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ELECTRON MICROSCOPIC STUDIES OF THE CHICK CHORIO-ALLANTOIS DURING EMBRYOGENESIS*

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The chorio-allantoic membrane of the developing chick embryo presents a suitable object for studies into histogenetic processes. Several papers contain light microscopic data on changes of the membrane during embryogenesis. Danchakoff (1917) e.g. described the development of vessels in the ectoderm of the chorio-allantois. Its structure has been recently studied in total preparations (Shtchelkounov, 1958, Petry, 1959, Kühnel, 1961). The present work discusses certain characteristics of the development of tissue structures in chick chorio-allantois, as revealed by the electron microscope.

Material and methods

Equatorial parts of the chorio-allantoic membrane (together with the shell membrane) from 9 to 21-day-old chick embryos were fixed for 18—20 hours in buffered 1 per cent osmic acid according to Palade, stained for 1 hour in 0.5 per cent uranyl acetate, dehydrated and embedded in methacrylate. Sections were cut on an LKB Ultrotome and viewed in an UEM-100 electron microscope, using a 60 kV beam and direct magnifications from 1500 to 15 000.

Results

The electron micrographs obtained have allowed the elucidation of certain aspects of the histogenetic processes in the chorio-allantois.

The chorio-allantois is known to consist of three morphologically distinct layers having different functions. The layers are the chorion, the mesodermal layer and the allantois (Hamilton, 1954, Ragozina, 1962).

An intimate connection of the external, chorial layer with the shell membrane could be demonstrated. The connection is established by means of a thin (approx. 50 mμ thick) osmiophilic fringed layer covering the epithelial surface and corresponding to the morphological continuation of the ooke-

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ratine fibrils in the inner shell membrane. If the latter is lifted away from the chorio-allantois, the chorial epithelial cells remain attached to the fringed layer. No structural changes are seen in these fibrils during embryonic development.

The most important changes are to be found in the chorial layer of the membrane. This is true first of all for the interrelationship of the epithelial layer with the blood vessels of the mesoderm. The vessels lie first isolated from each other and adhere from below to the two-layered epithelium at the 9th day of incubation. The upper layer contains some dark cells. In 10-day-old embryos the distance between the vessels decreases. The chorial epithelium becomes thinner, the capillaries shift to the shell membrane and "compress" the epithelial cells. By the 12th day the intercellular spaces widen in the epithelium. The epithelial cells move downward to occupy a place between the capillaries and below the vessels (Fig. 1).

By the end ot the 13th day the external layer adapted to gas exchange is completely formed in the chorio-allantois (Fig. 2). This adaptive transformation is expressed in the formation of a 150 to 200 mμ thick membrane

