IMPLANTATION, FOETAL MEMBRANES AND EARLY PLACENTATION OF THE AFRICAN ELEPHANT, *LOXODONTA AFRICANA*

By J. S. PERRY
Institute of Animal Physiology, Babraham, Cambridge

(*Communicated by E. C. Amoroso, F.R.S. – Received* 9 *April* 1974)

[Plates 1–7]

CONTENTS

The implantation of the blastocyst of *Loxodonta*, and the early development of the placenta, are described from material collected in Africa by Dr R. M. Laws. Implantation is central, the blastocyst settling in one of three or four deep longitudinal grooves in the uterine lumen. Its growth distends the uterine horn before it becomes attached to the uterine wall. As the bilaminar blastocyst continues to grow the trophoblast erodes the uterine epithelium over a zonary area and is there closely applied to the uterine stroma. It remains unattached over the embryonal and abembryonal poles, where the uterine epithelium is retained. As the yolk-sac approaches its maximum volume it is invested by mesoderm, forming an extensive trilaminar omphalopleur, the outer layer (trophoblast) of which immediately begins to invade the uterine stroma by peg-like proliferations that enter by the uterine glands. The latter undergo little change during these early stages and do not undergo extensive modification at any stage, but their basal portions become moderately distended after the gland openings are blocked by the trophoblast.

As the trophoblast of the yolk-sac wall invades the stroma the allantois reaches the chorion and from this time the yolk-sac is rapidly reduced in volume. The allantois soon fills the exocoel and occupies the whole cavity of the conceptus surrounding the embryo and amnion. The allantochorionic placenta develops (over three discrete areas in the specimen described) by the growth of villi which are formed as the earliest trophoblast proliferations acquire a mesenchymal core and become vascularized. There remain areas where the trophoblast does not attach to the uterine wall and in these areas the uterine epithelium proliferates in a characteristic manner and appears to shed cellular material into the residual uterine lumen.

As the allantochorionic villi develop, the underlying uterine stroma thickens, and large blood channels appear in it, lined by a shallow endothelium. These blood vessels, which have few branches, penetrate to the face of the placenta. Their investment by the advancing trophoblast leads to the 'vasochorial' condition described in an earlier account. The characteristic marginal haematomata of the elephant apparently form where an extending villous area meets an area of intact (although modified) uterine epithelium. This epithelium is undermined by lateral extension of the invading foetal tissue and some of the adventitious maternal blood vessels that reach the face of the placenta are disrupted and release blood into the uterine lumen where the stromal tissue is exposed between the advancing foetal villi and the surviving uterine epithelium. This blood is trapped in folds of the allantochorion, the trophoblast cells of which often contain maternal erythrocytes.

These developmental characters are discussed with reference to their functional significance, and compared with the corresponding changes in the Carnivora, most of which are also characterized by an endotheliochorial placenta of zonary form with haematomata, marginal or otherwise. It is suggested that their occurrence is related to the intermediate position of the endotheliochorial placenta between the epitheliochorial type, in which the uterine glands contribute more importantly to embryonic nourishment, and the haemochorial type, in which transfer from the maternal circulation to the trophoblast is facilitated by the direct contact between them. The mode of implantation is shown to be very different from that in hyrax, which superficially resembles *Loxodonta* in the morphology of the foetal membranes. Comparison is also made with the aard-vaak and the manatee. The aard-vaak has a zonary endothelial placenta, marginal haematomata and a quadrilocular allantois, but does not resemble *Loxodonta* closely in detailed placental structure. The mid-term placenta of the manatee, on the other hand, bears a very striking resemblance to that of the elephant in many respects, especially in the manner in which the trophoblast is modified where it invests large maternal blood vessels. The phylogenetic significance of these similarities and differences is briefly discussed.

INTRODUCTION

The earliest stage hitherto described in the development of the elephant was that of a conceptus collected in Uganda by Dr R. V. Short and described by Amoroso & Perry (1964). In that specimen the foetus was completely formed, with a crown-rump length of about 35 mm. The placenta and foetal membranes had already attained most of the features characteristic of the remainder of gestation, although nucleate erythrocytes still predominated in the foetal circulation. The allantois already filled the chorionic sac, having obliterated the yolk-sac and the exocoel, and the nature of the processes leading to this condition could not be deduced from this or from any of the more advanced specimens then available. These remarks apply equally to the Asian as to the African elephant and the early stages of pregnancy, around the time of implantation, have not hitherto been described for either. The same is unfortunately true of many of the species which most readily invite comparison with the elephant in placentation, such as hyrax, manatee and dugong. Nothing at all appears to have been published concerning the mode of implantation in the two latter genera, and comparison with the present observations is therefore confined to descriptions of early pregnancy in hyrax and in such members of the Carnivora as have been studied, particularly the dog and cat.

The material now available comprises a number of specimens collected by Dr R. M. Laws during conservation work in Kenya (Tsavo National Park) and Uganda (Murchison Falls Park), and the circumstances of this work were described by him (Laws 1969*b*). Emphasis lay on various aspects of the ecology, particularly the population dynamics, of the elephants in those areas, and the results have been summarized by Laws (1969*a*).

Material and methods

Autopsies were carried out in the field, under Dr Laws's supervision, and when a uterine swelling was seen it was excised and subjected to one of two procedures. Some were preserved entire, with a view to retaining the configuration of the foetal membranes for future examination in the laboratory. Others were opened, to allow more immediate penetration of the fixative (10 % formalin or Bouin's fluid) to the embryonic tissue and the uterine mucosa. The latter method has provided material for conventional histology by the excision of small pieces which were embedded and sectioned in paraffin wax. The topography of the membranes was, of course, lost by this treatment; it has been more readily elucidated from the specimens that were preserved intact and stored in 10 % formalin.

Dissection of such specimens, however, was found to be unprofitable because the membranes were often broken and were too easily displaced. One such specimen was impregnated with gelatine, hardened, and hand-sectioned into thin slices. This was fairly successful, but the slices were not suitable for examination under the microscope, and the account which follows is mainly based on specimens that were imbedded in celloidin and sectioned on a sledge microtome. For this purpose, most of the myometrium was dissected away, and the largest specimen so treated was pared down to an ovoid about 60 mm long. Dehydration of such specimens was by immersion in graded alcohols. From absolute alcohol, the specimen was placed in equal parts of alcohol and ether for about three days and then transferred to a 4 % solution of Necolloidine (B.D.H. Ltd) in ether and alcohol. After about 14 days in this mixture the specimen was transferred through 8 % (7 days) to a 16 % solution of Necolloidine. These stages were carried out in a covered, but not airtight, vessel to control the rate of evaporation, and further celloidin was added from time to time as required. The final stage of impregnation occupied about 6 weeks; after this, the block was hardened by evaporation to a rubbery consistency. Final hardening was by immersion in chloroform (24 h) and storage in 70 % ethanol.

Sections were cut transverse to the uterine axis, by means of a Leitz sledge microtome. The routine adopted was to cut 9 sections at 100 μm, followed by 5 sections at 20 μm. The first of the 20 μm sections in each set of five was stained and mounted, providing a series of such sections cut at intervals of 1 mm throughout the block. The remaining sections were retained in sequence by a rod passed through a hole drilled through one corner of the block before it was sectioned. These were stored in 70 % ethanol and were thus available to complete the sequence within any of the 1 mm intervals. Sections taken for immediate preparation were stained with cresyl fast violet, differentiated with acidified ethanol (1 % acetic acid). They were then cleared and mounted in the usual way, on slides of appropriate size.

The youngest specimen in this series was a bilaminar blastocyst, before the first attachment of the trophoblast to the uterine luminal epithelium. Between this and the 35 mm foetus referred to above are seven specimens which provide a relatively complete series covering the early development of the foetal membranes and the evolution of the placental attachment from its earliest stages, through the chorio-vitelline phase, to the establishment of the definitive chorio-allantoic placenta.

THE EMBRYONIC MEMBRANES

Amniogenesis

Implantation is of the central type, as described below, and the amnion is formed by a folding process. This material does not provide stages intermediate between that in which the embryonic shield is continuous with the surrounding (trophoblastic) ectoderm (figure 7, plate 2) and that in which the amnion is closed over the embryo (figure 12, plate 3).

The yolk-sac

The yolk-sac (blastocoel) is relatively large in early stages (figure 7) and continues to increase in volume as the mesoderm extends around it (figures 1, 12). Its trophoblastic covering layer destroys the uterine epithelium over more than half its surface, and begins to invade the underlying mucosa soon after the foetal mesoderm penetrates between it and the yolk-sac endoderm, that is to say, with the establishment of the trilaminar omphalopleur. As the exocoel extends around the yolk-sac the latter is separated from its connexion with the uterine wall at a relatively early stage of development and after this it shrinks rapidly and is not found in specimens in which the allantochorionic trophoblast has begun to proliferate (figure 21, plate 5).

The allantois

The allantois is vesicular and extensive. It was identified as an outgrowth of the hind-gut which just reached to the chorion in a specimen in which the yolk-sac was still near its maximum size and the exocoel was relatively small in volume (WLS 129, figures 1, 12). In its definitive form it is quadrilocular (Amoroso & Perry 1964) and this structure appears to have been attained by the stage shown in figure 21, plate 5, showing a section through a specimen in which the allantois had obliterated the exocoel and had come to fill the whole cavity of the loculus surrounding the embryo.

THE BLASTOCYST BEFORE ATTACHMENT
(WLS 216, figures 4–6, plate 1)

The lumen of the basal part of the uterine horn, where implantation usually occurs, characteristically extends into three deep longitudinal grooves, so that the apposed epithelia form, in transverse section, an irregular Y the arms of which may branch again. The blastocyst settles in one of these grooves and distends it as it grows. The other grooves are only slowly eliminated. Their epithelia, which are closely adherent so that the groove is completely closed, undergo a well defined modification and eventual dissolution. As will be described, they contribute a considerable amount of histiotrophic material that is gradually broken down and is apparently absorbed by the foetal tissue (trophoblast). The position of the 'unoccupied' grooves can still be clearly seen during the early stages of development of the allantochorionic placenta, and the amorphous material derived from them appears to persist into the middle stages of pregnancy.

The earliest implantation site so far examined, and the only specimen representing the pre-implantation condition, was detected at autopsy as a slight swelling of the lower (caudal) part of the uterine horn. The locular enlargement was removed entire and plunged into 10 % formalin for fixation, transport and storage. During the present investigation this specimen was imbedded in celloidin and sectioned in the manner described above. It contained a spherical blastocyst, lodged in and distending one of the luminal grooves (figure 4, plate 1). The blastocyst was about 1 cm in diameter after fixation, probably about 1.5 cm in life.

The deeper tissues, and the blastocyst itself, were poorly preserved in this specimen, and it was not possible to locate the embryonic disc or to deduce its orientation. It seems probable that this blastocyst was bilaminar throughout, for mesoderm formation had proceeded only a little way in another much larger specimen (WLS 129, figure 7, plate 2) described below which was well preserved and in which, by good fortune, sections cut transversely to the uterine axis were approximately sagittal to the embryonic shield.

In the youngest specimen the uterine epithelium had undergone no discernible modification in response to the presence of the blastocyst and the trophoblast was not seen to be attached to it at any point. Over most of its surface the primitive yolk-sac wall lay loosely over the luminal epithelium and across small folds in the latter (figure 5, plate 1). Elsewhere, however, the trophoblast cells were relatively tall, with basally located nuclei, and in such places (figure 6, plate 1) the embryonic tissue was closely applied over the mouths of uterine glands that appeared to be actively secreting.

The uterine glands remain simple, but undergo a moderate degree of enlargement during this stage, before there is any foetal-maternal attachment. The mucosa thickens and the radially disposed glands elongate in the immediate neighbourhood of the developing blastocyst. This thickening of the uterine wall contributes to the locular swelling, perhaps more than the distension of the blastocyst itself. There does not appear to be any regional specialization of the mucosa comparable to that in the rabbit (in which implantation is also of a 'central' type) nor do the uterine glands change dramatically as they do in the cat, which further resembles the elephant in possessing a zonary placenta.

THE CHORIO-VITELLINE PLACENTA

(WLS 33; WLS 129; CMU 254; MFP 245)

Early attachment

One specimen (WLS 33, figure 7, plate 2), a loculus which was preserved entire and imbedded in celloidin, represents a stage in which mesoderm formation has just begun. The embryonic disk is well defined in the sectioned material, which illustrates an early stage in the attachment of the trophoblast to the uterine wall. Figure 7 is of a section cut transverse to the longitudinal axis of the uterine horn, showing the embryonic disk in approximately sagittal section. Its position in relation to the insertion of the mesometrium, however, has been lost in the course of dissection, when most of the myometrium was removed. The embryonic disk faces, but does not attach to, the deepest part of the groove in which the implantation cavity has been formed. The blastocyst has expanded in comparison with that described above (figure 4) to the extent that the mucosal lining of the implantation cavity is slightly attenuated and is shallower over the embryonal than over the abembryonal region of the loculus.

Like the embryonic disk and the trophoblast immediately surrounding it, the abembryonic hemisphere of the blastocyst is free from the uterine wall. Over the intervening circumferential area, the trophoblast is closely applied to the uterine lining. The earliest attachment, therefore, is apparently oriented with respect to the position of the embryonic disk and is not, like the definitive placental band, circumferential to the uterine axis. This does not necessarily imply that the definitive zonary band is formed elsewhere. The latter is established relatively early in pregnancy and its position relative to the uterine axis may well be greatly altered by differential growth within the uterine wall.

The embryonic shield

The relations between the ectoderm, mesoderm and endoderm near the centre of the embryonic shield are shown in figures 8 and 9, plate 2. The ectoderm is four or five cells deep at the centre (figure 8), tapering gradually towards the limits of the shield, beyond which it is single-layered. Beneath the embryonic ectoderm, and extending beyond it in one direction, is the newly formed mesoderm, of irregular thickness, mainly one or two cells. Beyond the margin of the embryonic shield, local proliferations of the mesoderm form pockets or grooves which protrude into the yolk-sac, indenting its endodermal lining (figure 9). These clusters of cells constitute early stages in the formation of 'blood islands' (Islands of Pander), characteristic of the extending area of yolk-sac mesoderm.

The trophoblast

At the stage illustrated in figure 7, therefore, the blastocyst consists of a large primitive yolk-sac; much the greater part of it is as yet bilaminar, and about half its surface is closely applied to the uterine luminal epithelium. There is no 'inversion of the germ layers' or entypy, in the elephant. The yolk-sac reaches its maximum size early in development (see below, and figure 12, plate 3) and is thereafter rapidly reduced in volume as the exocoel extends around it (see below). Before this, and even before the mesoderm extends beyond the immediate vicinity of the embryonic shield, the uterine epithelium begins to undergo a process of attrition over the whole of the area where trophoblast is in contact with it.

The mode of attachment of the trophoblast to the uterine epithelium is in some respects reminiscent of that seen in the cat and dog (see, for example, Amoroso 1952), but it does not involve the extreme modification of the uterine glands that occurs in these species. Moreover, although the luminal epithelium of the loculus is destroyed over more than half its area at a quite early stage in development, a considerable proportion remains intact until much later. Over much of the area of attachment, the trophoblast appears to cause the dissolution of the underlying epithelium while it is merely apposed to its surface, without penetrating it and without any thickening or proliferation of the trophoblast layer. This is shown, for example, in figure 10, plate 2. On the right of the photograph, the luminal epithelium is intact and the trophoblast merely overlies it. On the left of the photograph, in contrast, the uterine epithelium has been lost and the trophoblast is closely applied to the uterine stroma. The uterine epithelium loses its basement membrane, becomes detached from the underlying stroma and is disorganized, often swollen, before its complete dissolution. The cells often appear to undergo nuclear division without cell division, so that they become large and binucleate, but the available material is not sufficiently well preserved to confirm this with certainty. The apical portions of the trophoblast cells become lobate and they probably absorb material resulting from the destruction of the uterine epithelium but, again, the preservation does not permit detailed cytological investigation. It has been found possible to re-imbed the celloidin-imbedded material in epoxy resin for electron microscopy, but it was found that cytoplasmic organelles were not preserved and subcellular structure was not discernible.

In many places within the same specimen, a different appearance is presented as the trophoblast actually undermines the luminal epithelium to some extent, penetrating between it and the underlying stroma (figure 11, plate 2). It has not yet penetrated the stroma itself, and does not appear to be more than single-layered at any point. The endoderm lining the primitive yolk-sac is also seen in these preparations and it is clear that the mesoderm has not yet reached this parietal region.

This process soon leads to a condition in which the trophoblast is very closely applied to the uterine wall over a broad band which (in this specimen) extends around the entire circumference of the conceptus, midway between the embryonic and abembryonic poles.

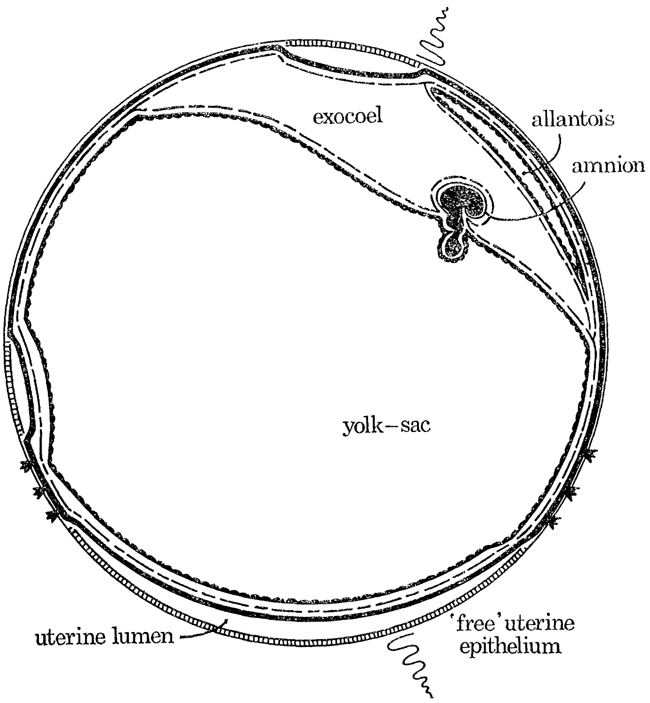


FIGURE 1. The foetal membranes at the stage of chorio-vitelline placentation. Diagram based on measurements of the specimen shown in figure 12, plate 3. It is possible that the mesoderm had not completely enveloped the yolk-sac as shown. The position of the 'unoccupied' luminal grooves is indicated (1 o'clock and 5 o'clock) and the two regions where penetration of the uterine stroma was just beginning are shown at 4 o'clock and 8 o'clock. Trophoblast is shown as a heavy solid line, uterine epithelium is hatched, mesoderm is shown as a broken line and endoderm as a dimpled line.

The fully developed yolk-sac (*trilaminar omphalopleur*) (*WLS* 129)

In the specimen shown in figure 12, plate 3 the yolk-sac has reached the greatest volume seen in this series, and must be near to the maximum size attained during development. It occupies almost the whole of a loculus 37 mm in diameter (after fixation; this probably represents a volume of about 50 ml in life). The mesoderm extends over about one third of the yolk-sac wall, and an exocoel is present. The allantois has just reached the chorion at one place, so the foetal-maternal connexion consists, at various points, of allantochorion, trilaminar omphalopleur or bilaminar omphalopleur, while the uterine lumen remains intact over considerable areas where the trophoblast has not yet become attached to it. These characteristics are illustrated in the drawing (figure 1) based on histological sections made at about the level of that shown in figure 12, plate 3.

By this time, the uterine glands (figure 15, plate 3) have become slightly more elaborate than in the earlier stages. They remain only slightly branched (if at all) but they are coiled and their basal portions are somewhat distended, presumably owing to the occlusion of the gland mouths. They appear to be evenly distributed around the wall of the implantation cavity. The development of the exocoel and allantois is described in more detail below (p. 117). The embryo has already undergone torsion and flexure; its overall length is about 5 mm and it possesses about 15 somites. It is therefore very small in comparison to the volume of the yolk-sac.

The trophoblast

In this specimen (figure 12) the groove in which the blastocyst first settled has been obliterated by the growth of the chorionic sac. Nearly all of this growth is accounted for by the increase in size of the yolk-sac. In figure 1 the proportions are derived from measurements made on serial sections (in celloidin) and it can be seen that considerable areas of the chorionic surface are still unattached. The mesoderm extends completely around the yolk-sac or nearly so, and its limits do not coincide with those of the trophoblast-uterine attachment, so that there are areas where the trilaminar yolk-sac is exposed to the residual uterine lumen.

Examination of the serial sections showed the trophoblast to be attached to the uterine wall over three discrete areas disposed around the 'equator' of the swelling. While they occupy more than two-thirds of the circumference of the middle section, they cover only about two-fifths of the surface of the implantation cavity in this specimen. It was earlier shown (Amoroso & Perry 1964) that the disposition of the placental attachment in its definitive form is very variable in this species. Two or all of the three discrete areas of attachment in the specimen under consideration may have been destined to fuse in the course of development. In later stages the placental band increases in length as the chorionic sac increases in girth, and it also increases in width, but the growth of the chorionic sac is so great that the placental area covers a progressively smaller proportion of its surface as pregnancy advances.

The margins of the areas of intact uterine epithelium are still being eroded by the trophoblast, but even where the trophoblast appears to be firmly attached to the uterine wall there are still remnants of disintegrating uterine epithelium in a few places. Apart from the greater relative extent of the areas of attachment 'implantation' has not proceeded far beyond the condition reached in the younger specimen (WLS 33, above). There are, however, minute areas which show the first signs of penetration of the uterine stroma by proliferative outgrowths of the trophoblast. They are few in number, and their formation and development is more readily followed in other specimens (below) which were not fixed in bulk.

The 'free' uterine epithelium

Of the three areas over which the uterine epithelium is still intact in the specimen (WLS 129) shown in figure 12, two adjoin the 'unoccupied' grooves of the uterine lumen and the third centres on the base of the 'occupied' groove, where the apposed epithelia have not yet been completely separated by the distention of the implantation chamber. In two specimens that were opened before fixation, at a slightly later stage of development, the epithelium was similarly intact in the vicinity of the occluded uterine grooves. There are now three areas of uterine attachment and three of free uterine epithelium because the yolk-sac has expanded beyond the groove in which the blastocyst first settled and its abembryonic trophoblast has made contact with the uterine wall between the two 'unoccupied' grooves. Thus the orientation of the attachment zone is changed and as the labyrinth develops, after the allantois has filled the yolk-sac, by trophoblastic proliferation over the areas of contact already established, it will form a band transverse to the uterine axis. The interruptions often seen in this band probably correspond in position to the uterine grooves, which may or may not be completely opened and obliterated.

The cells of the apposed epithelia within the occluded luminal grooves have a distinctive appearance (figure 13, plate 3). They are swollen, and pools of secretion are trapped between the apposed surfaces. As the epithelia are progressively separated by the growth of the chorionic sac, the material held between them is released and the cells become smaller. In many places the nearby uterine epithelium is thrown into folds with a fern-like appearance in section (figure 14, plate 3).

Formation of exocoel and allantois

The extent of the exocoel at the stage shown in figure 12, plate 3 is indicated in figure 1. It was represented in one other specimen, which was at about the same stage of development but was very poorly preserved. In all later stages the exocoel had been obliterated by the growth of the allantois, the mesoderm of which had fused with that of the chorion, thus effectively establishing the definitive condition (see below).

The specimen under consideration also illustrates a very early stage in the growth of the allantois. In the region shown in figure 12 (on which the diagram shown in figure 1 is based) the allantois appears as a narrow slit in the mesoderm, outside the exocoel. This cavity can be traced in serial sections as a crescentic vesicle (figure 16, plate 3) opening broadly, with little or no 'stalk', into the ventral surface of the embryo (figure 17). The former figure also shows the converging vitelline veins.

Early vascular changes in the uterine endometrium

Immediately after the establishment of the trilaminar omphalopleur, in the areas where the trophoblast is closely applied to the uterine stroma, there is a considerable increase in the capillary network that lies near the surface of the latter. The density of this network can be seen from the frequency of capillaries visible in this region in the section shown in figure 18, plate 4. This is the first indication of the immense development of uterine vessels which eventually supply the placental labyrinth.

As the photographs (figures 18–20, plate 4) show, the establishment of the trilaminar omphalopleur is accompanied also by a change in the appearance of the trophoblast cells. They assume a regular columnar form, with vesicular nuclei in the basal part of the cell. The apical parts of some of the cells contain large 'vacuoles' as though the cells had absorbed or engulfed material from the breakdown of the maternal epithelium, or from uterine secretion. The superficial maternal capillaries lie very near to the newly exposed surface of the stroma, but they appear to retain their integrity, and they do not yet release maternal blood as they do, locally, at a somewhat later stage in gestation after the establishment of the allantochorion.

