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Presence of an Endothelioid Tubelike Structure at the Interface of the Amniotic Membranes in Twins with Single and Double Placenta. Growth Factors Involvement

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Abstract. A histomorphological study of the amniotic membranes in full-term twins with double and single placenta was carried out by means of the silver impregnation staining technique suitably modified. Specimens of interface of amniotic membranes were prepared by means of sections. The constant presence of a tubelike structure was observed. Proceeding from the amniotic cavity, the following histological layers were noted: 1) single layer of amniotic cells; 2) amorphic substance with fibrocytes; 3) single layer of endothelial cells. The same order of single layer is present in the amniotic membrane of the second fetus. This tubelike structure is present only in cases of twins with double placenta. If the placenta is single with two umbilical cords, the tubelike structure is not present and only a central amorphic substance surrounded by two single layers of amniotic cells is observed, to confirm the single embryogenetic derivation (monovular). Therefore, through this histological method, we can recognize the true single placenta of twin pregnancy from the pseudosingle placenta so said for the presence of adherences of adjoining surfaces that make it appear single. On the contrary, by manual dissection it is possible to identify a twin pregnancy with two placentae. From the physiological point of view, the walls of the tubelike structure have probably the function to realize exchanges of amniotic liquids between the two fetuses, so as to obtain a balance of electrolytic ions and of intercavity pressure. Growth factors (vascular endothelial factor) are probably involved in the genesis of the endothelial tubelike structure.

Key words: Twin placentation, Endothelioid tubelike structure, Growth factors

INTRODUCTION

Any stimulus determines a cellular reaction that reflects the model of normal reactions as long as its significance is useful and specific.

Such a model still holds its relevance when the cell tries to respond to the changing environment through metaplasia, ie, the acquiring of different shapes. Exceptional or iterated stimuli, on the other hand, lead to the so-called process of dedifferentiation, during which the cell goes backwards in the evolutionary process, thus coming closer to the original cellular organisation. The cell reverts to the maximum degree of plasticity possessed by the merismatic cells at the beginning of their own evolution.

Such a vision at the cellular level was reported by one of us [3,4] as early as 1950, and may at present be integrated with the acknowledged recognition of the role played by the functions of the growth factor (GF) which is perhaps present in all types of tissue.

Up to the present, a number of growth factors have been recognised, such as: EGF (epithelial GF) [6], NGF (nerve GF) [10-12], TNF (tumor necrosis F) [8,9], PDGF (platelet derived GF) [15], TAF (tumor angigenesis F) [8,9], and, last, angigenin (endothelial vascular F) [1,16].

These factors have been identified as specific peptides. In fact, angigenin is a peptide formed by 123 aminoacids and Shapiro et al [16] were able to synthesize its gene, as well as to clone it. Furthermore, they found a structural analogy between angigenin and the enzyme ribonuclease. In the adult organism, it is probable that many types of cells, owing to particular changes in their environment, may be able to produce a GF by means of a specific protein synthesis.

It is also quite likely that GF may be produced in the course of embryogenesis so as to induce the differentiation of the various tissues. The process of differentiation of singular cellular stems during embryo development is due to the irreversible loss of the ability to transcribe these genes which are useless for the specialization of the various organs and systems. However, such a loss of genes may be potentially reversible, and in fact it was found to be so, by the discovery of genic inactivation, eg, by means of methylation. Steward [7] managed to give birth to full plant embryos starting from specific, but exclusively somatic cells, thereby proving that such cellular stems possess the characteristic of being totipotent.

It is well known that the genetic program responds to environmental stimuli by building particular proteins; the proteins themselves, chosen in both qualitative and quantitative terms, will play a significant role in modifying the morphological and physiological characteristics of the tissues. Therefore, Levi-Montalcini's hypothesis [10-12] that NGF be the unifying centre of the nervous, endocrine and immunologic systems could be extended as a valid model for the majority of systems and tissues; its unifying power would in this way be enhanced. The basis for such a unification, after all, lies in the operative program contained in the DNA; the genetic program is in fact frequently activated by regulatory substances (proteins) analogous for most systems.

