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A morphological study of the development of the allantois of rat embryos *in vivo*

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INTRODUCTION

The muridine allantois is purely mesodermal in origin (Mossman, 1937) and first appears as a bud of cells emerging from the caudal region of the embryo at the head-fold stage. The bud then becomes prolongated, extending from the embryo into the extra-embryonic coelomic cavity and towards the ectoplacental cone. It continues to grow until it makes contact, and fuses with the chorionic mesoderm (Amoroso, 1952). After the fusion of the allantois with the 'chorion', these tissues, together with cells of the ectoplacental cone, develop to form the fetal components of the chorio-allantoic placenta. The allantois remains intact throughout the remaining period of gestation connecting, and conveying fetal blood between, the fetus and the chorio-allantoic placenta.

The gross structural changes occurring during the development of the muridine allantois have been described for both the mouse (Amoroso, 1952; Snell & Stevens, 1966; Theiler, 1972) and the rat (Steven & Morriss, 1975) but there are no detailed descriptive studies.

In this paper the growth and differentiation of the allantois of the rat, as seen by direct observation of freshly dissected embryos, conventional histological techniques and scanning and electron microscopy, is described in detail.

MATERIALS AND METHODS

Animals

CFHB rats were used throughout this study. Females were caged with males overnight and those females with sperm in the vagina the following morning were regarded as half a day pregnant (0·5 day) at noon that day. The majority of the females were killed between 9·5 days and 10·5 days (see below for details). They were lightly anaesthetised with ether vapour and then killed by cervical dislocation. The embryos were dissected out as described by New (1971) and were either immediately processed for histology or were explanted and maintained in tissue culture for two hours prior to fixation.

Culture

Preliminary experiments indicated that the development of the allantois *in vitro* appeared to be similar in all respects to that occurring *in vivo*. In order to examine the distribution of cell division in the allantois, conceptuses with intact visceral yolk sacs but opened parietal yolk sacs were explanted, as previously described (New, 1971), at intervals between 9·5 and 10·5 days and were maintained in a rotator culture system (New & Cockroft, 1979) in undiluted, immediately-centrifuged heat-

inactivated serum (Steele & New, 1974) containing 0·1 % colchicine: this concentration of colchicine did not cause visible harm to the embryo. The gas phase used when culturing embryos younger than 10·5 days contained 5 % oxygen, 5 % carbon dioxide and 90 % nitrogen. The gas mixture used for 10·5 day embryos was 20 % oxygen, 5 % carbon dioxide and 75 % nitrogen. A total of 20 explants was cultured, four at each stage of development; all cultures were terminated after two hours, the specimens rinsed in saline and then processed for light microscopy.

Histology

This study was based on embryos from a total of 44 rats.

Light microscopy

The parietal yolk sacs were removed and the remaining embryonic and extra-embryonic components of the conceptus were rinsed in saline and either fixed in alcoholic Bouin's solution for at least 48 hours or processed as for electron microscopy. The Bouin-fixed conceptuses were dehydrated, cleared and embedded in paraffin wax. Blocks were sectioned at a thickness of 4 μm and sections were stained in haematoxylin and eosin for routine histology, and by the periodic acid–Schiff (PAS) technique to demonstrate the presence of PAS-positive carbohydrate. To calculate the mitotic index of the allantois of embryos cultured in the presence of colchicine, 5 μm thick sections were cut parallel to the longitudinal axis of the allantois. Ten sections of the allantois of each embryo were analysed; in each section one hundred cells were counted and the number of mitotic figures (n) noted. The mitotic index was calculated as the mean value of n.

Sections 1 μm thick were cut from the resin blocks prepared for electron microscopy and were stained with 1 % toluidine blue in borax.

Electron microscopy

Additional specimens were fixed in 3 % glutaraldehyde in phosphate buffer (300 m-osmol.) overnight at 4 °C. They were rinsed in buffer and specimens for transmission electron microscopy were postfixed in 1 % osmium tetroxide in phosphate buffer for two hours at room temperature. The fixed material was then block-stained in uranyl acetate, dehydrated in ethanol and propylene oxide, and embedded in either Araldite or Spurr's resin. Thin sections were stained in saturated uranyl acetate in 50 % ethanol and lead citrate (Reynolds, 1963).

