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The chorio-allantois of the chick. Light and electron microscopic observations at various times of incubation

BY T. S. LEESON[1] AND C. R. LEESON[2]

Departments of Anatomy, University of Toronto and Queen's University, Kingston, Ontario

INTRODUCTION

In the chick, the allantois appears as a ventral outgrowth of the endodermal hind-gut during the fourth day of incubation and thus corresponds in nature to the urinary bladder of the amphibia. Subsequently, it enlarges very rapidly up to the tenth day of incubation, by which time it has enveloped the embryo and the yolk-sac. In this process, the mesodermal layer of the allantois becomes fused with the adjacent mesodermal layer of the chorion. In this double layer of mesoderm a rich vascular network develops, which is connected with the embryonic circulation by the allantoic arteries and veins. By means of this circulation and by the position of the allantois immediately subjacent to the porous shell, this highly vascular chorio-allantoic membrane serves a respiratory function. In the study of the epigenetic processes involved in avian development, it is this membrane that has been used experimentally as a site for grafting small explants from younger embryos (e.g. Willier, 1930; Willier & Rawles, 1931). In addition to respiratory exchange, the allantois serves also as a reservoir for the waste products coming from the developing excretory organs of the embryo.

Romanoff (1960) reviewed the more general histological appearances of the chorio-allantois at various stages during incubation but there appears to have been little detailed investigation of this membrane in recent years. The present paper reports upon the appearance of the chorio-allantois at varying times of incubation, as revealed both by light and by electron microscopy. It was felt that these findings would be of interest in view of the functions of the membrane and of its homology with the chorion in the higher mammals.

MATERIALS AND METHODS

Small pieces of chorio-allantoic membrane, together with the shell membrane, were removed from fertile eggs (Leghorn) which had been incubated for 7, 11, 14, 16, 18 and 21 days.

For light microscopy, pieces of membrane were fixed in alcoholic Bouin solution and embedded in paraffin wax. The embedded membrane was sectioned transversely at 6μ and the following staining procedures employed: haematoxylin and eosin, iron haematoxylin–van Gieson, picro-Mallory, alcian blue, Weigert's elastin, toluidine blue, and the P.A.S. reaction.

For electron microscopy, portions of the membrane were fixed by immersion in

[1] Present address: Department of Anatomy, University of Alberta, Edmonton, Canada.
[2] Present address: Department of Anatomy, State University of Iowa, Iowa City, U.S.A.

2% osmium tetroxide mixture as recommended by Palade (1952). Adequate fixation was obtained after 45 min. Tissues were then dehydrated rapidly in increasing concentrations of ethyl alcohol. After dehydration, some material of each stage was embedded in a methacrylate mixture (20% methyl to 80% *n*-butyl) to which 1% benzoyl peroxide had been added as catalyst. The remainder was put through two changes of propylene oxide for 15–30 min. each and then embedded in Shell Epon 812 according to the method of Luft (1961). Sections were cut on either a Porter-Blum or a Huxley ultra-microtome with glass or diamond knives and examined in a Philips EM-100B or a Hitachi HS-6 electron microscope. Electron micrographs were taken at original magnifications of 1500–20,000 diameters and enlarged photographically as desired.

RESULTS

Light microscopy

After 7 days' incubation, the allantois has extended to cover the inner aspect of the chorion over more than half of its surface and the mesenchyme covering the allantoic sac has fused with that lining the chorion. Thus, there are three layers to the chorio-allantoic membrane: the chorionic epithelium, the conjoined layer of mesenchyme, and the allantoic endoderm (Pl. 1, fig. 1). The chorionic epithelium is composed of two layers of somewhat flattened cuboidal cells, which possess large oval nuclei, with prominent nucleoli. The cytoplasm of chorionic cells is deeply acidophilic, faintly positive with alcian blue and P.A.S., and exhibits faint metachromasia. The two layers of chorionic cells are separated by the chorionic blood sinus (Pl. 1, fig. 2). No basement membrane is apparent between the chorion and the underlying mesenchyme. The latter consists of cells which possess delicate, wavy cytoplasmic processes lying in an amorphous intercellular substance. This substance contains some fibrillar material which stains blue with Mallory's stain, and is faintly positive with alcian blue and P.A.S. Many large blood vessels course through the mesenchyme prior to their distribution to the rich capillary plexuses immediately beneath and within the chorion. The allantoic endoderm, which is separated from the mesenchyme by a thin, strongly P.A.S.-positive basement membrane, comprises a single layer of cuboidal cells with large rounded or oval nuclei and faintly basophilic cytoplasm.

After 11 days' incubation, the allantois has extended to cover the entire inner aspect of the chorion and the complete chorio-allantoic membrane is closely applied to the inner aspect of the shell membrane. Cells of the chorion appear more flattened and the chorionic blood sinus more marked than in the earlier stage. The features of both the mesenchyme and the allantois are unchanged from those present after 7 days' incubation.

By the fourteenth day of incubation, vascularization of the chorion is marked. In many regions, the chorionic blood sinus appears to be immediately subjacent to the shell membrane and persisting epithelial elements now lie beneath the chorionic blood sinus. Numerous capillaries can be seen passing from the mesenchyme, through the epithelium, to the chorionic sinus (Pl. 1, fig. 3). The shell membrane consists of coarse, deeply acidophilic fibres and is separated from the chorion by a thin membrane which stains intensely with alcian blue, is strongly P.A.S.-positive

and exhibits a positive staining with Weigert's elastin stain. The staining reactions of the chorionic epithelial cells and the features of the mesenchyme are similar to those reported for the earlier stages. Cells of the allantoic endoderm are more flattened. The cytoplasm of these cells appears granular, stains deeply with alcian blue, and is P.A.S.-positive (Pl. 1, fig. 4).

The appearance of the chorio-allantois after 16 days' incubation and after 18 days' incubation (Pl. 1, fig. 5) shows little change from that present after 14 days' incubation. However, the impression is gained that there is a further extension of the chorionic sinus immediately beneath the shell membrane and that the fibrillar content of the matrix of the mesenchyme is increased in amount.

After 21 days' incubation (immediately prior to hatching), the chorionic blood sinus beneath the shell membrane is a prominent feature (Pl. 1, fig. 6). Few areas can be found where chorionic epithelial cells can be seen interposed between the blood sinus and the shell membrane. Component cells of the chorion appear unchanged from the earlier stages. The mesenchyme shows more background staining of the matrix (Pl. 1, fig. 6), presumably due to an increase in formed fibre content. The allantoic endoderm is very irregular and many component cells show knob-like protrusions of cytoplasm into the allantoic sac. The cytoplasm of such cells is markedly vacuolated and again stains intensely with alcian blue and is P.A.S.-positive.