Trophoblastic penetration of the uterine stroma (MFP 245; CMU 254)

The next stage in the process of attachment consists of the penetration of the endometrium by outgrowths of the trophoblast in those areas where the latter is closely applied to the uterine wall. It begins soon after the extension of the extra-embryonic mesoderm between the trophoblast and the yolk-sac endoderm. The early stages of penetration may precede the extension of the exocoel into these areas, and the process continues after the establishment of the allantochorion, so that the invasive placenta is at first chorio-vitelline and subsequently chorio-allantoic.

Early stromal invasion can be seen in figures 18 and 20. These photographs are from sections of wax-imbedded material from a conceptus that was opened at autopsy, before being immersed in fixative. Spatial relations have therefore been sacrificed to obtain relatively good preservation of the remaining tissue. The trophoblastic outgrowths penetrate via the uterine glands, and during the stages shown in these photographs the interglandular stroma remains intact. Figure 19 shows the close apposition of trophoblast to the stromal surface where there are no glands and no penetration has occurred. The outgrowths are at first purely ectodermal; they soon acquire a mesodermal core, but the material affords no evidence of vascularization of these primitive villi by foetal blood vessels before the establishment of the chorio-allantoic condition.

THE ALLANTOCHORIONIC PLACENTA (WLS 114)

In the most advanced of the specimens that were imbedded and sectioned in celloidin, the embryo was about 20 mm long (figure 21, plate 5). Development has advanced considerably beyond the stage described in the preceding section, for the yolk-sac has shrunk to a minute size with the advance of the exocoel and the latter has, itself, been filled by the allantois, which is already compartmented as it is during the remainder of gestation. The architecture of the compartments cannot be reconstructed from this specimen, as the partitions, which are extremely thin and avascular, have fragmented during fixation and storage. The volume of the fluid-filled compartments of the conceptus (mainly allantois) is actually less at this stage than it was when the yolk-sac was at its maximum size. Penetration of the stroma has proceeded to the formation of areas of villous attachment which already have the essential character of the definitive placenta.

The section shown in figure 21 was cut approximately through the middle of the uterine swelling and approximately through the middle of two areas of allantochorionic penetration; there are also two regions where chorio-uterine attachment is still simple – trophoblast apposed to the unindented surface of the uterine stroma – and two other areas where no attachment has been accomplished and the uterine epithelium is still intact. These distinct regions, as related to the section in figure 21, are shown diagrammatically in figure 2. The 'villous' regions extend over almost one-third of the perimeter, the 'simple' attachment over about two-fifths.

The trophoblastic attachment

The three morphologically distinct regions of the chorionic surface, their differing relation to the underlying maternal tissue, and the transition between adjoining areas, are of special interest with regard to the development of the definitive placental labyrinth and its characteristic 'marginal haematomata'.

The two areas of intact uterine epithelium lie, as in younger specimens, over the 'unoccupied' luminal grooves. The apposed epithelia are by now separated almost to the base of each groove. The cells of the 'fern-like' epithelial projections shown at an earlier stage in figure 14, plate 3, are breaking down and the disintegrating tissue is shed into the residual uterine lumen together with secretion released from the occluded grooves (figure 13, plate 3) to form a dense coagulum trapped between the trophoblast and the uterine wall (figure 26, plate 6). All this amorphous material, secretion and cell breakdown products, appears to be absorbed by the cells of the overlying trophoblast.

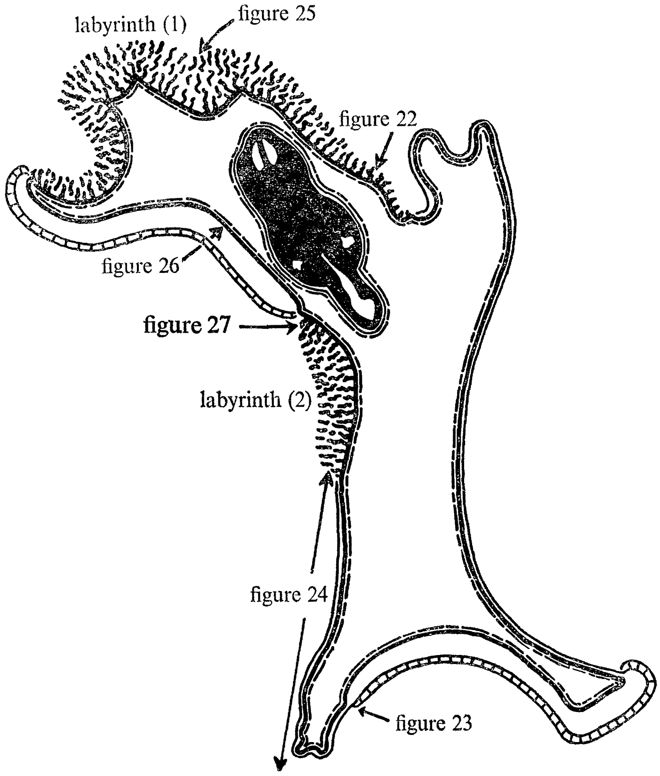


FIGURE 2. Early allantochorionic placentation. Outline of figure 21, plate 5, to show the position of the areas photographed at higher magnification in figures 22–26, plates 5 and 6. The allantois has fused with the chorion over the whole surface of the latter, and the allantochorion is attached to the uterine wall except in two areas where the uterine epithelium persists (hatched lines).

Figure 23, plate 5, shows a region (indicated on the diagram, figure 2) where an area of free uterine epithelium adjoins one in which the trophoblast is adherent to the uterine stroma. The trophoblast is single-layered here as elsewhere, and its cells are of cuboidal or low columnar form, with large round nuclei and prominent nucleoli. This area of 'simple' trophoblastic attachment merges very gradually with one of trophoblastic villous penetration, as shown in figure 24, plate 5. This in turn adjoins the second area around the remnants of an 'unoccupied' luminal groove where trophoblast is not attached to the uterine wall (figure 26, plate 6), and here the transition is abrupt (figure 27). For where the extending villous area (the developing labyrinth) advances into an area of 'simple' trophoblast attachment, the latter is gradually transformed by progressively deeper penetration of the villi into the stroma (figures 24, 22, 25, plates 5 and 6), but where the margin of the developing labyrinth impinges on an area of free uterine epithelium, the trophoblast appears to undermine the uterine epithelium, which is thrown up into folds. This process appears to explain the origin of the marginal haematomata, so characteristic of the mature placenta, and is described in greater detail below.

Continuing in a clockwise direction, as it were, around the perimeter of this section through the chorionic sac (i.e. around the inner surface of the uterine swelling) the next transition is again one where free uterine epithelium (figure 26) abuts on the edge of the developing labyrinth shown in figures 22 and 25. The farther margin of the latter merges gradually into a second region of 'simple' trophoblastic attachment and this adjoins the region of free uterine epithelium the farther boundary of which is that shown in figure 23.

Formation of the placental labyrinth

The earliest penetration of the uterine stroma by trophoblastic proliferations has already been described (p. 118, figures 18–20, plate 4). After the fusion of the allantois with the chorion, these outgrowths acquire a core of allantochorionic mesenchyme, carrying blood vessels into what may now be regarded as a true villus. As can be seen from figure 22, plate 5, these early villi are relatively broad and blunt, and this character is retained at the advancing front of the allantochorion throughout pregnancy. As the villi penetrate deeper into the uterine mucosa, their more proximal portions are progressively attenuated, forming the labyrinthine lamellae described by Amoroso & Perry (1964). As the mesenchymal core is reduced the cell boundaries between adjoining trophoblast cells become indistinct, at least as seen by optical microscopy.

As described above, the trophoblast first invades the uterine stroma by penetration of the uterine glands, and this process apparently continues, so that the glands are destroyed to the depth reached by the advancing foetal tissue. The basal portions of the glands remain, slightly less distended than they were at an earlier stage. The glands also become rather less coiled as the endometrium becomes much thicker in the region of the trophoblastic invasion, and large maternal blood channels form in this tissue and penetrate to within a very short distance of the advancing chorionic villi, and even between them. Thus large maternal vessels carry blood to and from the face of the placenta from a very early stage in its formation.

The appearance of these blood channels at this stage clarifies the interpretation of their nature as seen later in pregnancy (Amoroso & Perry 1964). All the channels have a very tenuous endothelium, and it is difficult to distinguish arteries from veins since there is little or no musculature associated with them. In their definitive condition they are bounded by the endothelium and a non-cellular matrix that is apparently derived from the reduced maternal stroma.

In the specimen under discussion (WLS 114, figure 21, plate 5) the chorionic villi have penetrated less than one quarter of the thickness of the mucosal cushion and the breadth of the villi is approximately similar to that of the intervening columns of maternal tissue.

The placental margin

The gradual transition between the developing labyrinth and an area of 'simple' trophoblastic attachment has already been referred to (p. 119 and figure 24, plate 5). This gradual transition bears little resemblance to the margin of the labyrinth where the latter abuts on one of the areas where the uterine epithelium is still intact and the trophoblast remains unattached to the uterine wall. It is here that the origins of the marginal haematomata of the mature placenta can be traced.

The mode of lateral growth of the labyrinth, and the way in which maternal blood comes to be released into trophoblastic enclosures, can be seen in the region illustrated in figure 27, plate 6, and shown diagrammatically in figure 3. As the earliest chorionic villi advance radially into the uterine mucosa, newly formed ones extend the placenta in a lateral plane. They undermine and destroy the uterine epithelium, the free edge of which is thrust upwards and outwards into the residual uterine lumen. The superficial layer of the uterine stroma is lifted with the epithelium and is therefore exposed to the uterine lumen, as shown at the point marked *x* in figure 3. Branches of the maternal blood vessels that now penetrate to the face of the placenta (see above) are often involved in this disruption. One such vessel is seen near the centre of figure 27, plate 6. It is the breakdown of these vessels that releases maternal blood at the placental margin. The blood escapes into the residual uterine lumen together with cellular detritus from the breakdown of epithelial and stromal tissue. Much of it is enclosed between the newly formed folds of chorion near the placental margin (figure 3, det.). The material derived from the disorganization of maternal tissue is apparently soon broken down, with the exception of the red blood cells. These survive longer and some of them at least are recognizable after they have been ingested by trophoblast cells (p. 122 and figure 30, plate 7).

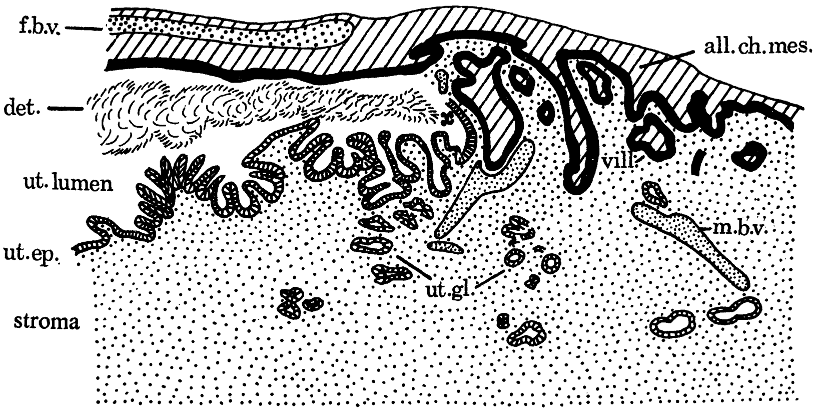


FIGURE 3. The placental margin. Outline of figure 27, plate 6. The allantochorionic mesenchyme (all. ch. mes.) is diagonally shaded and the marginal villi (vill.) are shown. The uterine epithelium is being undermined by the lateral advance of the villi and its limit is indicated at *x*. Maternal blood vessels are shaded by fine stippling, uterine stroma by coarse stippling. Cellular detritus (det.) is seen in the residual uterine lumen.

The marginal haematomata formed in this way differ from those of the cat and dog, by reason of the different architecture of the placenta, particularly with respect to the different rôle of the uterine glands. The comparison will be discussed in a later section (p. 127).

Establishment of the definitive zonary placenta

The present material includes several specimens of more advanced stages of development than the foregoing, but younger than most of those available to Amoroso & Perry (1964). A section through the placental band of one such specimen is shown in figure 28, plate 7. This section is transverse to the placental band and therefore parallel to the axis of the uterine horn. Its marginal region is shown at higher magnification in figure 29. This conceptus was dissected after fixation and storage in formalin and a block of tissue was excised, imbedded in paraffin and sectioned at 6 μm. The foetus was approximately 35 mm long – similar in size to the youngest specimen available to Amoroso & Perry (1964) but better preserved.

In this specimen the chorionic villi have penetrated further into the uterine mucosa, the placental band has increased in extent and now forms a definite girdle, and the conceptus has increased in volume. The margin of the placenta is still being extended laterally by the formation of new villi which bear the same relation to the adjoining uterine mucosa and epithelium as they did in those parts of the earlier specimen where the developing labyrinth met 'free' uterine epithelium. The nature of the uterine lining itself, however, is different. The surface is somewhat folded, but instead of the fern-like proliferations which, as we have seen, were formed before the trophoblast penetrated the stroma at any point, the folds shown in figure 29, plate 7 are covered by a simple cuboidal epithelium. This is evidently newly formed or secondary epithelium which now covers the whole of the extra-placental uterine wall. The chorionic sac can now be lifted or floated free of the uterus except where it is attached by the zonary placental band. The folds in the uterine surface in this region have the appearance of being formed by lateral compression of the stroma in the vicinity of the advancing villous area. The latter extends in the same manner as before, with a similar release of maternal blood. A short distance away from the actual placental margin, the trophoblast cells appear extremely active (figure 30, plate 7) and they evidently ingest maternal erythrocytes, which are to be seen within the apical part of some of the trophoblast cells, together with other large inclusions.

In this specimen the lamellae of endometrial tissue between adjacent trophoblastic villi are already becoming narrow in the 'oldest' part of the placenta – that is, near the centre of the placental band and near the face of the placenta. Otherwise, the villi are similar throughout their depth and there is as yet no differentiation between a basal 'junctional zone' and a 'syncitiotrophoblastic zone' nearer to the foetal side of the placenta. These terms were used by Amoroso & Perry (1964) in describing mid-term and subsequent stages. During the greater part of pregnancy most of the trophoblast is 'syncitial' in that lateral cell boundaries, if they exist, are not discernible by optical microscopy. The basal portions of the villi (distal to the foetus) remain blunt, or lobate, and are sheathed by trophoblast in which the intervening cell boundaries are very prominent. Throughout pregnancy these cells have every appearance of actively ingesting maternal blood cells in the marginal areas of the placenta. In the central region the maternal blood vessels, which are very large in the junctional zone of the mature placenta, are not disrupted, but much of the surrounding endometrial tissue is disorganized and forms a pabulum that is evidently absorbed by the trophoblast cells (Amoroso & Perry 1964, figure 53). In the fresh condition the placental attachment at this stage seems to consist of little but the maternal blood vessels (Amoroso & Perry, 1964, figure 14).

DESCRIPTION OF PLATE 1

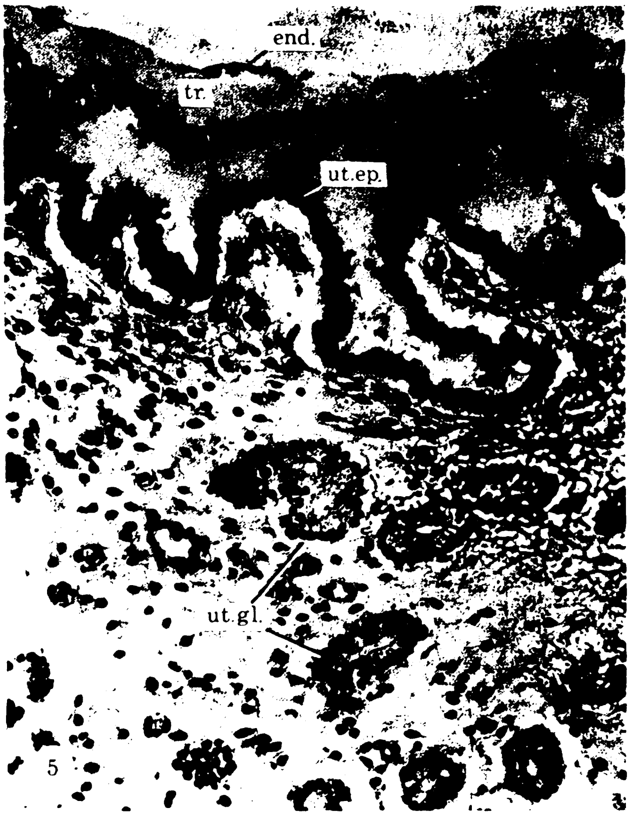
FIGURES 4–6. WLS 216

FIGURE 4. Transverse section through the implantation cavity and surrounding endometrium, in which the slightly coiled uterine glands are radially disposed. Two 'unoccupied' luminal grooves are seen at the bottom of the photograph. (Magn. × 2.5.)

FIGURE 5. Bilaminar yolk-sac wall lying across the mouth of a uterine gland. The trophoblast cells are simple and cuboidal. (Magn. × 100.)

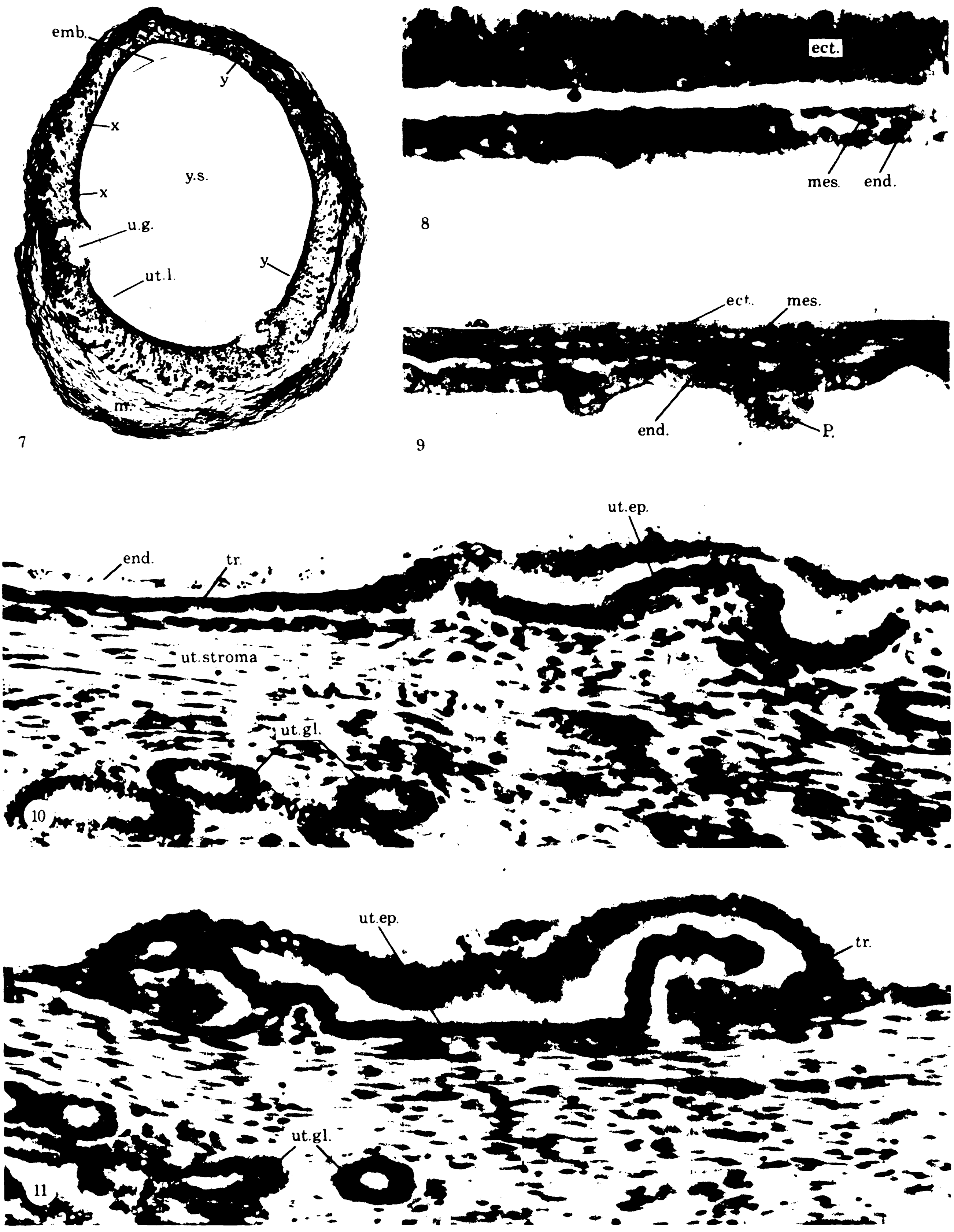
FIGURE 6. Another part of the yolk-sac wall, where the trophoblast cells are columnar, and are closely applied to the gland openings. (Magn. × 100.)


4


5


6

FIGURES 4–6. For description see opposite.



FIGURES 7–11. For description see opposite.

At the stage shown in figure 28, plate 7 (embryo c.r. length 35 mm) the basal portions of the uterine glands beneath the placental band are still intact. They are possibly less distended than at a slightly earlier stage. Somewhat later, but well before mid-term, the whole depth of the endometrium is penetrated and the glands of the placental zone are destroyed. Outside the area of attachment, however, the glands become further distended and the endometrium has a 'sponge-like' appearance. The role of the uterine glands in the early stages of pregnancy in the elephant contrasts strongly with corresponding events in the Carnivora, the placentation of which is strikingly similar in several respects. The present material allows us to pursue, further than was hitherto possible, the comparison of the elephant with certain other groups, with respect to the mode of implantation and the architecture of the placenta and the foetal membranes. Much more will be learnt, however, if it becomes possible to make more immediate investigations of elephant material, especially by the application of electron microscopy, and the comparison with other forms would be greatly enhanced if more were known about other groups, particularly the Hyracoidea and Sirenia.

Discussion

In comparing the foregoing description with the sequence of events in other species, particularly the carnivores and hyrax (*Procavia*), one is struck by a number of obvious similarities and a great many differences of detail. The areas of comparison are in the mode of implantation (the establishment of the placental attachment), the form of the definitive placenta, the origin and form of the 'marginal haematoma' and the disposition of the foetal membranes.

Comparison with the Carnivora

Implantation and early placentation

The elephant obviously resembles the cat and dog in that, in the terminology established by general use, the mode of implantation is central and the blastocyst is large before attachment occurs. Early attrition of the uterine luminal epithelium is common to all three species and is apparently characteristic of Carnivora in general. There is, however, a striking difference in that the uterine endometrium and glands undergo relatively little pre-implantation change in the elephant whereas they are remarkably modified in the carnivores.

Description of plate 2

Figures 7–11. WLS 33

Figure 7. Celloidin section (20 μm) through the conceptus, most of the myometrium having been removed before imbedding. The section is transverse to the uterine axis and passes through the embryonic disk. The trophoblast adheres to the uterine wall between the limits indicated (x- - -x and y - - - y) but the uterine epithelium remains intact over the embryonal pole and between the 'unoccupied' luminal grooves. (Magn. × 1.5.)

Figure 8. Sagittal section through the central part of the embryonic disk. (Magn. × 340.)

Figure 9. Early mesoderm proliferation and early stages in the formation of blood vessels (islands of Pander, P.) in the yolk-sac wall near the embryonic disk. (Magn. × 340.)

Figure 10. Part of the attachment area of figure 7. On the left-hand side of the photograph the trophoblast has destroyed and 'replaced' the uterine epithelium. On the right-hand side, uterine epithelium remains, but it is undergoing dissolution. (Magn. × 300.)

Figure 11. In other places, in the same specimen, the trophoblast forms short invasive pegs which undermine the uterine epithelium. The endoderm layer had become detached in the region photographed, presumably during fixation. (Magn. × 300.)