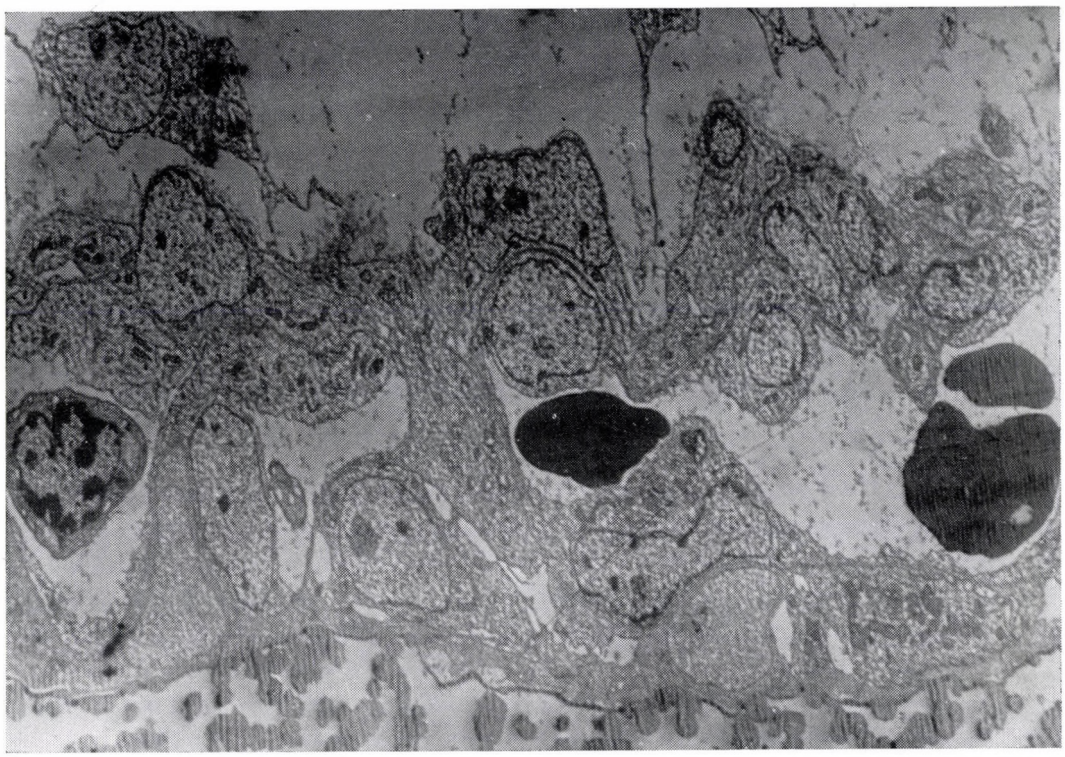

Fig. 1. Chorial layer of the chorio-allantois of a 12-day-old chick embryo. Shift of blood vessels to the shell membrane. 1 — shell membrane fibrils, 2 — ectodermal cells, 3 — endothelium, 4 — adventitial cells, 5 — capillaries, 6 — red blood cell

delimiting the vascular lumina from the air space between the fibrils of the shell membrane. Notwithstanding its extreme thinness, this membrane comprises four layers — the endothelial cytoplasm, a basement membrane, the cytoplasm of the ectodermal cells, and the fringed layer of the shell membrane.

The chorial layer attains its full development by the end of the 13th day and does not exhibit further changes throughout almost the whole period

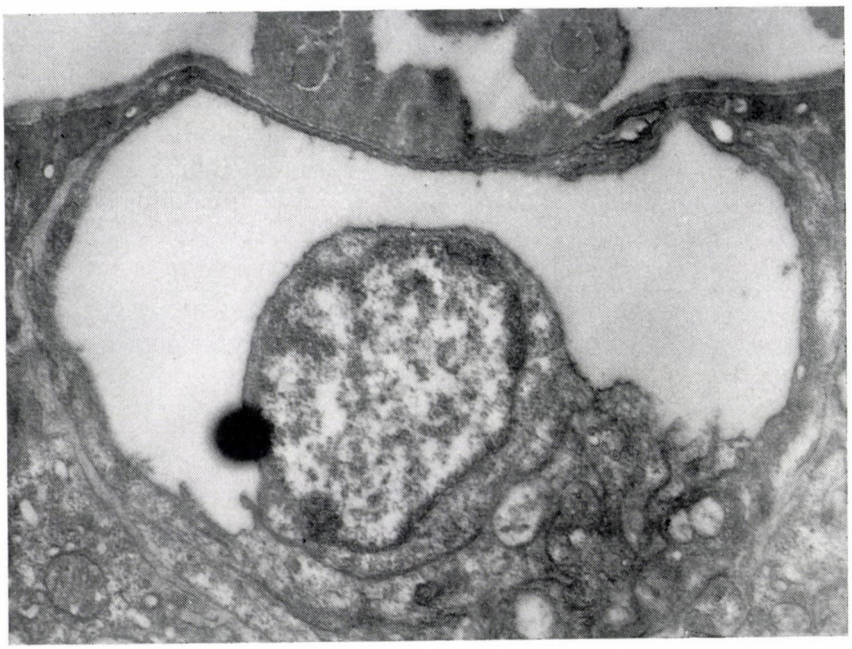

Fig. 2. Blood vessel in the chorial layer of a 13-day-old embryo. 1 — shell membrane fibrils, — fringed layer, 3 — cytoplasm of intercalated cell with osmiophilic substance, 4 — basal membrane of the vessel, 5 — cytoplasm of the endothelial cell, 6 — nucleus

of embryonal development (Fig. 3). The superficial layer is occupied by wide sinusoidal vessels lined with endothelial cells. The nuclei of the endothelial cells cause a bulging of the cell into the lumina. An increased chromatin content can be observed in the nuclei of certain endothelial cells which subsequently separate into the lumen. This represents most probably a haemopoietic proicess. The endothelial cells are supported by a 30 to 250 mμ thick basement membrane composed of two external dense and a middle grey layer. The membrane emits small thin processes having the same layered structure.

Below the blood sinuses a two-layered membrane consisting of epithelial and adventitial cells is seen, delimited against the mesoderm by a thin (approx. 20 mμ) basal membrane composed of a fibrillar network.

Some peculiar, very large intercalated cells are found between the sinusoids. They lie in the depth of the epithelial layer and may attain a size of 14 μ. Their nuclei have frequent deep invaginations and their cytoplasm contains a great number of large long mitochondria. The cytoplasm of these cells whose origin is probably ectodermal contributes to the formation of the upper wall of the blood sinusoids.

Beginning with the 13th day, numerous 60 to 200 mμ thick and 150 mμ to 3 μ long microvilli appear in the space between the intercalated cells and the fringed layer (Fig. 4). The villi have a dense core and a foamy margin. Immediately below the villi in the cytoplasm of the accessory cell a zone of small vesicles (50 to 200 mμ) with granular wall is found. The vesicles may appear in a great number thus giving the cytoplasm a foamy structure.