AIMS

The tendency towards a unification of the GF (UGF, united growth factor) has driven us to the study of amniotic membranes (consisting of continuously regenerating cells with a high metabolic rate) coming from twin pregnancies, both mono- and biplacental. According to Nylander [14], a careful histological examination of fetal membranes is the first way to obtain a diagnosis of zygosity in twins of the same sex, monochorionic placentation implying monozygosity. Different frequencies for the different types of placentation have been described [5,18]. The experience gained by one of us on the peritoneal serosa has brought us to the use of the same staining technique (Fig. 1, 2, 3), there being a structural histological affinity between amnion and the peritoneal membrane.

MATERIALS AND METHODS

The slides have been prepared with tissue taken immediately after the delivery. In case the specimens of amniotic membrane had been previously freezed, they were revived in physiological solution (37°C), and subsequently fixed with a 5% silver nitrate solution; the colour change was obtained through UV exposure, in accordance with the staining method modified by one of us [2].

The dehydrated specimens were set in balsam for their surface to be examined, or else included in paraffin to allow for the study of the central interamniotic space; the inclusion was followed by the sectioning at the microtome of the area in which the two amniotic walls come into contact. This method has been applied on fragments belonging to amnions of true single placentae, ie, placentae with two umbilical cords and two amnions. The fact must be stressed that sometimes what appears to be a monochorionic placenta is only a disguise: a careful manual dissection can easily show how well two bichorionic placentae can adhere to each other, thereby giving the impression of a single monochorionic entity [13].

DESCRIPTION OF THE FIGURES AND PERSONAL OBSERVATIONS

Observation of the Surface of Amniotic Fragments from True Monochorionic and from Bichorionic Placentae

Mono- or bichorionic origin notwithstanding, all slides show back lines interwoven in a mosaic pattern which typically brings to mind the histological structural aspect of the peritoneal membrane. The only difference lies in the size of the single cells, the amniotic cells being clearly smaller, about half the size (Fig. 1, 4). The cellular boundaries seem to be directly related to the metabolic condition of the amniotic cells, and therefore take on different shapes, just as the peritoneal cells do. Such various shapes and colours can be accounted for by the quasi-secretory function of the serous cells and by their being subject to continous mechanical, thermal and physiochemical stimuli. *As a conse-*

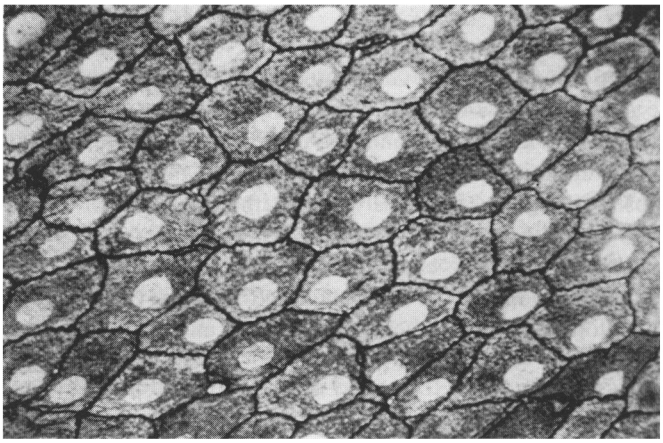

Fig. 1. Normal human peritoneal serosa in surface. Overstructure of blackish surrounding lines partially corresponding to real limits of the cells. Negative nuclear images. (Bondì staining; ×250).

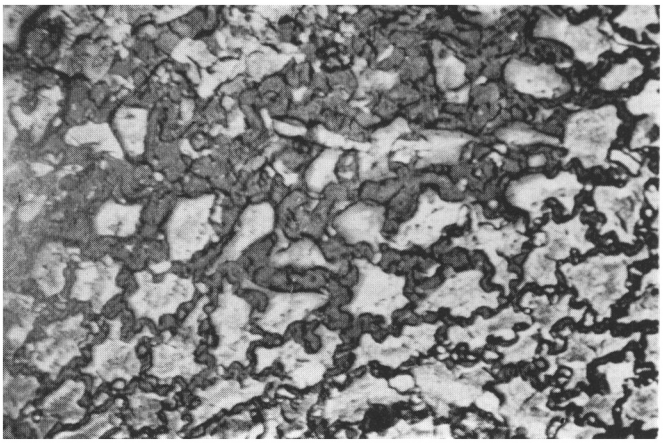

Fig. 2. Human peritoneal serosa in surface. Splitting of the boundaries owing to an inflammatory acute process. (Bondì staining; ×450).