The most complete description of the placental morphology of the carnivores appears to be that of Duval (1895) for the dog. This author described the corresponding changes in the cat in slightly less detail, and his accounts have been supplemented by Amoroso (1952), especially with reference to the cat. Professor Amoroso has made available to the present writer a collection of histological preparations covering these stages in the cat very completely. In this species (the dog is very like the cat in these features and other carnivores are broadly similar) the uterine lining becomes folded during oestrus to form a series of crypts of approximately equal depth, about one-tenth of the thickness of the mucosa. Radially disposed endometrial glands open into these crypts. The basal portions of the glands, near the myometrium, are slightly distended and coiled; they connect with the surface region by simple tubes. This condition persists during the early part of gestation, up to the time when a visible uterine swelling is caused by the distension of the blastocyst. As this occurs, the distal portions of the uterine glands, immediately beneath the uterine crypts, are enormously distended, forming a 'spongy zone' between the 'compact layer' of epithelial crypts and the underlying stroma. In the dog, 18 days p.c., the trophoblast lies in contact with the uterine epithelium of the inter-cryptal crests over the whole of the lining of the implantation chamber except in the vicinity of the embryonic shield (which is mesometrial in position) and in the areas facing the inter-locular uterine lumen. At this stage the mesoderm extends only a very little way beyond the embryonic shield. The disposition of the conceptus within the uterus is more symmetrical than in the elephant because the blastocyst is truly central and is not lodged in a uterine groove as it is in *Loxodonta*.

In the cat and dog, the uterine epithelium in contact with the trophoblast becomes extremely attenuated and soon disappears over the inter-cryptal crests; that which lines the mouths of the crypts is transformed into a symplasma. This term, introduced by Bonnet, was adopted by Amoroso (1952) to describe a form of tissue breakdown in which fragmented nuclei lie in a mass of homogeneous cytoplasm. This attrition of the uterine lining in the Canidae (and the process is very similar in other carnivores) is reminiscent of its destruction under the influence of the trophoblast in *Loxodonta*; here too the luminal epithelium loses its structure, but if the result can

---

DESCRIPTION OF PLATE 3

FIGURES 12–17. WLS 129

FIGURE 12. Celloidin section (20 μm) through the conceptus, transverse to the uterine axis, after removal of the muscle layers. The arrangement of the embryo and the foetal membranes, as reconstructed from this specimen, is shown in figure 1. The photograph indicates the large size of the yolk-sac in relation to that of the embryo, very little of the volume of the conceptus being occupied by exocoel and allantois at this stage. (Magn. × 1.5.)

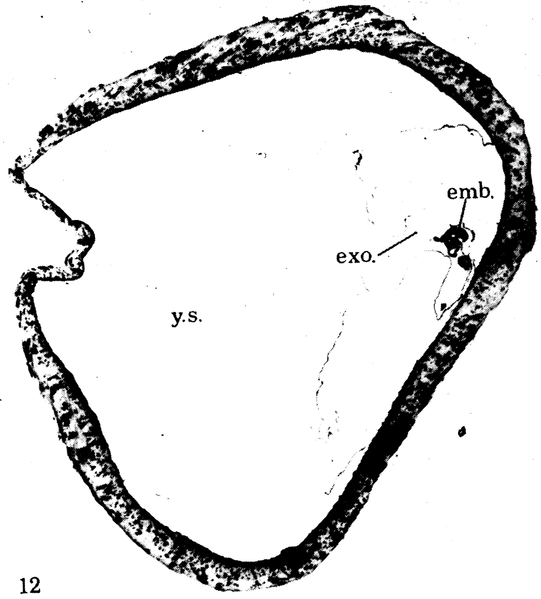
FIGURE 13. The apposed epithelia of the occluded 'unoccupied' luminal groove, with lakelets of secretion trapped between the epithelial cells, which are distinctly columnar. (Magn. × 500.)

FIGURE 14. The fern-like appearance of the uterine epithelium near to one of the 'unoccupied' grooves (see figure 13). The trophoblast is not closely applied to the uterine wall in this area. (Magn. × 300.)

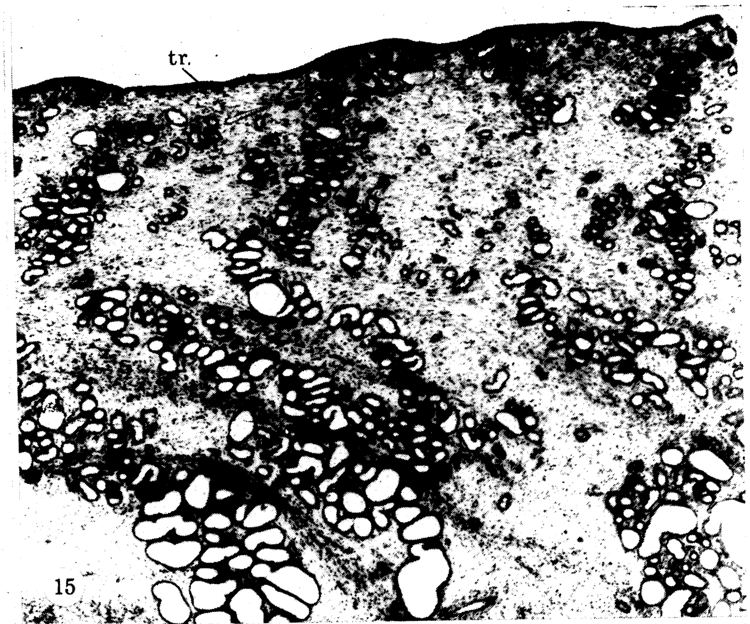
FIGURE 15. The uterine wall in the area of attachment. Trophoblast has 'replaced' the uterine epithelium. The basal portions of the uterine glands are slightly distended, presumably owing to the occlusion of the gland mouths by the trophoblast. (Magn. × 100.)

FIGURE 16. The lumbar region of the embryo in transverse section, showing the highly vascular allantois, and the junction of the vitelline veins. (Magn. × 80.)

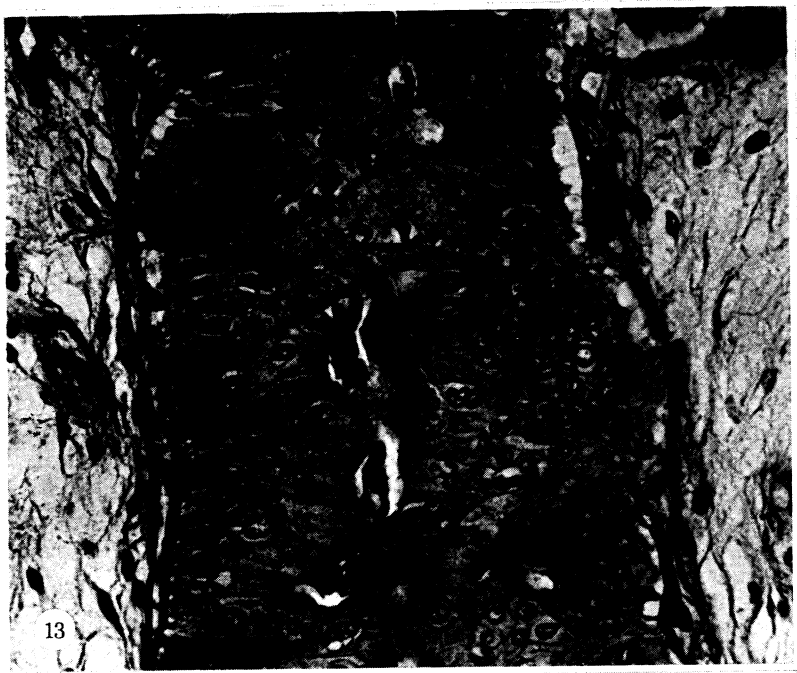
FIGURE 17. The allantois, which is shown dorsal to the embryo in figure 16, can be followed to its origin ventral to the embryo. (Magn. × 80.)



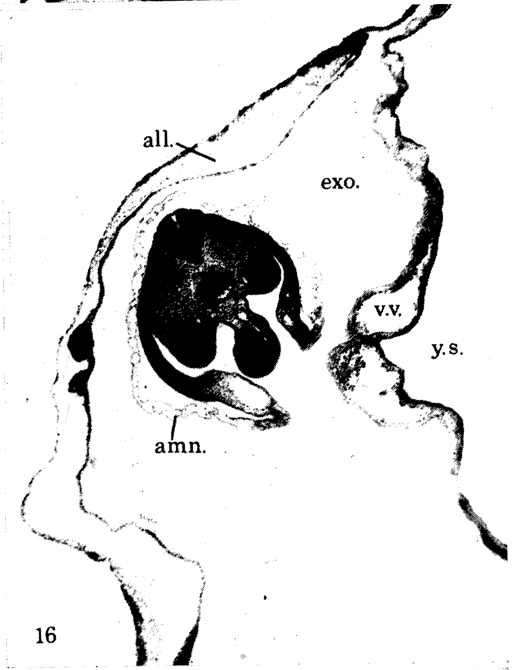
12



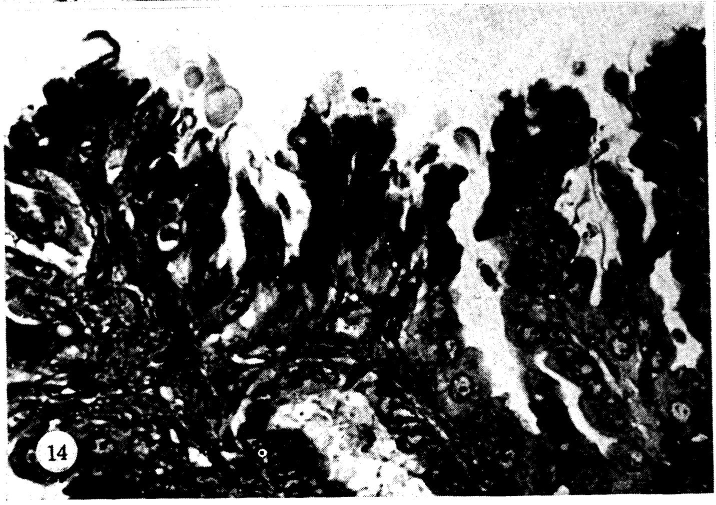
15



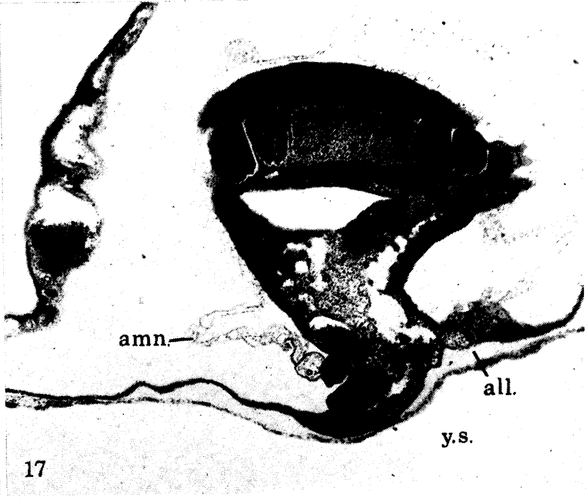
13



16



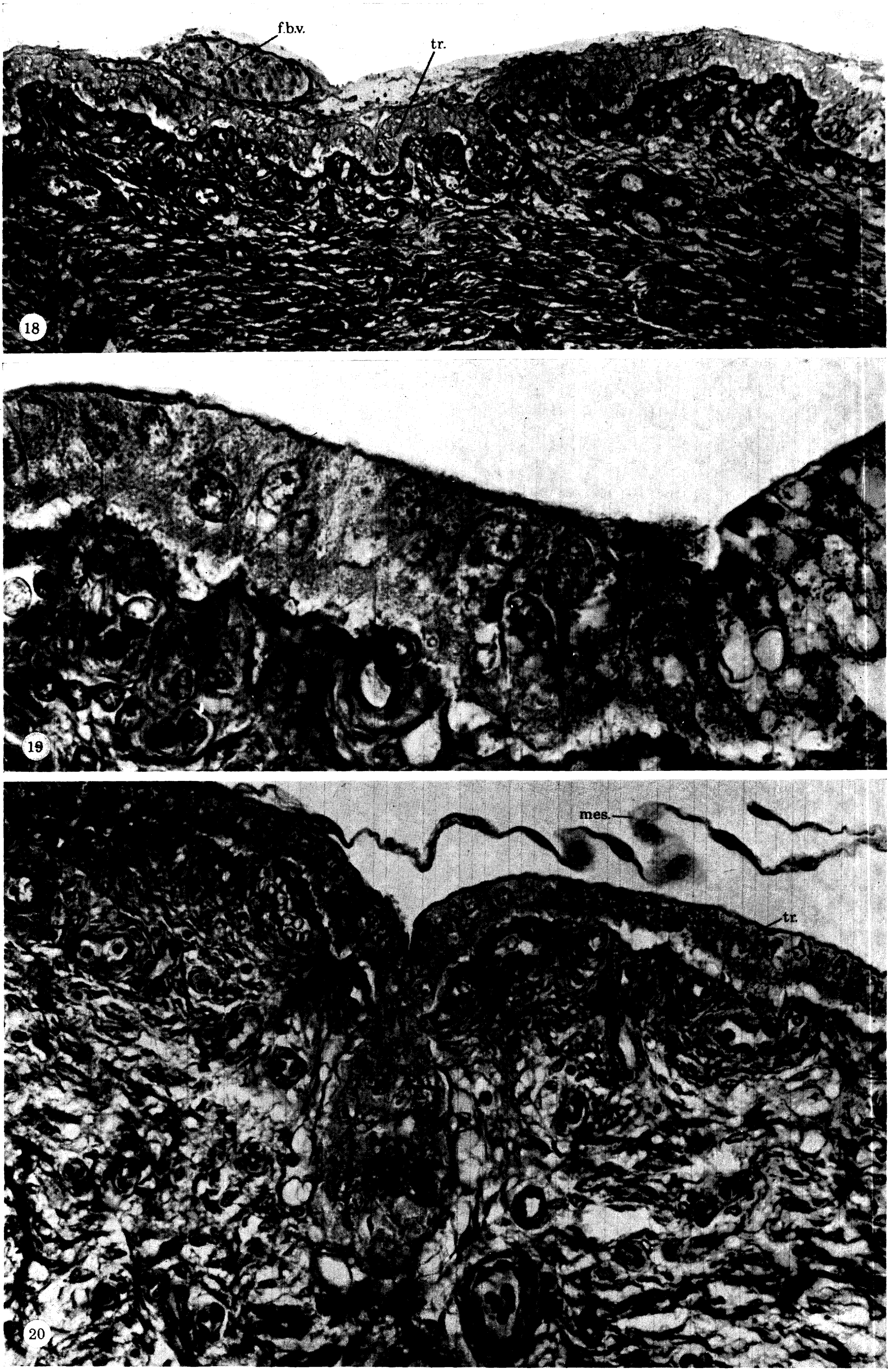
14



17

FIGURES 12–17. For description see opposite.

(*Facing p.* 124)



FIGURES 18–20. For description see opposite.

be described as a 'symplasma' its existence is transient whereas that of the carnivores, augmented by the continuing transformation of cryptal and glandular elements, constitutes a prominent feature of the placenta during at least the first half of pregnancy. Another and perhaps more fundamental difference lies in the fact that the uterine epithelium of the dog or cat undergoes a similar change in the area facing the embryonic shield where it is not in contact with the trophoblast. This fact, together with the degeneration of cryptal epithelium and glandular elements ahead of the trophoblastic penetration, suggests that the changes form part of the decidual reaction of the endometrium to the presence of the blastocyst, not necessarily dependent on a local activity of the trophoblast.

Very early in the development of the carnivore placenta a dense network of maternal capillaries is formed in the stroma of the inter-cryptal crests. This results in the formation of the 'angio-plasmode' of Duval, in which, as Amoroso says, 'the maternal capillaries remain intact, their endothelial walls, supported by a reticular stroma with occasional stromal cells, becoming completely surrounded by trophoblast'. The endometrial blood supply is locally increased and the main uterine vessels are progressively enlarged to accommodate the increasing demands of the conceptus as pregnancy advances. There appears, however, to be no equivalent in the canine uterus to the relatively sudden formation, in the elephant, of adventitious vessels in the endometrium which form the large channels carrying maternal blood through the labyrinth to the face of the placenta.

The placental labyrinth of the carnivores is formed (in those species for which information is available) by the extension of the trophoblastic sprouts to form penetrative villi. The early stages of this growth are similar over both the chorionic and chorio-vitelline areas. Three regions may be distinguished while part of the yolk-sac wall is still beyond the limits of the mesoderm (area vasculosa). After this, about half of the chorionic surface consists of a vascularized trilaminar omphalopleur (the chorio-vitelline placenta) while the remainder is avascular. As the exocoel extends, and progressively separates the yolk-sac from the uterine wall, the allantois makes contact with the chorion and a vascular allantochorionic placenta takes over from a vascular chorio-vitelline placenta, the former developing over the embryonic hemisphere of the chorionic sac while the latter retracts from the abembryonic hemisphere. The trophoblastic elements are similar throughout, their general form and distribution being determined while the bilaminar omphalopleur is still predominant. The elephant resembles the carnivores in the complete investment of the yolk-sac by the mesoderm without the formation of a sinus terminalis.

One outstanding difference between *Loxodonta* and the carnivores, in the mode of establishment of the definitive placenta, lies in the uniformity of the early foeto-maternal attachment in the latter, compared with its variety in the former. The carnivore placenta presents no areas

---

DESCRIPTION OF PLATE 4

FIGURES 18–20. MFP 245. Early stages in the invasion of the uterine stroma by trophoblastic outgrowths. Figure 18 shows, at low magnification (× 100), the indentations where peg-like proliferations of trophoblast are formed, and the numerous capillaries close to the uterine surface. Figure 19 shows, at higher magnification (× 1000), the single-layered trophoblast closely applied to the uterine stroma and possibly exhibiting a lytic or phagocytic activity. Figure 20 shows a slightly more advanced trophoblastic outgrowth penetrating the mouth of a uterine gland (× 150). A strand of mesoderm is seen above the trophoblast in this photograph, but the invading foetal protrusion has as yet no mesenchymal elements.

corresponding to the 'simple' trophoblastic attachment such as is seen, for example, in figures 10, 15, plates 2 and 3. Nor is there an area over which the uterine epithelium undergoes changes like those shown in figure 14, or where it breaks down to form a pabulum like that indicated in figure 27, plate 6 (unless one equates this with the symplasma into which trophoblastic outgrowths intrude over the whole surface of the carnivore placenta).

The definitive placenta

In the definitive placenta of the elephant (Amoroso & Perry 1964) a substantial part of the labyrinth near the base of the placenta is composed of blunt lobate villi covered by cellular trophoblast. This region was referred to as the 'junctional zone' by Amoroso & Perry (1964). Above it the villi or lamellae are slenderer and the margins of the trophoblast cells are indistinct in optical microscopy. The labyrinth of the dog's placenta resembles this 'syncitiotrophoblastic' zone through most of its depth in that an apparently syncitial trophoblast invests the maternal blood vessels. Electron microscopy, however, has revealed that cellular trophoblast also exists in this region in the dog (Anderson 1969) as it does in the cat, where it is more readily seen (Amoroso 1952). Electron microscopy has not yet been applied to the elephant placenta, but it seems more probable that the trophoblast of the labyrinth will prove to be single-layered, with inconspicuous boundaries between the cells, than that it will be found to be multi-layered, with occasional cytotrophoblastic elements interspersed in, or overlying, a syncitium which is only one cell thick. The penetrative apices of the foetal villi of the carnivores, like those of the elephant, are covered by actively phagocytic cytotrophoblast.

Throughout the elephant placenta the trophoblast is separated from the endothelium of the maternal vessels by material of maternal origin, which is homogeneous as seen under the optical microscope. It is conceivable that this material corresponds to the much less prominent 'intermediate layer' of the ferret placenta as described by Lawn & Chiquoine (1965). The trophoblast cells which abut onto this layer in the elephant are clearly distinct and are quite different in appearance from those which line the lamellae of the labyrinth.

Giant cells are present in the labyrinth of some carnivores, and are conspicuous in the cat, but not in the dog (Wislocki & Dempsey 1946; Amoroso 1952). There are no corresponding cells in the elephant placenta.

Description of plate 5

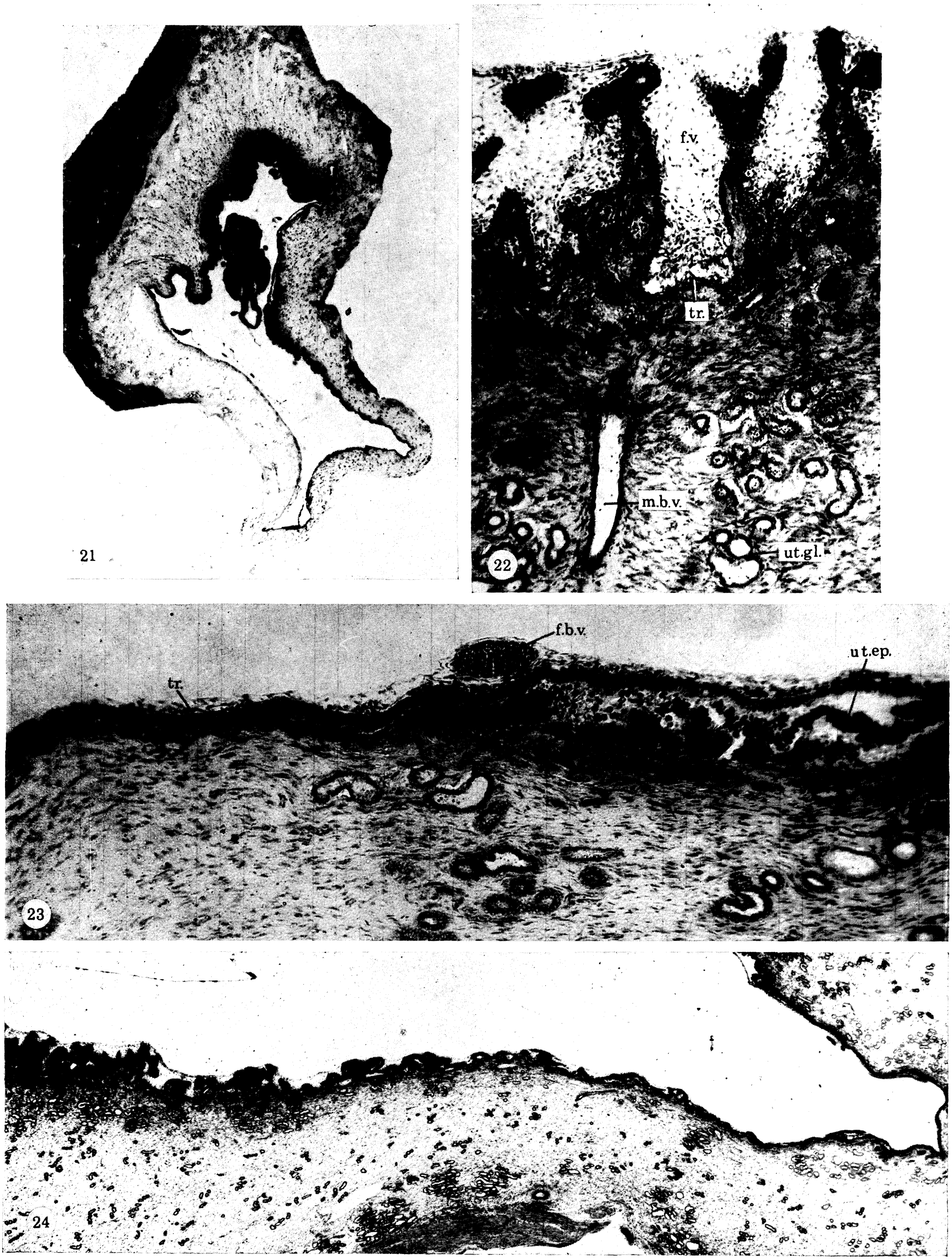
Figures 21–24. WLS 114

Figure 21. Celloidin section (20 μm) through the conceptus, transverse to the uterine axis, after removal of most of the myometrium. The areas of developing labyrinth, simple trophoblastic attachment and 'free' uterine epithelium are indicated in figure 2. The photograph shows the deepening of the endometrium over the region where the development of the labyrinth is most advanced. Large blood and lymph channels can be seen in the mucosa. (Magn. × 2.5.)