Electron microscopy

Sections were cut from at least ten blocks of tissue of each stage, orientated so that the plane of section was perpendicular to the chorio-allantois and shell membrane.

Chorion

Examination of the material from eggs incubated for 7 days revealed features virtually identical with those of the 11-day stage, a description of which follows. However, at the 7-day stage, the chorio-allantois is not closely apposed to the shell membrane and further artificial separation occurred during fixation and sectioning. Thus the 11-day material proved more satisfactory for analysis and illustration.

By incubation for 11 days, the chorion is well differentiated and consists of two or more layers of cells lying on a fine extracellular basement membrane (Pl. 2, fig. 7). Toward the shell membrane is the widely patent chorionic blood sinus, into which open vessels from the chorionic mesoderm. The shell membrane is lined by chorionic cells which intervene between the blood and the shell membrane. These cells are greatly thinned (Pl. 2, fig. 7), although in many regions the cells are cuboidal in type, forming a relatively thick membrane (Pl. 2, figs. 8, 9). The majority of cells of the chorion have a large oval nucleus, often with an irregular outline, and may show a prominent nucleolus. In a few cells, mitotic figures are seen. In the cytoplasm, mitochondria are numerous, pleomorphic, and variable in size. Cristae mitochondriales are of the transverse plate type (Pl. 2, figs. 8, 9). Granular endoplasmic reticulum is sparse. The majority of the RNP (Palade) granules are scattered separately and in small rosettes throughout the cytoplasm and are unassociated with membranes. The Golgi apparatus is often extensive and situated in a paranuclear position. In some cells, many microvesicles of the micropinocytotic type are present and appear to be more numerous in cells situated immediately subjacent

to the shell membrane. Cell interfaces vary greatly. They may be relatively straight or more complex with 'jig-saw' or 'zipper' interlockings, occasionally with desmosomes (Pl. 2, fig. 9).

The cells lining the chorionic blood sinus differ in no way from those not in the wall of the blood sinus, but in the region immediately subjacent to the shell membrane the plasma membrane may be complicated by fine, slender invaginations (Pl. 2, fig. 8). In some regions there are electron-dense, possibly degenerate chorionic cells lying beneath the shell membrane and slips of cytoplasm from these cells extend into the invaginations noted above (Pl. 2, fig. 9). Elsewhere, too, in the chorion are occasional electron-dense cells, many with vacuolated cytoplasm and distorted mitochondria. These also have the appearance of degenerating cells.

The chorionic mesoderm has all the appearances of an embryonic mesenchyme with cells of grossly irregular outline and long cytoplasmic processes scattered in a matrix containing very few unit fibrils of collagen (Pl. 2, fig. 7). Many of these cells contain an extensive granular endoplasmic reticulum.

After 14 days' incubation the chorion shows a more extensive blood sinus and is somewhat thicker (Pl. 3, fig. 10). There are other slight differences from the preceding stage. Nowhere is the shell membrane lined by cuboidal chorionic cells, as is seen at 11 days (Pl. 2, figs. 8, 9). Blood in the chorionic sinus is separated from the shell membrane only by a very thin layer of attenuated cytoplasm of chorionic cells, although 'bridges' connect this layer to the underlying chorionic membrane (Pl. 3, fig. 12). At the site of contact between chorionic cytoplasm and shell membrane there are regions where the chorionic plasma membrane forms many microvilli (Pl. 3, fig. 11). Throughout the chorion, cell interfaces are more complex and desmosomes are more frequent. The extent of the Golgi apparatus is impressive in some cells (Pl. 3, fig. 12) and dense intramitochondrial granules are seen occasionally. The chorionic mesoderm, as in the younger stage, has the appearance of embryonic mesenchyme and the granular endoplasmic reticulum in many cells is more extensive and suggestive of more active protein synthesis.

The general morphology of the chorion at 16 days shows no change from that present after 14 days' incubation, but there are some slight differences in the component cells. Mitochondria, in general, are larger (Pl. 4, figs. 13, 14) and more numerous, and small smooth-surfaced microvesicles also are more evident (Pl. 4, fig. 14), with a few cells containing multivesicular bodies. Cells lining the chorionic blood sinus are smaller than those which do not and show less prominent organelles. Desmosomes at cell interfaces are obvious and, in many regions, intercellular spaces are present, often with microvillous protrusions extending into them, somewhat in the nature of the bile capillaries of the liver (Pl. 4, fig. 13). A few dark, degenerate cells are present and also a few cells with very light cytoplasm. In the latter, mitochondria are small and numerous and elements of the Golgi apparatus are multiple and well developed (Pl. 4, fig. 15). These cells are possibly lymphocytes. Toward the shell membrane, the cytoplasm of chorionic sinus-lining cells is very attenuated, again with the complex microvillous arrangement described in earlier stages. Chorionic cells adjacent to mesoderm are supported by a fine extracellular basement membrane, itself associated closely with a few unit fibrils of collagen (Pl. 4, fig. 13).

The appearance after 18 days' incubation is virtually identical with that of the previous stage, although the thickness of the chorionic membrane is slightly greater (Pl. 4, fig. 16). It is worth noting that mitotic figures are seen occasionally even at this stage. In the mesenchyme, unit fibrils of collagen are more numerous and more closely packed.

There are many differences between the chorion after 21 days' incubation and that of earlier stages. The general architecture of the chorionic membrane and blood sinus is unchanged but low-power micrographs show distinct differences between cells lining the sinus and those toward the mesoderm. The former are smaller, have little cytoplasm and show nuclei with clumping of the chromatin. In the remainder of the chorion, many degenerate cells are present, with dark nuclei, vacuolation of the cytoplasm and often separation from surrounding cells by an extensive intercellular space. In some cells, large intracellular spaces or ducts lined by microvilli are apparent (Pl. 5, fig. 18), closely resembling those, for example, in parietal cells of the stomach. Elsewhere in the chorion, complex interfaces are seen, with desmosomes, separation of plasma membranes and formation of microvilli. The microvillous formation described previously in relation to the shell membrane is now seen elsewhere in the chorion, commonly in relation to the blood sinus (Pl. 5, fig. 19). In many cells, mitochondria appear degenerate with an electron-lucid matrix and crowding of cristae.

Allantois

After 11 days' incubation, the type of cell lining the allantoic sac varies from squamous to cuboidal (Pl. 6, fig. 20). Nuclei are irregularly oval in outline; mitochondria are small and numerous. Also present in many cells are large, dense, oval or spherical granules. The basal plasma membrane is irregular and supported by an extracellular basement membrane. Towards the lumen, a few short microvilli are present. Between adjacent cells, plasma membranes show desmosomes and in many regions are separated to form intercellular vesicles.