From the 13th day on, deposition of an osmiophilic substance starts to appear around the mitochondria which become swollen and show destroyed

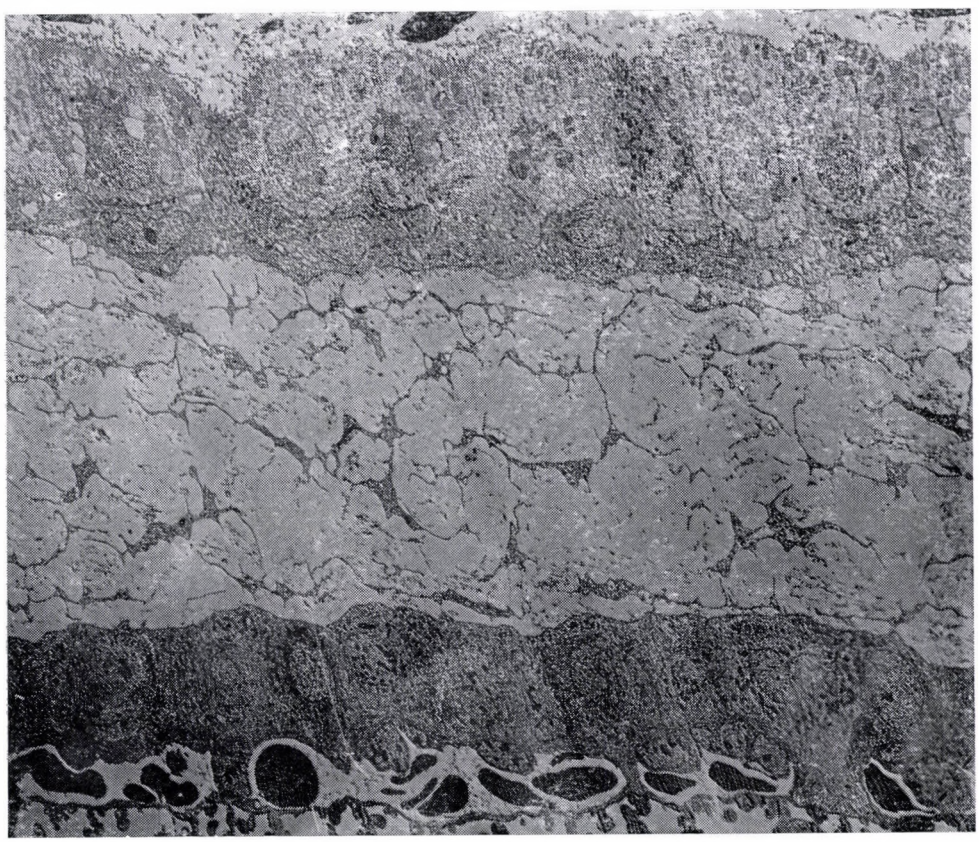

Fig. 3. Low power micrograph of the chorio-allantois of a 15-day-old embryo. 1 — shell membrane, 2 — chorial layer, 3 — blood vessels, 4 — mesodermal layer, 5 — basal and 6 — secreting cells of the allantois

