THE FOETAL MEMBRANES

A Review of the Anatomy of Normal Amnion and Chorion and Some Aspects of Their Function

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THE foetal membranes of the human are composed of the entire amnion and the non-placental chorion. The amnion is the innermost of the two human foetal membranes and, as such, is in contact with the contents of the amniotic sac, namely the amniotic fluid, the foetus and the umbilical cord. The chorionic membrane, which is attached to the outer surface of the amniotic membrane, separates the amnion from the decidua and the maternal uterus.

Throughout pregnancy the human foetus is surrounded by amniotic fluid which is enclosed within the sac formed by the foetal membranes. It is an important fact that both the amnion and chorion are composed entirely of foetal tissue. The close proximity of these structures to the maternal organism, as they line the inner surface of the uterus, may influence their structure or behaviour, but their chromosomal sex remains unaltered by this close association (Bourne, 1962; Klinger, 1957). It is now accepted that the fluid environment of the foetus should be regarded as a medium which is constantly being renewed or replaced, and perhaps, altered in composition. It is not, therefore, a static liquid but, similar to the other fluid compartments of the body, the liquor amnii has a circulation for which the term 'Amniotic Fluid Circulation' has been evolved. According to Plentl (1957), from work carried out by the use of deuterium, the exchange of water is about 450 ml. per hour at term and there are differential rates of transfer of sodium and potassium ions. The exact sites of origin of amniotic fluid are unknown nor is there any definite evidence to show how such large amounts of fluid are conveyed from the amniotic sac to the mother or to account for the passage of various ions at different rates. Undoubtedly some of the fluid passes into the foetus and thence to the mother via the foetal circulation and placenta, but it is very probable that some of the amniotic fluid exchange occurs via the foetal membranes into either the placenta itself or the uterus direct.

Theoretically, the foetus is in complete charge of its own environment since any fluid or sub-

stance that enters or leaves the amniotic compartment must pass through tissue of foetal origin. An understanding of the anatomy, physiology and pathology of the foetal membranes has therefore become important if pregnancy and its complications are to be better understood.

The Structure of the Amnion

It is now generally accepted that the human amnion is not merely an epithelial lining for the uterine contents, but that it is a complicated tissue constructed histologically of several different layers. Careful study of the amnion reveals that it is composed of five layers (Bourne, 1960). These are shown in a semi-diagrammatic manner in Fig. 1. The face of this illustration shows the layers as they appear in cut vertical sections, whilst the treads of the steps show how they are seen when membrane preparations of the tissue are examined. Confusion has arisen over the use of 'inner' and 'outer' in relation to the amnion and chorion; the term 'inner' refers to that part, or layer, nearest to the amniotic cavity and the term 'outer' refers to that part, or layer, nearest to the myometrium.

The amnion, which is normally 0.02 to 0.5 cm. in thickness, consists of five layers. These are, from within outwards:—

1. Epithelium.
2. Basement Membrane.
3. Compact Layer.
4. Fibroblast Layer.
5. Spongy Layer.

1. *The Epithelium* is the innermost layer and is in contact with the amniotic fluid. It consists of a single layer of cells which are usually cuboidal but may be columnar over the placenta or flattened into pavement cells over isolated areas of the remainder of the amnion. The apex or inner surface of the cells is slightly convex in shape. Small evaginations of the cell membrane protrude into the amniotic fluid from the free surface as microvilli to form a brush border (Fig. 2). The well-defined intercellular membrane is condensed at the apex to form terminal bars.

FIG. 1.—Composite diagram to illustrate the appearances of the layers of reflected amnion and chorion when examined by both membrane preparations and routine cut sections.

FIG. 2.—Amnion Epithelium. The upper, or free, margin of a portion of two columnar-shaped epithelial cells from the placental amnion is shown. Microvilli (M.V.) cover the surface of the cells. The nuclei (N) are irregular in outline. Inter-cellular canals (I.C.C.) extending from the free surface are dilated in places to form vacuolar-like spaces. Many smaller vacuoles are present in the cell cytoplasm. $\times 6,500$.