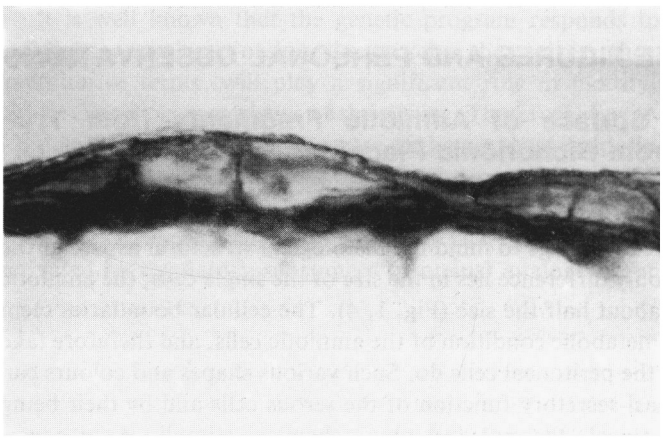

Fig. 3. Human normal peritoneal serosa. Paraffin inclusion followed by serial sections. Nucleus just divided. (Bondì staining; ×640).

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quence, we observe an overstructure only partially corresponding to real cellular limits (Fig. 2, 3). The cellular activity is particularly tumultuous in the amniotic membrane and this determines the frequent physiological splitting of the boundaries; when such a splitting is exhibited by the peritoneal cells, we are on the other hand confronted with an inflammatory process (Fig. 2, 4).

Furthermore, the basal layer, the capillary and lymphatic vessels and the nervous tissue are all absent in the amniotic membrane; it is likely that the cellular vitality is preserved through absorption off the chorion, which is a very well vascularised layer. The slides eventually reveal the presence of numerous syncytial and/or plasmodial centers, ie, multinucleate cells with 10 to 15 nuclei, the probable result of an intense metabolic activity (Fig. 5). The amnion seems to acquire the role of a filtrating membrane, introducing, as required, the variably dense liquid material, thus maintaining such parameters as pressure and hormonal and electrolytic concentration up to the optimum physiological level.

Observation of the Sections of Amniotic Fragments from True Double Placentae, Taken from the Contact Area of the Two Membranes

The slides show the following layers, proceeding from one amniotic cavity towards the membrane belonging to the other amnion: a) single layer of cuboid-cylindroid amniotic cells (Fig. 6, 7); b) amorphous substance with the presence of fibrocytes (Fig. 7); c) single layer of *endothelioid* cells (Fig. 7, 8) defining the limits of a *tubelike* structure.

The same ordered sequence of mentioned layers is met with when proceeding from the cavity of the second fetus outwards, ie, in the parallel and opposite direction. Directly below the layer of amniotic cells, a continuous line is present, though only on one versant (Fig. 7, 8); it can be interpreted as the result of a different pressure in the two cavities. An increase in the internal pressure of one cavity could lead to such a linear flattering and thickening of the amorphous substance and/or of the atrophic chorion. The presence of the above mentioned endothelioid space (Fig. 6, 7, 8) takes on a precise histological configuration, definable as *interamniotic tubelike structure* and identifiable as the result of a *metaplasia* developed in the formerly present decidual tissue.

Observation of the Sections of Amniotic Central Fragments from True Single, ie, Not Dissociable Placentae, with Two Umbilical Cords and Cavities

In these slides, the above mentioned tubelike structure is not present and the interamniotic space is obliterated (Fig. 9). The following layers can be found, proceeding from the amniotic cavity outwards: a) residual single layer of cuboid-cylindroid amniotic cells; b) amorphous substance; c) central flattened line; d) amorphous substance; e) residual single layer of amniotic cells, belonging to the second cavity. Both the chorion and the parietal reflexa decidua are absent. The interamniotic space is thus occupied by amorphous substance which thickens at the centre.