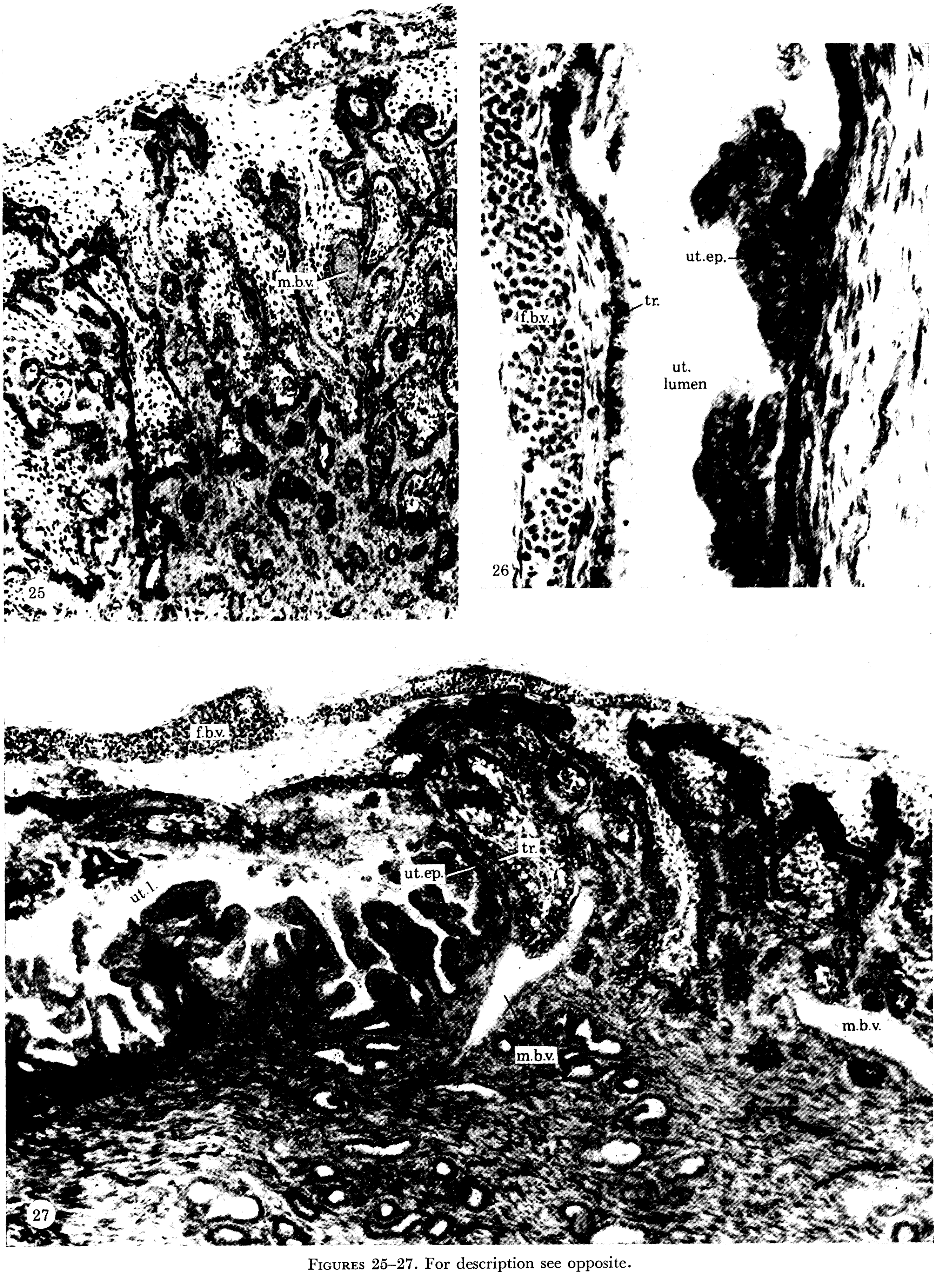
Figure 22. Allantochorionic villi in the region indicated on the diagram, figure 2. Each villus has a thick mesenchymal core (cf. the first trophoblastic proliferations shown in figures 18 and 20). Part of a large maternal blood vessel is seen approaching the face of the placenta. (Magn. × 80.)

Figure 23. Junction between an area of 'simple' trophoblastic attachment (left-hand side) and one of 'free' uterine epithelium (right-hand side); the latter undergoing dissolution. Taken at the position indicated on the diagram, figure 2. There are no large vascular channels in the uterine mucosa in this part of the conceptus. (Magn. × 100.)

Figure 24. Part of the section shown in figure 21, as indicated on the diagram, figure 2. It shows a gradual transition between a villous area and one of 'simple' allantochorionic attachment. (Magn. × 50.)



FIGURES 21–24. For description see opposite.



FIGURES 25–27. For description see opposite.

The marginal haematomata

Extravasation of maternal blood into lakelets trapped between the allantochorionic surface and the uterine wall is characteristic of the Carnivora and also of *Loxodonta*. The essential difference between them lies in the fact that the maternal vessels from which this blood derives are disrupted, in the elephant, by the invasive activity of the chorionic villi whereas, in the carnivores, maternal vessels reach the uterine surface outside the area of trophoblastic invasion and release blood directly into the uterine lumen. In the cat and dog this occurs along both margins of the placental band and the chorionic surface is modified in these areas so that the released blood is held in deep arcades lined by trophoblast. In the elephant the effusion of blood is relatively less and it is trapped between simple chorionic folds which are progressively reduced in height with increasing distance from the central band of attachment.

Here again, the difference between the condition found in *Loxodonta* and that described in various carnivore species can be attributed to the relatively passive rôle of the uterine tissues of the elephant in the reception of the blastocyst. The decidual reaction is much more pronounced in the Carnivora and the early breakdown of maternal tissue involves connective tissue and vascular elements as well as uterine and gland epithelium. In them the maternal tissue of the inter-cryptal crests is degraded to form a symplasma ahead of the advancing trophoblast, and only the capillary endothelium retains its integrity. Within the central and major part of the placenta these capillaries are invested and supported by the trophoblast, whereas in the 'marginal' regions the crests of maternal tissue, attenuated to thin lamellae, are not met by trophoblastic proliferations and therefore project freely into the uterine lumen. The breakdown of the endothelium of the capillaries at the extremities of these lamellae may be due to some lytic influence characteristic of the trophoblast of the marginal regions only. On the other hand, it may be that the capillary walls break down simply because they are not supported by investing trophoblast tissue after the degeneration of the perivascular stroma. As Amoroso (1952) remarked, 'a thorough investigation of the haematoma has yet to be made'.

By reason of its origin, the region of extravasated maternal blood is confined to the placental margins in the elephant, or to areas within the placenta that took origin at the margin. This is not the case in the Carnivora although marginal haematomata are characteristic of some suborders. In others, particularly the Mustelidae, the haematoma may take the form of a large sac formed by invagination of the allantochorion and filled with maternal blood which is released

---

Description of plate 6

Figures 25–27. WLS 114

Figure 25. The deepest part of the developing labyrinth, from the region in figure 21 indicated on the diagram, figure 2. As the villi advance (cf. figure 22) they branch and become more attenuated. The photograph shows the characteristic manner in which the advancing villus surrounds and envelops a large maternal blood vessel. Large foetal vessels are seen in the allantochorion. (Magn. × 55.)

Figure 26. The 'free' uterine epithelium at the point indicated in the diagram, figure 2. The epithelium is produced into outgrowths which appear to dehisce into the residual uterine lumen and contribute to a pabulum, part of which is seen at the top of the photograph. The allantochorion bears a very large foetal blood vessel. (Magn. × 100.)

Figure 27. The 'placental margin' at the junction of an area of developing labyrinth with one of 'free' uterine epithelium, at the point indicated in the diagram, figure 2, and explained in figure 3. The labyrinth, in extending laterally, destroys the uterine epithelium and releases blood from maternal vessels. The resulting cellular and vascular detritus collects in the residual uterine lumen. This is interpreted as the origin of the 'marginal haematoma' (see p. 121). (Magn. × 90.)

into it from maternal vessels over a restricted area of the uterine wall within the zonary placental band at a position determined in relation to the uterine axis. As in the marginal haematoma, the trophoblast of this area is non-invasive. This is the case, for example, in the mink (Enders 1957), raccoon (Biggers & Creed 1962; Creed & Biggers 1963, 1964), skunks (Creed & Biggers 1964), stoat and ferret (Gulamhusein 1974), wolverine (Wislocki & Amoroso 1956) and sea-otter (Sinha & Mossman 1966).

Gulamhusein (1974) adopted the term 'haemophagous organ' coined by Biggers & Creed (1962) to describe this sac in which maternal blood provides a histiotrophic contribution to the nourishment of the foetus. The latter authors claimed that although 'the general opinion is that these maternal blood spaces [haematomata] lie between the uterine epithelium and the chorion...[in] the raccoon these blood spaces are not so disposed'. It is, however, difficult to understand from their description how the structure could be morphologically different, although the pendulous form of the chorionic invagination in *Procyon* and in typical mustelids is very different from that of the marginal haematomata of such species as the dog and cat. Biggers & Creed (1962), indeed, elevated this structure to the status of a second placenta, of haemochorial type, but it is clear that this term is normally applied to a placenta in which maternal blood circulates through channels lined by trophoblast and is returned to the maternal circulation. Although Creed & Biggers (1963) stated that 'We are not yet in a position to tell whether the blood of the haemophagous organ is circulating' it seems clear that it is not. Erythrocytes trapped within this structure, and in the corresponding haematomata of other species, are destroyed there. Drabkin (1970), following earlier work on uteroverdin by Lemberg & Barcroft (1932), demonstrated that the placental haematoma of the dog possesses enzymes capable of degrading haemoglobin and related substances, and compared its activity with that of the liver in this respect. He remarked that 'The bile pigment production of the dog placenta is truly prodigious'.

The most detailed account of the development and structure of a haematoma of the sac-like type found among the mustelids is that of Sinha & Mossman (1966) relating to the sea-otter (*Enhydra*). From their description it is clear that in this species the haematoma arises in a restricted area of the placental band by the extravasation of blood from endometrial vessels under the trophoblast (chorion) into crypts or pockets in the uterine wall. The cavities of these crypts represent the uterine lumen. The extruded blood indents the chorion, which eventually forms the typical cylindrical sac filled with maternal blood cells, and chorionic outgrowths protrude into this mass. The trophoblast lining this sac and covering the chorionic ingrowths

---

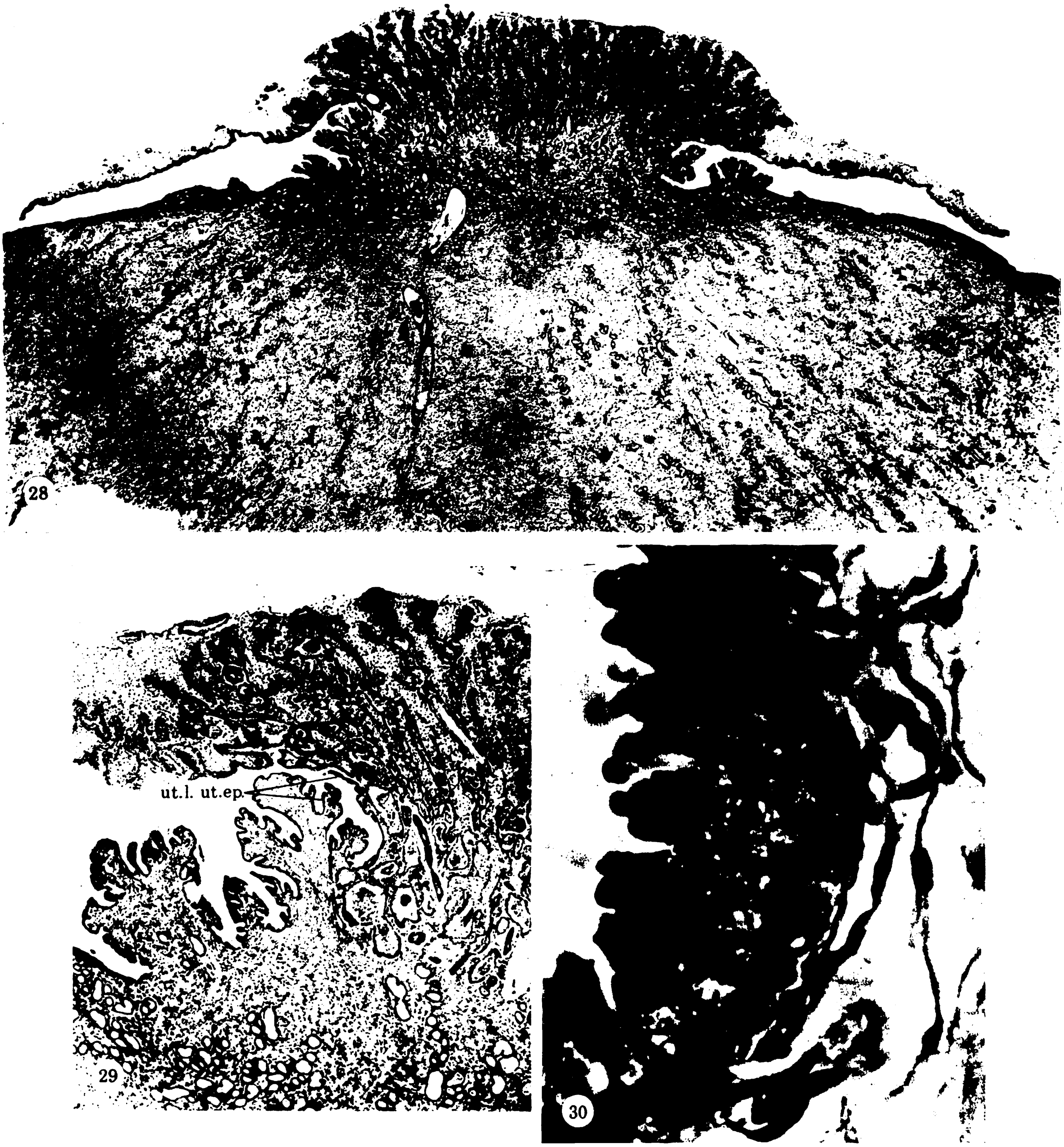
DESCRIPTION OF PLATE 7

FIGURES 28–30. Tsavo 193

FIGURE 28. A section transverse to the placental band soon after it has attained its definitive form (c.r. length of embryo about 35 mm). One large maternal blood channel is seen left of centre. Lateral shrinkage during fixation has compressed the placental villi, together with the necks of the uterine glands that provide the route of penetration. (Magn. × 7.)

FIGURE 29. The placental margin, sectioned in the same plane as figure 28. This is a later stage in the process shown in figure 27, plate 5. The uterine stroma is thrown into folds covered by a simple cuboidal epithelium (cf. the fern-like proliferations shown in figure 27). (Magn. × 50.)

FIGURE 30. At a short distance from the margin of the placenta the trophoblast consists of columnar cells with distinct cell boundaries. Their nuclei are basally located and the distal cytoplasm is heavily vacuolated, many of the vacuoles containing ingested erythrocytes. (Magn. × 100.)



FIGURES 28–30. For description see opposite.

is cellular, whereas that of the remainder of the placental band, where the maternal endothelia are retained, is syncitial.

Electron micrographs of the 'haemophagous organ' of the ferret (Gulamheusein 1974) confirm the phagocytosis of maternal blood cells by the trophoblast and show crowded accumulations of what appear to be lipid droplets in the basal parts of the cells (cf. figure 30, plate 7).

The pendulous type of haematoma is often greatly reduced in size towards the end of pregnancy. This may be due to the continued absorption of maternal blood cells after the cessation of extravasation. The marginal haematomata of the elephant persist to term but are relatively less prominent in the later part of gestation. Amoroso & Perry (1964) described the occlusion of maternal blood channels within the labyrinth in late pregnancy, and it may well be that the seepage of maternal blood at the margins is thereby reduced.

The hyena is unique among the species of Carnivora as yet described. It possesses a haemochorial placenta (Amoroso 1959) which was regarded by Wynn & Amoroso (1964) as more nearly akin to that of the New World monkeys than to the typical carnivore placenta. In this species there is no distinct haematoma such as is prominent in all the other carnivores; maternal blood which circulates in a large 'diffuse lake' on the foetal side of the placental labyrinth is returned to the maternal circulation. The morphology of this system does not suggest a close homology with the marginal or central haematomata of the Canidae and the bears, or with the 'haemophagous organ' of the Mustelidae and the raccoon.

It is probably significant that the placental haematoma is characteristic of the endothelio- or vasochorial placenta, allowing the phagocytosis of maternal blood elements by cytotrophoblast, supplementing the transfer of material to the foetus from the circulating maternal blood within the labyrinth. This is apparently unnecessary in the haemochorial placenta, where all such material is transferred directly from circulating maternal blood to the trophoblast cells or trophoblastic syncitium. The situation is different again in the epitheliochorial placenta of the ungulates, where there is little attrition of maternal tissue and no massive penetration or decidual formation. In them, the morphology varies greatly as between the cotyledonary placenta of the Bovidae, the diffuse placenta of the pig and the 'microcotyledonary' placenta of the horse, but it is characteristic of them all that the uterine glands play a prominent rôle and are more permanent than in the deciduate types of placenta. Thus one may perhaps regard the endotheliochorial or vasochorial placenta as intermediate between the epitheliochorial and the haemochorial types with regard to lessening dependence on uterine glandular activity and histiotrophic nourishment of the embryo, possibly concomitant with the attenuation of the 'placental barrier'.

This interpretation accords with the concept regarding the transfer of iron to the foetus put forward by Wislocki & Dempsey (1946) and endorsed by Huggett & Hammond (1952). On this view the principal transplacental route of iron in the pig is by glandular secretions into the chorionic alveoli, and further evidence of this has come from electron microscopic investigations in this laboratory (Crombie 1972; Crombie & Perry in preparation). In the Carnivora, iron transfer is apparently restricted to the haematomata and its absorption is a function of the cytotrophoblast (Wislocki & Dempsey 1946). Drabkin, whose work on the breakdown of haemoglobin within the haematoma has already been referred to, also subscribed to the view that the principal result of this process was the release of iron for the benefit of the foetus. In the haemochorial placenta the syncitiotrophoblast receives iron from the maternal blood that bathes its surface. That this is the case has been shown by histochemical methods and by radioisotopic labelling; the actual mode of transfer is not clear, but it does not involve the large-scale disruption of maternal erythrocytes.

The foetal membranes

Apart from the zonary form of the placenta, the elephant resembles the carnivores in that the amnion is formed by folding, the yolk-sac is at first large, and the allantois is vesicular and voluminous. The yolk-sac, however, is greatly reduced in size early in gestation, and the allantois is quadrilocular. Examination of the present material confirms the conclusion arrived at earlier (Amoroso & Perry 1964) with regard to the origin of this condition by expansion of the allantoic vesicle between four 'leashes' of umbilical vessels. The four lobes or loculi of the allantois are thus confluent in one region immediately beneath the point where the umbilicus leaves the amnion and divides into four. This region was described as an 'arcade' by Amoroso & Perry (1964) and it evidently corresponds to the 'pyramidal funnel' described by Mossman (1957) in the aard-vark.

Comparison with Orycteropus

The aard-vark, *Orycteropus*, like the elephant, has a zonary endotheliochorial placenta, marginal haematomata and a four-lobed allantois. Mossman (1957) described the labyrinth as extremely shallow and of simple structure, possibly having evolved with relatively little modification from a primitive epitheliochorial type. Scattered throughout the surface of the incomplete zonary band of his single specimen he found 'chorionic vesicles', in which the trophoblast was 'non-syncitial'. He compared these structures to the chorionic vesicles of the pig, the mare and the moles *Talpa* and *Scalopus*. With this in mind he described them as each 'presumably covering the mouth of a uterine gland, yet glands have not been seen in relation to them in the present specimen'. Mossman's photomicrographs do not show that these structures contain maternal blood, and they may be more nearly akin to the chorionic vesicles of the pig than to those of the cat, in which maternal blood cells are found. All such invaginations of the chorion, lined as they are by cytotrophoblast, are in large degree homologous; the variations within and between species may represent modifications in the histiotrophic nourishment of the embryo, ranging from total dependence on glandular secretion to active degradation of maternal blood. An intriguing question in respect of those in which maternal blood is released concerns the cause of the extravasation and the immunological problems that may be thought to arise from it. In this respect again, the placentae which bear haematomata may be thought to be intermediate between those in which no maternal blood comes into contact with the foetal tissue and those in which circulating maternal blood does so. The potential danger of antigen–antibody response in the haematomata is perhaps averted by the degradation of the maternal cells under the action of the powerful enzyme systems of the cytotrophoblast.

Comparison with hyrax

Procavia resembles *Loxodonta* in the possession of a zonary placenta and a quadrilocular vesicular allantois (Wislocki & van der Westhuysen 1940) but it has already been shown (Amoroso & Perry 1964) that these two genera differ very markedly in the detailed structure of the placenta. The present material shows that the mode of implantation and the formation of the foetal membranes is also very different, although implantation is 'central' in both. The hyrax blastocyst enlarges rapidly and the trophoblast becomes attached to the uterine lining of the implantation cavity over its whole surface. The trophoblast very soon becomes multilayered and lacunae, filled with maternal blood, form in the decidual tissue even before this. Amniogenesis is by cavitation and according to Sturgess (1948) an 'amnio-embryonic vesicle' is formed, projecting into the primitive yolk-sac cavity and so invaginating the endoderm in a form of partial inversion of the yolk-sac. Mesoderm is formed, according to the same author, between the ectoderm of the embryonic knot and that of the overlying trophoblast. After cavitation of the embryonic knot to form the amnio-embryonic vesicle the mesoderm extends around it, between the amnio-embryonic ectoderm and the endoderm. The amnio-embryonic vesicle then becomes separated from the overlying trophoblast, but is at the same time connected to it, by the development of a 'coagulated mass' the nature of which remains obscure. Meanwhile, the trophoblast of the yolk-sac area has advanced into the uterine tissue over its whole surface, forming a syncitium within which maternal blood occupies numerous lacunae. The advancing front of the trophoblast is cellular. This penetration, or rather destruction, of the endometrial tissue is not by trophoblastic sprouts or villi, as in the elephant and the carnivores, but by uniform thickening of the trophoblast. The resulting spherical cushion of trophoblast, enclosing maternal blood spaces, resembles the 'ectoplacental plasmodium' of the guinea-pig (Duval 1889) but it extends to an approximately equal depth around the whole surface of the conceptus. This combination of features appears to be unique among all the placental forms hitherto described, and *Procavia* further differs from the elephant, and more markedly from the carnivores, in that the uterine glands appear neither to hypertrophy nor to provide a route of entry for the invading trophoblast.

As far as I can ascertain, there has been no further investigation of the development and placentation of hyrax since the work of Sturgess (1948) in spite of the interesting features to which she drew attention in this widespread and relatively numerous genus.

It is clear that the zonary form of the hyrax placenta is arrived at in a manner different from that of the carnivores or of *Loxodonta* since, in hyrax, proliferation first occurs over the whole surface of the chorionic sac, including the embryonic and abembryonic poles. On the other hand, the compartmentation of the allantois seems to arise in a similar way in *Procavia* and *Loxodonta*. This character appears to have little if any phylogenetic significance and its functional significance is obscure. In the elephant, and perhaps in the manatee (Wislocki 1935) it might conceivably be related to the large volume of allantoic fluid and a need to control its movement, but this could not apply to hyrax and it is apparently unnecessary in other large mammals. The partitions between adjoining loculi are extremely thin and flexible, they are not vascularized and do not bear the 'allantoic pustules' that are prominent on the endodermal surface of the allantochorion (see, for example, Amoroso & Perry 1964). The compartmentation of the allantois thus appears to be simply a consequence of the early branching of the umbilical vessels and their being carried to the allantochorionic surface by the shortest route before the distension of the allantois.

Comparison with Sirenia

The only sirenian species for which any adequate account of the placenta exists is the Florida manatee, *Trichechus latirostris* (or *T. manatus*), a single specimen of which was described by Wislocki (1935). As the foetus was '44 cm from snout to tip of tail' it was presumably in the first half of gestation, but the placenta and foetal membranes had attained the definitive condition. No other specimens of this species or of any of the other sirenian species have been examined to date, if one excepts the 'necessarily brief' descriptions of 'rather poorly preserved specimens of *Halicore dugong*' by Harting (1878) and Turner (1889) cited by Wislocki.

