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Structure of the intra-chorionic blood sinus in the chick embryo

ROBERTO NARBAITZ

Department of Anatomy,
University of Ottawa

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INTRODUCTION

The avian egg contains most of the nutritive materials needed for the growth and differentiation of the embryo, and only respiratory gases and water vapour are exchanged with the environment during development. As the embryo grows this exchange becomes more active and during the second half of the incubation period calcium from the shell is resorbed by the embryo in order to cover its increasing requirements, particularly for bone mineralization (Simkiss, 1961).

In order to facilitate the increased gaseous exchange, and the resorption of calcium, the chorionic epithelium undergoes striking morphological changes. The deeply located capillaries appear to become more superficial, and by day 13 of incubation a very extensive network of intra-epithelial vascular spaces is present throughout the chorion (Lillie, 1952; Romanoff, 1960). In addition, highly specialized cells make their appearance; one cell type is characterized by the presence of numerous apical vacuoles and large microvilli directed towards the shell membrane, and has been named as the 'intercalate' cell (Skalinsky & Kondalenko, 1963), 'calcium-absorbing' cell (Owczarzak, 1971) or 'villus-cavity' cell (Coleman & Terepka, 1972*a*). Another cell type, known as the 'capillary-covering' cell (Coleman & Terepka, 1972*a*) has thin cytoplasmic processes which intercalate between the vascular spaces and the shell membrane. The chorionic epithelium contains, in addition, the regular 'undifferentiated' chorionic cells and a number of dark cells with distorted mitochondria which are probably degenerating (Skalinsky & Kodalenko, 1963; Narbaitz, 1972). Sethi & Brookes (1971) described a 'sustentacular' cell which partially or totally embraces the vascular elements. It is not clear if it constitutes a separate type of cell or represents a particular variation of a 'capillary-covering' cell.

The vascular spaces in the chorion were described initially as forming part of 'a single blood sinus interrupted by strands of tissue' (Fülleborn, 1895). In present day literature, however, there appears to be a great deal of confusion as to the real nature of these vascular spaces: they are variously designated as 'sinusoids' (Sethi & Brookes, 1971; Narbaitz, 1972), 'network of blood sinuses' (Owczarzak, 1972) and 'capillaries' (Coleman & Terepka, 1972*a*). This confusion appears to stem from the fact that most observations have been made on sections perpendicular or slightly oblique to the surface of the epithelium. The present electron microscopical study of the chorionic epithelium has made use of sections parallel to the surface of the epithelium in order to complement previous observations and try to clarify our understanding on the nature of the chorionic vascular structures.

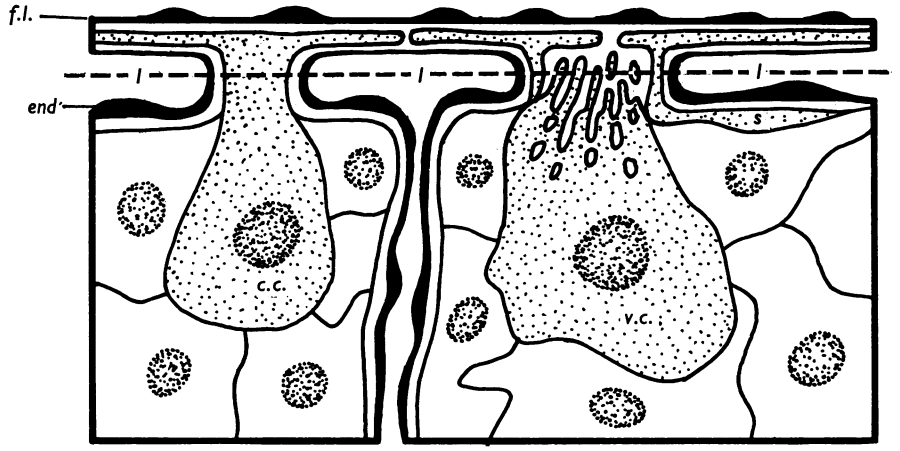


Fig. 1. Schematic representation of a cross section of the chorionic epithelium. *C.C.*, 'capillary-covering' cell; *V.C.*, 'villus-cavity' cell; *l*, lumen of vascular spaces; *end*, endothelium; *f.l.*, fringe layer of shell membranes; *S*, sustentacular cell.