By 14 days, allantoic cells are more regular, contain many dense granules in the apical cytoplasm and, as in the earlier stage, apical microvilli, intercellular vesicles, and, often, rows of desmosomes at cell interfaces (Pl. 6, fig. 21).

In the 16- and 18-day stages, changes are apparent leading to the state seen in the 21-day material. By 18 days, the allantois is composed of columnar cells, and large granules, desmosomes and intercellular spaces are apparent again. However, there is considerable overlapping and interlacing of cells as evidenced by the difficulty in obtaining a section where the epithelium appears simple and not pseudostratified in character.

By the 21-day stage, the allantois shows an appearance identical to 'hydropic degeneration' of light microscopy material. Cells are elongated with narrow bases and nuclei situated in apical protrusions of cytoplasm into the lumen of the allantoic sac (Pl. 6, fig. 22). Many large vacuoles are present in the cytoplasm and dense granules are absent or few in number. Beneath the epithelium is a relatively dense layer of dark cells with irregular nuclei and fibrillar cytoplasm, lying in a meshwork of closely packed unit fibrils of collagen. These cells have many of the characteristics of smooth muscle cells.

DISCUSSION

These observations upon the chick chorio-allantois after varying times of incubation indicate that there is considerable differentiation of the component layers of the membrane once it has become established. Essentially, the chorio-allantoic membrane consists of three distinct layers: (*a*) the chorionic layer of cells, with associated vascular elements, lying immediately subjacent to the shell membrane; (*b*) an intermediate layer of mesenchyme containing the main blood vessels; (*c*) the layer of allantoic endoderm. With regard to the chorion there is an extension of the chorionic blood sinus during the period of incubation from 7 to 21 days and a reduction in the cytoplasmic barrier between it and the overlying shell membrane. Changes in the intermediate layer of mesenchyme are not striking but there does appear to be a gradual increase, with time of incubation, in the fibre content of the matrix. The allantoic endoderm shows extensive changes. Initially, it is composed of a single layer of relatively undifferentiated cells which later show many degenerative changes, possibly associated with the gradual accumulation of harmful waste products within the allantoic sac.

The relationship of the chorionic blood sinus to the chorion itself requires further comment in view of previous observations and of functional considerations. A very adequate description of the chick chorion was given by Fülleborn (1895), as quoted by Lillie (1952). Fülleborn wrote, 'one could as well describe it (i.e. the respiratory network) as a great blood sinus interrupted by strands of tissue'. This description fits very well with the observations of the present study, particularly with regard to the cellular 'bridges' (see, for example, Pl. 2, fig. 7; Pl. 3, figs. 10, 12; Pl. 4, fig. 17). Lillie (1952), however, described the chorionic arteries as ending in 'an extraordinary fine-meshed capillary network interspersed with the ectodermal cells which they have largely displaced'. The phrase 'extraordinary fine-meshed capillary network' is, we feel, unfortunate since it does not appear to describe the vascular arrangement adequately. The chorionic blood sinus, even after only 11 days' incubation, is a large dilated blood space lined towards the shell membrane by much attenuated cytoplasm. Indeed, this very thin lining membrane often cannot be resolved by the light microscope as shown in this and previous studies. Romanoff (1960), for example, has stated that, by 13–15 days' incubation, the superficial layer of flat cells has mostly disappeared. However, the electron microscopic observations have shown that in no section is this much attenuated layer of cytoplasm absent, i.e. in no instance does blood appear to be directly in contact with the shell membrane. As Fülleborn (1895) described, the chorionic blood sinus is extraordinarily extensive and is interrupted only by relatively narrow 'bridges' of cytoplasm which connect the outer attenuated cytoplasmic layer to the main chorionic ectoderm lying deep to the sinus. A study of the micrographs of this investigation illustrates well the nature of the blood/air barrier which obviously allows of efficient gaseous exchange between blood within the sinus and the porous shell. Romanoff (1960) recognized this vascular pattern since he described it as being so fine and dense that it could be compared only to the 'capillary plexus found in the chorioid of the eye and the lungs of higher vertebrates'.

The findings of the present investigation with regard to the nature and extent of

the chorionic blood sinus are, however, in conflict with some earlier electron microscopic observations. In a brief report of the chick chorio-allantois by Rangan & Sirsat (1962), the chorionic blood sinus was described as consisting of scattered red blood cells *present in the deeper layers of the chorion.* Superficial to the deeper layers, these authors described a basement membrane which separated the deeper layers from two superficial layers of flattened chorionic cells. Such an arrangement probably could be found only at the site of entry of a large chorionic blood vessel into the blood sinus.

The nature of the cells lining the chorionic blood sinus is open to some doubt. Romanoff (1960), like Lillie (1952), considered the sinus to be a meshwork of capillaries with an endothelial lining. He emphasized that, by 13–15 days' incubation the capillaries lie directly under the shell membrane with no living tissue between them and the external atmosphere. Are the lining cells, in fact, endothelial or are they cells of the chorionic ectoderm? Danchakoff (1917) described the capillaries within the chorion as having their own walls of thin endothelium with numerous nuclei containing small, flattened, chromatic particles but no visible nucleoli. The electron microscope findings of the present study have shown that, whilst cells lining the chorionic blood sinus are much attenuated and perhaps contain fewer organelles than surrounding chorionic cells, they do not appear to be typically endothelial. Certainly, this applies to the early stages. It is only in the later stages that they can be distinguished readily, by their nuclear characteristics, from chorionic cells removed from the blood sinus. At no stage are the sinus-lining cells separated from surrounding chorionic cells by an extracellular basement membrane which would lead one to suspect them as being endothelial in type. Also, at the site of entry of a blood vessel into the chorionic sinus, the basement membrane of that vessel appears to be continuous with that lying subjacent to chorionic ectoderm. On the basis of these findings, we are of the opinion that the cells lining the chorionic blood sinus are of chorionic epithelial origin rather than of mesodermal origin as has been assumed from earlier light microscopy studies.

The consideration of the chick chorion to date has emphasized those structural modifications associated with the prime function of the chorion in respiratory exchange. That the chorion in birds might have further functional significance is less well established. There are some ultrastructural details present in chorionic cells which might indicate a greater functional complexity than is found normally in a purely 'respiratory' epithelium. Mitochondria are numerous and in many chorionic cells the Golgi apparatus is well developed. The distribution of RNP (Palade) granules, unassociated with membranes, does not suggest active protein synthesis for secretion, although it may indicate cellular division and differentiation (Howatson & Ham, 1955; Leeson, 1960, 1963). The presence of multivesicular bodies is suggestive perhaps of active fluid transport in bulk (Farquahar & Palade, 1962).