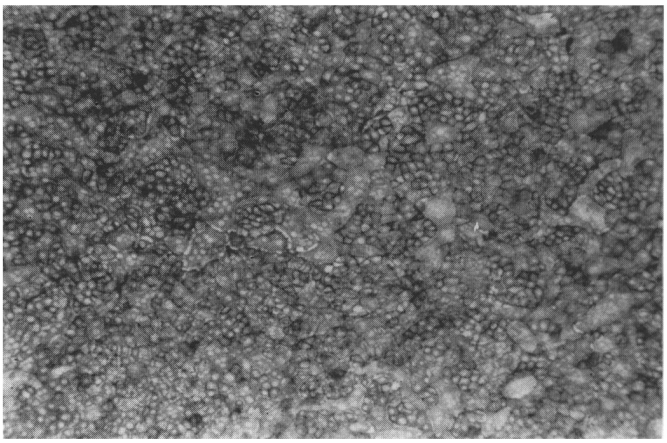

Fig. 4. Human amniotic membrane in surface. Tumultuous cellular activity. Splitting of the boundaries. Negative nuclei images. (Bondì staining; ×150).

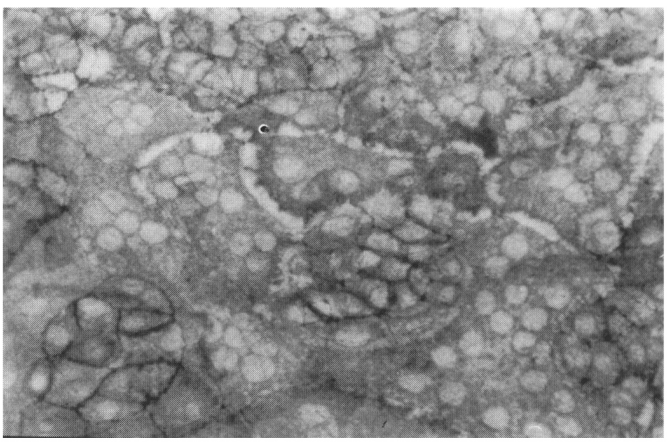

Fig. 5. Human normal amniotic membrane in surface. Multinucleate cells. Negative nuclei images. (Bondì staining; ×250).

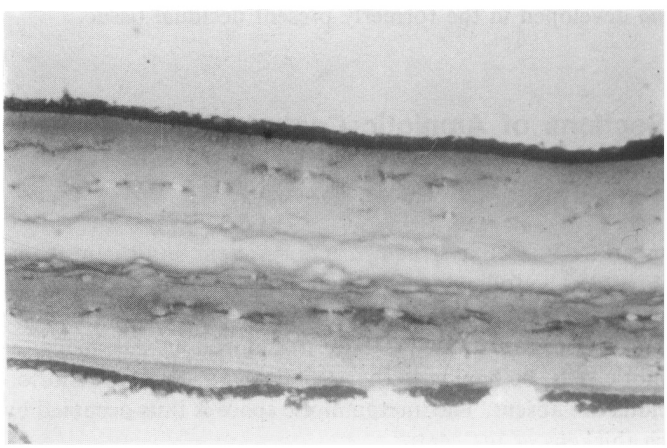

Fig. 6. Normal amniotic membranes of twins with true double placenta. Paraffin inclusion. Serial sections. Presence of an endothelioid tubelike structure in the interface of amniotic membranes. (Bondì staining; ×250).

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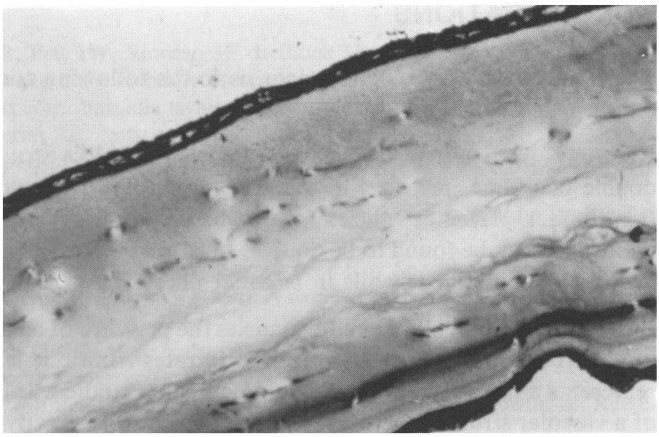

Fig. 7. Same as Fig. 6 (×450).