MATERIAL AND METHODS

Eggs from White Leghorn hens were obtained from a commercial source. They were incubated in a forced-air incubator. Ten eggs were opened on the fifteenth day of incubation; the chorio-allantoic membranes were separated together with the shell membranes, cut into small pieces and fixed in half-strength Karnovsky's fixative (Karnovsky, 1965). At least 10 different samples from the equatorial region of each membrane were processed. After 6 hours' fixation, tissues were washed in 0·1 M cacodylate buffer at pH 7·3 containing 0·2 M sucrose, post-fixed in 1 % osmium tetroxide, dehydrated and embedded in Araldite. The blocks were oriented in the ultramicrotome in such a way that the plane of section was parallel to the surface of the membrane (dotted line in Fig. 1). Sections 1 μm thick were stained with toluidine blue; thin sections were stained for 40 minutes with a saturated solution of uranyl acetate in 50 % alcohol and with lead citrate (Reynolds, 1963) and studied with a Philips 300 electron microscope.

RESULTS

The drawing in Figure 1 represents a cross section of the chorionic epithelium, and is based on previous descriptions. The dotted line indicates the general direction of the sections analysed in the present study. It can be seen that this type of section should show the lumina of the vascular spaces and the strands or columns of tissue separating them.

Figure 2 is a photomicrograph of a section 1 μm thick stained with toluidine blue. It shows that the lumen of the vascular spaces is a single space interrupted at regular intervals by the cross sections of narrow cylindrical 'columns'. Dark, oval red blood cells are seen between the columns. Sections from all zones of the chorionic epithelium show similar features, and no septum was ever found subdividing in any way the wide vascular lumen. On this basis one must conclude that the blood sinus is a single space extending over the entire chorion of each embryo!

Figure 3 is a low magnification electron micrograph of a section following a similar parallel plane. It also shows the wide single lumen of the blood sinus containing red blood cells. Details of the structure of the columns can now be seen; each

column is enveloped by endothelium, a basal lamina intervening. The three columns in Figure 3 contain apical portions of 'villus-cavity' cells.

A section of a column observed at higher magnification is shown in the micrograph in Figure 4. The endothelial cells covering the column are seen to overlap extensively so that in some zones more than one layer appears to be present. Numerous pinocytotic vesicles are seen in these endothelial cells.

The arrows in Figure 4 indicate the location of cell junctions connecting the chorionic cells which form the core of the column; it can be seen that several different chorionic cells contribute to the formation of this core. The central part of the core is occupied by cross sections of microvilli belonging to a 'villus-cavity' cell as well as by a portion of the apical, vacuole-filled zone of the same cell. Some of the portions of the chorionic cells which are in contact with the basal lamina may correspond to 'capillary-covering' cells. In the left lower corner of Figure 4 an oblique section of an endothelial cell in the floor of the sinus can be observed; it presents numerous vesicles, apparently of pinocytotic nature.

The column in Figure 5 has been sectioned close to its upper end; it contains a large cavity with few microvilli. Note that the cells lining the cavity, and probably corresponding to 'capillary-covering' cells, have a very dense cytoplasm; this characteristic was frequently observed. The arrows indicating the location of cell junctions show again that more than one cell contributes to the formation of the column's core. Figure 6 shows a section of a column which does not contain 'villus-cavity' cells, the core of the column being formed only by 'capillary-covering' cells.

The section shown in Figure 7 is in a very superficial plane so that it cuts through the sinus-covering cytoplasmic layer rather than the lumen of the sinus. This cytoplasmic layer is seen to contain numerous microfilaments; it is continuous with the chorionic cells forming the core of a column, which can be identified by the presence of a cavity with numerous microvilli.