The base or outer edge of the cells is in contact with the basement membrane. At high magnification the basal region of the cell is complex and irregular in outline forming irregularly-shaped basal processes of various sizes (Fig. 3). These basal processes are in intimate contact with the basement membrane to which they are densely adherent.

Along the lateral aspect, the two membranes of adjacent cells delimit a series of irregularly shaped vacuoles (Fig. 2). Some of these vacuoles simply appear to extend into adjacent ones; others are connected by fine channels formed by the close apposition of the two cell membranes. Whilst the majority of these vacuoles are located in the regions between adjacent cells (that is, hollowed out into the side of the cells) a few are seen lying

deeper in the cytoplasm. Projecting into some of the vacuoles are a variable number of microvilli, generally less per unit area than those seen on the upper surface of the cells.

When viewed from above, as in membrane preparations, the cells are seen to be polygonal in shape, appearing as an irregularly arranged mosaic.

The epithelial cells normally contain a single nucleus which is irregular in outline and sometimes fenestrated. The cytoplasm at high magnification is dense and granular and contains many vacuoles which vary both in size and content (Fig. 2). Mitochondria are scarce and small. A Golgi apparatus has been described by some workers but has not been seen by others. Studies of the amniotic epithelial cells at high resolution

FIG. 3.—Amnion Epithelium. The base of an epithelial cell (E.C.) showing the basal processes which form a complex arrangement with the basement membrane (B.M.). Intercellular canals (I.C.C.) extend upwards between the cells. The compact layer (C.L.) of the amnion lies at the bottom of the photograph. $\times 8,500$.

and magnification demonstrate that comparatively large areas of the cytoplasm contain numerous paired parallel lines and circular elements (Fig. 4) (Bourne and Lacy, 1960). These observations are interpreted as showing the presence of membranes arranged mainly in the form of fine canals with some occurring as parallel sheets. The membranes are each about $60 \text{ \AA}$ thick, the distance between them (canal lumen) is about $100 \text{ \AA}$. Examination of representative parts of the cell surface, where it borders the lateral and basal system of vacuoles referred to above, reveals many circular apertures (about $75\text{--}100 \text{ \AA}$ internal diameter) which communicate with the intracellular channels and canals. The evidence suggests, therefore, that amniotic epithelial cells contain an extensive system of canals and channels which communicate directly with the extracellular 'space'.

2. *The Basement Membrane.* This is a thin layer composed of a network of reticular fibres and is well marked over both the placental and the reflected parts of the amnion. The superficial or inner aspect of this layer has a complex relationship with the epithelial cells. Short, blunt processes from the bases of the epithelial cells interdigitate with similar processes that arise from the basement membrane.

3. *The Compact Layer.* This relatively dense

layer is almost completely devoid of cells and consists of a complex network of reticular fibres. The outline of the mesh alters as tension is applied to it. This layer, which is probably the strongest of the amniotic layers, is rarely thickened by oedema, and it appears to resist, to some extent, penetration by leucocytes when the amnion is inflamed.

4. *The Fibroblast Layer.* This is the thickest layer of the amnion. It is composed of a loose fibroblast network embedded in a mass of reticulin. The cells occasionally show phagocytic activity.

5. *The Spongy Layer.* The tissue of the extra-embryonic coelom is compressed between the amnion and the chorion to form the spongy layer. Its wavy bundles of reticulin, bathed in mucin, render routine staining difficult, but these bundles are seen by phase microscopy as branching fibres having triangular shaped nodes at the junctions. A few isolated fibroblasts are present in this layer. It frequently becomes oedematous and, as such, accounts for the increase in thickness which often occurs in the amnion. This layer permits the amnion to slide upon the underlying chorion which is firmly adherent to the maternal decidua (Fig. 5).

Macrophages (Hofbauer Cells). These were described in the placenta by Hofbauer in 1905, since when they have frequently been known as

FIG. 4.—Micrograph of the numerous membranes found in various regions of the cytoplasm of amniotic epithelial cells. The membranes are arranged mainly in the form of canals which appear to open on the cell surface and communicate with the extracellular space. $\times 100,000$.

