medial plane than that seen in the figure, is the cauda epididymis. Note that the width of the testis is no greater than that of the gubernacular mass. In fact, the gubernaculum is far more bulky than this particular section suggests. In a more lateral section the caudal pole of the testis is fully in contact with the gubernaculum.

External to the lining of the sac, muscle fibres can be faintly visible. They are not well marked as in the rat. The sac wall is more fibrous in nature.

This appearance of the testis in the sac preceded by a bulky gubernaculum might very well be similar to what occurs in the human. The bulky gubernaculum, controlled by hormones perhaps, grows steadily downwards along the path nature intended towards the prepared loose area within the scrotal swelling. The testis, with the abdominal pressure to aid it has no alternative but to follow the wide pathway created. There is no fibrous or muscular tissue within the gubernacular mass which directly pulls upon the testis.

255 MM. C.R. LENGTH HUMAN EMBRYO.

This specimen was dissected and a thick walled sac extending from the mid-inguinal region, obliquely downwards, forwards and medially, as far as the scrotal swelling was found. The loose mesenchyme between the fundus of the sac and the skin of the scrotal swelling formed a wide layer. The sac could be easily lifted off this bed of mesenchyme.

In this particular specimen, the sac was patent and its opening fairly wide. On opening the sac through the anterior wall, the testis was found to lie attached to the dorso-medial wall more than 10 mm. above the bottom of the sac. The testis and the adjacent dorso-medial wall was cut out, prepared, sectioned and stained. Sagittal sections showed that the area where the testis was fixed to the wall, was much thicker than elsewhere. In that thickened area the cauda epididymis coils were seen to be embedded. Mallory's method of staining for collagen and muscle fibres showed no muscle fibres in this part of the wall of the sac. There was plenty of fibrous tissue, but in the thickened region where the epididymis was embedded, there was little or none.

The wall of the sac was thinnest and longest opposite the ventro-lateral aspect of the testis, just like in the rat.

DISCUSSION.

Although there are numerous theories regarding the descent of the testis, every one of them, even though a few appear grossly contradictory, are linked together by certain facts. These facts, however, do not by themselves form a complete picture and the gaps that exist have resulted in many unsubstantiated surmises. With more modern methods of investigation more and more facts are being uncovered and many of the views held in the past have had to give way to new ones which take into account all the later known facts.

Although much is known about the subject from the work of past investigators some aspects of it are still controversial.

The contributions made by the present investigation are discussed under the following headings:-

1. The Praenatal Position of the Testis.

2. The Early Development of the Gubernaculum.

3. The Later Development of the Gubernaculum.

4. The Formation of the Processus Vaginalis.

5./

5. The Development of the Cremaster.

6. The Development of the Scrotum.

The Pre-natal Position of the Testis.

When the embryo is orientated so that its C.R. axis is vertical it is found that from the appearance of the gonadal blastema till the time the testis leaves the abdomen, the male gonad is situated in the floor of the coelomic cavity. As the embryo is very markedly curved the cephalic end of the gonadal blastema reaches the level of the middle thoracic vertebrae while the caudal end is in the region of the sacral segments in the early stages of the embryo. As development proceeds certain changes occur. First the cephalic portion of the gonadal blastema, and later of the gonad, degenerates. Secondly, a long mesogenitale forms, the cephalic part of which steadily disappears allowing the testis to retain its position in the caudal curve of the coelomic cavity and thirdly, the unrolling of the caudal curvature of the embryo changes the relation of the testis to the vertebral column. Thus, if it is considered in relation to the vertebrae, the testis appears to have 'descended' within the abdomen; but in relation to the caudal/

caudal curvature of the coelomic cavity, the positon of the testis has remained constant. Similarly, in the case of the ovary, owing to its short mesogenitale and the relative slowness of the degeneration of the cephalic part of the gonadal blastema, the ovary 'descends' less, in relation to the vertebrae while in relation to the caudal curvature it would appear to have 'ascended' somewhat, being at a higher horizontal level than the testis of the same stage.