The significance of two other features of the chorion, namely the microvillous structures and the intracellular ducts, is unknown. The latter appear similar in form to those found, for example, in parietal cells of the stomach (Ito, 1961). The arrangement of the microvillous structures subjacent to the shell membrane resembles, in some respects, that of glomerular pedicels (Hall, 1954; Leeson, 1959)

and functionally these structures represent an increase in surface area of the plasma membrane of chorionic cells for some function such as fluid transport, an indication of which has been noted already in the presence of multivesicular bodies. In the literature, most discussions of the chorion have concentrated upon it as the site of respiratory exchange and ignored the fluid requirements of the developing embryo, which must be considerable. Both microvillous structures and multivesicular bodies might indicate that the chorion is of importance not only in respiratory exchange but also in the acquisition of the fluid requirements of the embryo. Undoubtedly, however, the majority of this requirement is met by yolk metabolism, particularly in the young embryo.

Danchakoff (1917), in a light microscopy study, was unable to visualise cell boundaries in chorionic ectoderm. However, such boundaries can be resolved clearly with the electron microscope. The interface between cells may be quite regular or show considerable interlocking. Desmosomes were found frequently in the chorion in the present study, although they were not reported by Rangan & Sirsat (1962). The presence of desmosomes does not indicate necessarily, as has been assumed by many workers, that there is a firm adhesion between cells. Both Fawcett (1958), in the stratum spinosum of epidermis, and Leeson (1962), in transitional epithelium of the rat urinary bladder, have reported their presence in epithelia assumed to be quite labile with regard to orientation of cells.

It is interesting to note that the present study indicates that there are many similarities in fine structure between component cells of the chick chorion and cells of the implantation site in the rabbit (Larsen, 1961). One further point about the chorion is that dark, probably degenerate cells have been seen in all stages in the present investigation. This finding is in agreement with that of Romanoff (1960), who noted degeneration of chorionic cells as early as after 10 days' incubation.

Romanoff (1960) also described hydropic degeneration of the allantois in which the cells became spheroidal, with clear cytoplasm and deformed or displaced nuclei. As numbers of such cells increased, they protruded into the allantoic cavity and irregular spaces appeared between them and the deeper elements. The protruding cells then degenerated completely, according to Romanoff (1960), and fell into the allantoic sac. Such hydropic degeneration has been noted also in the present study, notably after 21 days' incubation. The dense bodies seen in the apical region of allantoic cells, particularly marked in stages prior to 21 days' incubation, are possibly lipid in nature, as has been suggested by Borysko & Bang (1953) and, more recently, by Rangan & Sirsat (1962). The latter authors were of the opinion that such granules might represent a reaction of the cells to the presence of toxic materials in the allantoic sac. Such may be the case, although it should be noted that the granules were not found to be a prominent feature of allantoic cells after 21 days' incubation in the present study, a period at which reasonably one might expect the toxicity of the allantoic contents to be maximal.

SUMMARY

1. Descriptions are given of the histology and fine structure of the chorio-allantois in the chick after incubation times of 7, 11, 14, 16, 18 and 21 days.

2. The chorio-allantoic membrane consists of three distinct layers: (*a*) chorionic ectoderm, with associated vascular elements, lying immediately subjacent to the shell membrane; (*b*) an intermediate layer of mesenchyme in which course the main blood vessels; (*c*) allantoic endoderm.

3. Within the chorion, there is an extension of the chorionic blood sinus and a reduction in the cytoplasmic barrier between it and the overlying shell membrane with increase in time of incubation.

4. The fibre content of the intermediate layer of mesenchyme increases from 7 to 21 days' incubation.

5. Cells of the allantoic endoderm show extensive changes during incubation, leading to the appearance of hydropic degeneration just prior to hatching.

6. The nature and extent of the chorionic blood sinus are discussed in detail. It is concluded that the lining of the sinus is composed of cells of chorionic epithelial origin.

7. Fine structural details within chorionic cells, viz. multivesicular bodies and microvillous structures, suggest that the chorion is of functional importance not only in respiratory exchange but also in the acquisition of the fluid requirements of the embryo.

8. The presence of dense granules, possibly lipid in nature, within cells of allantoic endoderm and of degenerative changes are thought to represent a reaction of the cells to the accumulation of toxic materials within the allantoic sac.

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EXPLANATION OF PLATES

The following abbreviations are used in the figures: *a*, allantoic endoderm; *b*, chorionic blood sinus; *bm*, basement membrane; *c*, chorionic epithelium; *d*, desmosome; *e*, endoplasmic reticulum; *f*, unit fibril of collagen; *g*, Golgi apparatus; *h*, mitochondrion; *i*, infolding of plasma membrane; *m*, mesenchyme; *n*, nucleus; *nm*, nuclear membrane; *p*, Palade granule; *r*, red blood cell; *s*, shell membrane; *t*, chorionic blood sinus-lining cell; *u*, vesicle; *v*, microvillus; *w*, microvesicle; *z*, multivesicular body. With the exception of those in Pl. 1, all figures are electron micrographs.

PLATE 1

Fig. 1. Seven days' incubation, to show the three components of the membrane: chorionic epithelium, mesenchyme and allantoic endoderm. A large allantoic blood vessel is present in the mesenchyme. Iron haematoxylin–van Gieson, × 250.

Fig. 2. High-power micrograph of chorionic epithelium after 7 days' incubation. Note the small blood vessel passing through the basal layer of chorionic epithelium to join the chorionic blood sinus. Haematoxylin and eosin, × 1200.

Fig. 3. Fourteen days' incubation. Above is the shell membrane separated by an artefactual gap from the underlying chorion. Numerous capillaries lie in close association with the chorion. Toluidine blue, × 200.

Fig. 4. Fourteen days' incubation. The shell membrane and the allantoic endoderm are strongly P.A.S.-positive. On the surface of the allantoic endoderm there is a layer of amorphous material, possibly the result of fixation of some protein content of the allantoic sac. P.A.S. reaction, × 225.

Fig. 5. Eighteen days' incubation. The fibrillar content of the mesenchyme is more marked than in the earlier stages. Haematoxylin and eosin, × 200.