The cellular composition of more than 200 columns was analysed in detail. In only one case did the core of a column appear to be constituted by a single cell (capillary-covering cell). In all other cases portions of from two to five different cells contributed to the formation of the core. In about 90 % of the columns, portions of at least one 'villus-cavity' cell were found. Portions of cytoplasm with a very high electron density and somewhat distorted contours were frequently found, and were probably from degenerating cells.

DISCUSSION

Fülleborn (1895) described the vascular arrangement of the chick chorion as 'a great blood sinus interrupted by strands of tissue'. Despite quoting Fülleborn, Lillie (1952) defined the system as a 'fine-meshed capillary network'. In their electron microscopical study of the chorionic epithelium, Leeson & Leeson (1963) agreed with Fülleborn's concept but did not supply adequate proof to support it. Later papers refer to the vascular spaces as 'chorionic sinuses' (Owczarzak, 1971) 'sinusoids' (Sethi & Brookes, 1971; Narbaitz, 1972) or 'capillaries' (Coleman & Terepka, 1972*a*, *b*). The present observations have shown, in agreement with Fülleborn, that a single blood sinus, only interrupted by cellular columns, occupies the whole extension of the chorionic epithelium. Contrary to Fülleborn's description, however, the columns are known to be formed by chorionic cells of various types and not by mesodermal cells. The surface of the chorion on the tenth day of