Bramann (1884) drew attention to the fact that the testis was never more than 1 mm. away from the groin. Wyndham (1943) in his first table showed that in embryos of C.R. lengths between 20 and 56 mm. the maximum distance between the testis and the groins was 1.8 mm. In all the rat and human specimens examined by the writer, the testis was found to be in the lowest part of the caudal curvature of the coelomic cavity, when the embryo was orientated so that its C.R. axis was vertical, and therefore never far from the groin. In fact the site of the inguinal cone gradually descended as the caudal curvature of the embryo unrolled, bringing it closer to the caudal pole of the testis. According to Wells (1943), by the third month there is an impression of a descent of the testis which

is/

is "an apparent rather than a real descent, since the cranial portion of the testis undergoes developmental involution". He finds that the testis is very mobile, having a long mesentery. Lockwood (1885) noting the constant caudal position of the testis emphasized the fact by showing its relation to the acetabulum and he states that for sometime their relative positions remained unchanged.

In man, during the 5th and 6th months, the testis recedes from the internal abdominal ring. Thus Wyndham (1943) in his second table shows that the separation amounts to 1.5 mm. at 4 months, 3.4 mm. at 5 months and 6.5 mm. at 6½ months. This apparent 'ascent', so called by Bramann (1884) is due to the massive enlargement of the gubernaculum which pushes inwards through the internal ring and carries the testis away from it (fig. 78).

Sometime during the 7th month the testis begins its descent through the inguinal canal into the inguinal bursa (Bramann 1884), and reaches its final positon within the scrotum during the 8th month.

The Early Stages of the Gubernaculum till the Appearance of the External Ring.

The present investigation has indicated that what has been commonly termed the gubernaculum is essentially a combination of the "inguinal fold" and the "inguinal cone". In the rat these two structures can be recognised right up to the time when the testis is well within the sac. In man, in the early stages they are clearly recognised but the subsequent modification of the two structures has led to many controversial theories.

There is little or no disagreement about the presence of the inguinal fold and cone in the early stages, as one continuous structure. However, Felix (1912) believed that in the early stages there were two components of the gubernaculum, the "inguinal fold" and the "inguinal crest" which approached each other and fused. This has been rightly denied by Mosskowicz (1935) who maintains that the ridge is a single structure from the time of its first appearance and moreover considered that this ridge is composed of dense mesenchyme that is covered with a layer of peritoneum. He is supported by most of the recent investigators (Wyndham 1943, Backhouse and Butler 1956).

In/

In the very early stages the cellular condensation extends from the mesonephric ridge to the mesoderm immediately subjacent to the conical swelling of the inguinal cone (fig. 30 A). It does not extend beyond the base of the swelling and therefore cannot be justly described as reaching the future scrotal area. At this stage there appear no muscle condensations in this region (figs. 30, 30A, 31, 31A) and this has been mentioned by Bramann (1884) and Wyndham (1943).

As development proceeds, the cellular condensation (C.C.) extends ventro-laterally while simultaneously the condensations of the ventral abdominal musculature are being laid down in the neighbourhood. Reaching the vicinity of the external oblique layer, this condensation visibly fades. In the rat it appears to end rather abruptly and gives rise to doubts as to whether it goes past the plane of the external oblique layer or not (figs. 50 and 55). However, whether it goes past or not, a definite deficiency appears in the external oblique, the infero-lateral margin of which is clearly defined over this region (figs. 55, 58, 59). In man the deficiency is small and unless sections passing obliquely ventro-caudally through this deficiency are viewed, the/

the condensation of cells passing through will not be seen. The writer has seen the condensation 'passing through' the 'opening' but it does not extend far. It fades very rapidly and blends with the loose subcutaneous mesenchyme. Wells (1943) in examining young embryos of the Minessota collection found a cellular condensation extending beyond the external oblique layer in serially sectioned embryos, but he could not demonstrate them in older specimens. The explanation for the latter will be discussed in the next section. It is perhaps this condensation seen outside the external oblique layer that made Felix (1912) believe in a 'pars scrotalis' which fused with the intra mural part through the external ring. Moszkowicz (1935) terms this same cellular condensation, the 'pars subcutanea'. Bramann (1884) on the other hand by dissection tried to look for the circumscribed string, that he says Burkner had described reaching the scrotum from the internal ring. This he did not find. Instead he found only loose connective tissue. However, he saw a rounded white mass in the region of the external ring and so came to the conclusion that this, the ventral end of the gubernaculum, did not perforate the external oblique aponeurosis. At this stage, 3 to 4 months foetus, if the external oblique aponeurosis is clearly seen as a thin and transparent/