FIG. 5.—Human amnion and chorion. A vertical histologic section through amnion (above) and chorion (below). Compare with Fig. 1, A = epithelium, C = compact layer, D = fibroblast layer, E = spongy layer, G = reticular layer, I = trophoblast, OV = obliterated villus. Note the fine reticular tissue connecting the spongy layer with its adjacent structures. Haematoxylin-eosin $\times 90$.

Hofbauer cells, though they had been previously described and are probably the cells discussed by Müller in 1847. They are single nucleated macrophages varying from 15 to 35 μ in diameter and having a foamy or vacuolated cytoplasm. Their single nucleus is eccentrically situated and often reniform in shape. The cellular outline varies, being usually oval or round, but, not infrequently, pseudopodia are present. Hofbauer cells are normally present in the fibroblast and spongy layers of the amnion and in the cellular, reticular and trophoblast layers of the chorion. They are occasionally seen in the compact layer of the amnion, especially if they are actively phagocytic. *In vitro*, these cells readily take up dye which they concentrate in their vacuoles and *in vivo* they commonly contain relatively large quantities of meconium.

The Chorion

The chorion being the outermost of the two fœtal membranes is in contact with the amnion on its inner aspect and the maternal decidua on its outer aspect. The placenta is composed of chorion and is formed by the hypertrophied villi of the chorion frondosum. The chorionic villi over the remainder of the chorion (chorion læve) atrophy and may be recognized in histological sections as obliterated or ghost villi (Fig. 5). The non-placental chorion consists of four layers. These are, from within outward:

6. Cellular Layer.
7. Reticular Layer.
8. Pseudo-basement Membrane.
9. Trophoblast.
6. *Cellular Layer*. This is a thin layer consisting of an interlacing fibroblast network. It is frequently imperfect or completely absent from the chorion when examined at term, but is more easily recognized in early pregnancy.
7. *Reticular Layer*. This forms the majority of the thickness of the chorion and consists of a reticular network, the fibres of which tend to be parallel. Nodes are present on the fibres at those places where branching occurs. A few fibroblasts are present, together with many Hofbauer cells.
8. *Pseudo-basement Membrane*. This forms a basement membrane for the trophoblast. It is a layer of dense argyrophil connective tissue that is firmly adherent to the reticular layer above and which sends anchoring and branching fibres down into the trophoblast.
9. *Trophoblast*. The deepest layer of the chorion consists of from two to 10 layers of trophoblast cells in contact, on their deeper aspect, with maternal decidua. This layer contains the obliterated chorionic villi, one of which is shown diagrammatically in Fig. 1.

Blood Supply

The only blood vessels present in the fœtal membranes are those in the region of the placenta itself. The umbilical vessels, on reaching the placenta, spread out and radiate over its surface, travelling within the substance of the reticular layer of the chorion. Although this layer contains the fœtal vessels, it does not receive a capillary blood supply from them, either over the placenta or in the reflected part of the membrane. The amnion has no blood supply of its own, nor does it contain blood vessels even over the placenta.

Amniotic Fluid Exchange

From the above description and from the experimental work that has so far been performed it seems reasonable to conclude that the amnion and chorion do influence the fluid environment of the fœtus. The structure of the amniotic epithelium indicates that it is physiologically capable of taking an active part in some of the amniotic fluid exchange mechanism.

So far as can be determined on a purely morphological basis, it seems likely that some of the amniotic fluid which passes from the fœtus to the mother, and the accompanying differential rate of turnover of various ions, may be brought about by the amnion. As a working hypothesis it has been suggested that the fluid enters the amnion via (i) the opening between adjacent cells where it passes into the lateral system of vacuoles and (ii) the upper surface, the area of which is greatly increased by the presence of microvilli. Within the cells the fluid presumably enters the complex system of fine canals and channels. These communicate with the lateral and basal vacuoles and hence with the extracellular space; this results in an enormous increase in surface area. Within the vacuoles there is presumably some mixing of any solution which may have entered via the two principal pathways. Assuming that some ions travel more readily along one route than the other, then this could offer an explanation (although much over-simplified) of the differential rate of turnover of different ions. Having passed through the epithelial layer the fluid must permeate the connective tissue layers. Since these layers consist mainly of networks of fibres there is no objection to this on morphological grounds.

