MORPHOLOGY OF THE AMNIOTIC EPITHELIUM IN RELATION TO THE PROBLEM OF FORMATION AND RESORPTION OF AMNIOTIC FLUID

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Most of the studies of the amniotic epithelium have been related to the problem of the origin of the amniotic fluid. Recently published papers have described in considerable detail [7, 8, 10] the process of formation of vacuoles in the cytoplasm of the amniotic epithelial cells, thus confirming the original view [9, 11] that the amniotic fluid of humans and of some other mammals is of secretory origin. There is, however, evidence of the transudative origin of the fluid [1] in the human, from the chorionic vessels, based on the observation that vacuoles are rarely encountered in the epithelial cells earlier than the 16th week of gestation. In view of this contradictory evidence, we undertook the study of the amniotic epithelium of such animals as would, because of the morphological peculiarities of their membranes, present the most favorable conditions for transudation. We chose the allanto-amnion of swine, which has a vascular supply, and we studied it at the stage of development at which vascularization was at a maximum.

The vascular supply of the allanto-amnion of ruminants reaches maximum development about half way through the gestational period. It has been shown [3] that the vessels of the fetal membrane are situated closer to the amniotic surface. We considered that transudation of fluid from the blood vessels would be greatest at this stage of pregnancy. It was for this reason that we chose this stage for the study of the amniotic epithelium of swine allanto-amnion.

The boundaries of the amniotic epithelium appear in the form of festoons, and the thickness of the epithelial layer varies at different locations of the surface of the sac, in sections treated with silver nitrate according to Ranvier. This procedure regularly reveals nonnucleated cytoplasmic regions of various forms, which may possibly be sites of resorption of amniotic fluid. The presence of similar sectors in the epithelium of the other membranes [6] has been related to the process of resorption of amniotic fluid. The number and dimensions of such formations are highly variable (Figure 1). After short treatment (one to two minutes) with dilute (0.1-0.25%) solutions of silver nitrate, such sectors differ very little from the cytoplasm of the surrounding cells. With longer treatment, or using more concentrated solutions of silver nitrate, such nonnucleated sectors actively take up silver, and become stained brown or black.

Our attention was directed chiefly to the cytoplasm of the epithelial cells. Treatment with silver nitrate revealed the boundary between the endo- and the ectoplasm. We have been unable to find any mention of a similar effect in the epithelial or endothelial membranes. Silver is not deposited in the cytoplasm of all the epithelial cells. Where this does occur, the boundary between the endo- and the ectoplasm can be clearly distinguished. In sections stained with iron-hematoxylin the endoplasm is strongly basophilic, and is in clear contrast to the faintly-stained ectoplasmic zone. As will be shown later , it is in the endoplasm that continuous formation of vacuoles takes place, followed by their secretion into the amniotic sac. We believe that so clear-

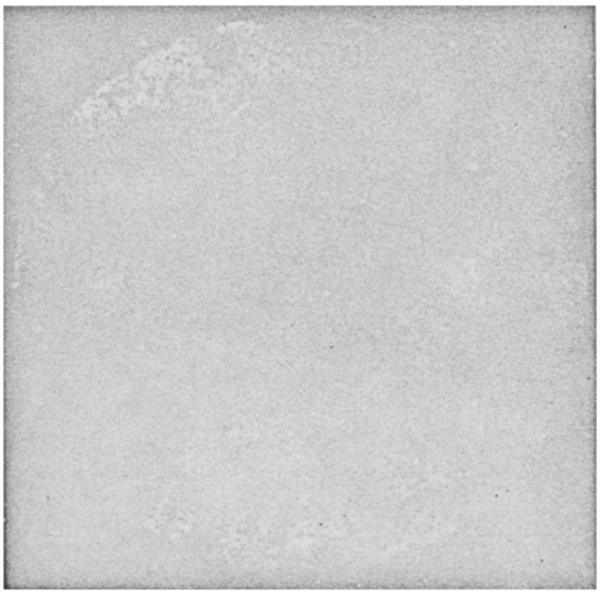

Fig. 1. Nonnucleated cytoplasmic sectors of the amniotic epithelium of swine. One of the cells contains a clump of closely aggregated nuclei. Impregnated with silver nitrate, counterstained with iron hematoxylin. Photomicrograph. Objective x90, ocular x7.

cut a delineation of the endoplasm from the ectoplasm must in some way point to a difference between the roles of these structures in exchange processes affecting the amniotic fluid. Whereas the endoplasm is responsible for production and secretion of fluid, the ectoplasm would fulfill the opposite function of resorption of amniotic fluid.

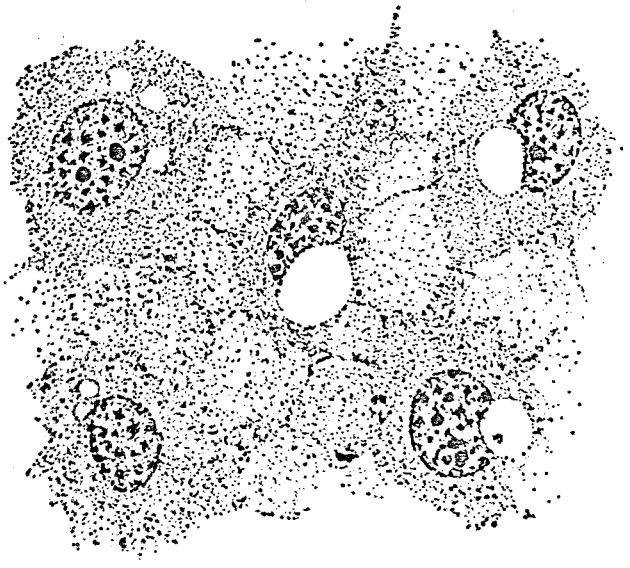

Fig. 2. Secretory vacuoles in swine amnion epithelium. Phase contrast. Objective x90, ocular x10.