transparent membrane, as he says, then histological sections must show it up even more definitely. This is not so, and special stains have failed to show the continuity of the external oblique over the surface of the gubernaculum. Fig. 75 is a section of a 3½ month embryo and the break in the external oblique is clearly seen. Many have challenged and refuted Bramann's statement that the external oblique was not perforated, and amongst the more recent investigators are (Moszkowicz (1935), Pearson (1955), Backhouse and Butler (1955). They found clearly defined openings which give passage to the gubernacular mesenchyme, the genital branch of the genito-femoral nerve and the ilio-inguinal nerve. Pearson, proved it by using special stains to show up the nerves and fibrous tissue.

Later Stages of the Gubernaculum before Decensus Occurs.

The cellular condensation within the gubernaculum which had migrated to the plane of the external oblique gradually begins to lose its deeply staining property and dense character. Once the deficiency in the external oblique has been established, the caudal end of the gubernacular cellular condensation begins to proliferate and becomes loose/

loose and gelatinous. Cells from the caudal end of this proliferating mass pass out through the external ring everting the margins in the process. The rest of the cells extending in all directions becomes a globular and later an olive shaped mass (fig. 75). This proliferation extends cranially along the gubernaculum and gradually all of the deeply stained cellular condensation disappears and the gubernaculum becomes a single mass of jelly like tissue. Wells (1943), it will be remembered, found cellular condensations in young embryos but not in older specimens.

In the process (depicted in fig. 77) the caudal margins of the internal oblique (I.O.) and Transversus (Tr.) are raised due to the thickening of the gubernaculum and at the same time the fibres cephalic to the (caudal) margins are pushed inwards by the globular caudal end of the gubernacular mass (G.M.C.) (figs. 74 and 75). These fibres therefore overlie the newly developing processus vaginalis (P.V.), covering its lateral and superior surfaces and encroaching on to the infero-lateral and supero-medial surfaces. Neither the processus nor the gubernaculum is completely surrounded by muscle fibres and they never are.

As/

As the gubernaculum grows larger, the cavity of the processus vaginalis is gradually obliterated (fig. 78). The further enlargement of the gubernaculum causes it to protrude into the abdominal cavity and push the testis away from its position close to the abdominal wall.

The proliferation of the cephalic extremity of the gubernaculum tends to invest the rapidly lengthening coils of the cauda epididymis (Cau. Ep.). This mass gives the appearance of a cone with its apex directed towards the processus vaginalis (P.V.) (fig. 74, left side). The proliferating caudal end of the gubernaculum spreading cranially eventually takes in this conical mass and together they appear as one large gelatinous mass wider in diameter than the testis (cf. figs. 78 and 79). The testis is naturally in contact with the cephalic end of the gubernacular mass, having been pushed backwards away from the internal ring by it. It is not, however, fused to this mass, but is separated by a short mesorchium, the part usually termed the ligamentum testis.

No fibrous tissue has been seen so far, running from the testis or cauda epididymis along the gubernaculum. The muscle fibres/

fibres present, lie on the surface of the gubernaculum distal to the processus vaginalis and are derived from the transversus abdominis and internal oblique muscles.

Before Bramann (1884) and Weil (1885) most of the investigators believed that the gubernaculum pulled the testis down towards the scrotum and therefore tried to find the cause for this. Hunter pulling up the testis with the attached gubernaculum in an early embryo in which the processus vaginalis had just begun to form, everted the processus and to quote his own words "..... there is no appearance of an aperture or passage down towards the scrotum: but when the scrotum and ligament are drawn downwards, the loose doubled part of the peritoneum descends with the ligament, and then there is an aperture from the cavity of the abdomen all around the forepart of the ligament, which seems ready to receive the testis." He thus came to the conclusion that the ligament was attached to the scrotum and that when it contracted it pulled on the 'conical' cephalic end of the gubernaculum which was in contact with the testis. This "pyramidal or wedge like ligament was first drawn down, in order not only to direct but to make room for the testis which must follow it." From dissection Hunter could not find muscle/

muscle or fibrous tissue either within the gubernaculum or extending towards the scrotum but because he had seen muscle fibres in the inguinal cone of the hedgehog he inferred their presence in the gubernaculum of man.