Following glutaraldehyde fixation, specimens for scanning electron microscopy were thoroughly rinsed in buffer, a large window made in the visceral yolk sac and then part of the ectoplacental cone dissected away. The specimens were then post-fixed in osmium tetroxide, dehydrated in ethanol, critical-point dried and gold coated prior to being viewed in a JEOL scanning electron microscope.

The allantoic bud became clearly defined from the mesoderm of the primitive streak by 9·5 days. The description is based primarily on embryos explanted at 9·5 days and at four subsequent six hourly intervals. In order to analyse the early development of the allantois in more detail, embryos were also fixed (for light microscopy only) at 9·375 and 9·625 days.

Table 1. *The mitotic index of cells of the allantois during its early development*

Embryos were cultured for two hours in medium containing 0·1% colchicine. The mitotic index is calculated as mean number of mitotic figures/100 cells.

Age of embryos at onset of culture (days)	Number of embryos (number of sections)	Mitotic index (mean ± S.E.)
9·5	4 (40)	11·9±0·8
9·75	4 (40)	9·5±1·1
10·0	4 (40)	6·4±0·5*
10·25	4 (40)	5·4±0·4**
10·5	4 (40)	5·3±0·8*

* Significantly different from the mitotic index of 9·5 day embryos $P < 0{\cdot}01$.
** Significantly different from the mitotic index of 9·5 day embryos $P < 0{\cdot}001$.

OBSERVATIONS

The morphological variation between embryos fixed at the same stage of gestation, whether they were littermates or from different mothers, was relatively small. This account is based on the 'typical' development for each stage of gestation; an indication of the variation is also given. For each stage the gross morphology is described first, followed by descriptions of the cell morphology, histochemistry, the pattern of cell division, the development of vascularisation where appropriate and, finally, the ultrastructure of the cells.

9·5 *days*

By 9·5 days a small clump of cells had formed, just distal to the caudal region of the primitive streak, in the angle between the amnion and the yolk sac. The cluster of cells was several cells thick in the centre but became thinner near the edges. In a few of the more advanced embryos the cell cluster was beginning to elongate and project into the extra-embryonic coelom and away from the visceral yolk sac. PAS-positive droplets were present in a few cells at the distal tip of the most advanced allantois.

Cell divisions occurred throughout the cluster (Fig. 1*a*) and the mitotic index was high (Table 1).

As seen with the electron microscope, there was little obvious differentiation amongst the presumptive allantois cells. The cytoplasm was granular and contained few organelles, predominantly small mitochondria and short strands of granular endoplasmic reticulum. The outer surfaces of the cells abutting the extra-embryonic coelom were smooth. The inner borders of these outer cells and all parts of the borders of the inner cells had small cell processes projecting into the extracellular space and, occasionally, onto the surface of a neighbouring cell. No extracellular matrix was observed between cells. The morphological development is summarised in Table 2 and in Figure 2.

9·75 *days*

At this stage, the distal end of the allantois extended as a thin column of cells projecting into, and across the cavity of the extra-embryonic coelom. The most advanced allantois observed in this group extended two thirds of the way across the