The generalization proposed in an earlier paragraph (p. 129), that haematomata or comparable structures characterize the placentae of endothelial type, is at first sight compromised by Wislocki's description of the placenta of the manatee, since he concluded, with some reservation, that it must be considered to be haemochorial, and he also found areas 'the equivalent of the haematomata...of carnivores'. The latter comprised 'a series of recesses or lacunae containing stagnant maternal blood which is being actively phagocytized by trophoblastic cells. These recesses containing extravasated maternal blood constitute a narrow zone covering the entire placenta. Thus provision is made in this region for histiotrophic nourishment of the fetus by the destruction and assimilation of stagnated maternal blood.' In classifying the manatee placenta as haemochorial, Wislocki remarked that 'The decision has not been simple to make, because of the complexity of the tenuous layer of cells between the two circulations'. It is evident from his description, and particularly from his photomicrographs, that the relation of the trophoblast to the maternal vessels within the labyrinth in the manatee is remarkably like that found in *Loxodonta*. Wislocki's Fig. 19 is virtually identical with, for example, figure 42 in the description of the elephant placenta by Amoroso & Perry (1964). In both, the trophoblast is modified wherever it encloses a maternal vessel and the latter does not possess a typical endothelium. In the elephant, however, what appears to be a discontinuous endothelium, resting upon a collagenous basement membrane, interposes between the maternal blood and the trophoblast. Thus it was stated that 'It is apparent that the extent to which the endothelium is preserved intact varies a good deal' along the walls of the maternal vessels. Similarly, Wislocki remarked that 'some of the homogeneous cells making up the lamina [in the manatee] may be swollen and altered maternal endothelium'. His photographs show what might be the collagenous membrane more clearly seen in our elephant material. In both cases there was hesitation before the manatee was declared 'haemochorial' and the elephant 'endotheliochorial' (or 'vasochorial'). Earlier stages in the development of the manatee placenta should prove most interesting, for it is possible that its haemochorial condition is not acquired until some time later than the first development of the labyrinth, and the fact that the enclosures of extravasated maternal blood are confined to the (foetal) surface of the placenta may indicate that they are formed early and are perhaps reduced as the labyrinth acquires its haemochorial condition and the placenta becomes less dependent upon histiotrophic supplementation.

The resemblance between the manatee and the elephant is further emphasized by the variations in trophoblastic cell form within the labyrinth in each of them. In Wislocki's words: 'The trophoblast cells lining the pockets of [extravasated maternal blood] are unlike the trophoblastic cells seen in the form of syncitium in the bulk of the placental labyrinth or as small-celled cytotrophoblast described at the growing base of the placenta.' The three cell types he described are exactly matched in the elephant placenta.

A curious feature of the manatee placenta examined by Wislocki (1935) was the presence near the placental margin of 'about a dozen minute, scattered areas, some two to four millimeters in diameter, in which chorion and uterine epithelium are fused, leading to the formation of minute, accessory placental areas.' Wislocki describes them as having a villous structure, similar to that of the main placental band, with chorionic villi penetrating a 'cushion of proliferated endometrium' which, in his illustration, appears very like an early stage in the development of the labyrinth of *Loxodonta*. In the absence of any specimens of the manatee representing earlier stages of development it is not possible to determine the origin of these discrete villous patches but their presence, together with the fact that in the manatee, as in the elephant, 'the uterine glands play no conspicuous rôle in the formation of the placenta', and the similarity of the two species in the way in which the umbilical vessels reach the allantochorion, suggests that the early placentation of the manatee is more likely to resemble that of the elephant than that of the carnivores.

Phylogenetic significance?

In comparing the placentation of the elephant with various other species or groups of mammals whose placental morphology resembles it in some degree, I have not so far attempted to attribute phylogenetic significance to the similarities or dissimilarities. In my view these characteristics are of limited value in assessing phylogenetic affinities, especially as it becomes evident that important differences in placental function are not necessarily reflected in discernible morphological differences. This caveat notwithstanding, placental similarities that accompany similarities in characters of greater phylogenetic value must be regarded as additional evidence of such affinity. Wislocki & van der Westhuysen (1940) presented evidence of this kind concerning hyrax and the manatee. From the comparison they concluded that 'It becomes apparent at once that the placentae of these two forms are very intimately related. The manatee placenta which was described [Wislocki 1935] corresponds almost exactly to that stage in *Procavia* in which the definitive zonary placenta has formed. In regard to the topography of the fetal membranes, as well as of the placenta, the two agree completely, leaving no doubt as to their close kinship.' These authors went on to say that the relationship of *Procavia* to other groups was much less clear. They suggested that information about the early stages of hyrax development might throw light on this problem, but the later work of Sturgess (1948), which provided this information, certainly did not fulfil this hope. Early stages of manatee development have not yet been described; when this is done it will be interesting to learn how far they resemble those of hyrax and to what extent they bear out the conclusion drawn from comparison of the fully differentiated placentae.

Wislocki & van der Westhuysen also suggested that the elephant, manatee and hyrax should be regarded as a group of closely related forms, but the subsequent description of the elephant placenta by Amoroso & Perry (1964) showed that its structure was not what had been supposed, and the present investigation has shown that *Loxodonta* and *Procavia* also differ markedly in their implantation and early development. Wislocki (1935) rightly attached little taxonomic weight to the common possession of a zonary placenta, and Wislocki & van der Westhuysen regarded the possession of a quadrilocular allantois by the lemurs as no evidence of relationship to other forms in which it occurs. In this case, this item is not associated with other points of similarity and they concluded that such coincidences 'probably represent independent but parallel developments in quite unrelated groups'.

At the present time, morphology is studied with a view to elucidating function rather than establishing phylogenetic affinities, and it is from this standpoint that the placenta of the elephant has been compared with those of the Carnivora in the foregoing discussion. It is tempting to suppose that the progressively intimate relations between foetal and maternal tissue, seen in the sequence from epitheliochorial to haemochorial types, represents an advance, possibly related to the development of increasingly effective ways of meeting the problems posed by the presence of the foetus as an allograft. The fact that all three types of placental structure are distributed among the living orders of mammals in a way that appears to bear little relationship to their phylogenetic affinities, suggests that, in Wislocki's words, they 'represent independent but parallel developments'.

The diversity of placental types is obviously associated to some extent with differences in the mode of implantation, and this in turn seems likely to be related to the 'recognition' of pregnancy – the alteration of the reproductive rhythm to accommodate gestation, generally involving the modification of luteal function. As the functional corpus luteum appears to be a specifically mammalian attribute it is the more surprising to find such diversity as exists in the way in which it is controlled and the uses to which it is put (see, for example, Heap, Perry & Challis 1973). Although the placenta plays a key rôle in the hormonal maintenance of pregnancy, the variations in function are not clearly related to differences in structure, even at the cellular level. As an example; the epitheliochorial cotyledonary placenta of the sheep produces progesterone, whereas the very similar placenta of the closely related goat does not (Linzell & Heap 1968). Progesterone is pre-eminently the hormone of pregnancy and the elephant has already been shown to be exceptional in that its luteal tissue has remarkably little capacity to synthesize this steroid (Smith, Hanks & Short 1969) and the circulating levels of progesterone are extremely low. Whether the elephant placenta synthesizes progesterone, which is perhaps metabolized within the uterus, remains to be determined. Thus, although it is safe to assume that the extravasated maternal blood trapped in the marginal haematomata of the elephant placenta will be utilized in a way very similar to that in the corresponding structures in the carnivore placenta, the reproductive hormonal mechanism cannot be inferred by comparison and requires to be investigated in the species concerned. It is to be hoped that this will prove possible, in the case of *Loxodonta*, in the laboratories now established in Africa.

I am greatly indebted to Dr R. M. Laws for putting at my disposal the material on which this account is based. The circumstances of its collection have been briefly described in the Introduction. I am also greatly indebted to Professor E. C. Amoroso, C.B.E., F.R.S., for his continued interest in the investigation and his help in the interpretation of the histological material and in the preparation of this account. Mr L. G. Jarvis and Mr I. S. King gave valuable advice on histological techniques, Mr King prepared the large celloidin sections and Mr A. L. Gallup prepared a number of the photomicrographs.

REFERENCES

Amoroso, E. C. 1952 Placentation. In *Marshall's physiology of reproduction* (ed. A. S. Parkes), 3rd ed., vol. 2, 127–311.

Amoroso, E. C. 1959 Structural and functional aspects of the placenta. *Ann. N.Y. Acad. Sci.* **75**, 855–872.

Amoroso, E. C. & Perry, J. S. 1964 The foetal membranes and placenta of the African elephant (*Loxodonta africana*). *Phil. Trans. R. Soc.* B, **248**, 1–34.

Anderson, J. W. 1969 Ultrastructure of the placenta and foetal membranes of the dog. I. The placental labyrinth. *Anat. Rec.* **165**, 15–35.

Biggers, J. D. & Creed, R. F. S. 1962 Two morphological types of placentae in the raccoon. *Nature, Lond.* **194**, 103–105.

Creed, R. F. S. & Biggers, J. D. 1963 Development of the raccoon placenta. *Am. J. Anat.* **113**, 417–445.

Creed, R. F. S. & Biggers, J. D. 1964 Placental haemophagous organs in the Procyonidae and Mustelidae. *J. Reprod. Fert.* **8**, 133–137.

Crombie, P. R. 1972 The morphology and ultrastructure of the pig's placenta throughout pregnancy. Thesis, University of Cambridge.

Drabkin, D. 1970 Hemophagous organ of the placenta and *in vitro* studies of endogenous carbon monoxide production. *Ann. N.Y. Acad. Sci.* **174**, 49–63.

Duval, M. 1889–92 Le placenta des rongeurs. *J. Anat. Physiol., Paris* **25**, 309–342, 573–627; **26**, 1–48, 273–344, 521–592; **27**, 24–73, 344–395, 515–612; **28**, 58–98, 333–453.

Duval, M. 1893–95 Le placenta des carnassiers. *J. Anat. Physiol., Paris* **29**, 249–340, 426–465, 663–729; **30**, 189–240, 649–715; **31**, 38–80.

Enders, A. C. 1957 Histological observations on the chorio-allantoic placenta of the mink. *Anat. Rec.* **127**, 231–245.

Gulamhusein, A. P. 1974 Studies of reproduction in the Mustelidae. Thesis, University of London.

Heap, R. B., Perry, J. S. & Challis, J. R. G. 1973 Hormonal maintenance of pregnancy. In *Handbook of physiology*, §7 (*endocrinology*), vol. 2, pt. 2 (ed. R. O. Greep), 217–260.

Huggett, A. St G. & Hammond, J. 1952 Physiology of the placenta. In *Marshall's physiology of reproduction* (ed. A. S. Parkes), 3rd ed., vol. 2, 312–397.

Lawn, A. M. & Chiquoine, A. D. 1965 The ultrastructure of the placental labyrinth of the ferret (*Mustela putorius furo*). *J. Anat.* **99**, 47–69.

Laws, R. M. 1969*a* Aspects of reproduction in the African elephant, *Loxodonta africana*. *J. Reprod. Fert.* Suppl. **6**, 193–217.

Laws, R. M. 1969*b* The Tsavo Research Project. *J. Reprod. Fert.* Suppl. **6**, 495–531.

Lemberg, R. & Barcroft, J. 1932 Uteroverdin, the green pigment of dog's placenta. *Proc. R. Soc. Lond.* B **110**, 362–372.

Linzell, J. L. & Heap, R. B. 1968 A comparison of progesterone metabolism in the pregnant sheep and goat: sources of production and an estimation of uptake by some target organs. *J. Endocr.* **41**, 433–438.

Mossman, H. W. 1957 The foetal membranes of the aard-vark. *Mitt. Natf. Ges. Ber.* (N.F.) **14**, 119–127.

Sinha, A. A. & Mossman, H. W. 1966 Placentation in the sea otter. *Am. J. Anat.* **119**, 521–553.

Smith, J. G., Hanks, J. & Short, R. V. 1969 Biochemical observations on the corpora lutea of the African elephant, *Loxodonta africana*. *J. Reprod. Fert.* **20**, 111–117.

Sturgess, I. 1948 The early embryology and placentation of *Procavia capensis*. (Thesis, University of Witwatersrand, 1947). *Acta zool., Stockh.* **29**, 393–479.

Wislocki, G. B. 1935 The placentation of the manatee (*Trichechus latirostris*). *Mem. Mus. comp. Zool., Harvard* **54**, 159–178.

Wislocki, G. B. & Amoroso, E. C. 1956 The placenta of the wolverine (*Gulu gulu luscus* (Linnaeus)). *Bull. Mus. comp. Zool., Harvard University* **114**, 91–100.

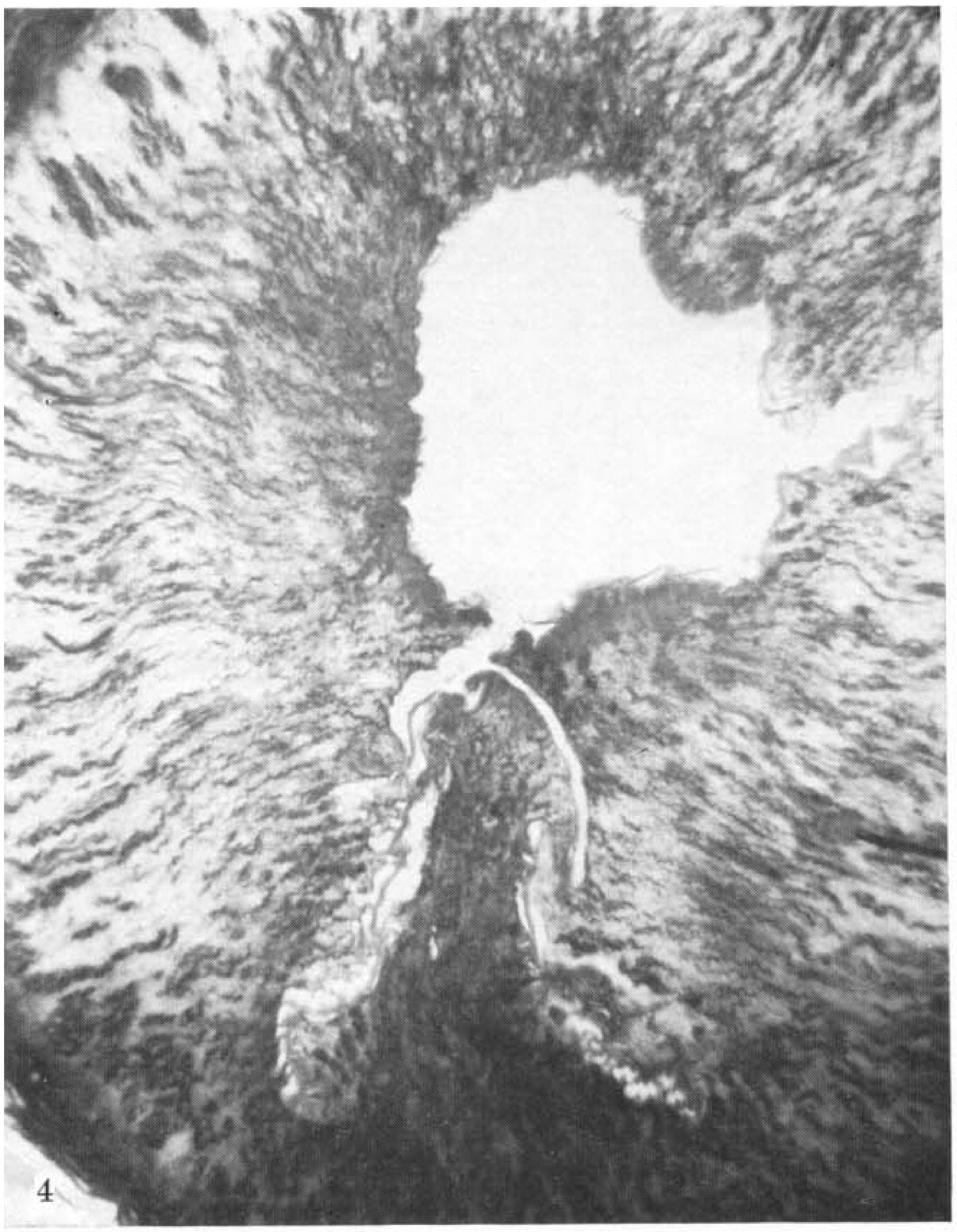
Wislocki, G. B. & Dempsey, E. W. 1946 Histochemical reactions in the placenta of the cat. *Am. J. Anat.* **78**, 1–45.

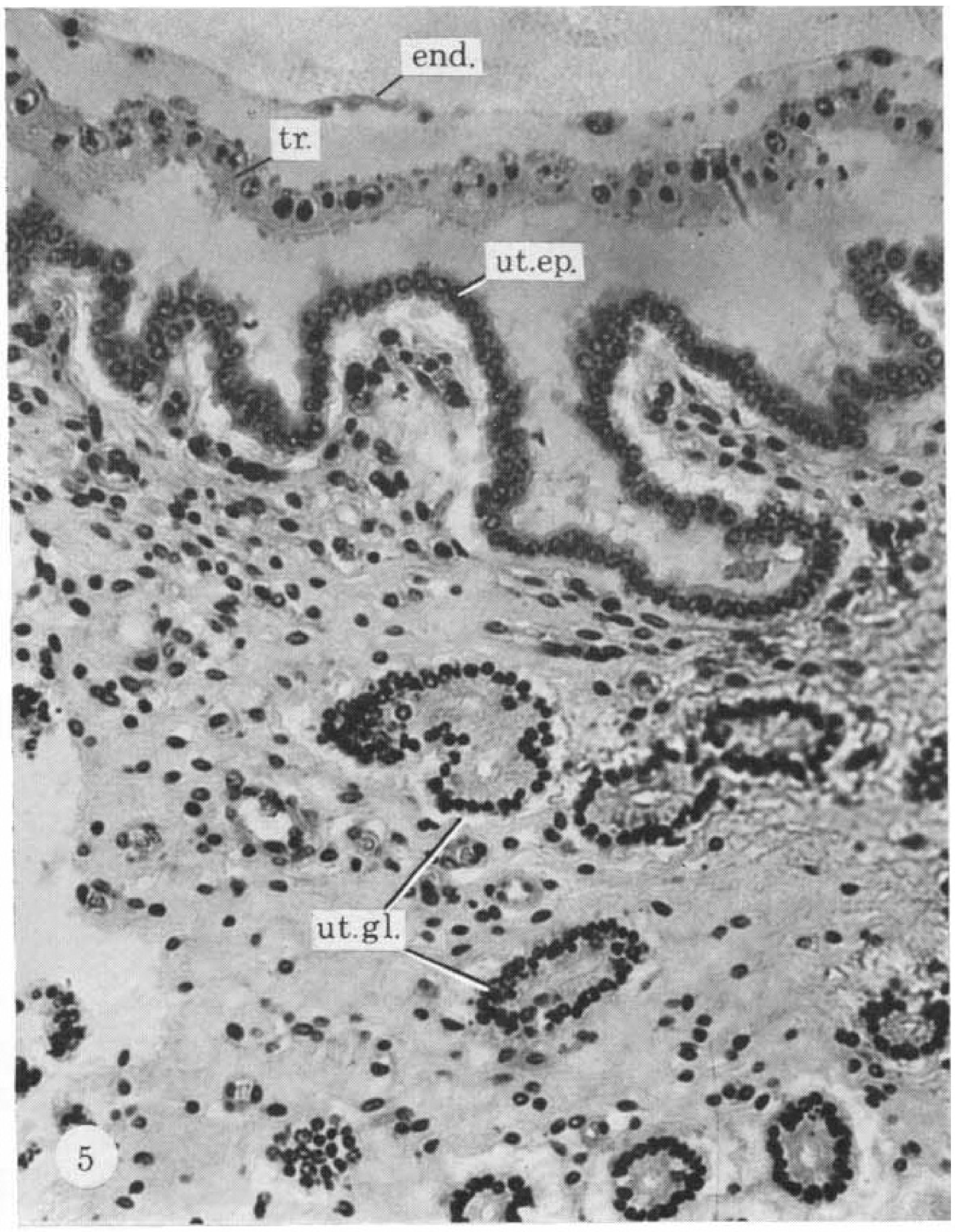
Wislocki, G. B. & van der Westhuysen, O. P. 1940 The placentation of *Procavia capensis* with a discussion of the placental affinities of the Hyracoidea. *Contr. Embryol. Carneg. Instn* **28**, 65–68.

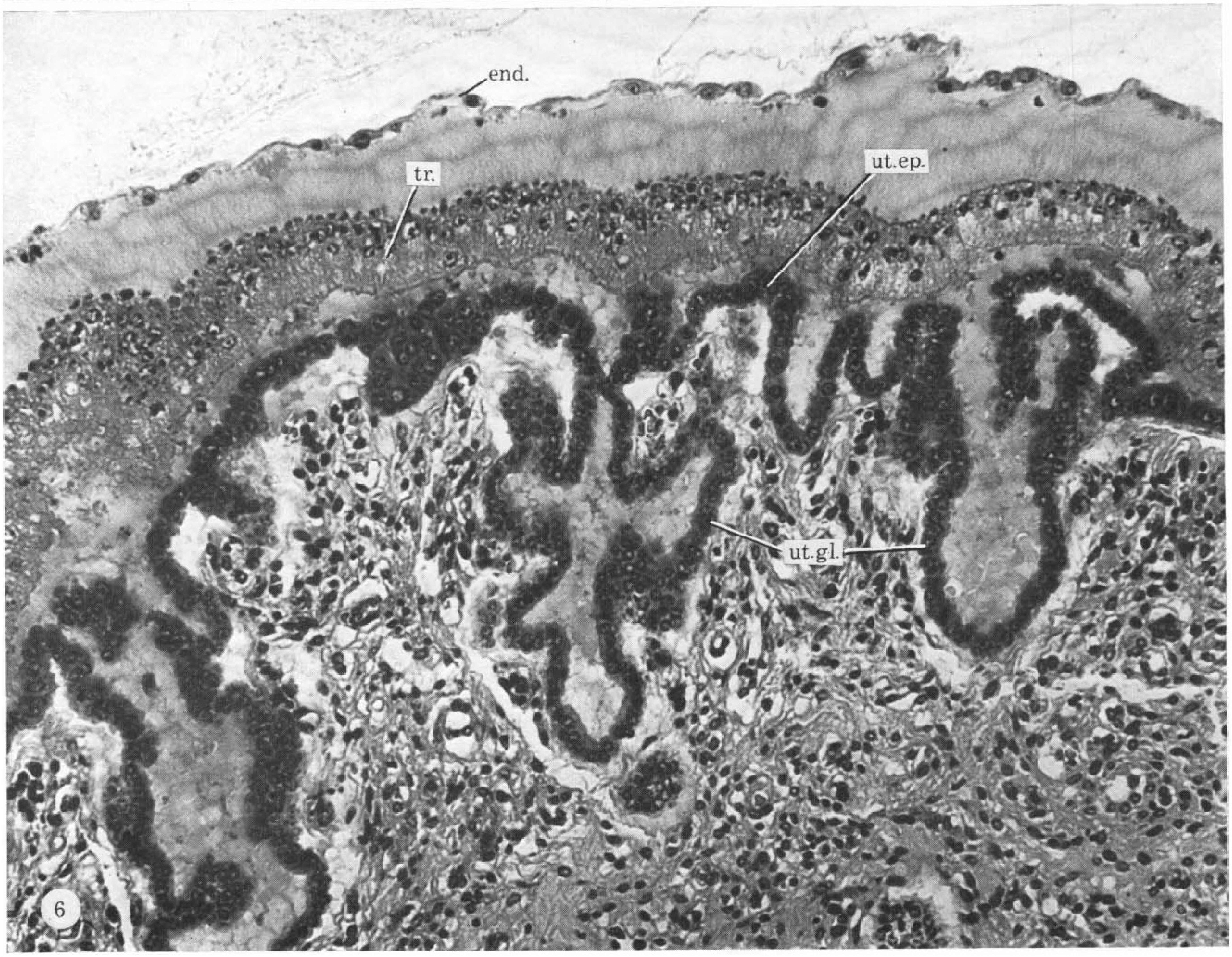
Wynn, R. M. & Amoroso, E. C. 1964 Placentation in the spotted hyena (*Crocuta crocuta*, Erxleben) with particular reference to the circulation. *Am. J. Anat.* **115**, 327–361.