Transfer of Meconium

Meconium is passed by the fœtus at times of stress, especially when it is subjected to anoxia, and the presence of meconium in the amniotic fluid is accepted by most obstetricians as an indication of anoxia or hypoxia, although the exact level at which this occurs is uncertain. Meconium absorbed by the amnion and chorion,

FIG. 6.—Meconium-stained membrane. Dense inclusions of dark brownish-green meconium are present in the degenerating epithelial layer. Meconium is also present in the cytoplasm of three fibroblasts in the amniotic connective tissue. Formalin fixed paraffin embedded tissue. Very light eosin stain. $\times 435$.

stains the membranes green. Histological sections of such meconium-stained membranes reveals that meconium may be present in the epithelium and the Hofbauer cells of the amnion (Fig. 6). In deeply stained membranes the meconium penetrates not only the reticular and trophoblast layers of the chorion but also into the decidua, where it may be found in cells adjacent to maternal capillaries (Fig. 7). This passage of meconium, from amniotic cavity to decidua, is further proof of the movement of material from the foetus to the mother across the membranes.

Amnion Nodosum

This is a pathological condition of the amnion in which the foetal surface of the amniotic membrane is studded with multiple greyish nodules. The term 'Amnion Nodosum' was coined by Landing (1950) when he reported eight cases, five of which suffered from a major congenital abnormality of the renal tract and three, who were stillborn, were not examined by autopsy, although two suffered from multiple external abnormalities. Recently Jeffcoate and Scott (1959), in a critical review of polyhydramnios and oligohydramnios, reported their findings in 14 cases and came to the conclusion that the lesion is associated primarily with a shortage of liquor, rather than directly with the renal defect. The author has seen amnion nodosum five times and on each occasion it was associated with oligohydramnios and renal agenesis.

That the incidence of amnion nodosum is much greater than the 43 reported cases would

imply, is indicated by the fact that Jeffcoate and Scott collected 14 cases in less than two years. The paucity of the recorded material probably results from inadequate examination of the amniotic membrane immediately after delivery. It is also possible that the small nodules are not seen, in that their significance is not appreciated, so that the secundines are destroyed before the clinical condition of renal agenesis is recognized.

Macroscopic Appearance. The lesions are small, circular nodules situated on the foetal surface of the amniotic membrane and the epithelium of the umbilical cord. They vary in colour from white to dark grey, or red, but are mostly pale grey with a faint yellowish tinge. The junction of the umbilical cord with the placenta is the centre of their area of distribution and it is around this point that the nodules are most frequently found. Whilst the nodules are commonest over the placental amnion they also occur on the juxta-placental amnion, although they are found less frequently in areas further from the umbilical cord.

The nodules vary in size from microscopic to 3 or 4 millimetres in diameter and, especially near the placental-cord junction they frequently coalesce to form irregularly shaped plaques but, individually, each nodule has a circular outline of almost geometric precision (Fig. 8). A characteristic feature of the nodules is that they can be picked off the underlying amnion leaving a semi-translucent saucer-shaped depression. The nodules are composed of a soft, waxy but granular material which is friable and may be crushed easily.

FIG. 7.—Meconium-stained chorion. The reticular layer of the chorion (R) above and the trophoblast (T) and decidua (D) below. The shrunken and disintegrating stromal cells lie within empty clefts in the reticular tissue. The small round dark inclusions are meconium (M). Note how the meconium has penetrated the membrane and lies within the substance of the maternal decidua. Hæmatoxylin and Eosin $\times 400$.