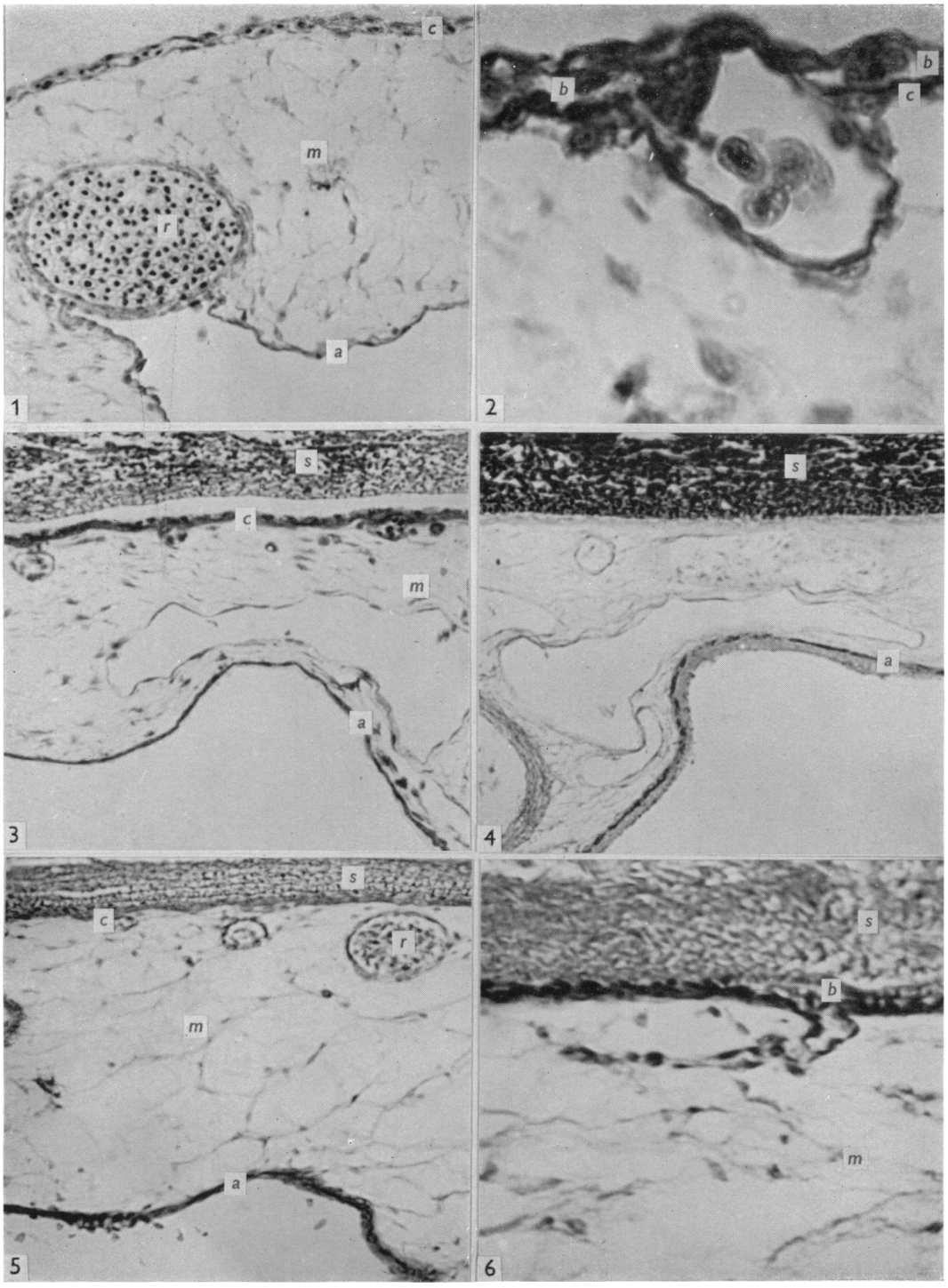
Fig. 6. Twenty-one days' incubation, showing shell membrane, chorion and a portion of the mesenchyme. Note the blood vessel passing through the chorion to end in the chorionic blood sinus immediately subjacent to the shell membrane. Iron haematoxylin–van Gieson, × 350.

PLATE 2

11 days' incubation

Fig. 7. Low-power micrograph to show the shell membrane, chorionic cells, chorionic blood sinus containing a portion of a red blood cell, and mesenchyme. In the chorionic epithelium, nuclei and mitochondria are obvious. × 2700.