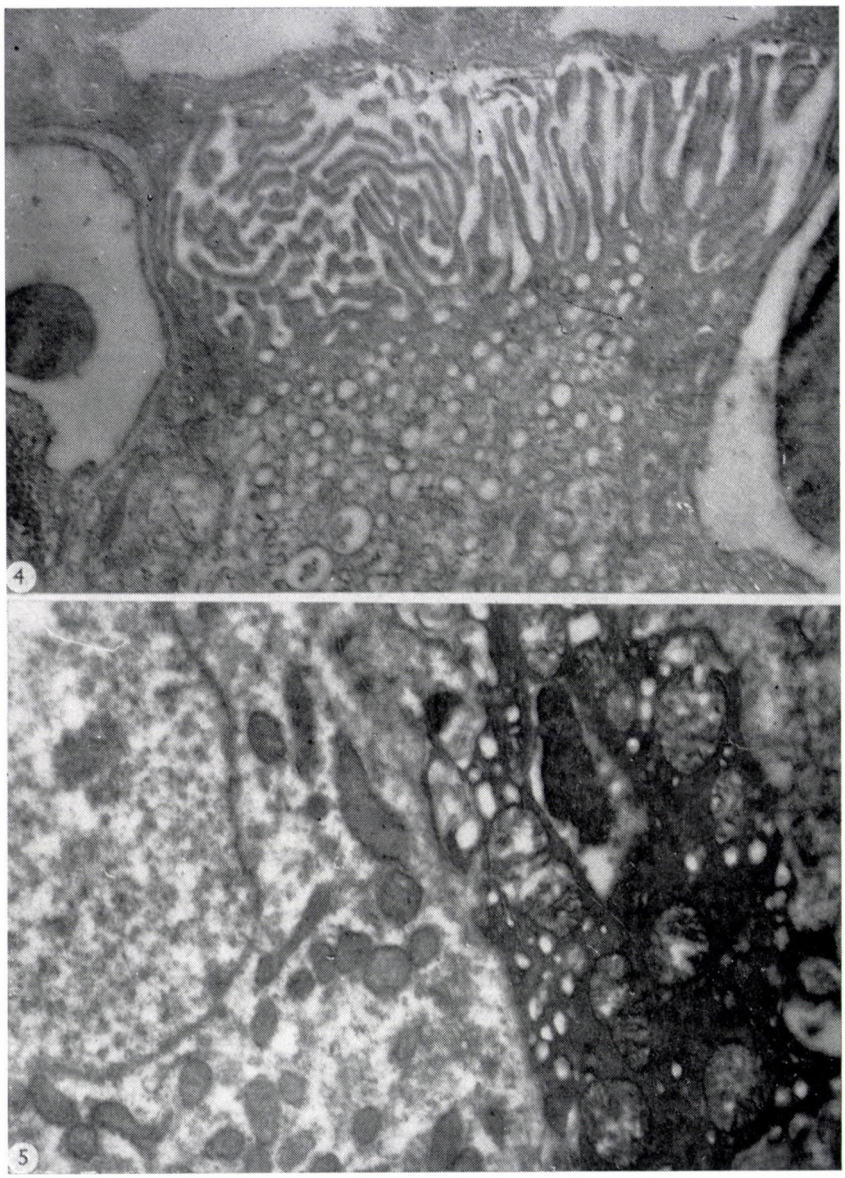

Fig. 4. Upper part of an intercalated cell. 1 — shell membrane fibrils, 2 — fringed layer, 3 — microvilli of the intercalated cell, 4 — zone of the small vesicles, 5 — sinus lumen

Fig. 5. Part of a cell of the chorial layer with osmiophilic substance (1) and swollen mitochondria (2). Part of the cytoplasm contains undamaged mitochondria (3). 4 — nucleus

cristae in the wall of the sinuses and in the cytoplasm of the chorial cells. The osmiophilic substance occupies the whole cytoplasm of certain cells and contains only scattered destroyed mitochondria (Fig. 5). Such changes were