LIST OF ABBREVIATIONS USED ON PLATES 1–7

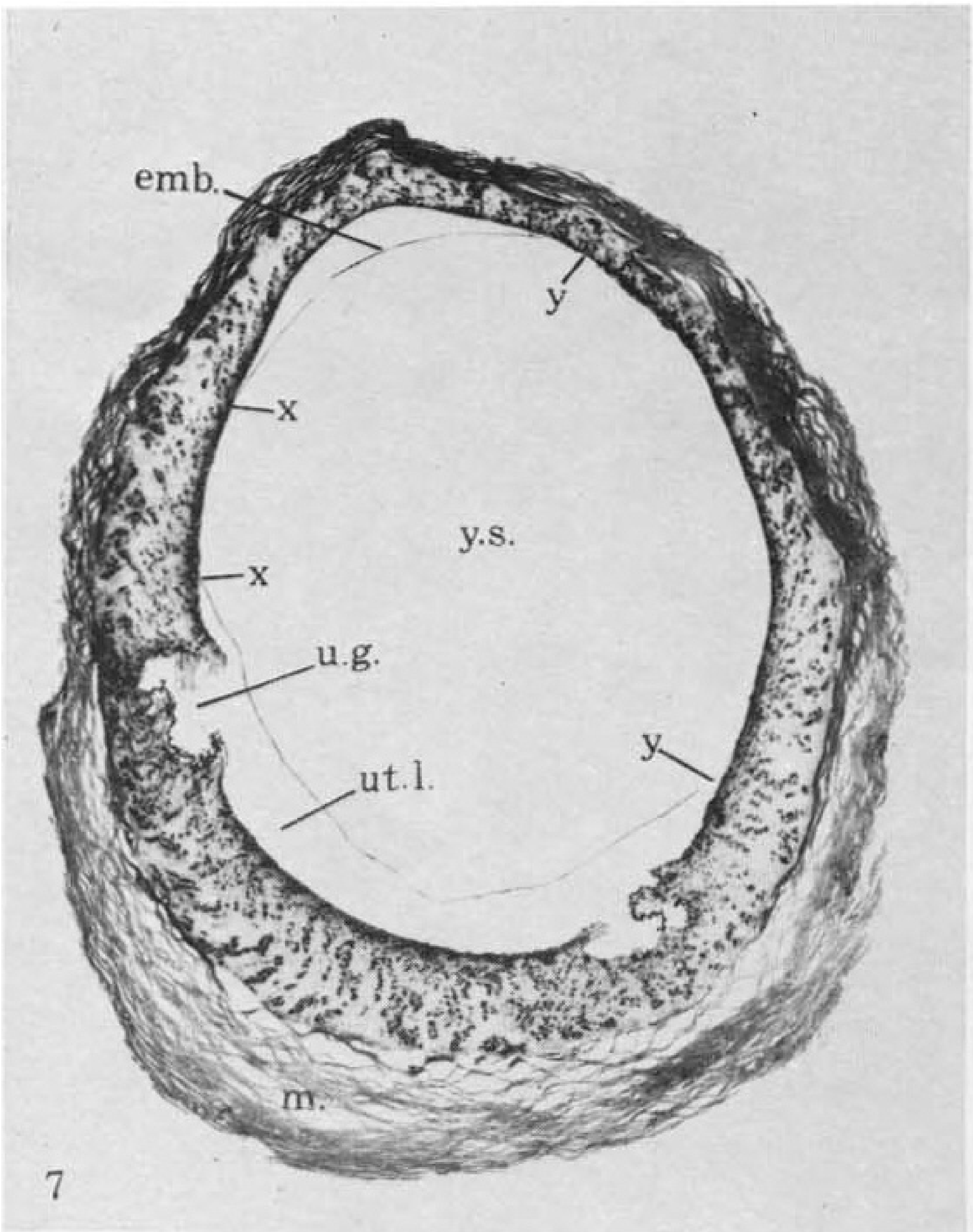
all.	allantois	mes.	mesoderm
amn.	amnion	P.	island of Pander
ect.	ectoderm	tr.	trophoblast
emb.	embryo	u.g.	‘unoccupied’ groove
end.	endoderm	ut. ep.	uterine epithelium
exo.	exocoel	ut. gl.	uterine gland
f.b.v.	foetal blood vessel	ut. l.	uterine lumen
f.v.	foetal villus	v. v.	vitelline vein
m.b.v.	maternal blood vessel	y. s.	yolk-sac
m.	myometrium		

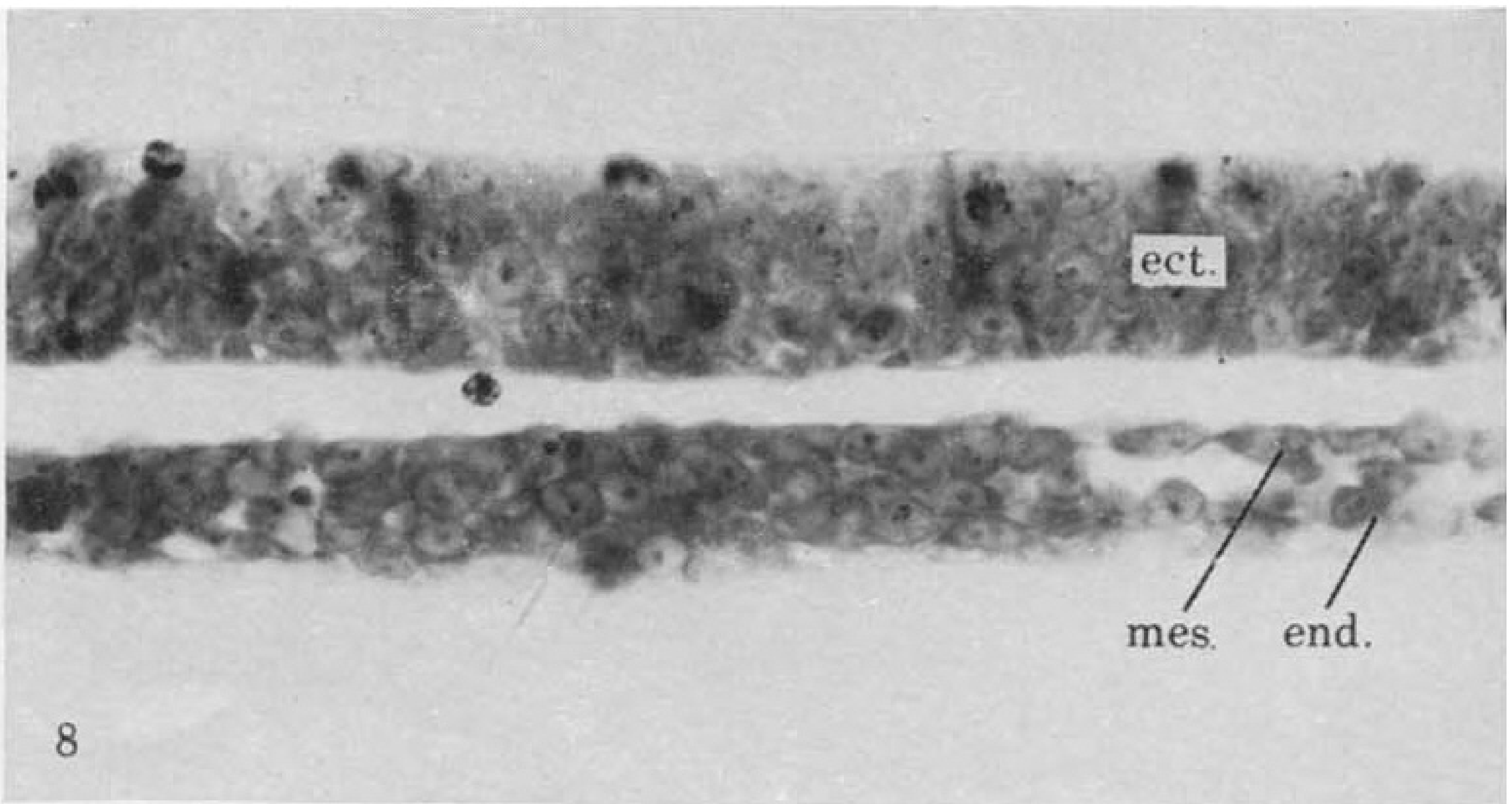


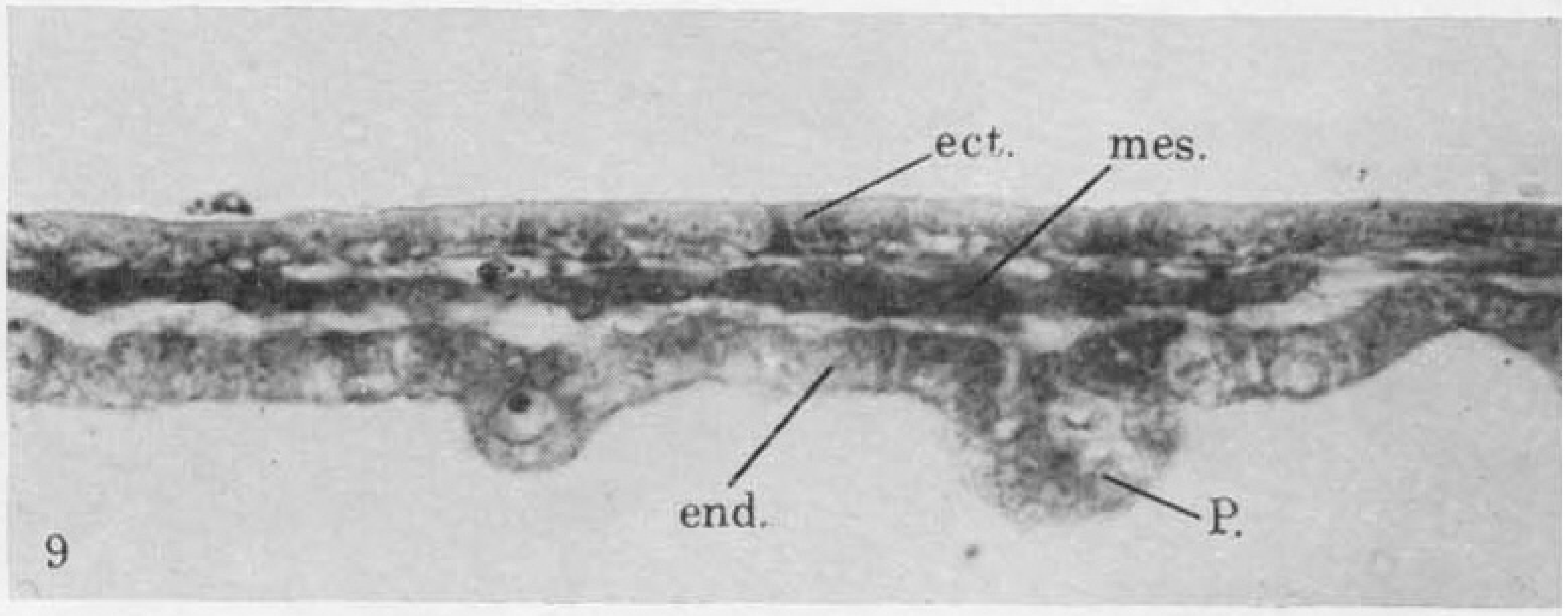


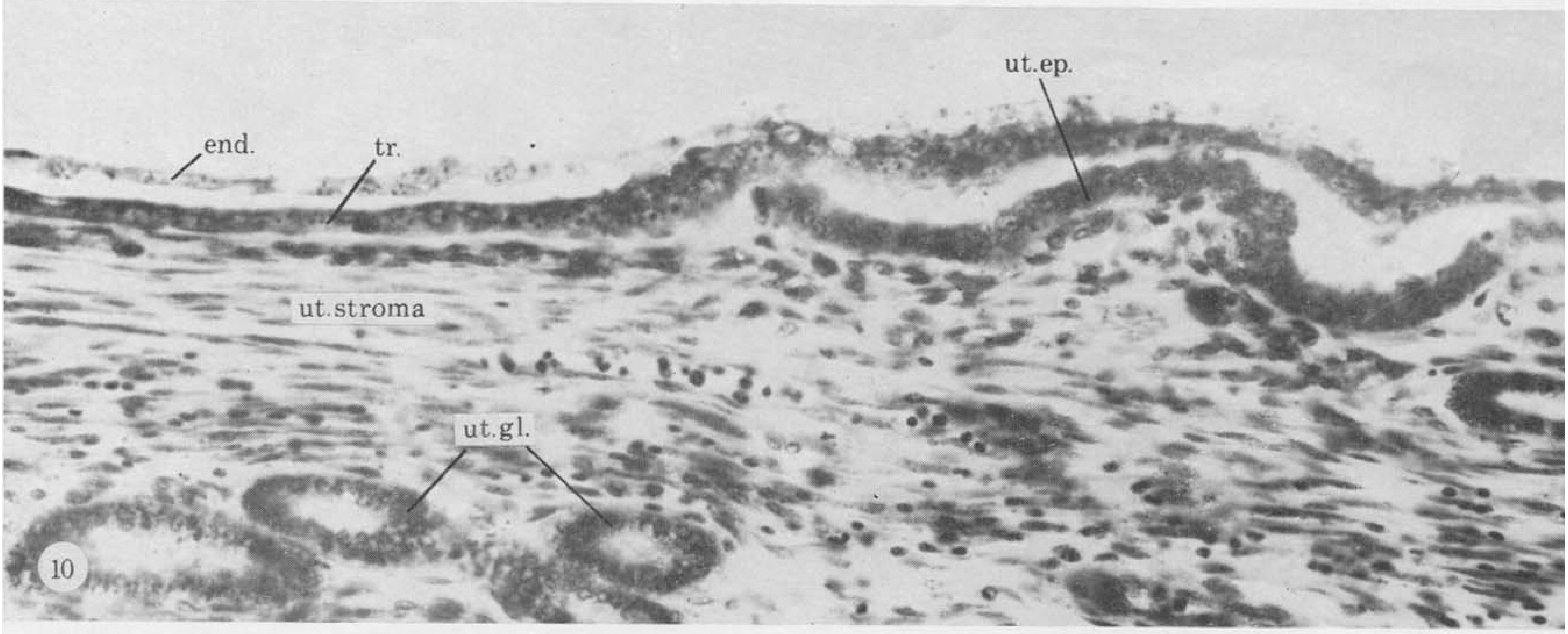


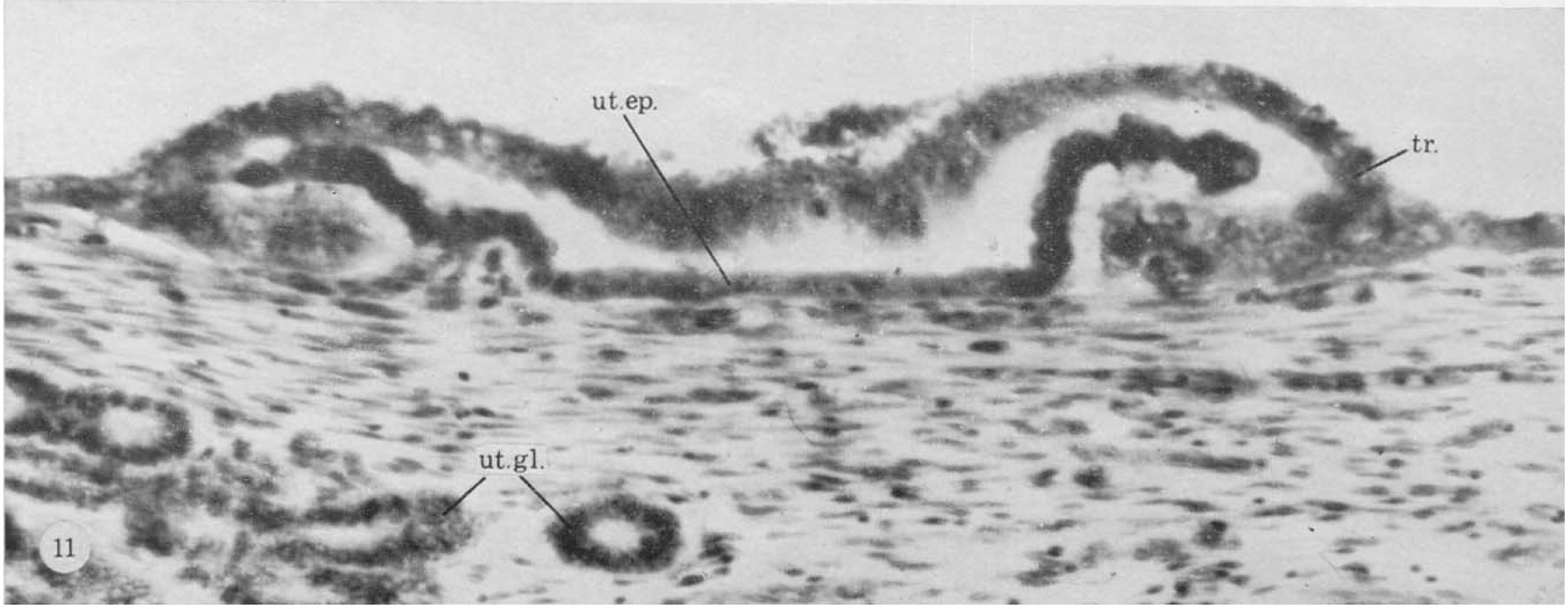
FIGURES 4–6. For description see opposite.