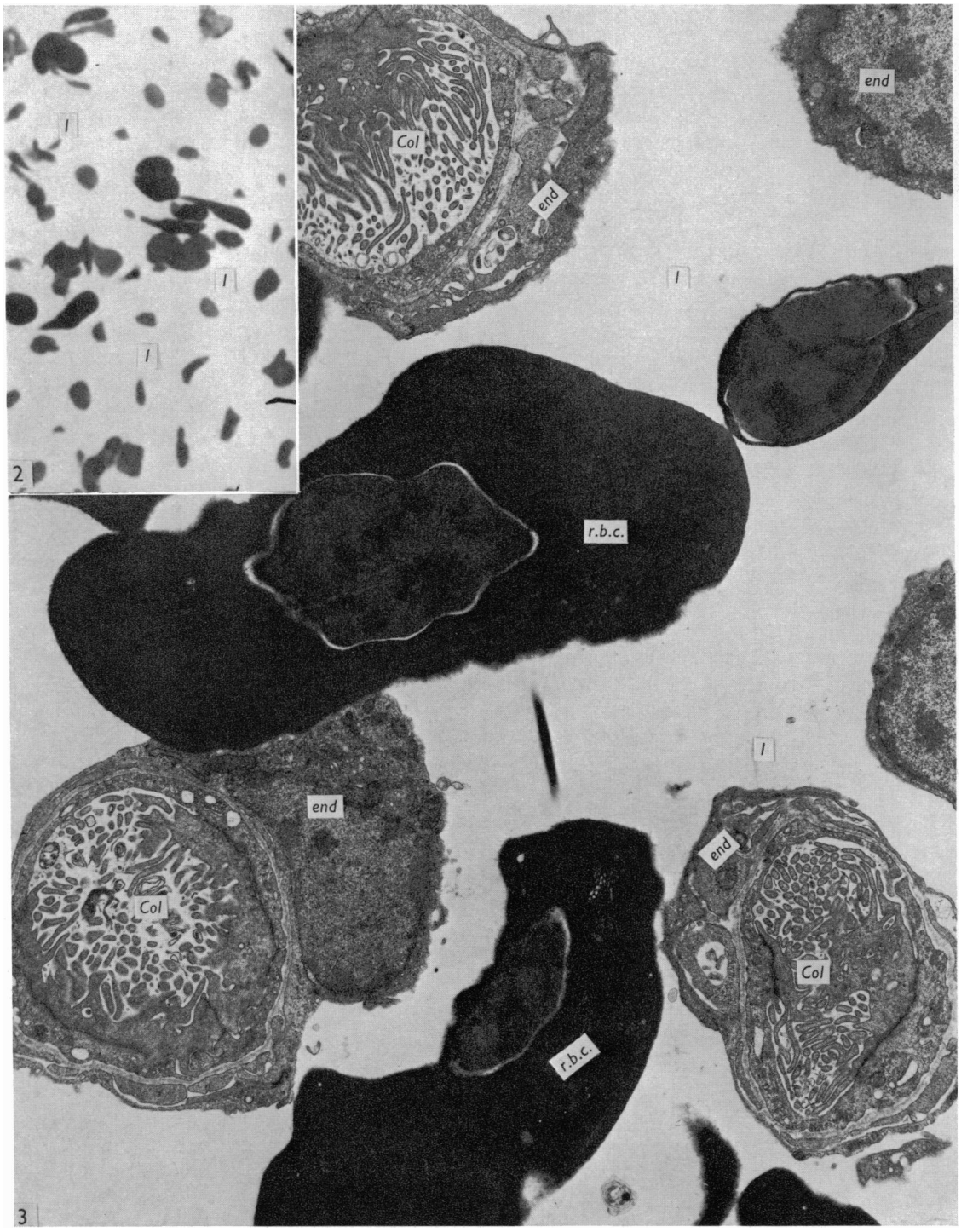
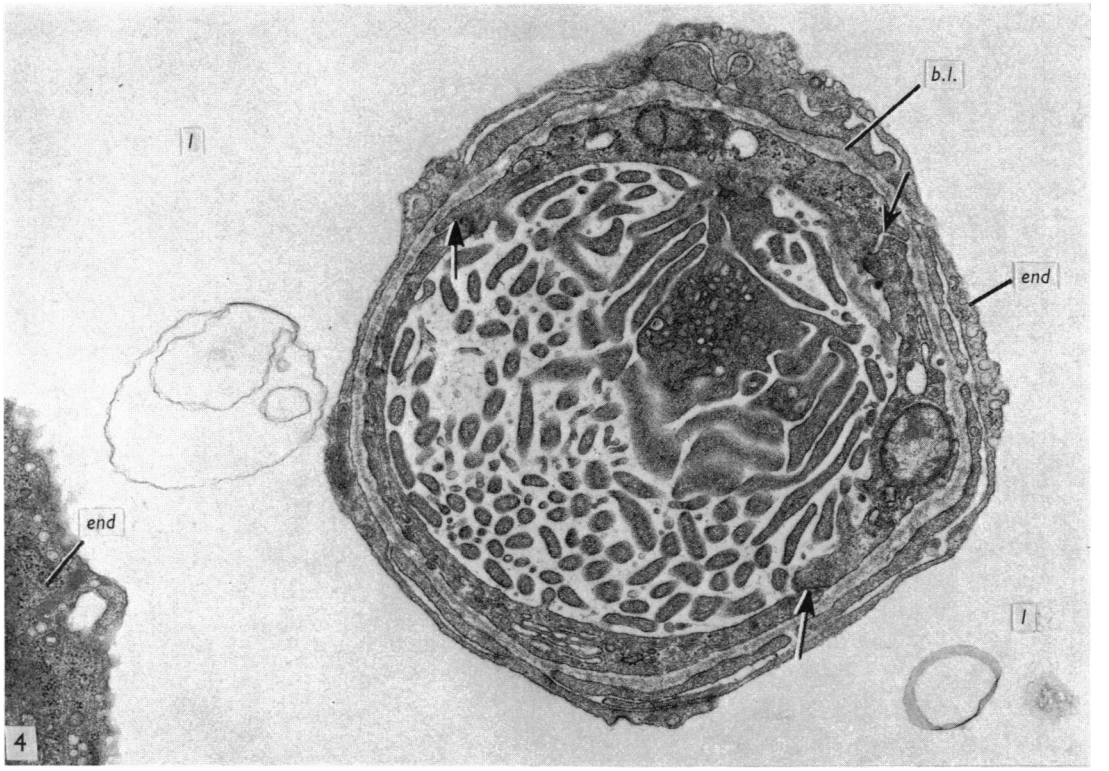


Fig. 2. Photomicrograph from a section of the chorion, parallel to its surface. A continuous lumen (*l*) is only interrupted by the cross sections of columns; dark red blood cells can be observed in the lumen. Toluidine blue stain. ×950.