Seiler (1817) noting the presence of the plica gubernatrix saw within it dense connective tissue running from the testis, through the abdominal ring to the cellular tissue external to the external oblique, where it divided into two branches, one going to the pubis and the other directed forwards. This arrangement is similar to that seen by Wells (1943) and by the present writer. Seiler believed this condensation to be the ligament to which Hunter referred. Furthermore, noting the incurving condensations of the internal oblique (I.O.) and transversus (Tr.) (fig. 73) he believed that they surrounded the cellular mass in the gubernaculum. These condensations he called the cremaster muscle and believed that they were mainly responsible for drawing down the testis and everting the gubernaculum in the process.

Rathke (1832) from researches in ruminants and pigs, found the lower end of the gubernaculum to be mucoid and described/

described this part as forming an olive shaped swelling (cf. fig. 75).

Bramann (1884) found no fibrous or muscular connection between the scrotum and the gubernaculum. He performed a similar experiment as Hunter did, but this time he pulled on the gubernaculum to see if the scrotum followed. It didn't. Instead there was a dimpling in the region of the external ring. He therefore concluded that the gubernaculum was attached to the abdominal wall and not to the floor of the scrotum.

He found that the gubernaculum increased in thickness and length and became attached to the caudal pole of the testis. In embryos, 4 to 5 months of age, he found the upper end of the gubernaculum 2 mm. across and where it entered the abdominal wall it was 1 mm. By the 6th month the gubernaculum had reached its maximum size and was 2 - 4 mm. wide just below the testis. From a section, bulk stained in borax carmine, he describes an upper whitish part and a lower brownish part. In the lower part from without in, he found a peritoneal layer, a thin layer of connective tissue with no orderly arrangement, a brownish layer consisting of connective tissue and bundles of muscle fibres and lastly a/

a core of spongy vascular tissue. In the upper lighter part of the gubernaculum, he found no muscle fibres but only spongy connective tissue such as that in the core of the lower part. This description is much like that seen in fig. 78. His observations in the rat cone led him to believe that the muscle completely invested the core of the spongy tissue, but this as the writer has shown is not true. In a sagittal section Bramann found that the muscle layer formed half the thickness of the gubernaculum (cf. rabbit, fig. 71A) becoming thinner towards its upper end. This might appear to conflict with what most other investigators have found, namely a very thin layer of muscle fibres in man. However, when it is remembered that the gubernaculum is directed obliquely medially and downwards, then a sagittal section must necessarily cut through the muscle layer in an oblique fashion to produce a wider layer in the lower or caudal region. Fig. 78 is an example. Here in spite of a deliberate oblique section downwards and forwards, because the gubernaculum curves downwards the section still shows a 'thick layer' of muscle fibres in the lower (caudal) end, with the layer 'thinning out' cranially.

Weil (1885) using serial sections was able to correct many of the older theories. Most of his descriptions have been/

been corroborated by the present investigation. He describes the gubernaculum in a 5 month embryo as "a connective tissue cord which only, so far as it is covered with peritoneum, shows an exact boundary and an approximate round form; that part which lies between the abdominal muscles is indistinctly formed and contained several bundles of striped muscle in an irregular arrangement. Beyond the belly wall (muscles), its outline becomes more and more indistinct and it loses itself almost completely in the jellylike tissue under the skin in the neighbourhood of the symphysis. The main mass of the cord is formed from a soft, often very vascular jelly like tissue in which one frequently finds more or less extravasation of blood and irregularity shaped spaces which results from the falling apart of the surrounding connective tissue and which are partially filled with detritus and blood cells." (cf. figs. 75, 76 and 78). Irregular spaces were also noted in the present investigation but they appeared to occur in those specimens in which fixation was not good and they have consequently been interpreted as artefacts. Weil disagreed with those authors who stated that all the muscle bundles ran in a longitudinal direction or that these were arranged in three separate bundles (Curling 1841)/

(Curling 1841) of which the middle goes to the floor of the scrotum and the lateral ones to the inguinal ligament and pubis.