Fig. 8. Higher magnification of a portion of a chorionic epithelial cell adjacent to the shell membrane. Nucleus, nuclear membrane, mitochondria, some granular endoplasmic reticulum and Palade (RNP) granules are seen. Note also the infoldings of the plasma membrane. × 18,900.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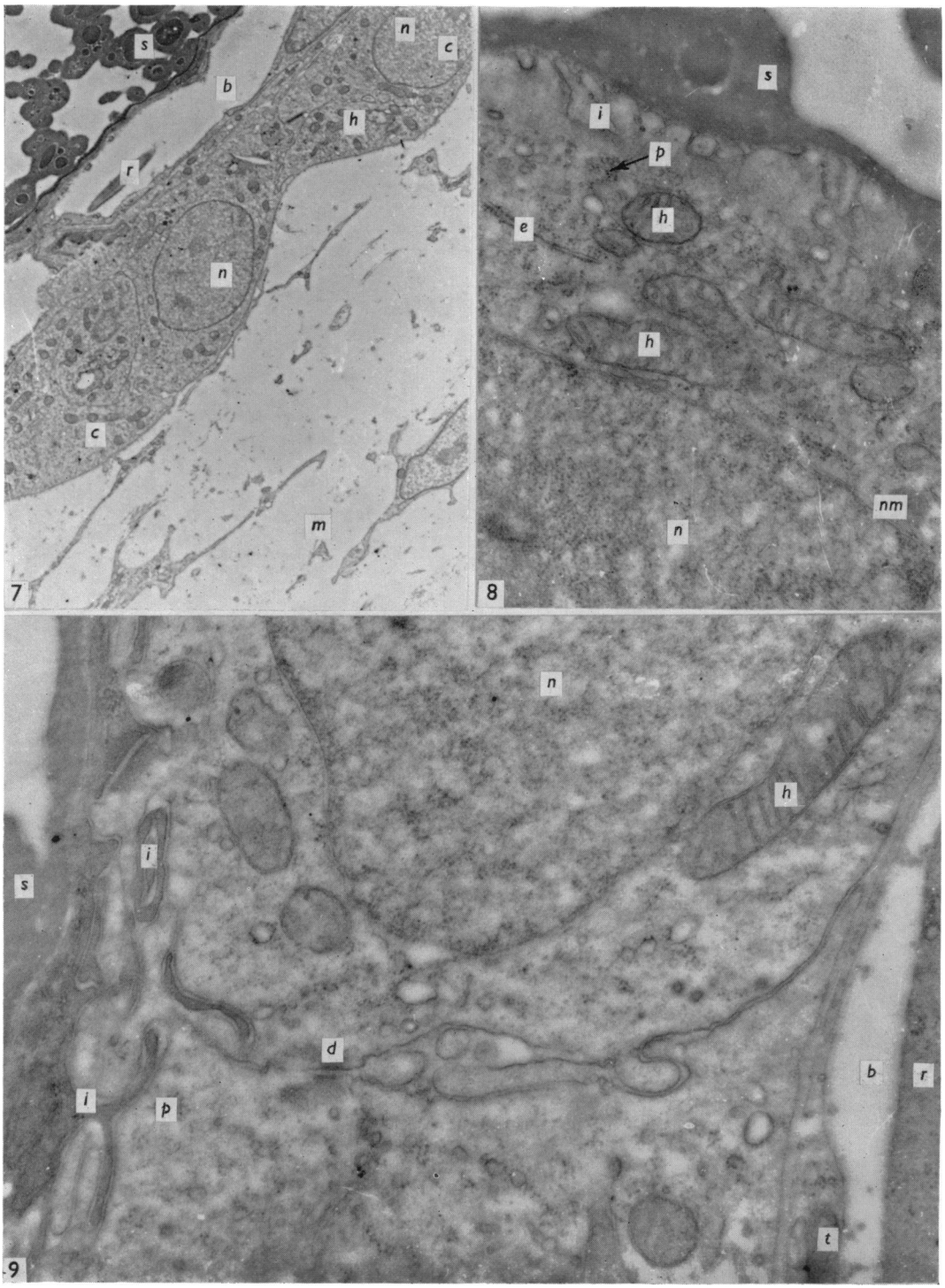
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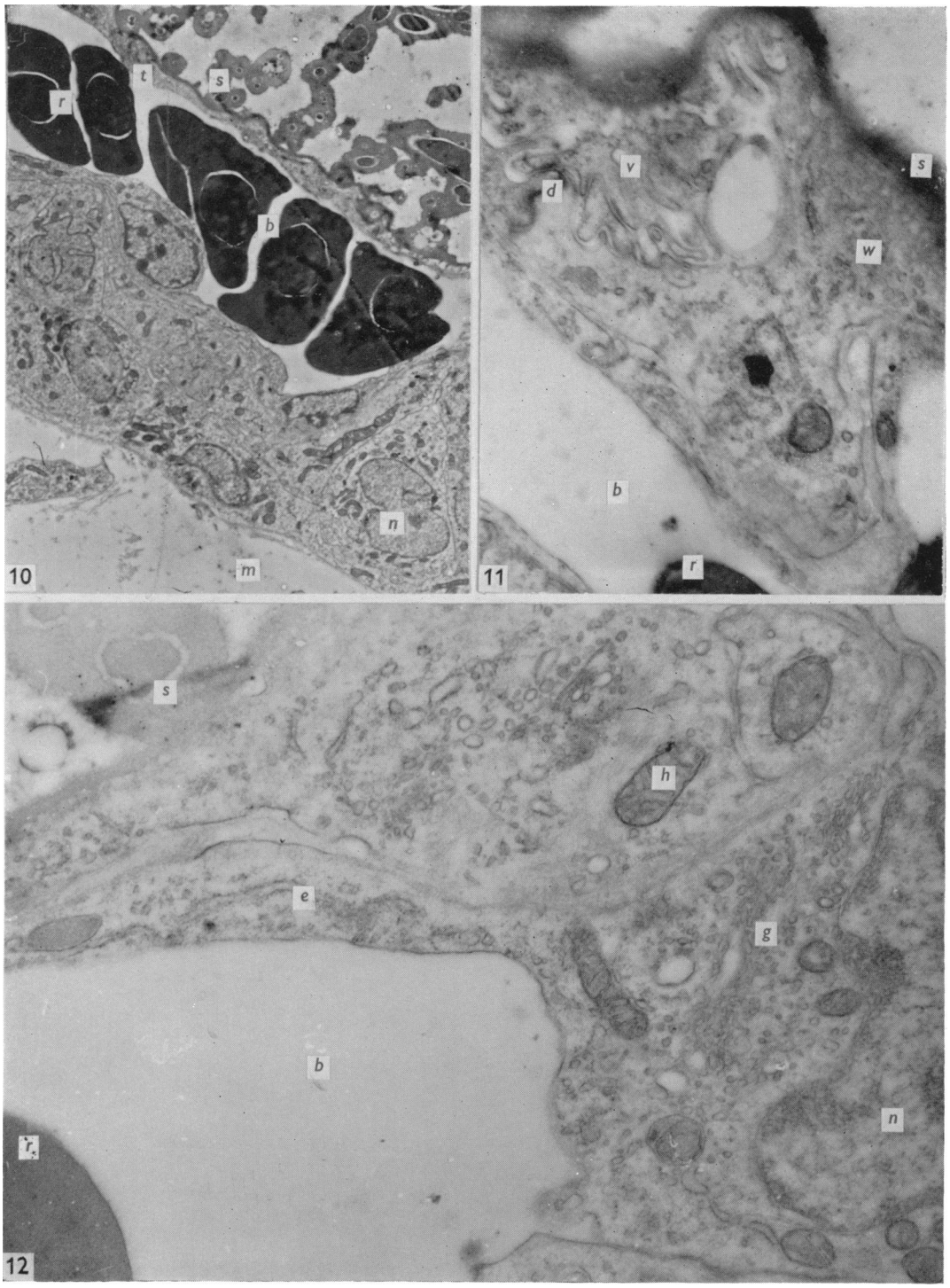
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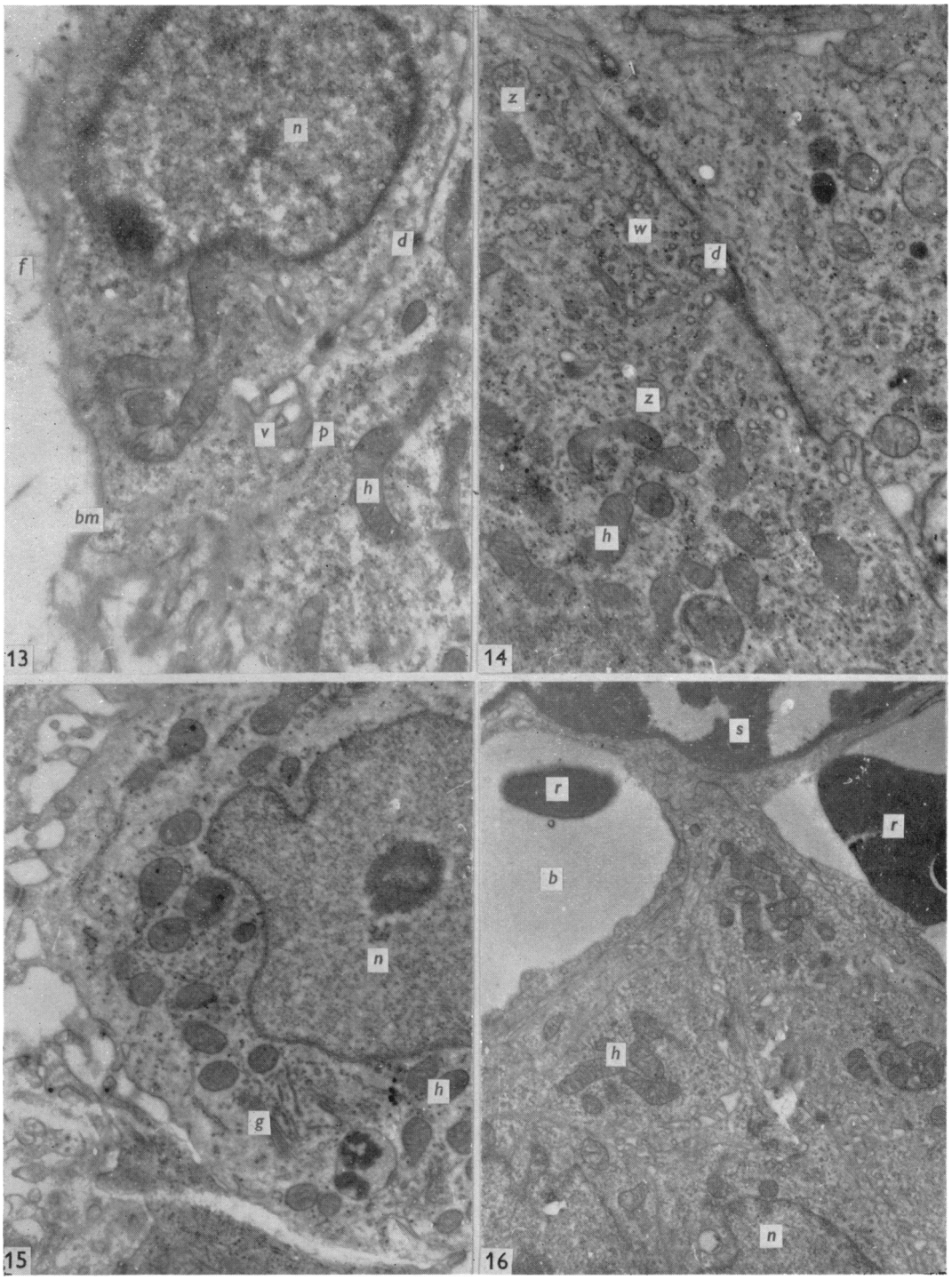