Fig. 3. Electron micrograph of a similarly oriented section, *l*, lumen; *r.b.c.*, red blood cell; *end*, endothelium; *col*, column ×11400.



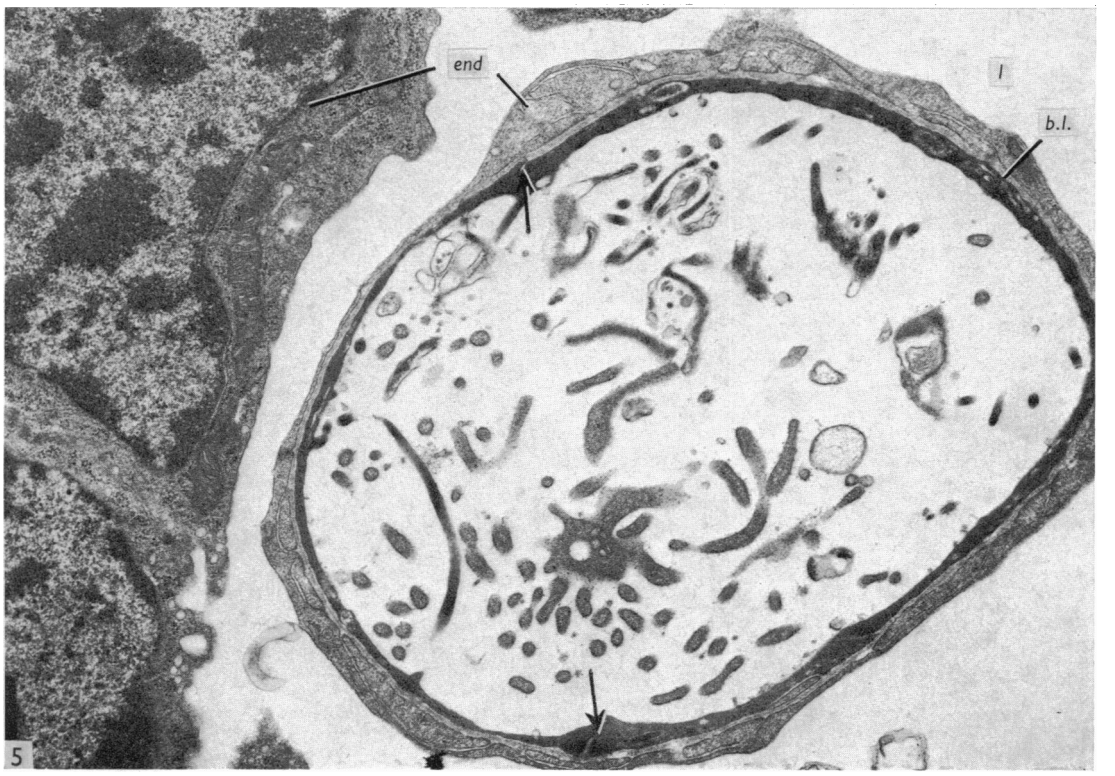
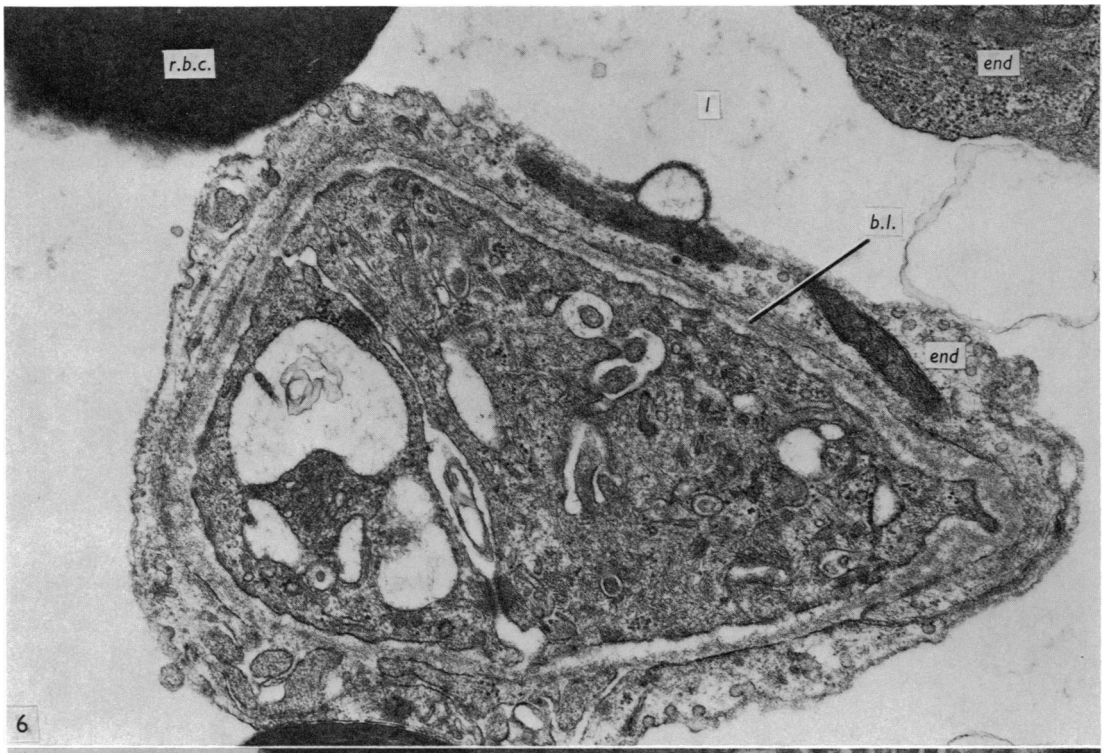