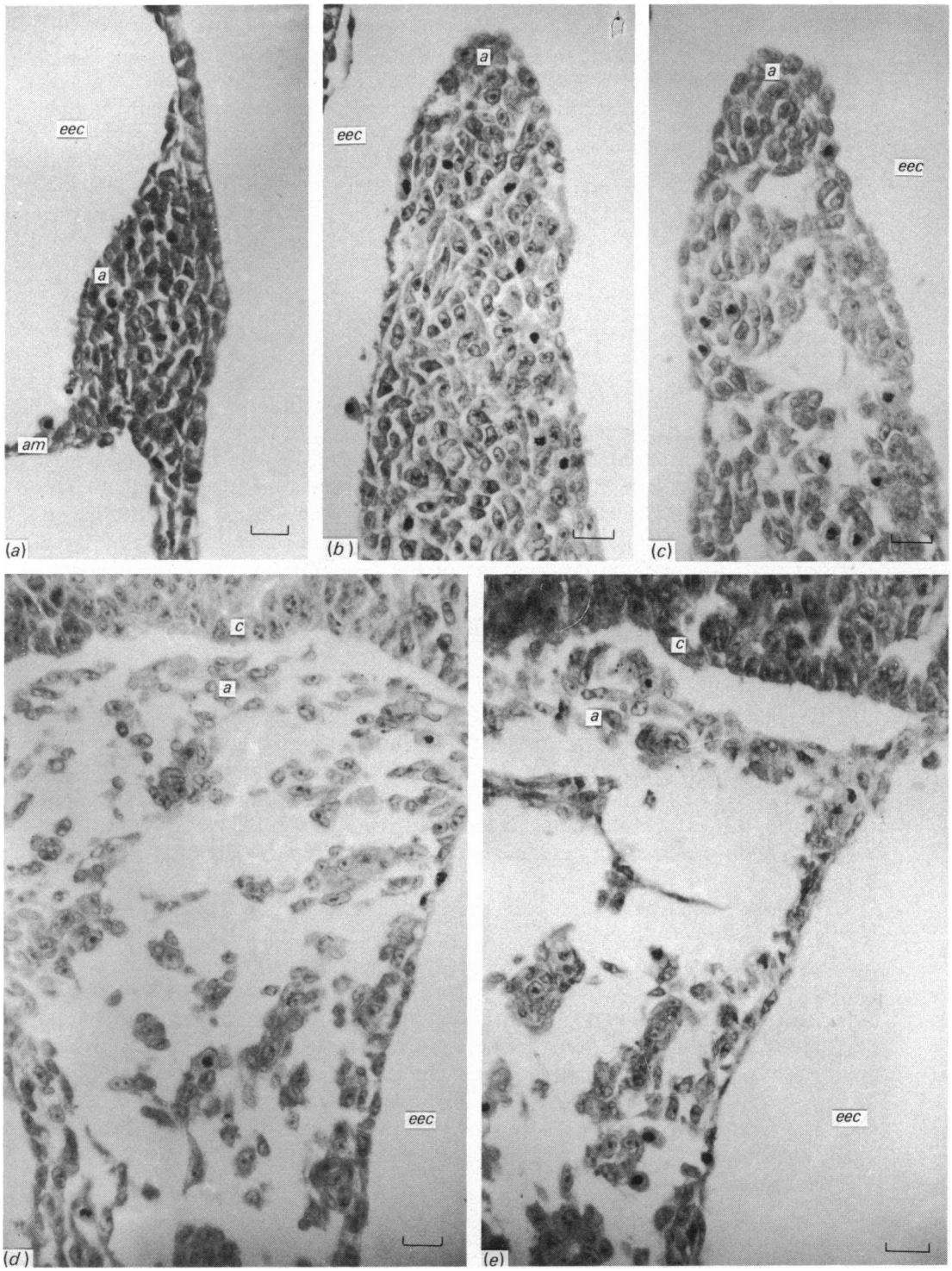

Fig. 1(*a–e*). Light micrographs of the distal tip of the allantois of embryos after two hours culture in medium containing 0·1% colchicine to show the relative number and the distribution dividing cells at each stage. (*a*)–(*e*) are of embryos put into culture at 9·5, 9·75, 10, 10·25 and 10·5 days respectively. Scale bar: 10 μm. See p. 11 for abbreviations.

Table 2. *The development of the allantois from the stage of its first appearance to the formation of a functional vascular system*

All measurements were taken from Bouin-fixed specimens sectioned either parallel to, or perpendicular to, the longitudinal axis of the allantois.

Age of embryo (days)	Length of allantois μm ± S.E. (number)	Diameter of allantois μm ± S.E. (number)	% of embryos with fused chorion and allantois	Carbohydrate present (PAS+)	Vascularisation
9·375	148·6 ± 30·0 (4)	123·6 ± 7·3 (8)	0	NT	Absent
9·5	147·4 ± 5·8 (6)	143·7 ± 8·1 (9)	0	Absent	Absent
9·625	203 ± 0 (3)	153·7 ± 8·7 (5)	0	NT	Absent
9·75	630·7 ± 145·5 (3)	120·8 ± 21·1 (3)	0	Distal tip only	Absent
10·0	770·3 ± 98·5 (6)	178·4 ± 2·5 (6)	0	Distal tip + some other cells	Rudiments of blood vessels
10·25	960·3 ± 48·2 (9)	218·8 ± 12·7 (9)	~ 75*	Most cells	Blood vessels + blood cells
10·5	—	—	100	Scattered cells only	Blood vessels + blood cells

* Only approximate value because of difficulty of establishing precise relationship of tissues prior to dissection and histological processing.
NT, not tested.

extra-embryonic coelom; most were considerably shorter. A few cells at the distal tip of the allantois and occasional cells amongst the inner and outer cells of the allantois contained PAS-positive droplets in the cytoplasm. Mitotic figures were distributed evenly throughout the allantois but the mitotic index was slightly lower than at 9·5 days (Table 1).