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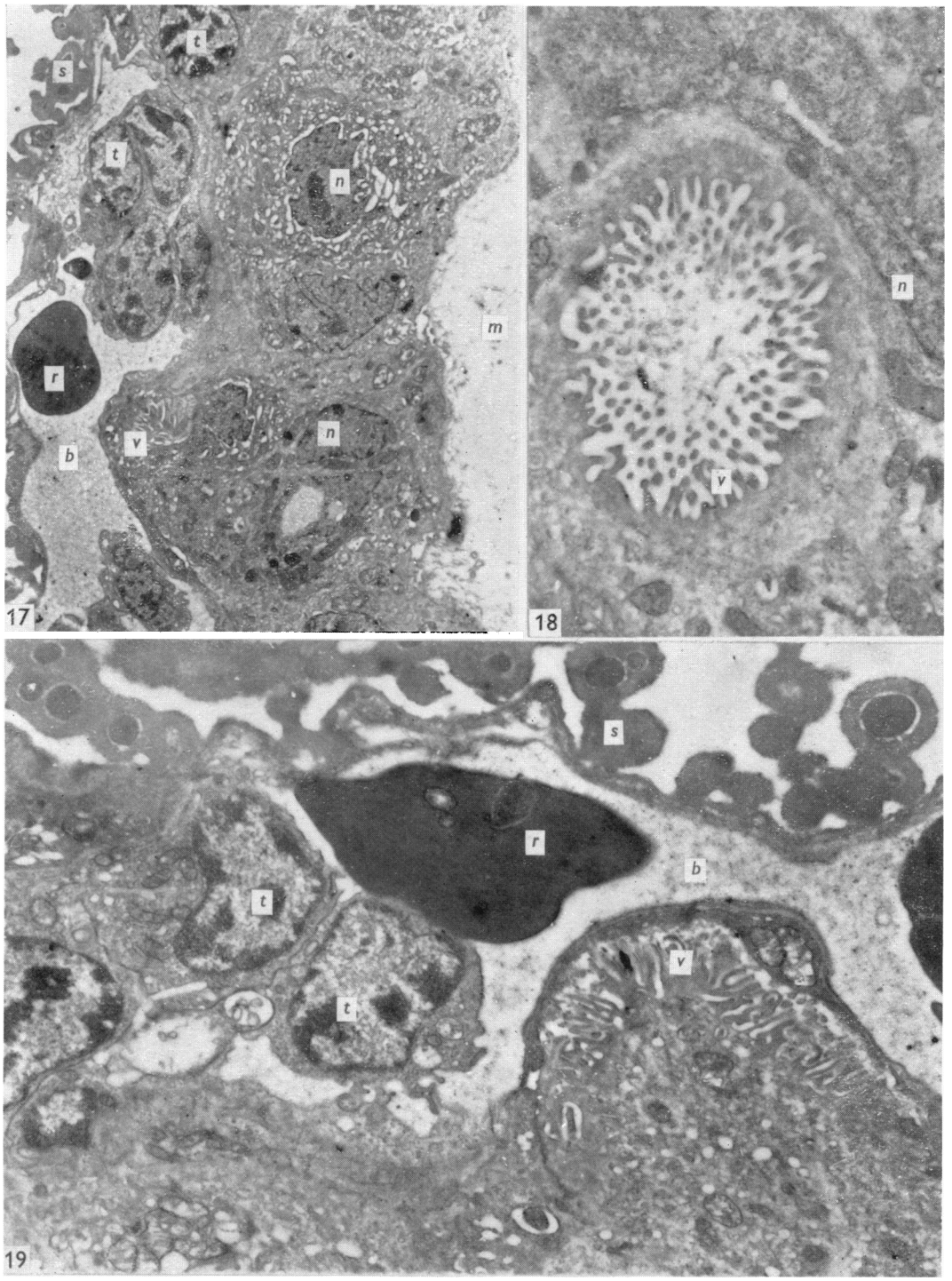
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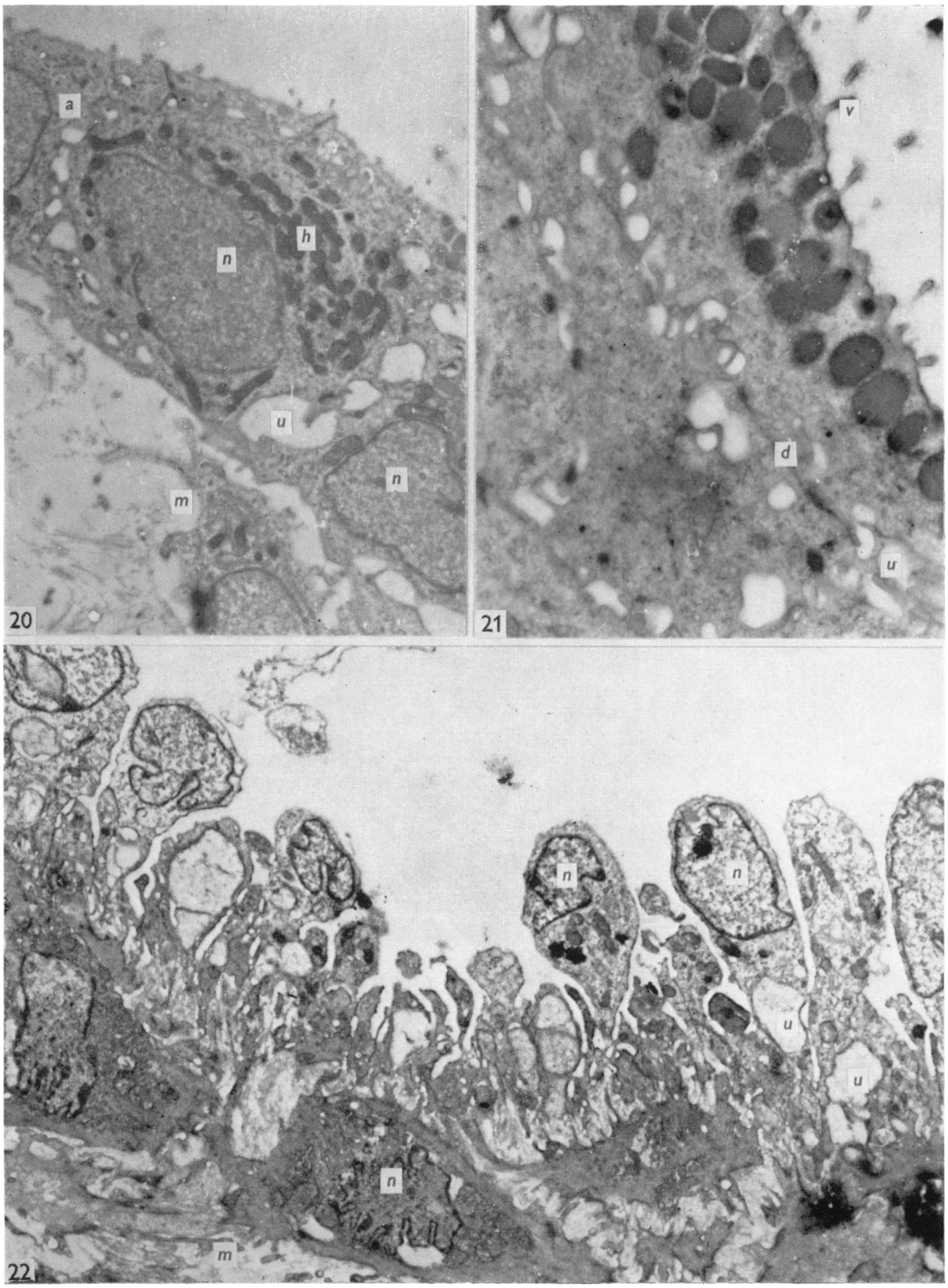
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Fig. 9. A similar region to Pl. 2, fig. 8, but with infoldings containing slips of dark cytoplasm. A cell interface with a desmosome is present, and part of the chorionic blood sinus, lined by attenuated cytoplasm, can be seen to the right. × 24,500.