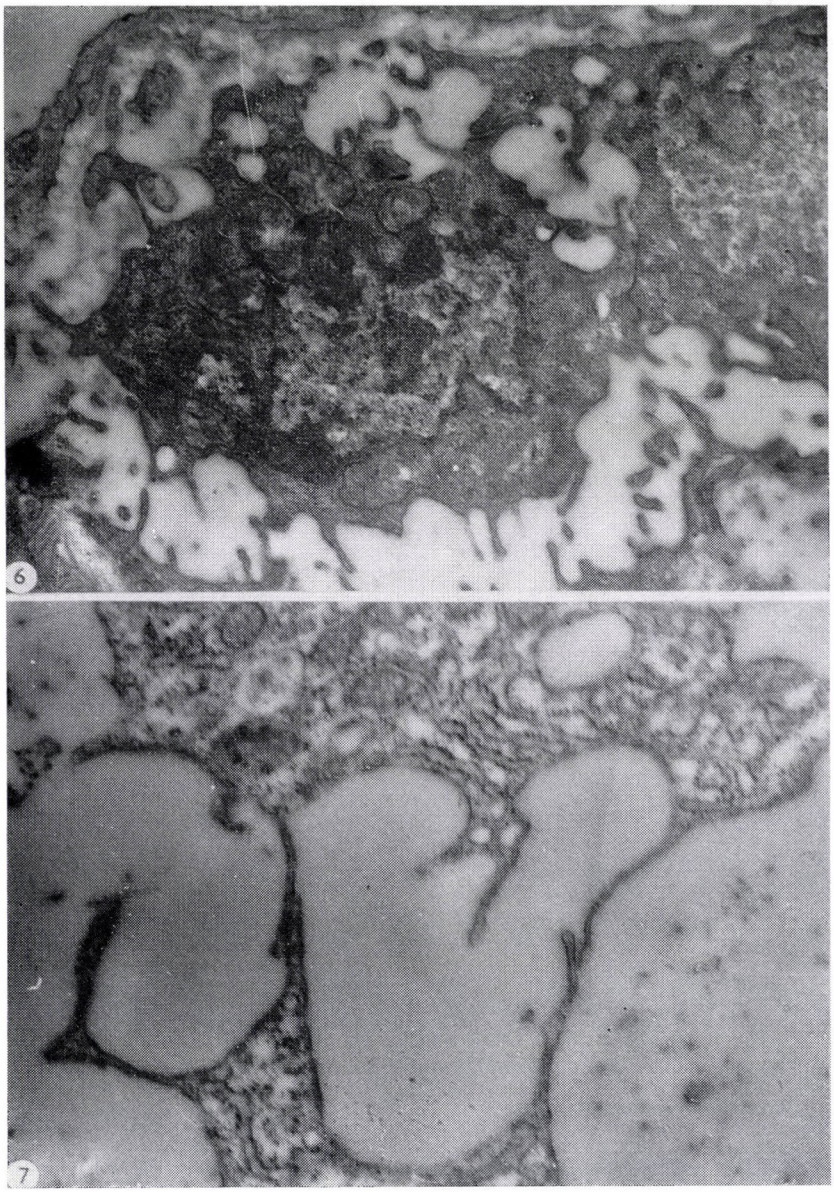

Fig. 6. Shrunken cell of the chorial layer of a 21-day-old embryo. 1 — cytoplasmic processes, 2 — nucleus, 3 — ground substance

Fig. 7. Contacts of the protoplasmic processes of mesenchymal cells in the mesodermal layer

found in chorial cells of embryos on the 13th, 16th, 19th, 21st days of development. In the same stages, swelling of mitochondria without osmiophilic deposits could be observed in the cells of the chorial layer with the exception

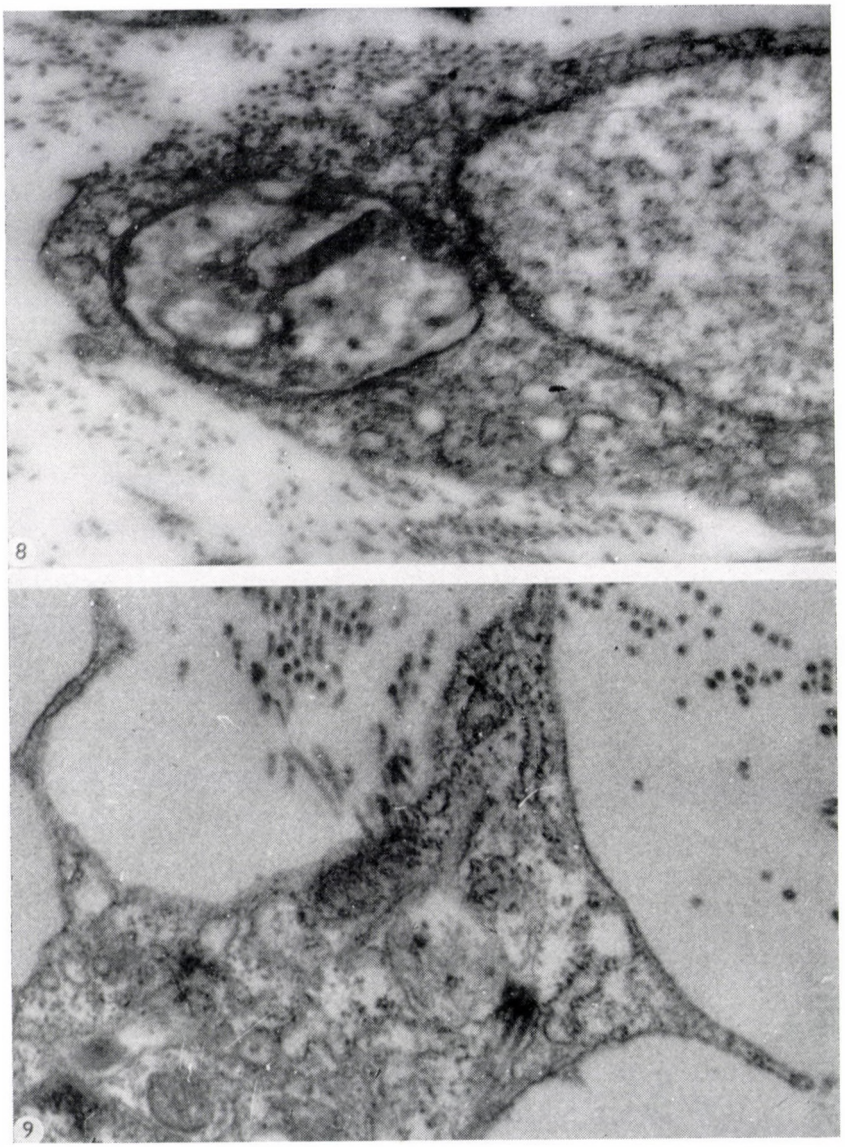

Fig. 8. Fibroblast of the mesodermal layer surrounded by adhering microfibrils
Fig. 9. Fibroblast containing microfibrils without cross striation

of the intercalated cells. No similar changes were found in other stages of development.