By this stage of development there were obvious regional differences in the allantois. The distribution of the cells in the proximal part of the allantois resembled that in the allantois of 9·5 day embryos, although by 9·75 days most of the outer cells were joined to one another by desmosomes, thus forming a complete layer of cells enclosing the loosely packed inner cells. Although cell contacts did occur between inner cells, no specialised junctions were observed.

In the distal part of the allantois, the outer cells were either completely flattened or had large, flattened, cytoplasmic sheets radiating from the perinuclear region; neighbouring cells were joined by desmosomes. The inner cells were widely dispersed so that, in the more advanced allantois, the distal tips appeared almost hollow.

As at 9·5 days, the cytoplasm of the majority of cells contained few organelles, predominantly small mitochondria and short strands of granular endoplasmic reticulum (Fig. 4).

10 *days*

Between 9·75 and 10 days, the allantois elongated slightly but the most marked morphological change was the increase in diameter (Table 2). A slight constriction formed in the allantois near the distal end, giving the distal tip a bulbous appearance.

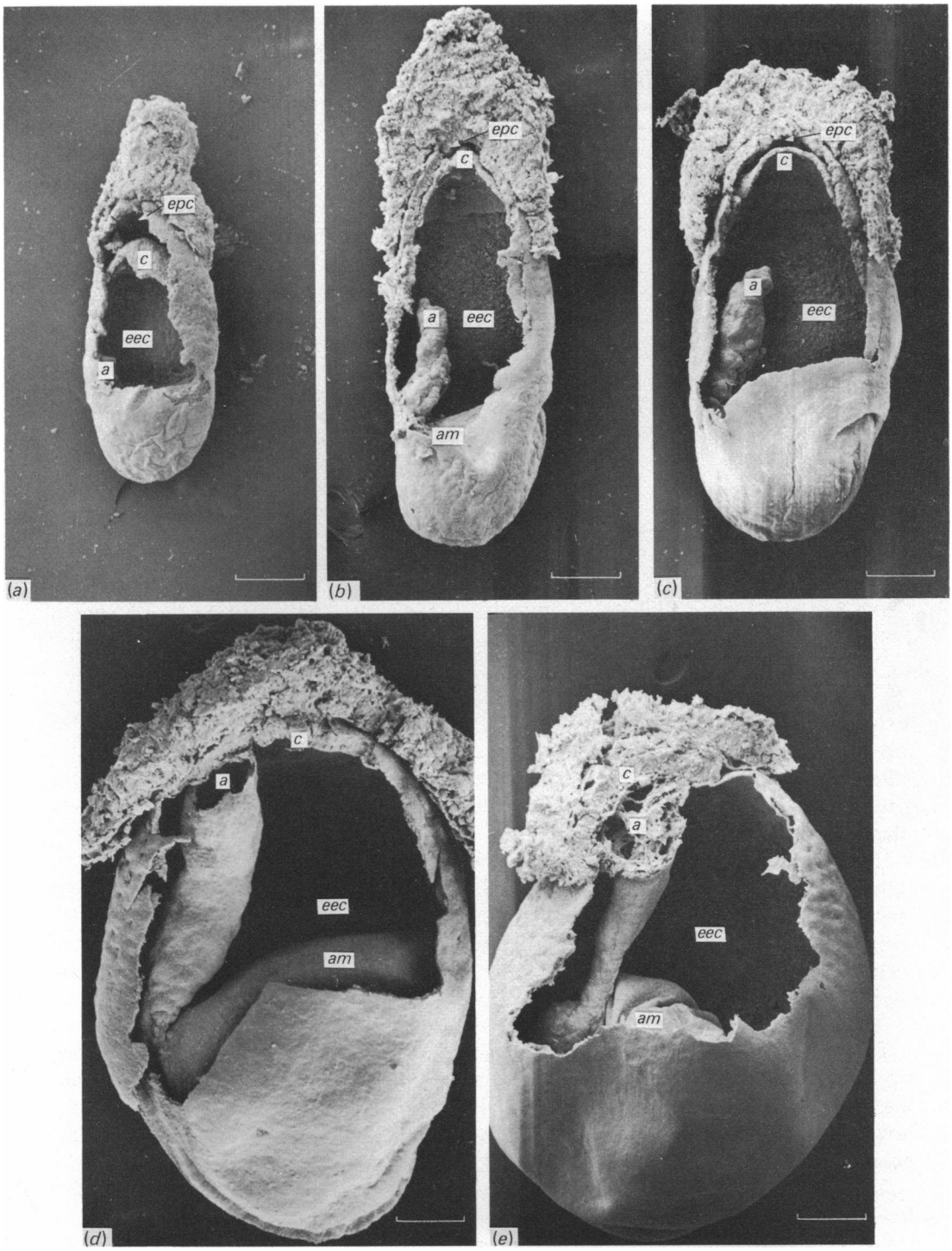

Fig. 2(*a–e*). Scanning electron micrographs to show the development of the allantois of rat embryos from 9·5 to 10·5 days. (*a*)–(*e*) are of embryos of 9·5, 9·75, 10, 10·25 and 10·5 days respectively. Scale bar: 200 μm. See p. 11 for abbreviations.