Lockwood (1888), contemporary of Weil, gave a remarkable series of lectures on the development and transition of the testis, his investigations being carried out mainly through dissections and stained sections. The latter were not serial. Many, though not all, of his findings have been found to be true and have been corroborated by many of the present day investigators. However, it is to his famous 'tails' that the present writer would like to direct attention. Lockwood believed that the muscle fibres of the gubernaculum were responsible for pulling the testis down to the scrotum. He believed the condensation he saw within the gubernaculum in the early stages, developed into muscle fibres. From the appearance of the direction of the condensation, as described by Seiler (1817), and suggested by many previous investigators (fig. 73) he deduced that there were 3 bundles of muscle fibres, one going medially to the pubis, another to the floor of the scrotum and the third going laterally to the inguinal ligament. He also dissected out addition bundles running into the femoral and perineal regions. He then correlated these five 'tails' of the gubernaculum with malpositions of the/

the testis. None of this work has ever been confirmed and recent investigators have emphatically denied the existence of either fibrous or muscular tissue extending from the bulbous end of the gubernaculum towards the scrotum or anywhere else. (Forsner 1928, Moszkowicz 1935, Wyndham, 1943, Wells 1943, Backhouse and Butler 1954). Backhouse and Butler studying pig, sheep and ox factuses state that in none could the gubernaculum be describe as fibro-muscular and that in all, the testis could be easily lifted out of the sac, there being no 'tails of Lockwood'. To quote Forsner, who finds no muscle and observes progressive transformation of the gubernaculum into mucoid tissue, he says "the usual description of descensus that a central core of connective tissue and unstriped muscle, the chorda gubernaculi, is formed in the inguinal ligament, growing down to the bottom of the scrotum and by contractions pulling the testis down, is not founded on facts and ought to be regarded as pure invention." (Wells, 1943).

In fairness to those investigators who have genuinely seen muscle fibres in the gubernaculum (Bramann 1884, Weil 1885) one must admit that a section through the gubernaculum at the stage after the latter has enlarged and obliterated the early processus vaginalis, must show muscle fibres in what appears the periphery of the gubernaculum. The fibres cut at a tangent near the surface must show a disordered arrangement as Weil described (figs. 75, 76 and 78).

Development of the Processus Vaginalis.

Almost all the investigators in the past realised that the processus vaginalis preceeded the descent of the testis as an extension of the peritoneal cavity. They also recognised that the testis, during development, never reached the caudal extremity of the sac and that during descent a portion of the processus always preceded the testis.

Most investigators explain the formation of the processus vaginalis in one of the two following ways. In the one view the testis was pulled down by some means from below and in the process, dragged down the peritoneal lining of the abdominal cavity. In the other view some force from above pushed down the testis evaginating the abdominal wall before it in the region of the external ring.

Weil, alone disagreed with both views. He believed that the jelly-like tissue of the gubernaculum degenerated producing resorption spaces which eventually flowed together and led to the formation of the long canal of the procdssus vaginalis.

In the present investigation it has been found that right up to the 35 mm. stage there is little difference in the/

the process in man and in the rat. In the rat, towards the later stages it has been shown that the whole thickness of the abdominal wall, deep to the external ring, herniates through this opening. This herniation was likened to a balloon which could not expand uniformly, being restricted on one aspect. This restriction in the case of the herniation in the rat was the attachment of the inguinal cone on the dorso-medial aspect of the sac (figs. 62, 62A).

By the 35 mm. stage in man, there has appeared a shallow crescentic depression on the deep aspect of the ventral abdominal wall surrounding the base of the gubernaculum, on all sides except dorso-medially. This depression, the early processus vaginalis (P.V.) deepens extending ventro-medially towards the external ring. The caudal end of the gubernaculum keeps pace with the caudal blind end of the processus and by the 110 mm. stage the latter appears, in a transverse section (fig. 76) as a long canal closed ventrally but having a wide opening into the abdominal cavity dorsally. The gubernaculum (Gub.) appears as a thick round cord within the canal but is attached along its whole length to the dorso-medial wall of the canal. The growth of the processus (P.V.) is greater on the ventro-lateral aspect and in the 110 mm. stage/

stage (fig. 76) the distance from the internal abdominal opening to the blind end of the processus is 5 mm. long on the lateral aspect as compared with 2.5 mm. on the medial aspect of the gubernaculum.