On the 21st day, i.e. before hatching, a dehydration of the chorio-allantois is observed. The fringed layer desquamates at several sites. The cells contract, wide intercellular spaces appear which contain the processes of the cells. Some cells lie completely isolated (Fig. 6).

At early stages (6 to 7 days) the middle mesodermal layer of the chorio-allantois contains loosely arranged mesenchymal cells with round nucleus, and a thin cytoplasmic layer with vesicular cysterns of the endoplasmic reticulum. These cells become later stellate with large eccentric nuclei and long curiously branching membraneous processes establishing intimate contacts (Fig. 7). A similar attachment was observed also by other authors (BARGMANN and KNOOP, 1959). These cells contain numerous flattened cysterns of the

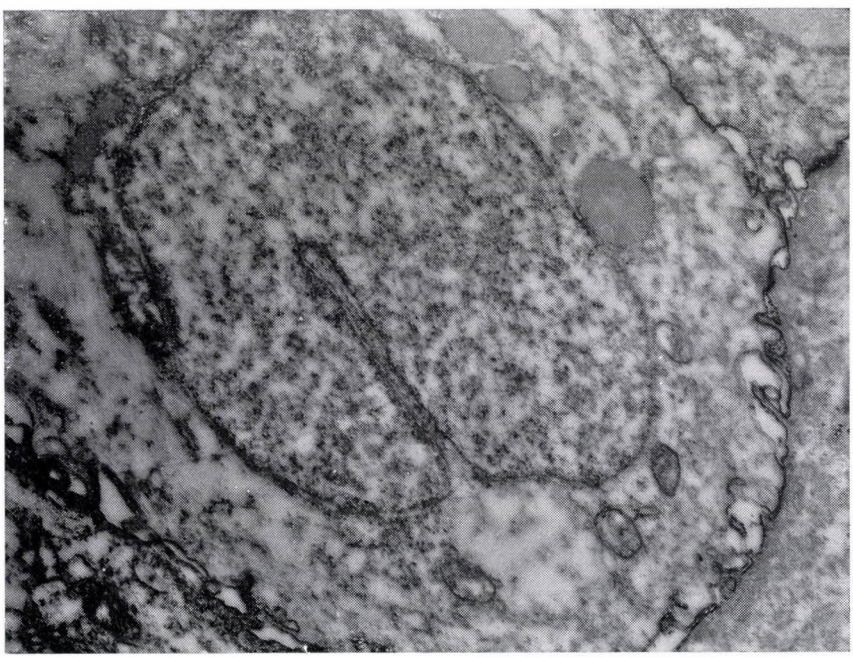

Fig. 10. Part of a secreting cell in the allantoidal layer. 1 — nucleus, 2 — cell border, 3 — cloudy part of the cytoplasm, 4 — vacuoles with dense content

endoplasmic reticulum. In certain parts of some cells the cell membrane is lacking and in these regions tensely attached microfibrils are seen (Fig. 8). Fig. 9. shows their longitudinal sections within the cytoplasm. These fibrils have the thickness of fully developed fibrils but are devoid of cross striation.

At the end of the development (21st day), large spaces and small osmiophilic inclusions appear in the cytoplasm of the mesenchymal cells. Many 15 to 30 mμ thick microfibrils with a cross striation of 20 to 65 mμ periodicity are seen in the intercellular spaces.

Arteries, veins and lymphatics are found in the mesodermal layer. The arteries and veins usually run together. The lymphatics lie mostly alone or accompany the arteries. The arteries have the thickest walls. The nucleated parts of their endothelial cells protrude into the lumen. A homogeneous base-

ment membrane is seen between the endothelial and adventitial cells. The veins have thinner walls. The wall of the lymphatics irrespective of the vessel's size, is composed of 200 to 300 mμ thin membrane-like cytoplasmic parts of the endothelial cells.

Mesenchymal cells aggregate in concentric layers around the blood vessels, especially the arteries, or contribute to the formation of the wall. The