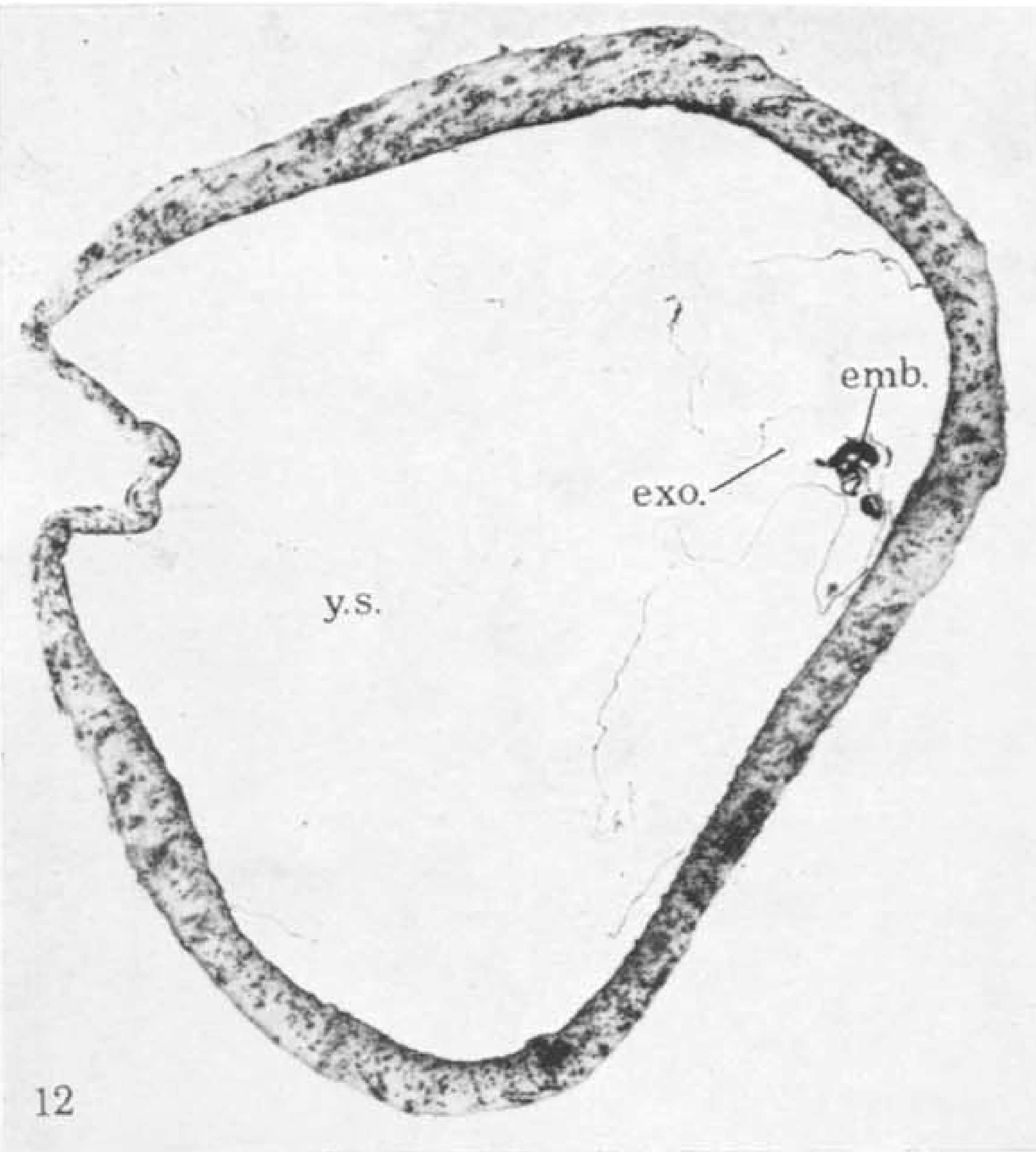


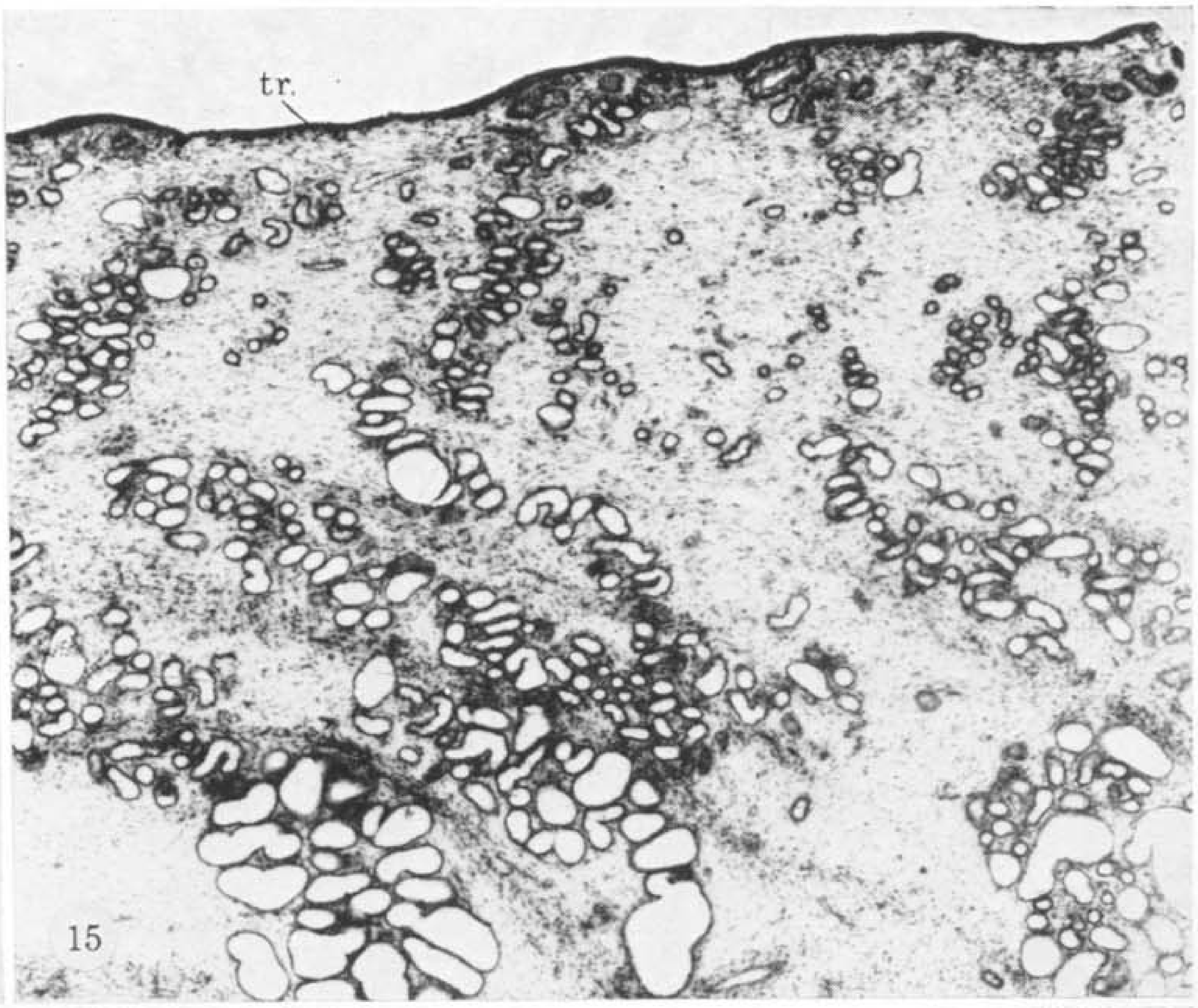


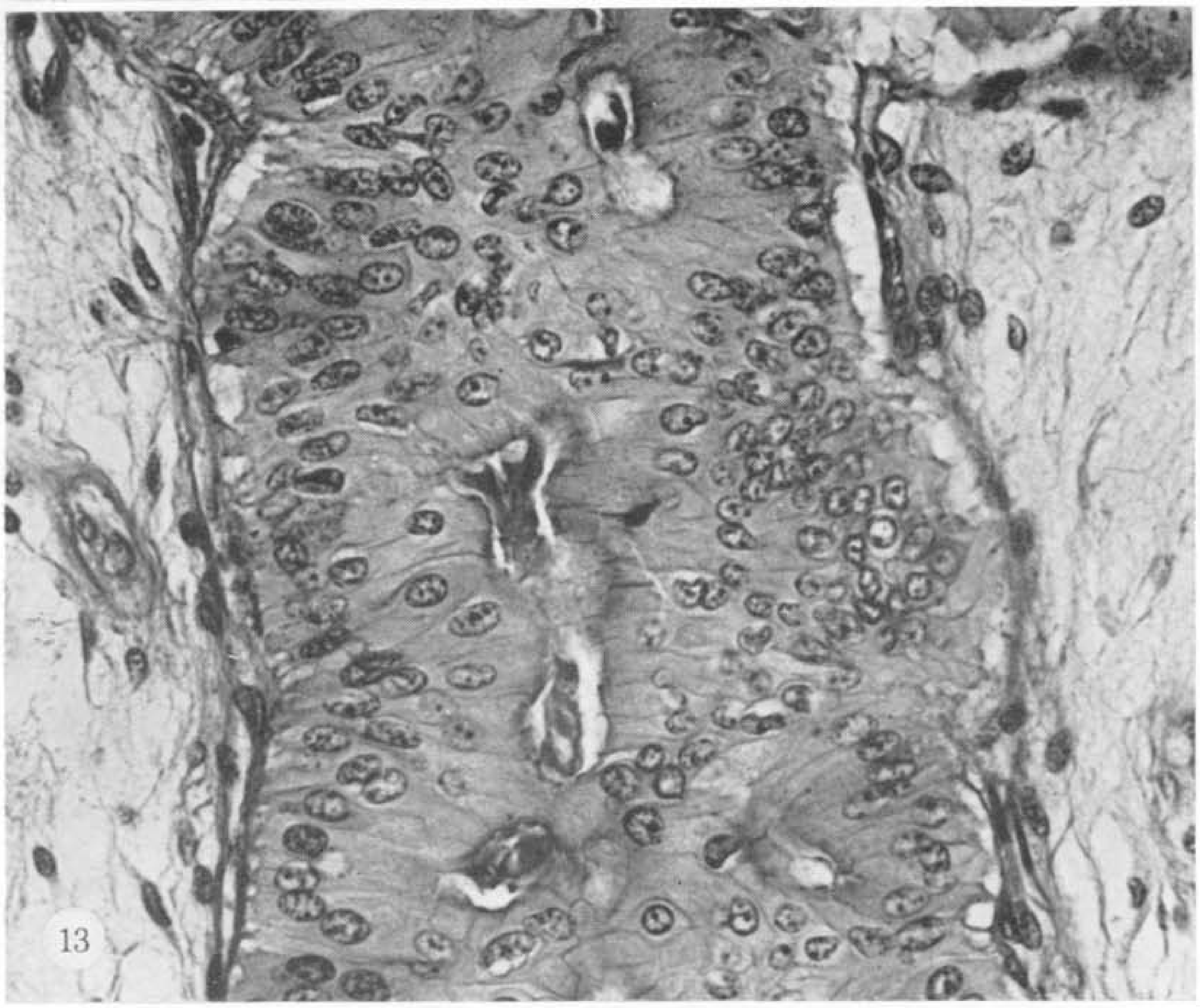


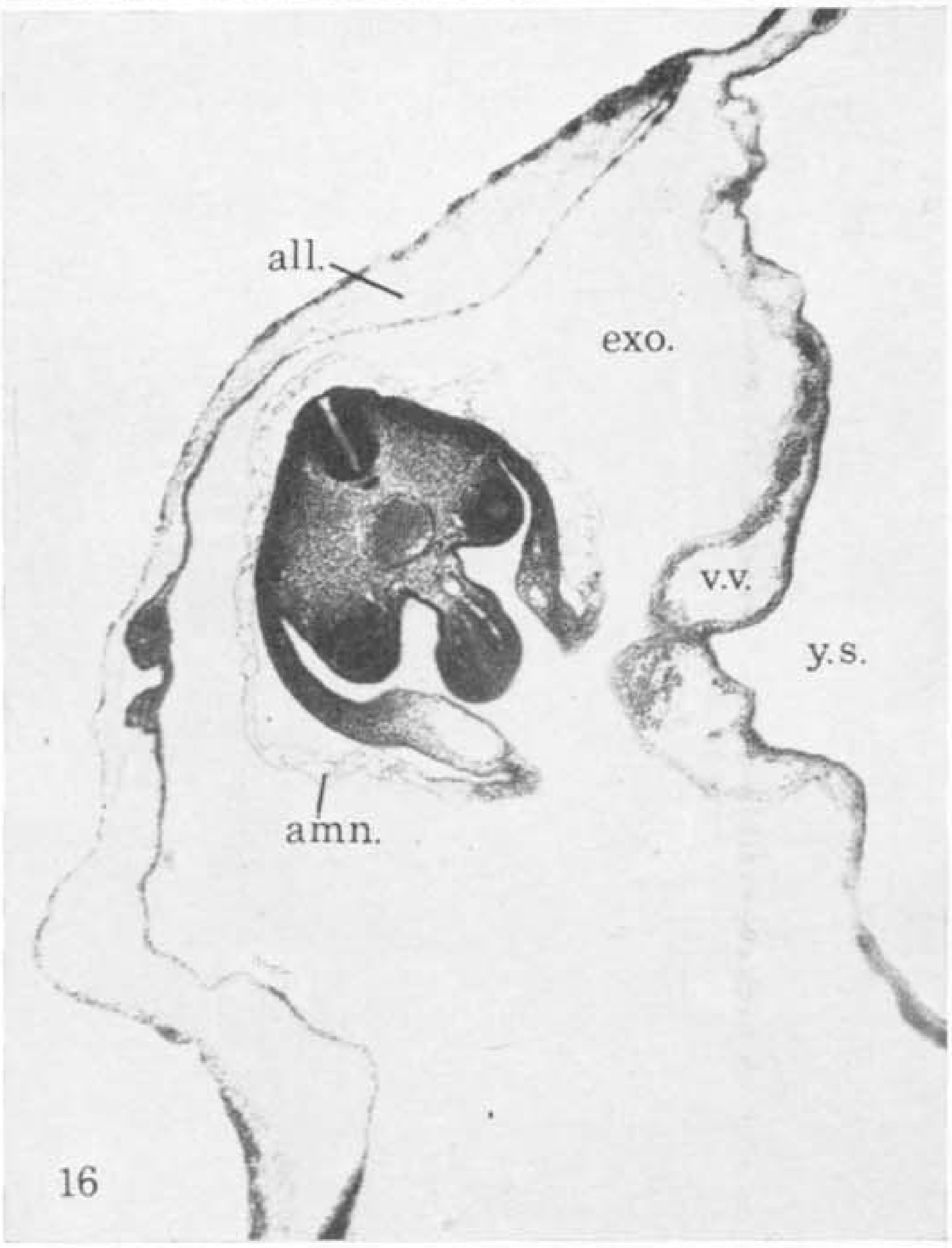


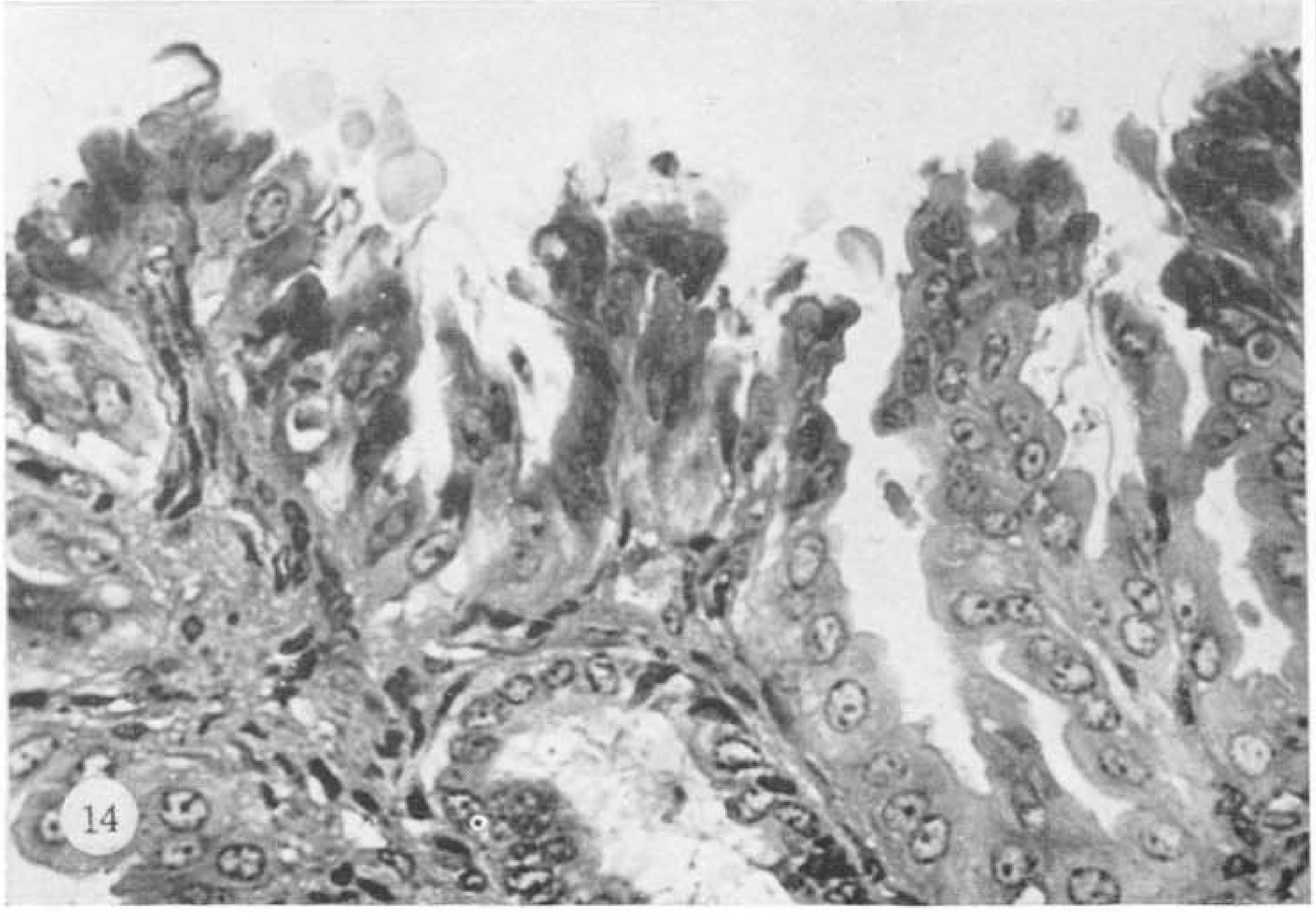
FIGURES 7–11. For description see opposite.