Fig. 4. Section of a column through its lower half. *b.l.*, basal lamina; *end*, endothelium; *l*, lumen; *arrows*, cell junctions between chorionic cells. × 21 500.

Fig. 5. Section of a column through its upper half. *b.l.*, basal lamina; *end*, endothelium; *l*, lumen; *arrows*, cell junctions. × 20000.



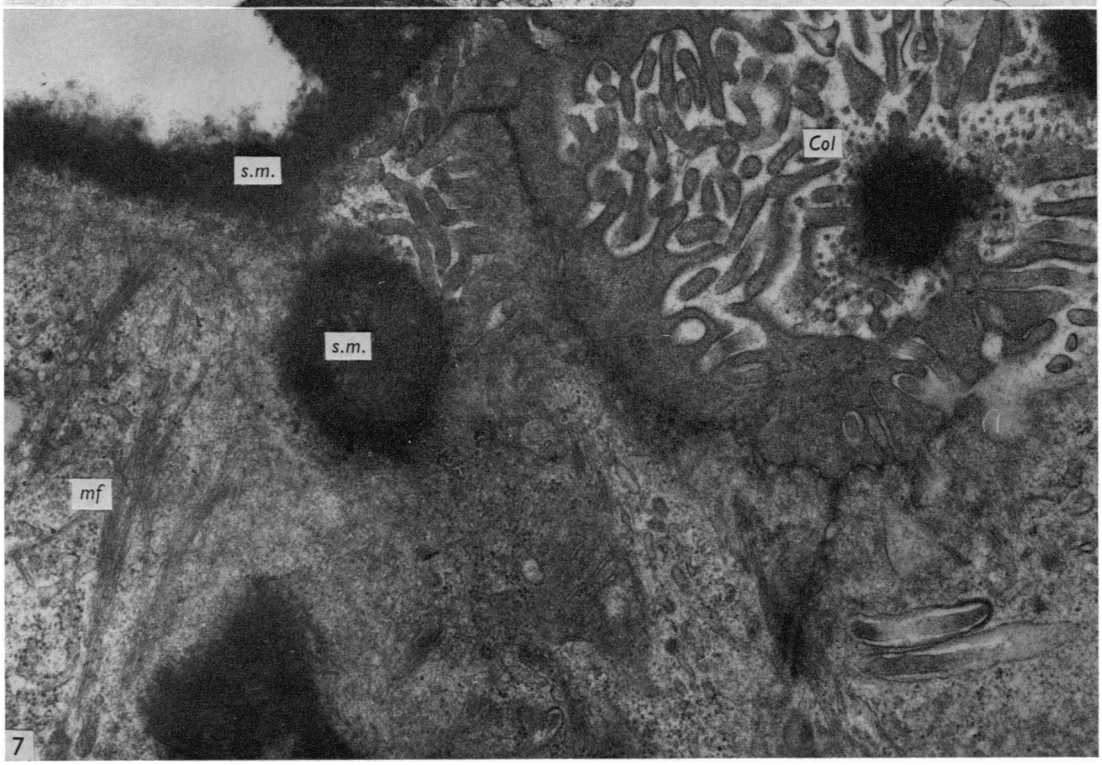


Fig. 6. Section of a column not containing 'villus-cavity' cells. *b.l.*, basal lamina; *end*, endothelium; *l*, lumen; *r.b.c.*, red blood cell. ×17000.

Fig. 7. Section through the sinus-covering cellular process and upper end of a column. *col*, column; *s.m.*, shell membrane; *mf*, microfilaments. ×25600.

incubation has been calculated to be about 58 sq.cm (Romanoff, 1960). The blood sinus which occupies the whole extension of the chorion is clearly very large indeed, and the blood probably circulates rather slowly throughout, favouring efficient respiratory exchanges with the environment.

Thin cellular processes always separate the roof of the sinus from the shell membrane (Skalinsky & Kondalenko, 1963). The present study has shown that the cellular processes contain a large number of microfilaments: these probably serve for mechanical support. Coleman & Terepka (1972*a*) maintain that these cytoplasmic processes are always connected to specialized cells, which they named 'capillary-covering' cells. The body of these cells has also been shown to contain numerous microfilaments (Coleman & Terepka, 1972*a*; Narbaitz, 1972). In view of the present results showing that the vascular arrangement consists of a single blood sinus, and not of a network of capillaries, it would seem more appropriate to rename these cells 'sinus-covering' cells.

It is clear that active pinocytosis takes place in the endothelial cells which form the floor of the sinus and surround the columns. This is in accord with the fact that while exchanges with the environment take place through the roof of the sinus, nutritive exchanges with the chorionic cells must take place through the thicker portions of the wall of the sinus.

Coleman & Terepka (1972*b*) conducted an electron probe analysis of the calcium distribution in the chorionic epithelium. They found calcium in the zone overlying the blood sinus, and this could suggest that the ion is absorbed directly through the sinus-covering cellular processes. However, they also found large concentrations of calcium in the space 'between respiratory capillaries' (i.e. columns) and they interpreted this finding as meaning that the ion was accumulated in the body of the 'capillary-covering' cells. This interpretation can be challenged in view of the present results which have shown that most of the columns are composed of portions of two to five different chorionic cells, 'villus-cavity' cells being included among them. This fact is important since other authors (Skalinsky & Kondalenko, 1963; Owczarzak, 1971; Narbaitz, 1972) have postulated that 'villus-cavity' ('intercalated', 'calcium-absorbing') cells are responsible for calcium absorption. This aspect of the problem must await further clarification.

SUMMARY

Portions of the chorio-allantoic membranes from 15 day old chick embryos were processed for electron microscopical examination.

The analysis of both 1 μm thick sections stained with toluidine blue, and of thin sections stained with uranyl acetate and lead citrate, showed that the lumen of the intraepithelial vascular spaces in the chorion constitutes a single cavity extending over the whole membrane. The vascular arrangement can thus best be described as a single blood sinus, and not as a network of capillaries or sinusoids. The large lumen of the sinus is interrupted by cylindrical columns connecting its floor with its roof. Each column is enveloped in a layer of endothelium, a basal lamina intervening. The core of the column is formed by cytoplasm from two to five different cells ('villus-cavity' cells, 'capillary-covering' cells or various combinations of both).

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