The process of vacuolization of the epithelial cells is of particular interest. Vacuoles filled with a clear secretion are to be found in practically all the cells, in greater or smaller amount. Similar pictures have been

described by other authors, for the avascular human amnion [10]. In this case there can be no doubt as to the secretory nature of the vacuoles, and we attach particular importance to this morphological similarity.

The vacuoles begin to form in the perinuclear zone. We have been unable to confirm the observations of certain authors [10] who reported intranuclear formation of vacuoles; we could perceive them only in the cytoplasm, or more precisely, in the endoplasm of the cells. The earliest stage of their formation is the appearance of one or two clear droplets, in close contact with the nucleus. Their number rises progressively (Figure 2), and they run together to form large droplets, bulging into the lumen of the amniotic sac. All these processes take place in the perinuclear zone. The nuclei of some cells undergo deformation, due to the pressure of the secretion forming in the vacuoles. Giant cells, resembling fat cells, appear sometimes (Figure 3); their nucleus

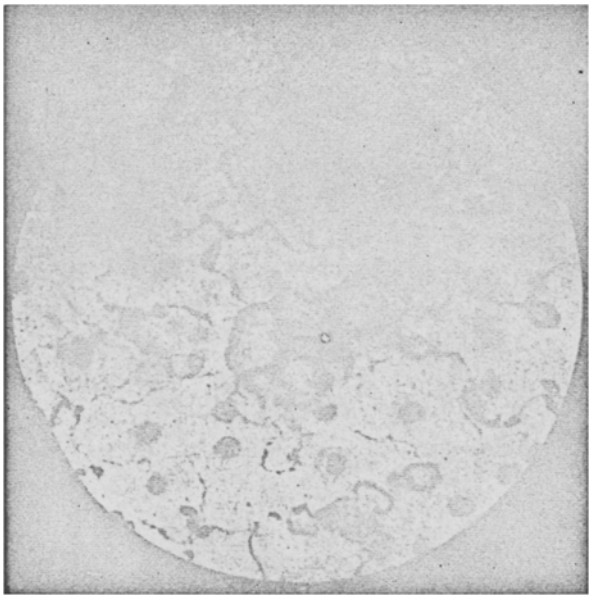

Fig. 3. Giant vacuoles in cells of swine amniotic epithelium. Nonnucleated cytoplasmic sectors, staining intensively with silver nitrate. Counterstained with iron hematoxylin. Photomicrograph. Objective x90, ocular x7.

or nuclei (these are more frequently large multinuclear cells) are squeezed out towards the periphery, and the whole of the cell is filled with the giant vacuole. We were unable to observe the formation of extracellular giant vacuoles, as reported by Bautzmann [8]. We invariably found nuclear matter, in one form or another, in the extracellular vacuoles. For this reason we consider that "extracellular vacuoles" should be regarded as being highly deformed uni- or multinuclear cells of the amniotic epithelium.

The conclusion may be drawn from our findings respecting the morphology of the amniotic epithelium of a highly vascular fetal sac that the secretory theory of the origin of the amniotic fluid is the most probable one. It is probable that production of amniotic fluid takes place as a result of the secretory activity of the amniotic epithelial cells in the early stages of gestation of the human and of animals in which blood vessels are absent from the membrane or from its vicinity.

During our investigation we made an observation relating to the regeneration of amniotic epithelium. It is known [4] that at an early stage of pregnancy the amniotic epithelium is regenerated by means of karyokinetic division. We observed isolated cases of karyokinesis at half-term in swine. Direct division of epithelial cells was even less frequently seen. At the same time, we more than once saw pictures closely resembling the end-stages of so-called endoamitosis. Something similar has been reported of recent years for human embryonic membranes [2, 5]. These authors reported the division of the large nucleus of the cells into a number of small nuclei, which

remained enclosed within the nuclear membrane for a certain time. In the terminal stages of this process the nuclear membrane disappears, but the daughter nuclei remain grouped together, so that each group of nuclei resembles a single giant nucleus of a cell. We have several times seen similar pictures, morphologically corresponding very closely with the terminal stage of endoamitosis, in the amniotic sac of swine (see Figure 1). While we record these observations, we by no means insist on the same explanation of their occurrence as has been proposed by the above authors [2, 5]. There can, however, be no doubt that what we saw in the amniotic epithelium very closely resembles the picture reported for endoamitosis. We would add, however, that the presence of endoamitotic division should in no way be considered to exclude the possibility of amitotic division or of karyokinesis.

SUMMARY

Considerable vacuolization of the epithelial cells of the amnion was found in swine during the stage of the greatest vascularization of the membrane. This supports the secretory theory of the origin of amniotic fluid. It is possible that resorption of amniotic fluid takes place through the cytoplasmic anuclear sites of various size at the area of the cellular boundaries.

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