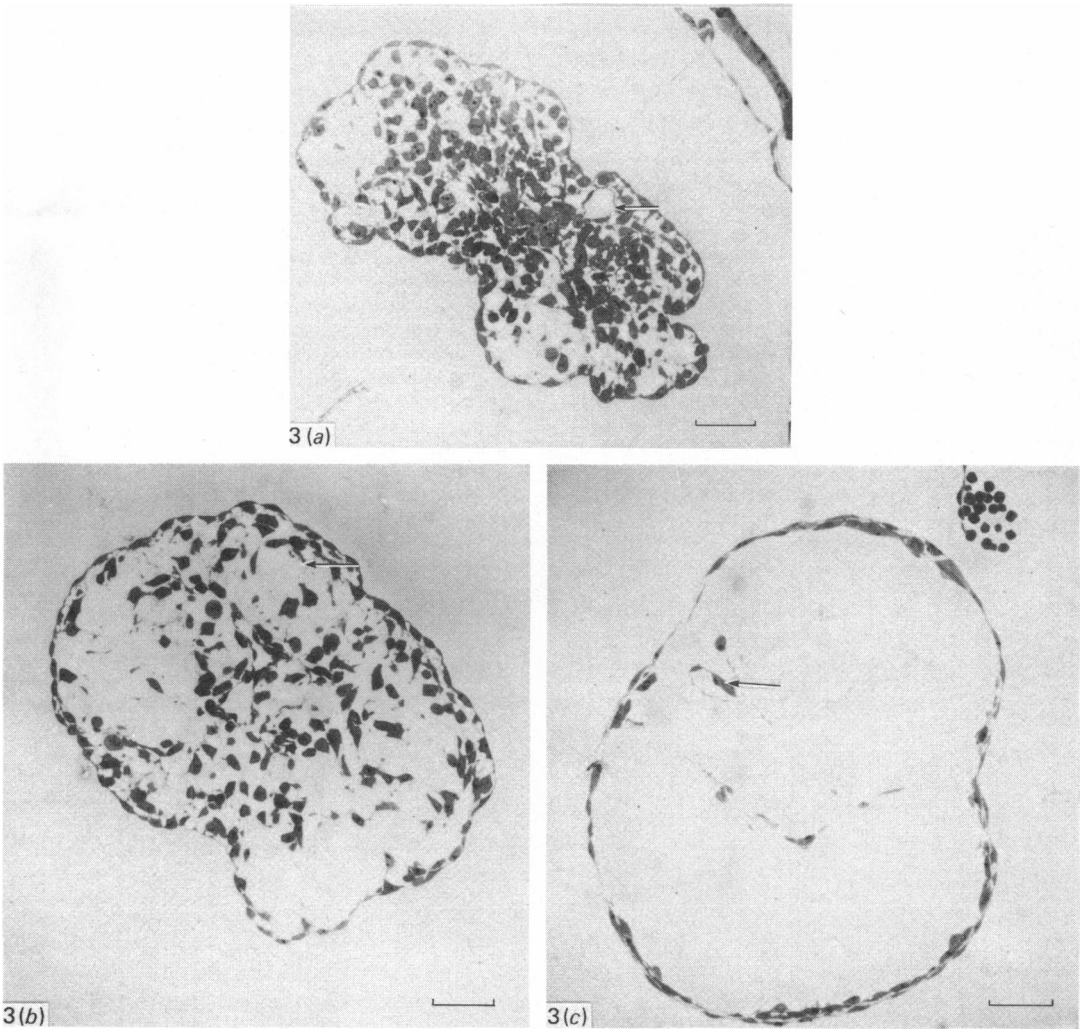

Fig. 3

Fig. 3 (*a–c*). Transverse sections from the allantois of a 10 day rat embryo. Section (*a*) was taken from the proximal region, (*b*) from the mid region and (*c*) from the distal tip of the allantois. The sections show the structural variation present in a single allantois and the differences between the inner and the outer cells of the allantois. Developing blood islands are arrowed. Scale bar: 50 μm.

The regional differences described for the 9·75 day allantois were more conspicuous by 10 days (Fig. 3). The proximal region resembled that of 9·75 day embryos. The distal region with its flattened outer cells and widely dispersed inner cells had, however, increased both in its overall length and diameter. Cells containing PAS-positive droplets were largely restricted to the distal tip.

The number of dividing cells was still high but the mitotic index was significantly lower than in 9·5 day embryos (Table 1).

By 10 days, rudiments of the vascular system of the allantois were beginning to form. Some of the inner allantoic cells developed long thin cytoplasmic processes which joined with similar processes from neighbouring cells to form small vesicles (these later coalesced to form the blood vessels of the allantois).