The above description is reminiscent of the process in the rat where the ventro-lateral wall of the processus vaginalis is longer than the dorso-medial. However, the cavity itself is not wide, as it is in the rat, and as further development continues in man, the gubernaculum grow larger in diameter and gradually obliterates it.

After the gubernaculum has reached its maximum size, it appears as a long wide gelatinous mass, wider in diameter than the testis and lying obliquely along the path of the future spermatic cord. As already described, the gubernaculum obliterates the early processus vaginalis and its cranial end comes in contact with the testis (fig. 78 and 79).

With further development, it would appear that this gelatinous mass moves down along a prepared path of very loose mesenchyme towards the scrotum carrying with it a new processus vaginalis. Though none of the previous investigators have actually seen this stage of the process whereby/

whereby the gubernaculum gets past the external ring followed by the testis, it is believed to take place during the 7th month (Bramann, 1884, Wyndham, 1943).

By the 8th month the testis is well outside the external ring and near the caudal end of the sac. However, as shown in the 255 mm. embryo the testis is actually a good distance, 10 mm. in this case, away from the lower-most part of the sac and is attached by a very short stalk to the dorso-medial wall, suggesting that like the rat, the sac is developing grew more rapidly on the ventro-lateral aspect.

It has been suggested that the expansion of the gubernaculum was a means of widening the internal abdominal orifice (Bramann, 1884, Klaatsch 1890, Frankl 1900, Forsner 1928, Moszkowicz 1935). Certainly the gubernaculum is very wide, but it contains jelly like tissue which does look delicate in comparison with the surrounding muscle and connective tissue. There must exist therefore some control whereby the osmotic tension within this mesenchymous mass would resist any external pressure that might tend to obliterate the passage created by the presence of the gubernaculum.

Soon after the gubernaculum and testis have passed through/

through the abdominal wall, its passage, which is now termed the inguinal canal, narrows and soon gets obliterated. In the 255 mm. specimen examined, the passage was patent.

According to data collected by Scammon (1923) more than 80% of the cases show partial or complete obliteration by the 10 - 20th post-natal days. The obliteration has been observed to begin in the middle third of the canal and advance rapidly upwards and downwards from this level (Frankl 1895). Lockwood (1888) believed that the closure began at two points, namely near the abdominal ring and near the superior margin of the testis.

Development of the Cremaster.

In man the development of the cremaster is similar to that in the rat and the 'loops' of cremasteric fibres seen in man are derivatives of the internal oblique and transversus layers.

The situation of the testis in the rat is very vulnerable to injury. Hence, the existence of a protective mechanism. This is in the form of a widely patent sac surrounded by well developed muscle, the cremaster. At the slightest indication of possible injury to the testis, the muscular sac contracts and the mobile testis is sent out of danger into the abdominal cavity. The writer has found that in the white rat the testes are always within their sacs all year round. When the rat is handled or frightened, the testes are seen to disappear from the sac. Engle (1932) states that the testes descend into the scrotum only during the breeding season in those animals with patent sacs and that this is "directly correlated with the pituitary-gonadal relationship". This may be true, in which case the rat must be considered as being capable of breeding all year round. This also was found to be so, as the writer has mated rats throughout the year and got results.

The/

The cremaster in man is believed by the writer to be a vestigial structure. As the testis is not capable of moving back into the abdomen and as other mechanisms for its protection from injury are present, the cremaster must necessarily degenerate from disuse. The few fibres that are left certainly show a reflex contraction, but does that produce any useful result? It is only a reminder of its one time function of sending the testis back into the abdomen.

It has been generally agreed that the cremaster in man is developed from the internal oblique and transversus layers. Recently, however, doubts were raised, as in pigs, sheep and most quadrupeds, there usually is a strong single band of muscle in the wall of the sac running downwards and appearing to be at right angles to the caudal fibres of the internal oblique and transversus that arch over the spermatic cord (Backhouse & Butler, 1957). Ruth Millar (1937) in her comparative study by dissection of various primates found varying number of bands. The lateral bundles according to her, taking origin either from the internal oblique, transversus or both, ran down on the lateral or dorso-lateral side of the spermatic cord. The medial bands usually blended with the pubic tubercle, rectus sheath or adjacent part of the/

the conjoined tendon. Again the fibres 'appear' to be at right angles to the general direction of the internal oblique and transversus muscle fibres.