PLATE 3

14 days' incubation

Fig. 10. Low-power survey micrograph of the chorionic membrane. × 2600.

Fig. 11. A portion of a chorionic epithelial cell adjacent to the shell membrane. Note the extensive microvillus formation. × 12,500.

Fig. 12. A similar area to Pl. 3, fig. 11, showing portions of two chorionic cells, one containing a well-developed Golgi apparatus. × 18,900.

PLATE 4

Fig. 13. Sixteen days' incubation. The basal region of the chorionic membrane, illustrating an interface with desmosomes, and separation of the opposed plasma membranes, with microvilli. Also seen are the basement membrane and a few unit fibrils of collagen. × 10,900.

Fig. 14. Sixteen days' incubation. Portions of two epithelial cells, with separation of plasma membranes at an interface (lower right), numerous microvesicles, and a multivesicular body. × 10,900.

Fig. 15. Sixteen days' incubation, showing part of a cell, possibly a lymphocyte, with a well-developed Golgi apparatus, separated from surrounding cells by a wide intercellular space. This cell also contains an inclusion, with dark granular content, near the Golgi apparatus. × 10,900.

Fig. 16. Eighteen days' incubation. The chorionic membrane and blood sinus beneath the shell membrane. There is a 'bridge' of tissue crossing the blood sinus. × 3400.

PLATE 5

21 days' incubation

Fig. 17. Low-power survey micrograph showing shell membrane, chorionic blood sinus lined by small cells with nuclei showing chromatin masses, and the chorionic membrane containing two vacuolated, dark, possibly degenerate cells. In the region immediately beneath the blood sinus there is a complex development of microvilli. × 2600.

Fig. 18. Part of a chorionic cell showing an intracellular duct or vacuole lined by microvilli. × 8500.

Fig. 19. The chorionic blood sinus at higher magnification. Note the extensive development of microvilli, centre right. × 4200.

PLATE 6

Allantoic endoderm

Fig. 20. Eleven days' incubation, showing allantoic endoderm and subjacent mesoderm. In the endoderm cells, mitochondria and intercellular vesicles are prominent. × 3800.

Fig. 21. Fourteen days' incubation. The apex of an endodermal cell, which contains microvilli, dense granules, intercellular vesicles and desmosomes. × 16,100.

Fig. 22. Twenty-one days' incubation, showing the protrusions of endodermal cells into the allantoic cavity, with nuclei, vesicles and some lipid. The dark mesodermal cells beneath the endoderm resemble smooth muscle. × 3100.