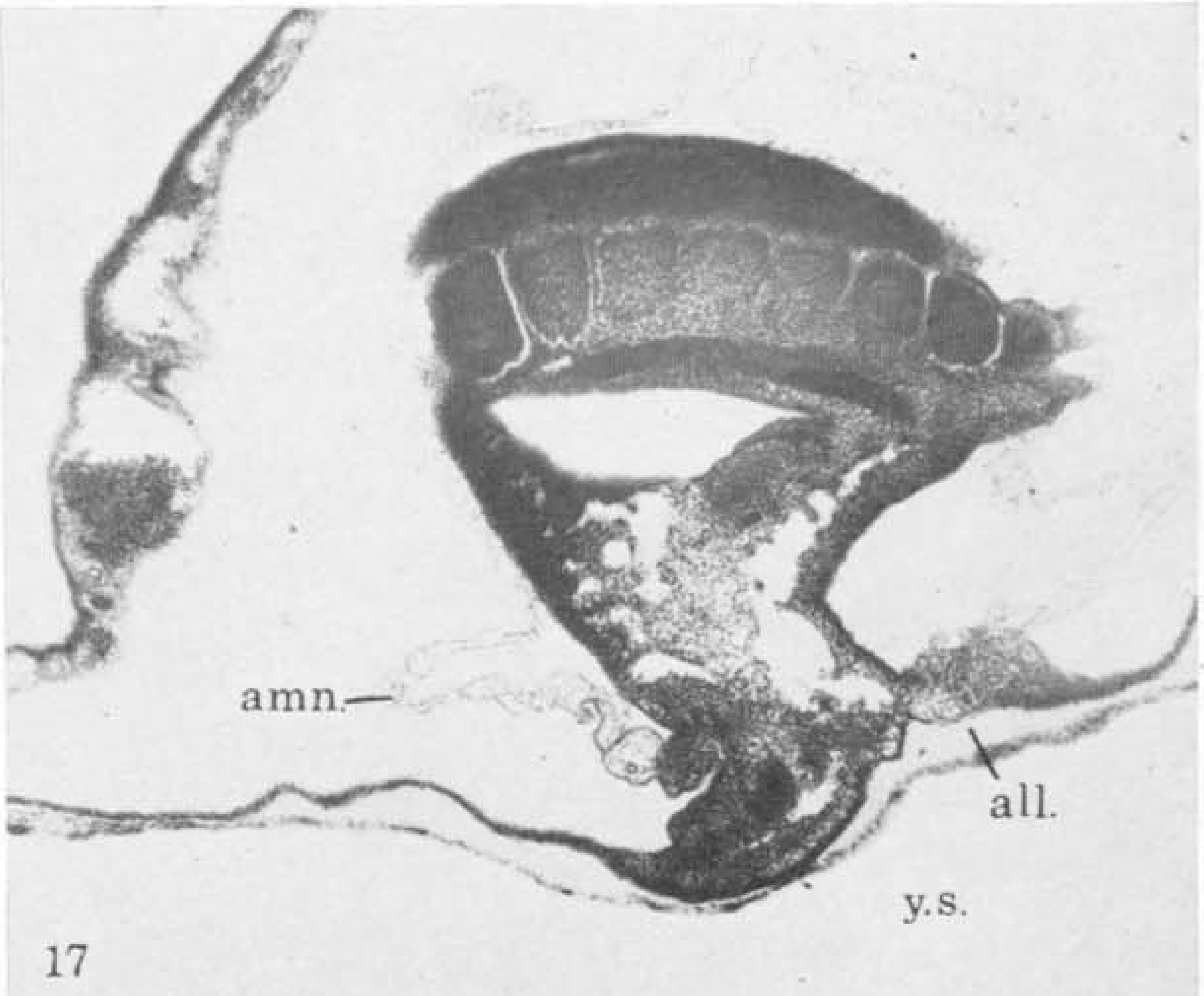

12


15

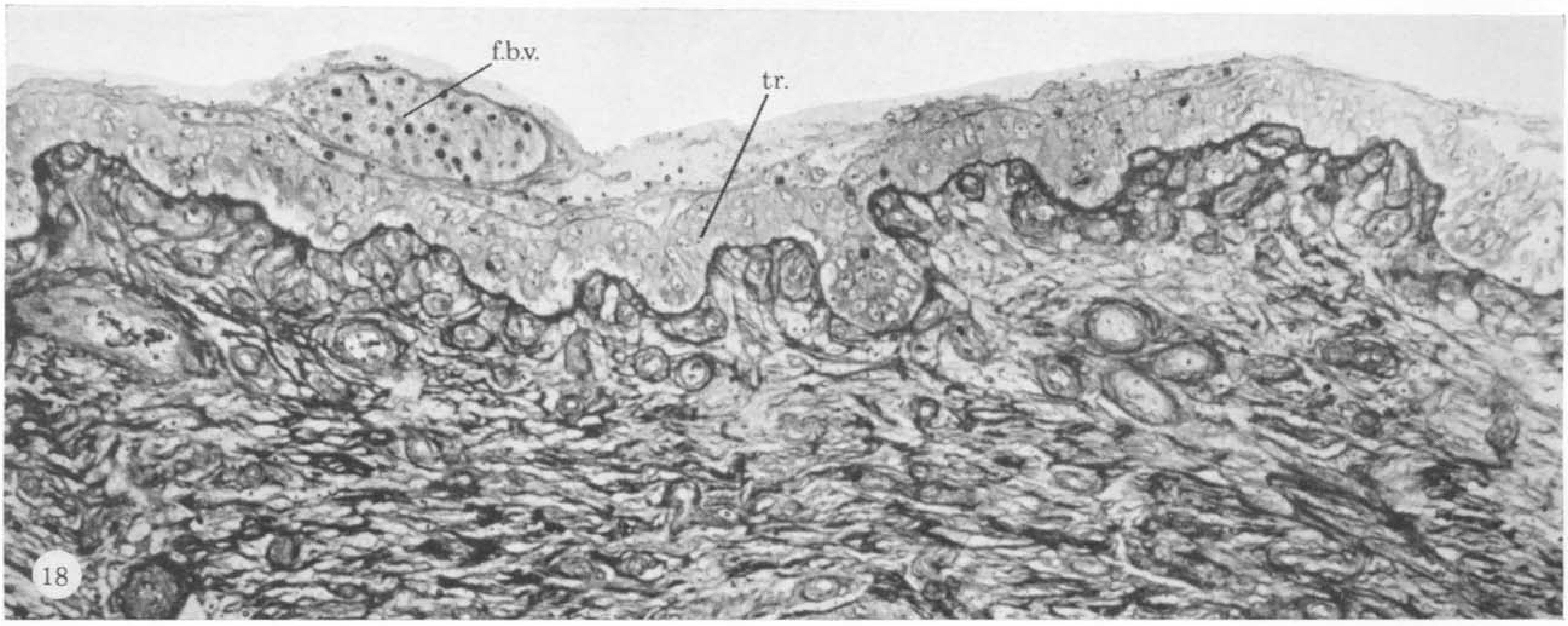

13

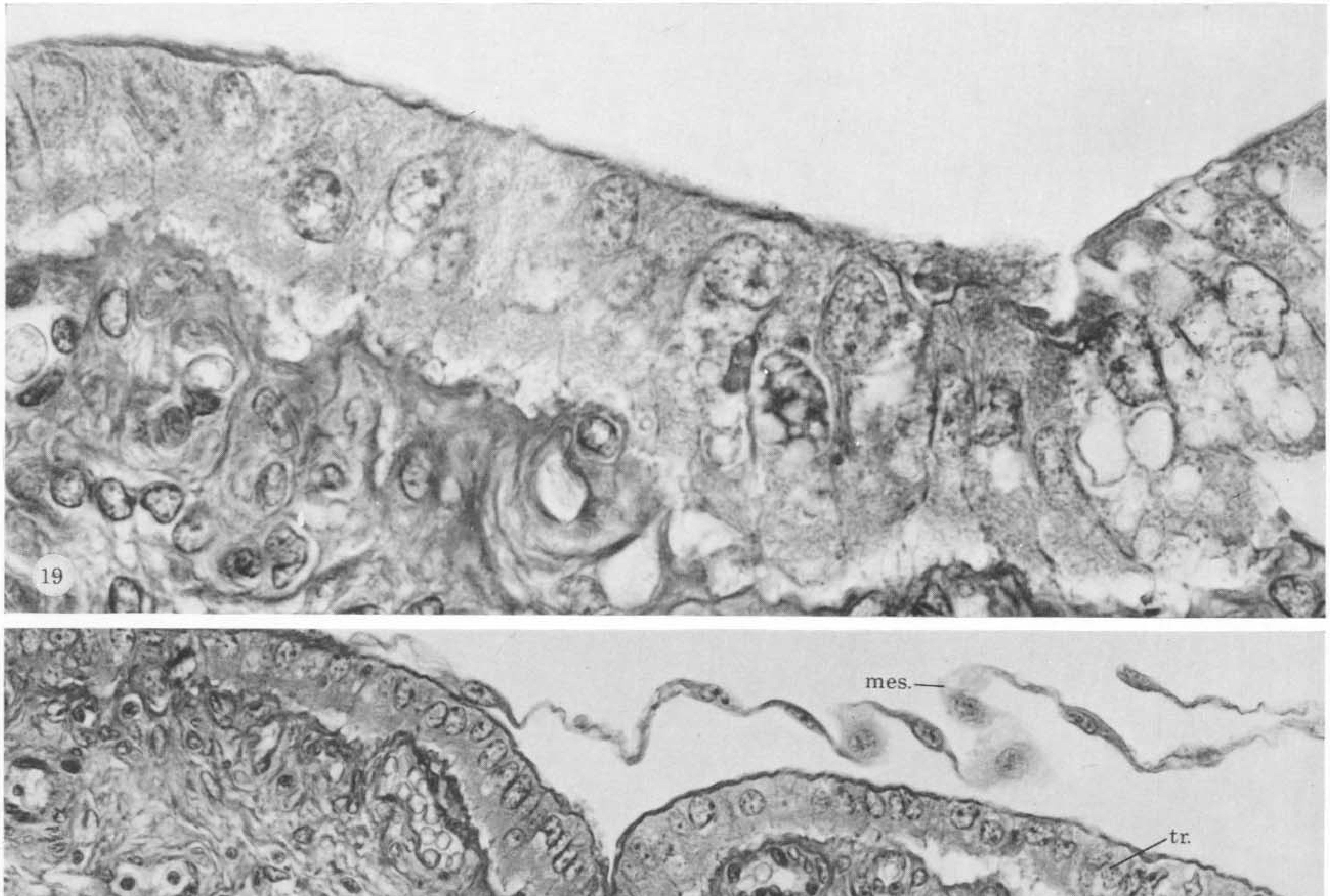

16

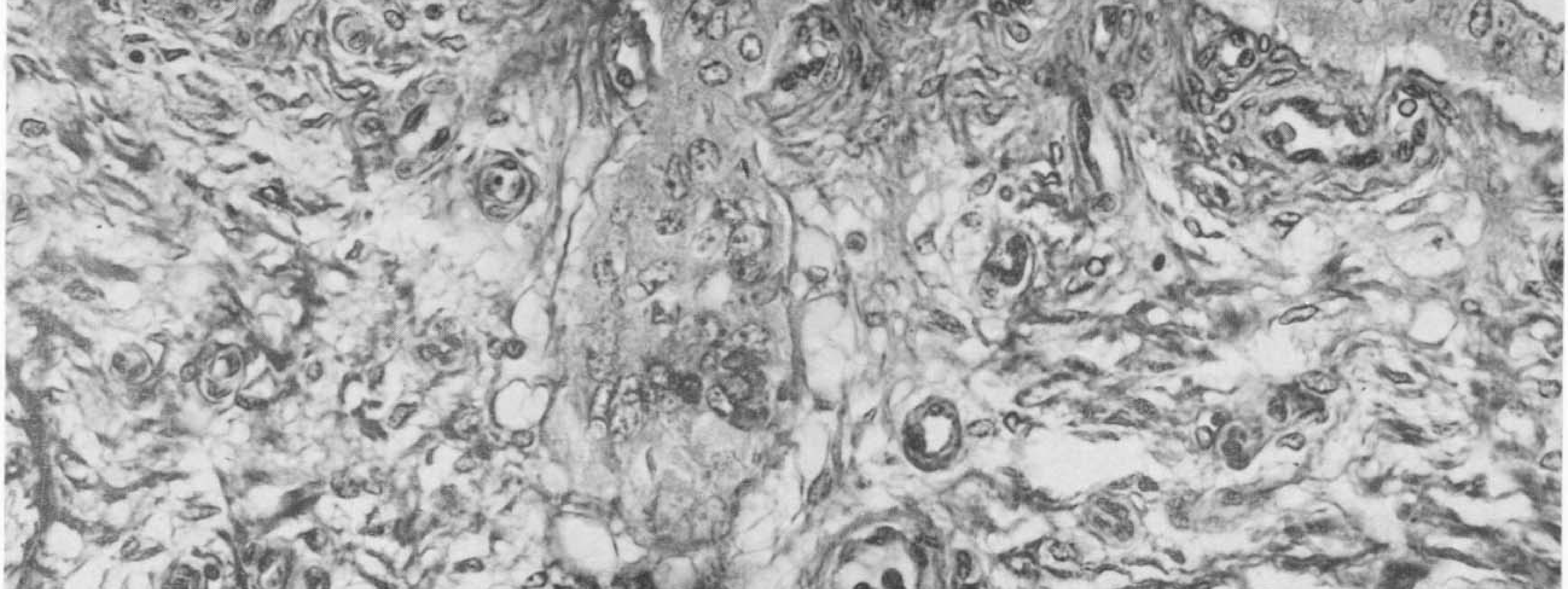

14


17

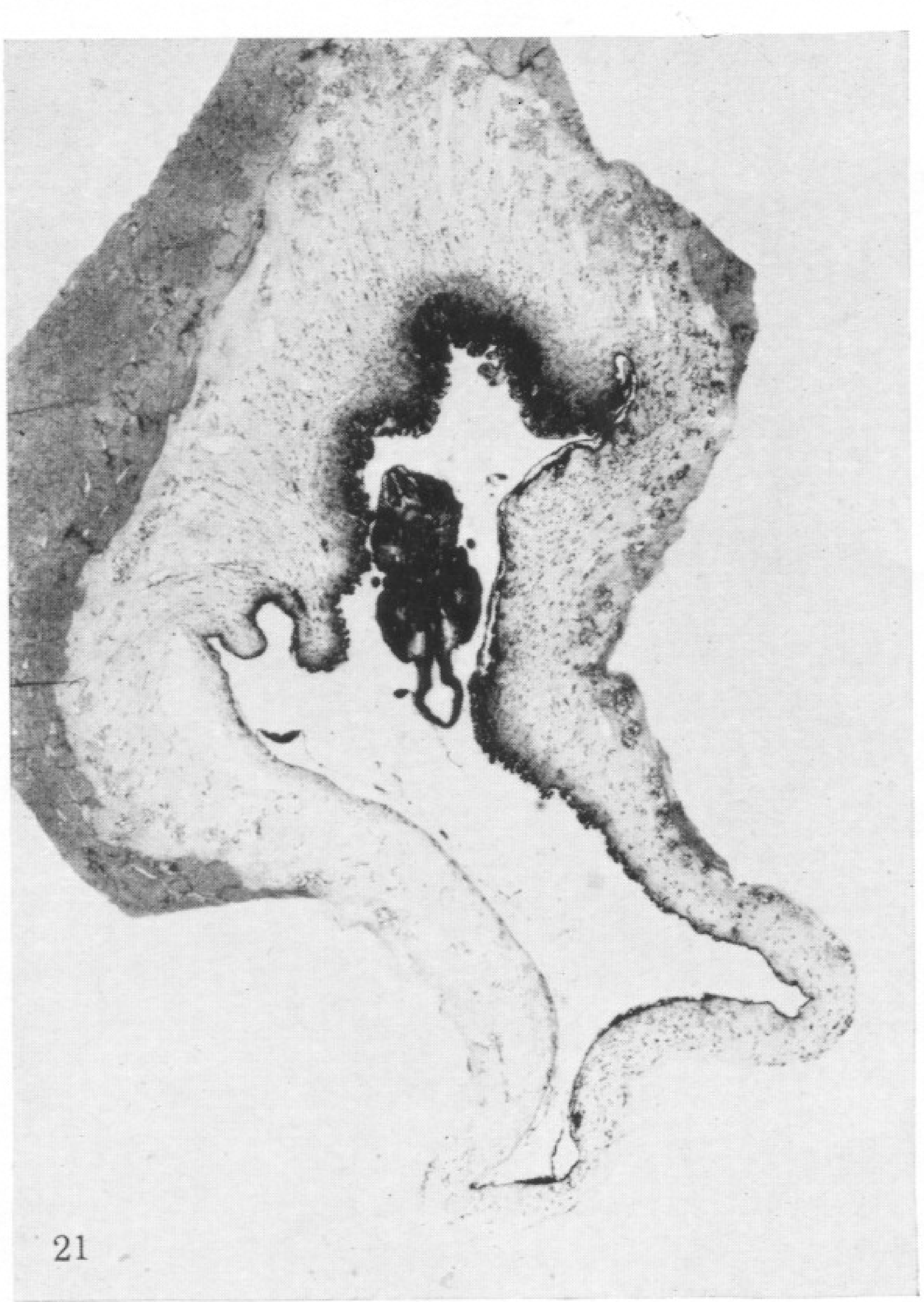
FIGURES 12–17. For description see opposite.

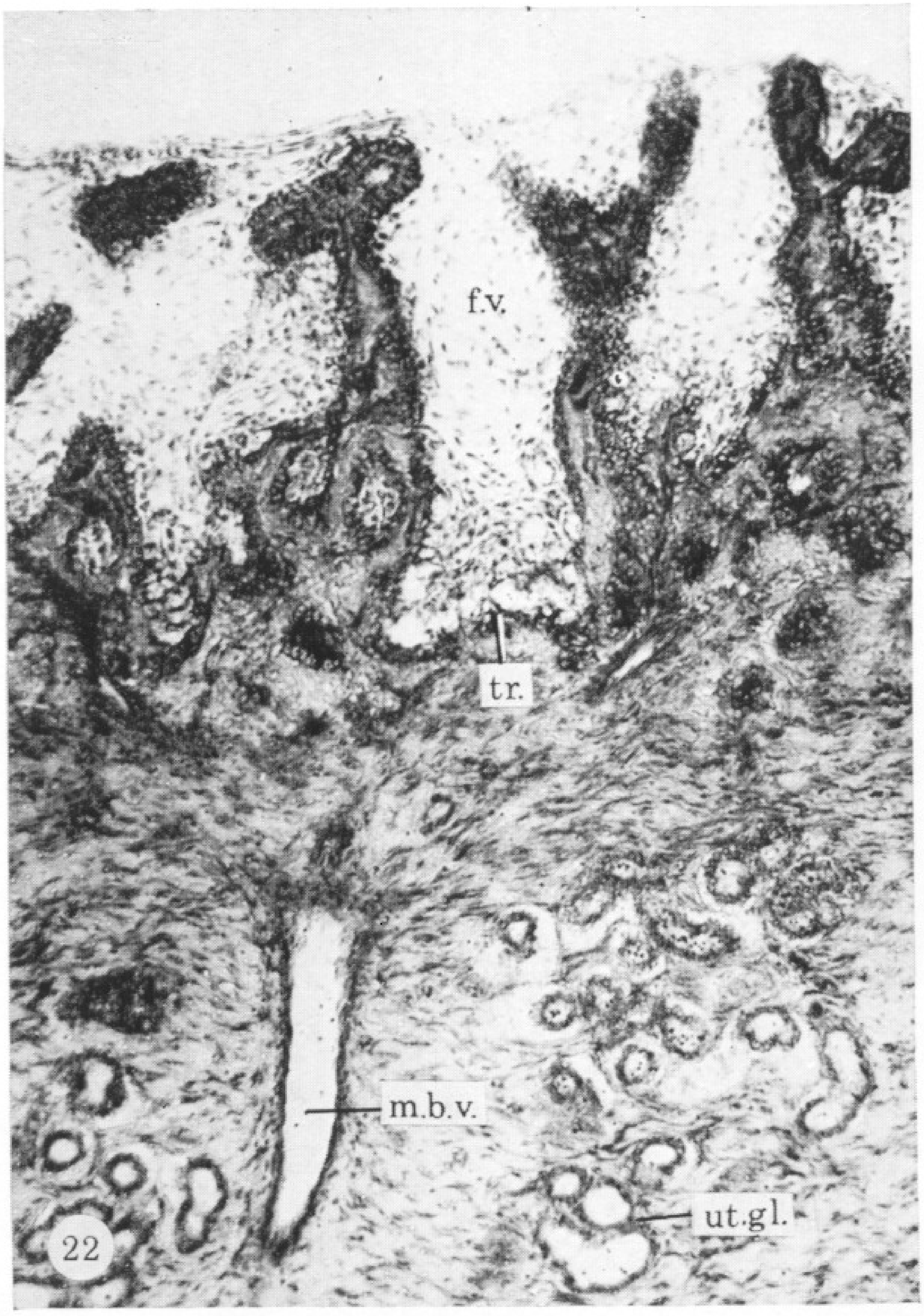


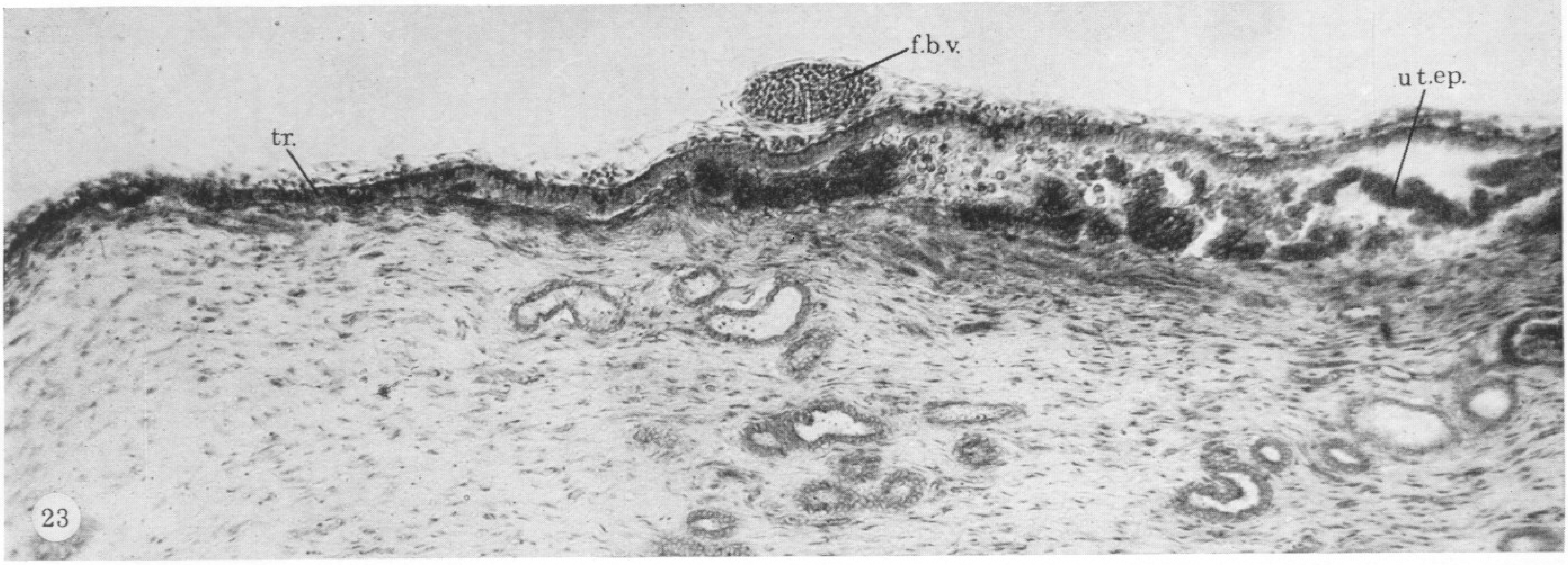


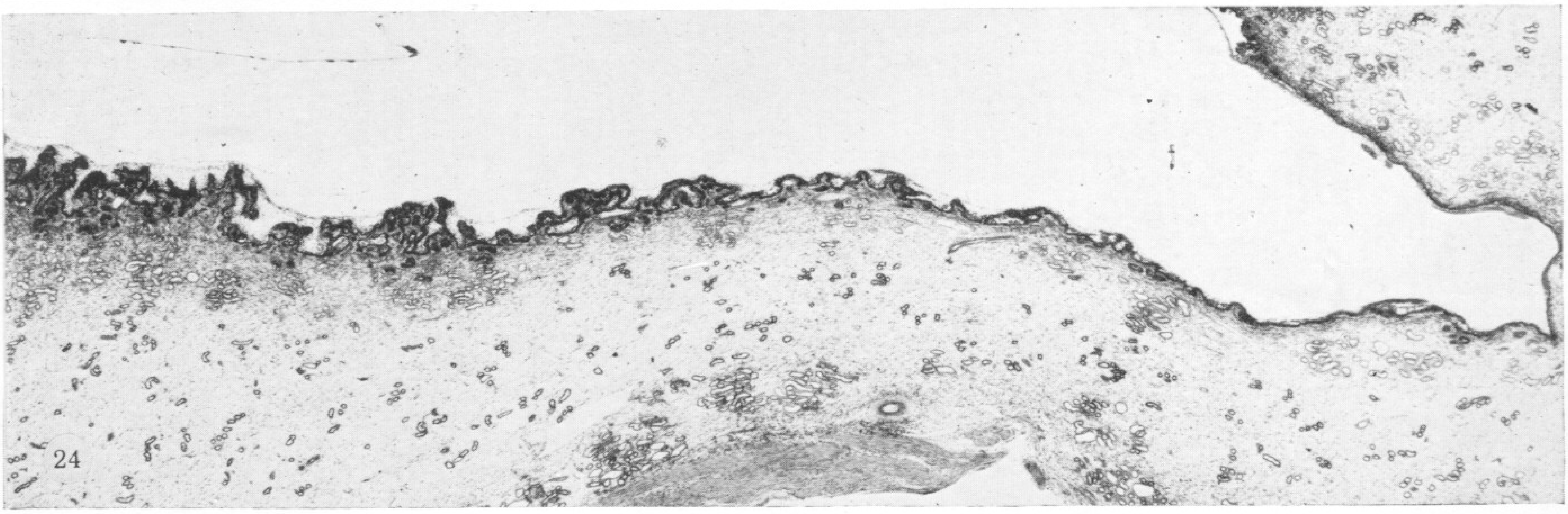


FIGURES 18–20. For description see opposite.

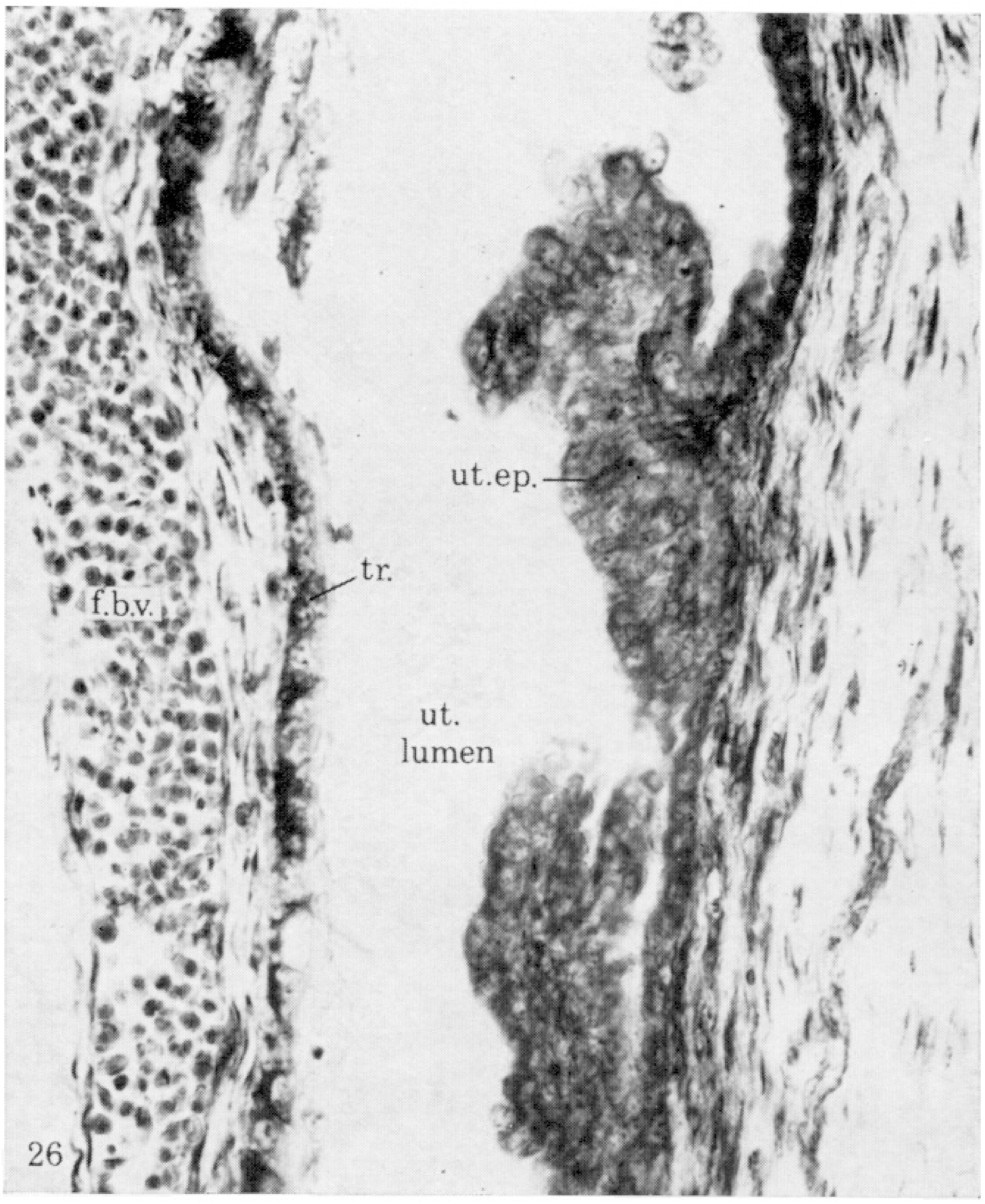




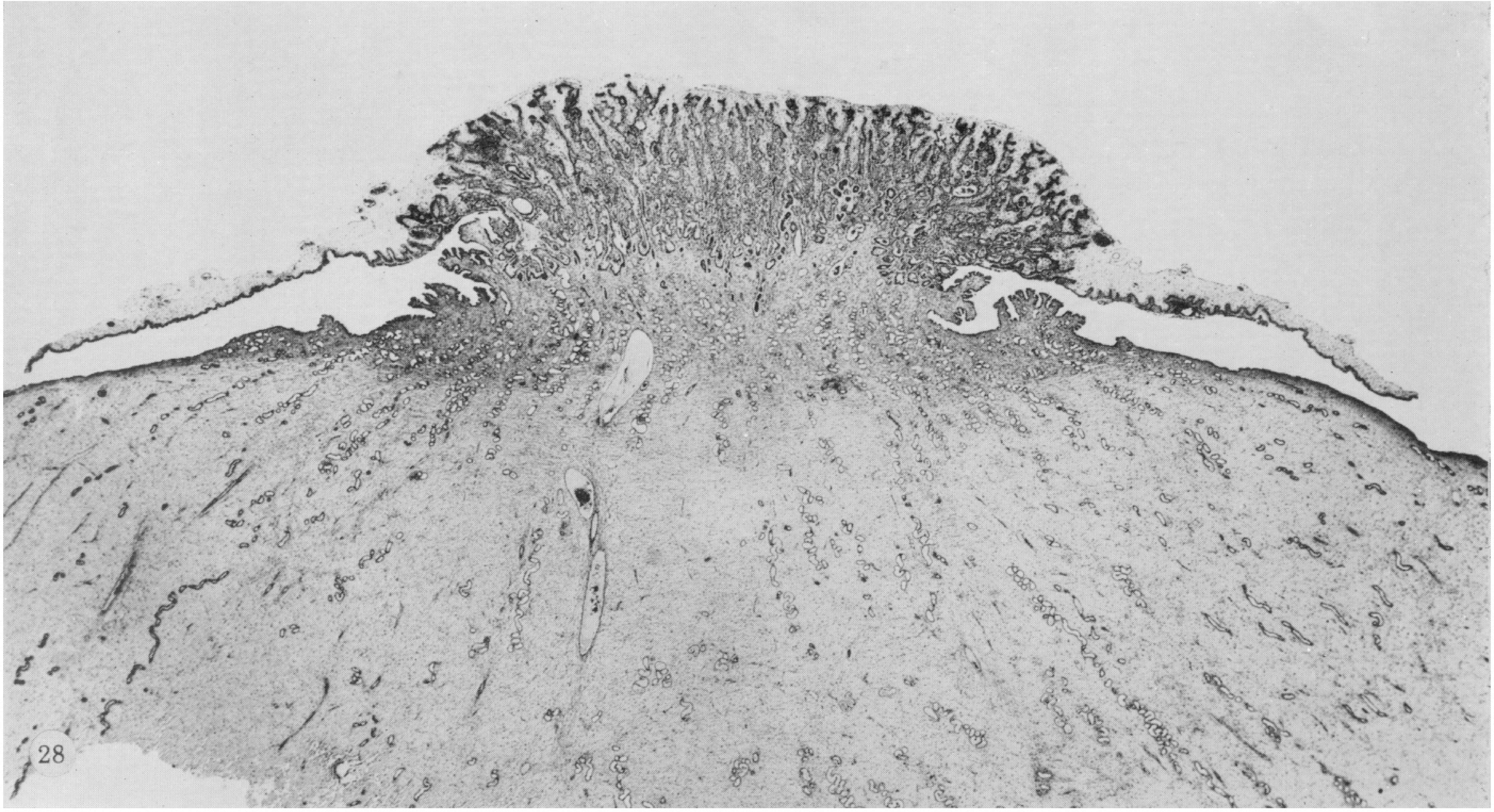


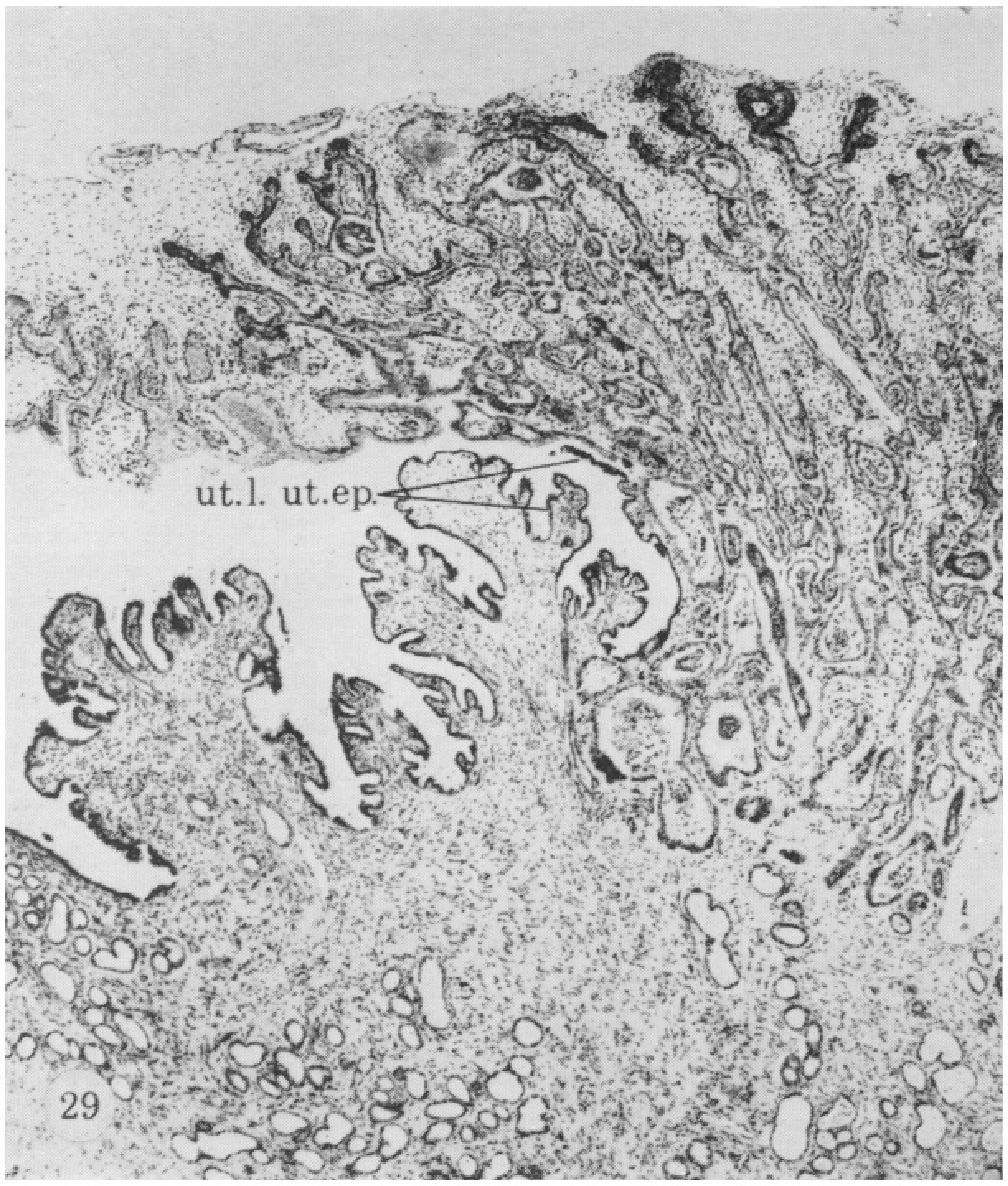


FIGURES 21–24. For description see opposite.



FIGURES 25–27. For description see opposite.





Figures 28–30. For description see opposite.