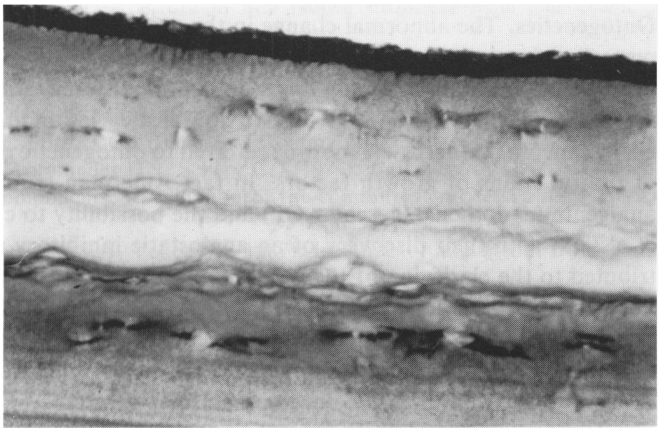

Fig. 8. Same as Fig. 6 (×640). Nuclear division of endothelioid cell.

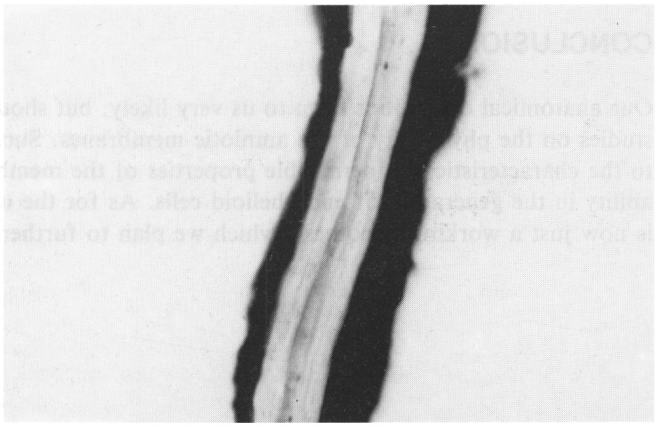

Fig. 9. Normal amniotic membranes of twins with true single not dissociable placenta and two amniotic cavities. Tubelike structure is not present. Interamniotic space is obliterated. (Bondì staining; ×250).

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CONSIDERATIONS

The observation of the slides brings us to the following anatomical, physiological and ontogenetic deductions.

Anatomy. The existence of an interamniotic tubelike structure, only evident in truly double placentae, provides histological support to macroscopic postpartum examination. In fact, placentae which appear to be single at a superficial examination, if suitably dissociated can be found to be double. In case of monovular placentae, the interamniotic space is obliterated, while in biovular ones it is present.

Physiology. In biovular twin pregnancies the presence of an interamniotic tubelike structure, through which a balanced liquid filtrates from one amniotic cavity to the other, represents a new element that should be further studied. It is possible that the presence of a vascular structure between two membranes with greatly developed surfaces, constitutes both a mechanical sliding interface-device as well as an apparatus securing the humoral and biochemical interchange between the liquids that surround the two fetuses.

Ontogenetics. The abnormal change in the nature of a tissue (metaplasia), explains the presence of endothelioid-like cells inside a space bounded by two layers of epithelial amniotic cells, which constitute a lining in continuous and tumultuous reproduction. The concept of metaplasia, described up to now as a variation in cell shape owing to specific environmental requests, can now be referred to ontogenetic reasons since the recognition of the functions of growth factors. In fact Alderman et al [1] with their discovery of angigenin (endothelial/vascular GF) and the possibility to clone its gene, and Folkman et al [8,9] with their discovery of an angiostatic inhibitory steroid GF, have both contributed to the clarifying of the concept of metaplasia. In this way, we can understand how particular environmental situations, such as the contact between two layers of epithelial amniotic cells in tumultuous regeneration, can determine a singular favourable circumstance supporting protein synthesis. Such a synthesis could lead to the production of a vascular GF and to the development of a new type of cell, which in our research we identify with the endothelial cell of the interamniotic tubelike structure.

CONCLUSIONS

Our anatomical deductions seem to us very likely, but should be supported by further studies on the physiology of the amniotic membranes. Such studies should refer both to the characteristic semipermeable properties of the membranes, and to their genetic ability in the generation of endothelioid cells. As for the unification of GF (UGF), it is now just a working hypothesis which we plan to further explore.

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