In the present investigation it has already been noted that the fibres derived from the transversus were aggregated on the dorsal and dorso-lateral aspect of the sac and gave an appearance of running vertically downwards (figs. 11, 12 and 13). Similarly, on the ventro-medial aspect of the sac, the fibres derived from the internal oblique were bunched together as they approached their insertion to the pubis and lower end of the rectus sheath (fig. 10). In fig. 70, where the very young sac is cut through transversely a little above its middle, the transversus layer is extremely thick dorsally while the internal oblique layer is likewise thick on the ventro-medial aspect. Note that in both these regions the fibres have been cut across transversely. It is suggested that selective regional degeneration on this basic pattern may account for much of the variations in different species. Thus in the pig and sheep, the dorsal fibres of the transversus only remain and therefore appear as a single band. The variations may be studied in the descriptions given by Ruth Millar (1937). Generally it is found that the main bundles lie on the/

the lateral side of the spermatic cord, arising from the internal and transversus layers, and on the ventro-medial side usually being inserted into the pubis or adjacent region. In man there are no muscle fibres on the dorsal aspect of the spermatic cord, but on the lateral and ventro-medial aspects only. Below they appear irregularly to 'form loops' or 'interdigitate'. Evidently, the fibres from the transversus have undergone greater degenerationas the cremaster is more difficult to trace into this layer.

The Development of the Scrotum.

The development of the scrotum appears to be a simple process which is primarily concerned with preparing a pathway for and later receiving the testis.

From an early stage,at about the time the caudal end of the gubernaculum begins to proliferate,the subcutaneous mesenchyme in the infra umbilical and pudendal regions is a very wide layer. This subcutaneous mesenchyme begins to proliferate and becomes more loose at the region ventral and caudal to the external ring. As if anticipating the bulbous end of the gubernaculum, this loose area begins to spread caudally between the lateral buttress of the genital tubercle and the lower limb. The limbs being wide apart in the rat the overlying skin does not appear as a prominent swelling (L.S.S.) (figs. 35 and 36). In man, however, the proliferating loose tissue raises a prominent labio-scrotal swelling. As development proceeds the loose mesenchyme extends caudally and proliferates more rapidly. By the time the proliferation reaches the base of the penis, the urethral groove has closed in man and the mesenchyme is able to unite with that of the opposite side, no demarcation being/

being present. In the male rat no breakdown of the urogenital membrane has been seen by the writer. A common uniform swelling appears in the pudendal region, caudal to the developing penis, containing a mass of loose mesenchyme and blood vessels. Later a median septum (Sep.) differentiates within this mass which extends from the caudal surface of the penis to the skin of the swelling which is now properly called the scrotum (Scr.) (figures 76 and 78). In the rat there is no such prominent swelling, as the pudendal region is very wide and the subcutaneous loose mesenchyme does not need to localise itself into a swelling near the median plane.

This scrotal swelling is not the mechanical result of the descending gubernaculum or testis. It occurs long before the gubernaculum has begun to descend. Fig. 77 shows a 110 mm. stage (4 months) human embryo transverse section in which the scrotum has formed and even shows a median septum (Sep.) while the gubernaculum has not yet enlarged to its full extent. In figure 78 the gubernaculum has reached its maximum size and the scrotum (Scr.) shows a vast area of loose mesenchymous tissue ready to offer little or no resistance to any structure that will enter therein.

It/

It has been shown that hormones control and maintain the development of the scrotum (Engle 1932 and Hamilton 1936). Engle (1932) using either A.P. extract or the water soluble fraction of pregnancy urine found that the scrotum enlarged in size and fulness; much more so with pregnancy urine that A.P. extract. The scrotal sac became filled with mucoid like tissue similar to Wharton's jelly.

It is thus reasonable to assume that the tissue within the labio-scrotal swellings and later the scrotum, under hormonal control is specially prepared for the reception of the descending testes and its coverings.

Fibres of the panniculus carnosus underlying the scrotal skin develop in the long axis of the scrotum and specialise in its function, reacting to temperature changes. This sensitivity to temperature changes increases as the animal matures (Phillips & Andrews, 1936-39). This increase in size and sensitivity was shown to be under the control of the male hormone. Hans Selye (1943) has, however, shown that the presence of either the testis or its epididymis is an essential additional factor in the later development of the scrotum and dartos.