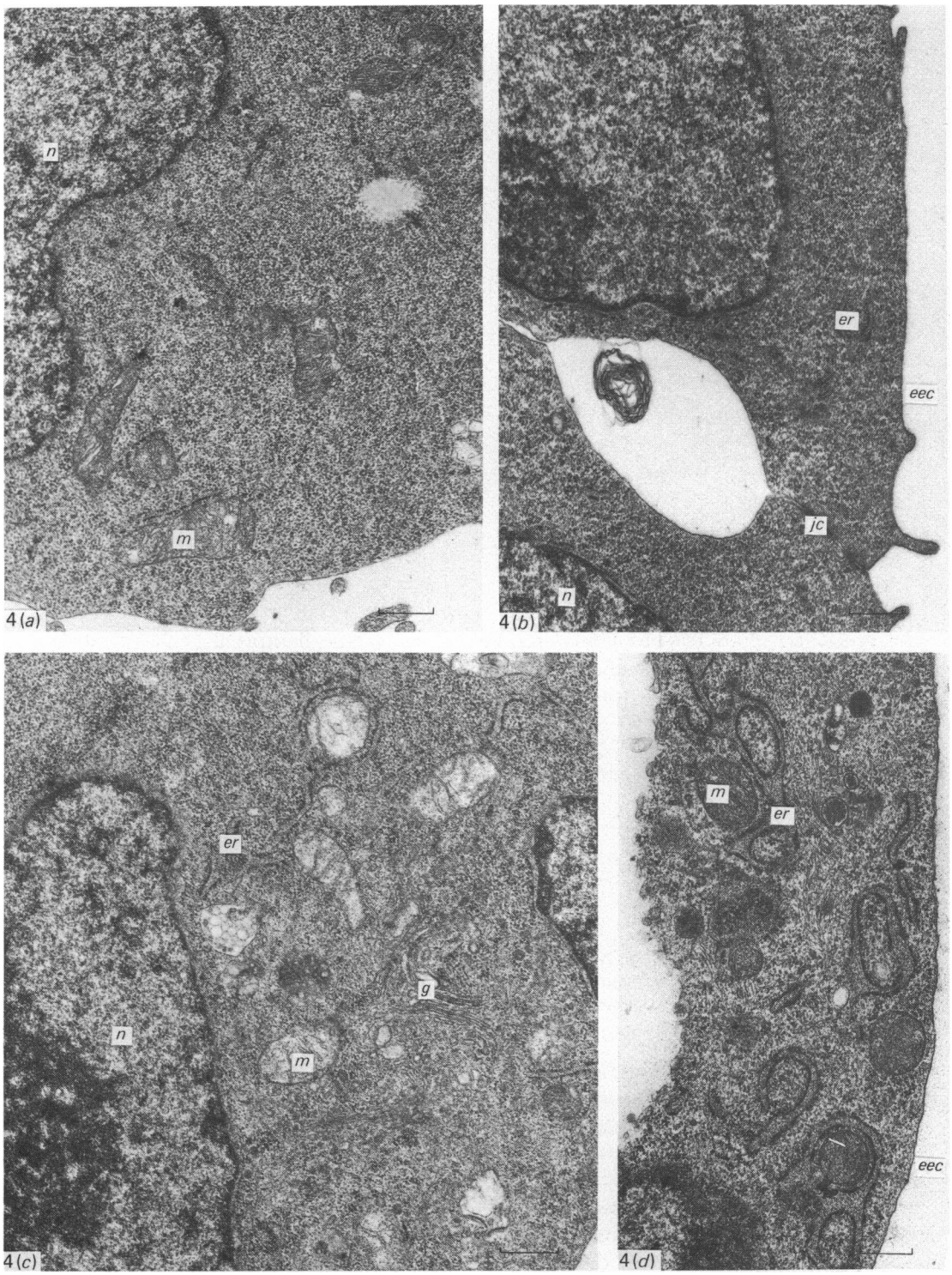

Fig. 4(*a–d*). Transmission electron micrographs of cells from the allantois to show cellular changes between 9·75 and 10·25 days. (*a*) and (*b*) are inner and outer cells respectively from a 9·75 day embryo. (*c*) and (*d*) are inner and outer cells respectively from a 10·25 day embryo. Scale bar: 0·5 μm. See p. 11 for abbreviations.

In spite of the gross structural changes observed amongst cells of the allantois, the majority of the cells showed little cytodifferentiation. Those that were beginning to undergo differentiation contained numerous small Golgi complexes and increasing numbers of mitochondria. The network of granular endoplasmic reticulum became more extensive and the cisternae were slightly distended. Droplets within the cytoplasm (diameter: 0·5–1·0 μm) contained a substance resembling that in the cisternae of the endoplasmic reticulum.

10·25 *days*

By 10·25 days, the allantois of most embryos extended across the extra-embryonic coelom and contacted the 'chorion'.

Cell structural appearance was essentially similar to that of the 10 day allantois except at the distal tip. The outer layer of the distal tip was several cells thick, the outermost cells were flattened, the inner ones more rounded. Once the allantois had made contact with the 'chorion' it became difficult to distinguish the cells of the allantois from the cells of the chorionic mesoderm. Within the area of contact between the allantois and 'chorion', rounded mesoderm cells became closely associated with the chorionic endoderm cells.

Almost all the cells in the allantois contained PAS-positive droplets and the outer surface of the allantois had a thin covering of a PAS-positive substance.

Dividing cells could still be seen throughout the allantois but the mitotic index was significantly lower than that in 9·5 day embryos (Table 1).

The vesicles had fused to form blood vessels within the allantois and blood vessels at the proximal end of the allantois became confluent with the network of the yolk sac. Nucleated blood cells (erythroblasts) could now be seen in the blood vessels of the allantois.

Throughout the allantois, the cytoplasm of the cells contained small Golgi complexes, mitochondria, granular endoplasmic reticulum with distended cisternae and small secretory droplets (Fig. 4).

10·5 *days*

Once the allantois had fused with the chorion, the distal end tended to splay out to cover more of the surface of the chorion and the remaining part of the allantois became thinner. There was rapid development of the vascular system.

As the distal tip spread over the surface of the chorion, the cells in the tip became more widely dispersed and they elongated, extending strands of cytoplasm towards the chorion. The majority of the cells did not stain using the PAS technique.

There was a continuation of cell division throughout the allantois but the mitotic index was lower than at earlier stages of development (Table 1).