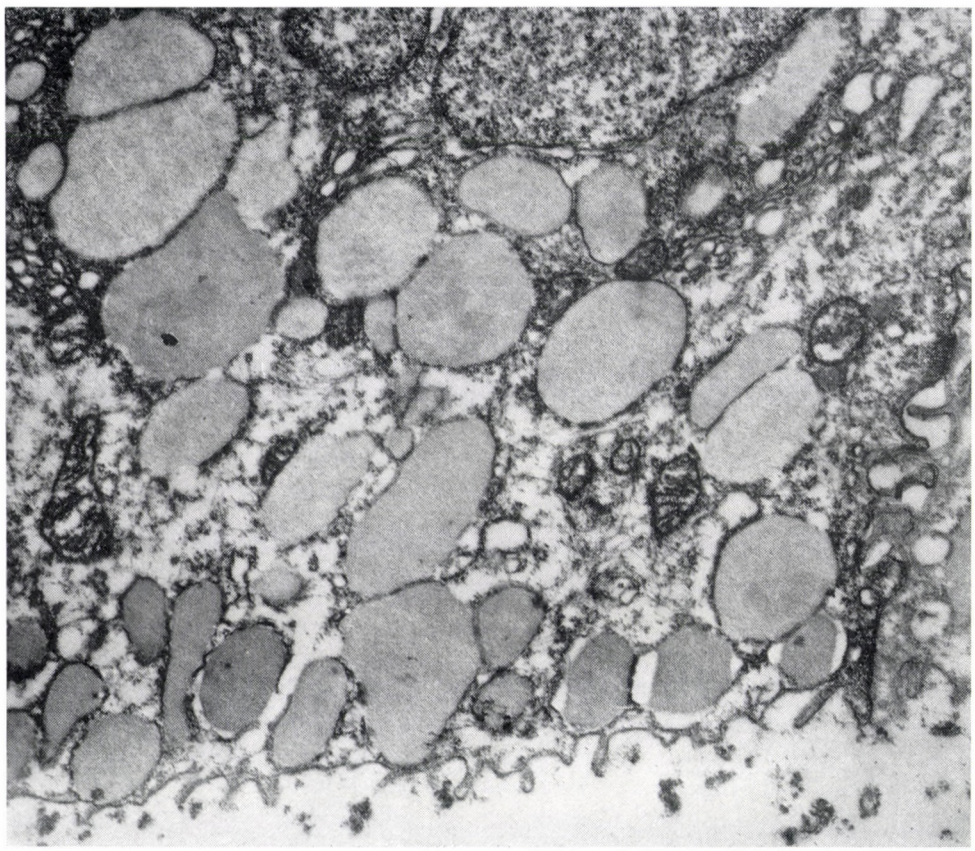

Fig. 11. Vacuoles in a secreting allantoidal cell. 1 — nucleus, 2 — vacuoles, 3 — intercellular space, 4 — microvilli

vessels are surrounded by bundles of microfibrils. The micrographs suggest a very intense fibrillogenesis to occur in this region, by fibrillar transformation of the processes emerging from mesenchymal cells. In addition, the extrusion of a grey homogenoeus substance from the cytoplasmic cell processes may be observed.

The allantoidal layer of 6-day-old embryos consists of almost cuboidal cells containing vacuoles and microvilli. On the 9th day the allantoid is composed of chains of elongated flat cells containing no vacuoles. The allantois

gradually becomes double-layered; its cells, however, remain elongated. Only few of them attain a cuboidal shape and become vacuolated by the 11th day.

Full development of the allantois is observed on the 13th day when it comprises two cell layers. The lower one is composed of vacuolated cuboidal cells, the upper one of a row of basal cells without vacuoles but often having a cloudy cytoplasm. This structure persists till the 20th day.

Cloudy cytoplasmic regions around the nuclei are formed at the beginning of the secretion (Fig. 10). Light areas are also developing which are first partially but later completely delimited from the cytoplasm by a membrane. The cells contain vacuoles attaining a size of one third of the nuclear diameter. These have light or dense contents. Smaller and dark vacuoles are situated in the vicinity of the cell membrane at the allantoidal surface (Fig. 11). A great number of vacuoles may be present in the cytoplasm of allantoidal cells of the lower row. The content of many vacuoles is granular.

The allantoidal surface of the cells, the one in contact with the fluid medium, has short microvilli (60 mμ in diameter and 300 mμ or less in length). On certain cells the villi are arranged more densely and may attain a length of 800 mμ.

Regressive phenomena begin to appear in the membrane on the 20th and especially on the 21st day of development. The intercellular spaces between the upper and lower cell rows increase and thus a great number of cytoplasmic processes appear in the ground substance. The cells in the upper row become darker. The number of vacuoles in the lower row somewhat decreases.

Discussion

Electron microscopy allows a detailed elucidation of cellular interactions in the tissues. The present results e.g. may contribute to the solution of the problem concerning the formation of the respiratory layer in the chorio-allantois (see DONDUA, 1962). In variance with FÜLLEBORN (1895), we could not observe any signs of degeneration in the ectodermal epithelium, but only its shift below the vessels. DONDUA (1962) found a participation of ectodermal cells in the formation of the upper wall of sinusoids. His light microscopic findings have been confirmed by us. Most probably all papers on the formation of the respiratory layer contain but part of the truth. We accept the view of DANCHAKOFF (1917) that the blood vessels grow into the ectoderm whereas the latter shifts downward. In addition, we often observed mesenchymal cells grown to the walls of blood vessels which run to the ectoderm from below. In the chorio-allantois of 11-day-old embryos the wall of the vessels situated below the chorial layer is formed by endothelial cells; mesenchymal cells forming the adventitia also attach to them. The two layers are separated by a basal membrane. After the ingrowth of the vessels into the

chorial layer these adventitial cells contribute to the layer below the vessels. This finding partially corroborates the view of FÜLLEBORN (1895) that the layer below the capillaries is formed by the mesenchyme. It is impossible to recognize the cells of mesenchymal or ectodermal origin in the layer below the vessels after the full development of the respiratory chorial layer at the end of the 13th day. This time coincides with the beginning of the foetal period in the development of the chick (RAGOZINA, 1962).