LIST OF ABBREVIATIONS.

A.	Allantois; Allantoic diverticulum.
Acet.	Acetabulum.
Ao.	Aorta.
Art.	Artery.
A.M.	Anal membrane.
A.S.I.S.	Anterior Superior Iliac Spine.
Ab. Cav.	Abdominal Cavity.
Art.	Artery.
Ap.R.	Aponeurotic raphe.
B.	Bulb.
B.S.	Blood sinus.
B.Vs.	Blood vessels.
C.	Cone.
C.C.	Cellular condensation. Cone condensation.
C.R.L.	Crown Rump length.
Cap. Ep.	Caput Epididymis.
Cau. Ep.	Cauda Epididymis.
Cl.	Cloaca.
Cl.M.	Cloacal membrane.
Cl.P.	Cloacal passage.
Cr. Ca.	Crux Cavernosum.

E.O./

E.O.	External Oblique.
E.Rg	External ring.
Ep.	Epididymis.
F.B.G.F.N.	Femoral branch of the genito-femoral nerve.
G.	Gonad.
G.B.	Gonadal blastema.
G.F.	Genital fold.
G.F.N.	Genito-femoral nerve.
G.M.C.	Glomular mass of cells;
	Gubernacular mass of cells.
G.P.	Glans penis.
G.T.	Genital tubercle.
Gub.	Gubernaculum.
I.F.	Inguinal fold.
I.lig.	Inguinal ligament.
I.O.	Internal oblique.
I.U.A.W.	Infra-umbilical abdominal wall.
Is.	Ischium.
K.	Kidney.
L.B.	Lateral buttress.
L.B.A.	Limb bud artery.
L.C.B.	Lateral coelomic bay.
L.E.	Lateral eminence.
L.S.S.	Labio-scrotal swelling.

L.W./

L.W.	Lateral wall.
M.	Mesorchium.
M.C.	Mesenchymal cell.
M.C.B.	Medial Coelomic bay.
M.G.	Meso-genitale.
M.M.	Mesonephric Mesentery.
M.R.	Mesonephric ridge.
M.S.	Muscular sac.
M.T.	Mesonephric tubules.
M.W.	Medial wall.
Mam. G.	Mammary gland.
Me.	Mesenchyme.
Mem. l.	Membranous layer.
Met. C.	Metanephric cap.
Mu.	Mullerian duct.
Mu. T.	Mullerian tubercle.
N.	Neck (of sac).
N.P.U.G.S.	Non-phallic part of urogenital sinus.
N.S.	Neck of sac.
O.	Ovary.
Ob. M.	Obturator membrane.
Op.	Opening.
P.	Prepuce.
P.C.V.	Posterior Cardinal Vein.

P.D./

P.D.	Plica diaphragmatica.
P.E.	Pudic-epigastric.
P.E.A.	Pudic-epigastric artery.
P.E.Vs.	Pudic-epigastric vessels.
P.G.	Preputial gland.
P.U.	Plica urogenitalis.
P.U.G.S.	Phallic part of urogenital sinus.
P.V.	Processus vaginalis.
Pe.	Penis.
Pub.	Pubis.
R.	Rectus abdominis.
R.S.	Rectus sheath.
Rm.	Rectum.
S.	Swelling.
S.C.	Sex cords.
S.L.	Suspensory ligament.
Sep.	Septum.
Scr.	Scrotum; scrotal swelling.
T.	Testis.
T.S.	Transverse section.
T.V.	Testicular vessels.
Tr.	Transversus abdominis.
Tu.A.	Tunica Albuginea.
U.	Ureter.
U.A.	Umbilical artery.

Ur.B./

Ur. B.	Ureteric bud.
U.G.M.	Urogenital membrane.
U.G.R.	Uro-genital ridge.
U.G.S.	Urogenital sinus.
U.P.	Urethral plate.
U.S.	Urorectal septum.
Ur. Bl.	Urinary bladder.
V.	Vas deferens.
V.E.	Vasa efferentia.
W.	Wolffian duct.

A C K N O W L E D G E M E N T.

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