Many of the inner cells of the allantois were incorporated into the vascular system, which developed a network of capillaries extending almost to the surface of the chorion. Vessels in all parts of the allantois contained blood cells. The remaining inner cells formed a loose mesh of connective tissue cells around the blood vessels; the outer cells formed a continuous layer around the allantois.

There appeared to be a reduction in the synthetic activity of the allantoic cells, compared with those of 10·25 day embryos, as indicated both by the organelles present in the cytoplasm and the quantity of PAS material associated with the cells. The cells around the bood vessels contained small, rounded, mitochondria, Golgi

complexes and short strands of granular endoplasmic reticulum. Outer cells contained elongated mitochondria, very small Golgi complexes and a small amount of granular endoplasmic reticulum. Some cells contained a few irregularly shaped droplets, but these were uncommon.

DISCUSSION

The above account describes in detail the morphological development of the rat allantois from its first appearance as a small cluster of cells attached to the caudal region of the embryo, through its growth across the extra-embryonic coelom and until its fusion with the 'chorion' and the development of a functional blood supply. During its development, the allantois initially increases in length; there is then also a rapid increase in diameter associated with an increase in the extracellular space inside the allantois and distention of the outer cells. After the fusion of allantois and chorion and the initiation of a blood circulation through the allantois, the diameter decreases again (Fig. 2*d*, *e*). The increase in size of the allantois is partially due to an increase in cell number, with very rapid proliferation of cells especially during the earlier stages of its development. After colchicine treatment, mitotic figures can be seen throughout the allantois where they are evenly distributed between the regions and amongst both inner and outer cells.

It seems likely that another important factor in the growth of the allantois could be the presence of a relatively high hydrostatic pressure in its cavity compared with that in the surrounding extra-embryonic coelom. Both the stiff, turgid appearance of the intact allantois and of its component cells, coupled with the absence of any obvious extracellular supporting fibres, are indicative of fluid accumulation within the tissue. The allantois may be functioning as a storage organ for embryonic excretory products until they can pass into the maternal blood on the initiation of placental circulation.

As observed by Jolly & Lieure (1938), the rudiments of the vascular system in the allantois develop from the allantoic mesoderm rather than by invasion from the yolk sac. Small vesicles develop independently in several parts of the allantois and then coalesce to form capillary-like vessels. In the proximal end of the allantois the allantoic and visceral yolk sac vessels become confluent and, only at this stage, erythroblasts appear within the allantoic vascular system. The site of formation of the erythroblasts is not certain but it seems probable that they are formed in the blood islands of the yolk sac.

Ultrastructural studies show relatively little cytodifferentiation in the allantoic cells until 10 days when cells, especially in the distal tip, contain increasing numbers of mitochondria, Golgi complexes and secretory droplets, and more extensive granular endoplasmic reticulum. PAS-positive material accumulates, from 10 days, as intracellular droplets and also as a layer covering the outer surfaces of the outer allantoic cells. The cytodifferentiation and accumulation of PAS material continues between 10 days and 10·25 days and then regresses rapidly. The transient appearance of a synthetic apparatus and of PAS-positive material is coincidental with the fusion of the allantois with the chorion. The PAS-positive material may be essential for normal fusion to occur.

SUMMARY

The development of the allantois of the rat embryo has been studied from its first appearance as a cluster of cells near the caudal region of the embryo, through its growth across the extra-embryonic coelom, until its fusion with the chorion and the development of a functional vascular system.

Development and growth of the allantois is extremely fast; in the first 18 hours after its initial appearance there is rapid structural differentiation, followed, in the next few hours, by cytodifferentiation and development of the vascular system. Dividing cells occur throughout the allantois at all stages and the mitotic index, initially very high, falls rapidly as the distal tip of the allantois approaches the chorion. The structural features suggest that the extension of the allantois across the extra-embryonic coelom could be caused by a relatively high hydrostatic pressure within the allantois and by rapid proliferation of allantoic cells.

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ABBREVIATIONS FOR ALL FIGURES

a,	distal tip of allantois	*er*,	endoplasmic reticulum
am,	amnion	*g*,	Golgi complex
c,	chorion	*jc*,	junctional complex
eec,	extra-embryonic coelom	*m*,	mitochondria
epc,	ectoplacental cavity	*n*,	nucleus