The so-called intercalated (bulbous) cells are a conspicuous feature of the chorial layer. These cells have peculiar cytoplasmic processes which reach the shell membrane. Physiological observations have shown the absorption by the chorio-allantois of anorganic salts of the shell, beginning from the 16th day of development. These salts are used as a building material for the embryo's skeleton (SAJNER, 1955, 1957).

The intercalated cells probably represent the absorbing elements which start working as early as the 13th day of development. In this connection the dark osmiophilic structures in the cytoplasm of the chorial cells should be mentioned. These are well developed on a certain phase (13, 16, 18, 21 days) and disappear almost completely in others. The dark structures probably correspond to calcium deposits which accumulate gradually and then are absorbed again. The absence of the deposits in certain periods might alternatively be explained by an asynchronous function of different but adjacent areas of the membrane.

The synthesis of collagen in the endoplasmic reticulum of fibroblasts seems to be a firmly established fact. This is not true as to the form and mode of extrusion of collagen from the cells. CHAPMAN (1961), and YARDLEY et al. (1960) assume that the collagen accumulates in the cell along the membrane which disappears at this place. KARRER (1960), MERKER (1961) and SCHWARZ (1960) demonstrated external openings of the intracysternal space of the endoplasmic reticulum. SCHWARZ, MERKER and KUTZSCHE (1962) found no disappearance of cell membranes in explanted fibroblasts and describe accumulated fine fibrils (of smaller size than that of fully developed fibrils) on the external surface of cells. Fully formed fibrils are found at a certain distance from the cells. Our micrographs show some cells with interrupted cell membrane in different areas and fully formed fibrils in their immediate vicinity (Fig. 8), and the presence of unstriated fibrils 25 mμ in diameter within the cytoplasm (Fig. 9).

In the allantoidal layer the presence of a high number of vacuoles is a remarkable feature of the covering cells. Their variety points to certain processes of their development. The nuclei also play a part in this process, as revealed by the frequent deep invaginations on the nuclei and the presence of cytoplasmic areas in their vicinity, highly differing from other areas of the cytoplasm. Some authors (e.g. KNORRE, 1959) assume that the allantoidal

epithelium takes up and secretes the nitrogen metabolism end-products into the allantoidal cavity. In our opinion, these products are concentrated in the above mentioned vacuoles. On the lower surface of the allantoidal cells a great number of microvilli is found. Microvilli are characteristic of cells performing absorption (intestinal epithelium, kidney tubules, peritoneal epithelium etc.). The microvilli of the allantois participate most probably in the uptake of fluids from the allantoidal cavity.

Many aspects of the functional significance of the chorio-allantoic fine structure still remain to be elucidated.

Summary

The equatorial region of the chorio-allantois of 9 to 21-day-old chick embryos was studied under the electron microscope after osmic acid fixation and uranyl acetate staining. The participation of both ectodermal epithelial cells and mesenchymal cells in the formation of the chorial layer was demonstrated. Cytoplasmic processes were found in the intercalated chorial cells. Deposition of an osmiophilic substance in close connection with the mitochondria was observed in the cells limiting the blood vessels. These cells most probably reabsorb the calcium salts from the shell. The structure of the secreting allantoidal epithelium has been described.

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ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ ХОРИОНАЛЛАНТОИСНОЙ ОБОЛОЧКИ КУРИНОГО ЗАРОДЫША В ТЕЧЕНИЕ ЭМБРИОГЕНЕЗА

Е. И. СКАЛИНСКИЙ и В. Ф. КОНДАЛЕНКО

Исследовали экваториальные участки хорионаллантоисной оболочки 9—21 дневного срока развития куриного эмбриона. Материал фиксировали по Паладе с докрашиванием уранилацетатом. Выявлено участие в образовании хорионального слоя как эпителиальных клеток эктодермы, так и клеток мезенхимы. Во вставочных клетках хориона обнаружено наличие цитоплазматических выростов, а в клетках, подстилающих сосуды, отложения осмиофильного вещества, имеющего непосредственную связь с митохондриями. По-видимому, эти клетки резорбируют соли кальция из скорлуповой оболочки. Описано строение секретирующего аллантоисного эпителия.

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