Microscopic Examination. Many more nodules are present than can normally be seen by the naked eye. Histological sections reveal many tiny nodes which may be as small as 0.1 millimetres in diameter. They consist of masses of keratinized squamous cells embedded in an amorphous acidophil matrix. The nodes lie directly upon the connective tissue of the amnion, forming a minute saucer-shaped depression within the superficial layers of the amnion. At the site of their attachment the amniotic epithelium has, for the most part, disappeared though occasionally a few cells remain. The structure of the nodes in vertical section gives the appearance of crude and inefficient whorl formation the centre of which, in the smaller lesions, is near the centre of the nodule (Fig. 9).

FIG. 8.—Amnion Nodosum. A portion of amnion nodosum photographed from above and trans-illuminated from below. The nodules are circular except where they are distorted by pressure from neighbouring nodes. Occasionally they coalesce to form oval or irregularly-shaped areas. Note that most of the nodes appear to be umbilicated and that they are surrounded by a relatively translucent area. An area from which the nodules have been removed is present and demonstrates how the lesions may be 'picked off' without disturbing the underlying amnion or the neighbouring nodules. Fresh specimen $\times 25$.

Aetiology. Most of the reported instances of amnion nodosum have been in pregnancies in which there has been very little or no amniotic fluid. Any abnormality that prevents the secretion of foetal urine may be associated with absence of amniotic fluid. Renal agenesis is the lesion most frequently associated with amnion nodosum and, in fact, has been present in nearly every recorded instance of amnion nodosum. Professor Jeffcoate and J. S. Scott (1959) consider, from an analysis of their 14 cases, that amnion nodosum is not a developmental error of genetic origin associated specifically with renal agenesis. Their conclusion

FIG. 9.—Amnion Nodosum. A typical nodule of amnion nodosum showing attempts at whorl formation. The lesion is not attached to the amnion, part of whose epithelium persists at the edges, beneath it. This particular node gives the impression of having been deposited upon the amnion as a pre-formed nodule. The remainder of the amnion and the chorion (C) are normal in this instance, except for extensive deposition of fibrin in the trophoblast. T = Trophoblast. D = Decidua. B.V. = Maternal blood vessel. Hæmatoxylin and Eosin $\times 75$.

FIG. 10.—Necrosis of the cervical amnion in a patient whose membranes ruptured spontaneously at 34 weeks' gestation. The epithelium (E) is reduced to a continuous narrow band of eosinophilic amorphous tissue in which the remnants of three nuclei are visible. The remaining layers form a relatively continuous pale-staining strip of reticular tissue containing no cells. Compare with Fig. 5. Hæmatoxylin and Eosin $\times 390$.

is supported by finding one instance of amnion nodosum in the presence of a normal renal tract, although the remaining 13 infants had renal agenesis. They consider that the lesion is actually caused by, or associated with, oligohydramnios rather than being the direct result of congenital renal abnormality.

The exact mechanism whereby the nodules are formed is uncertain. Squamous cells, normally shed from the foetal skin, float freely within the amniotic fluid, unless or until, they are swallowed by the foetus. When a shortage of liquor prevents free movement of these cells they coalesce into tiny rounded coagula which become adherent to

the amnion producing secondary degenerative changes in the amniotic epithelium.

Premature Rupture of the Membranes

The ætiology of premature labour is inadequately understood and many investigations have been undertaken in attempts to find the cause or causes. Unfortunately there has not been agreement regarding the classification of the ætiological factors involved, with the result that correlation of published findings is very difficult. Most authors stress the importance of premature rupture of the foetal membranes as a causative factor in the onset of premature labour. A search

through the copious literature shows that premature rupture of membranes is thought to be a major ætiological factor in from 6% to 60% of all cases of prematurity although some authors do not even include it amongst the possible causes.

The author found that premature rupture of membranes, as a major causative factor, occurred in 34% of a series of 1,000 premature infants delivered at the City of London Maternity Hospital and the North Middlesex Hospital. In a number of these cases there were both maternal and fœtal factors which contributed to the onset of labour, such as pre-eclampsia, multiple pregnancy, anæmia, fœtal abnormality and malnutrition, etc. but, even so, in 34% the membranes ruptured before the onset of true labour and must therefore be considered to be a major factor in its onset.

One of the main functions of the amnion and chorion is to maintain the fluid environment upon which the fœtus depends for its survival *in utero*. There is little correlation between their premature rupture and other known factors, although the association between the young primigravida and the under privileged patient has been noted; so also has its recurrence in subsequent pregnancies of a patient so afflicted in an earlier pregnancy. There is, however, still no definite link with any known clinical condition to account for the majority of instances.

The incompetent cervix is now associated with a syndrome that includes painless rupture of the membranes in the second trimester of pregnancy. In some patients the membranes may rupture when the cervix is only one finger dilated, whilst in others the bulging membranes may fill the vagina before they finally break. Similarly, patients who abort during this period may rupture their membranes early, whilst others deliver the fœtus within a complete sac. These apparent inconsistencies are not entirely explained by the protection which the well-placed presenting part is supposed to give to the foremembranes.

Similar conditions may be found at any subsequent stage of pregnancy. Why should the

membranes rupture at 32 weeks, or at term, in a patient whose cervix is shut tightly and not yet taken up, whilst in another patient of similar maturity they need to be artificially ruptured at full dilatation of the cervix? It is this apparent incompatibility that has led many investigators to consider that such accidents are the result of an inherent weakness of the membranes themselves.

Examination of the tensile strength of the membranes has failed to demonstrate any significant difference between the strength of those membranes that rupture prematurely and those that do not. It is impossible for these estimations of tensile strength to be undertaken upon the precise area of rupture, partly because it cannot be exactly defined but also because it has already ruptured.

A method has been devised (Bourne, 1962) by which the membranes presenting at the internal cervical os can be stained *in situ* and then studied histologically after delivery. This means that the amnion and chorion can be studied at their exact site of rupture. Using a satisfactory series of controls it has been demonstrated that about 90% of the membranes that rupture prematurely, between the 30th and 34th week of pregnancy, have previously undergone extensive degenerative changes at their precise site of rupture over the internal cervical os (Bourne, 1962).

Such atrophic and degenerative changes in the amnion and chorion (Fig. 10) overlying the internal cervical os would almost certainly change both the function and strength of the membranes in this area. It seems reasonable to suppose that such changes would weaken their tensile strength and hence lead to their premature rupture. The reason for the necrosis of this particular area of the membranes is unknown and work is proceeding in an attempt to isolate the ætiological factors.

These findings await confirmation from other workers, but they do, perhaps, provide a new and interesting approach to the ever-increasing problem of prematurity.

Figs. 1, 2, 3, 4, 5, 6, 9 and 10 are reproduced from 'The Human Amnion and Chorion' by kind permission of Lloyd-Luke (Medical Books) Ltd.

REFERENCES

- BOURNE, G. L. (1962): *The Human Amnion and Chorion*. London: Lloyd-Luke (Medical Books).
 — (1960): The Microscopic Anatomy of the Human Amnion and Chorion, *Amer. J. Obstet. Gynec.*, **79**, 1070.
 —, and LACY, D. (1960): Ultrastructure of Human Amnion and Its Possible Relation to the Circulation of Amniotic Fluid, *Nature* (Lond.), **186**, 952.
 HOFBAUER, J. (1905): *Grundzüge einer Biologie der menschlichen Plazenta*. Leipzig: W. Braumüller.
 JEFFCOATE, T. N. A., and SCOTT, J. S. (1959): Polyhydramnios and Oligohydramnios, *Canad. med. Ass. J.*, **80**, 77.
 KLINGER, H. P. (1957): Sex Chromatin in Fetal and Maternal Portions of the Human Placenta, *Acta. anat. (Basel)*, **30**, 371.
 LANDING, B. H. (1950): Amnion Nodosum: A Lesion of the Placenta apparently associated with Deficient Secretion of the Fetal Urine, *Amer. J. Obstet. Gynec.*, **60**, 1339.
 MÜLLER, H. (1847): *Abhandlung über den Bau der Molen*. Würzburg: Bonitas-Bauer.
 PLENTL, A. A. (1957): In *Gestation: Transactions of the Fourth Conference*. Ed. C. A. Villee. New York: Josiah Macy Jr. Foundation, p. 71.