formation of the amnion on mechanical principles, but we must probably seek its cause as an evolutionary development of a structure useful to the organism.

The development of the embryos of fishes and amphibia takes place for the most part in water, a medium

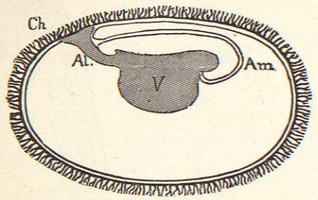

FIG. 103.—Earliest Observed Stage of Human Amnion.

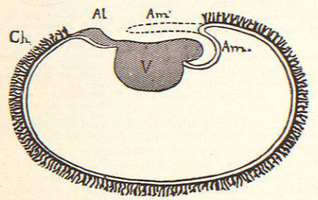

FIG. 104.—Diagram Illustrating His's Theory as to the Formation of the Human Amnion by the Growth Backward of an Amnion Fold from the Anterior Extremity of the Embryo.

FIGS. 103 AND 104.—Early Stage of Human Amnion. *Am*, amnion; *Al*, allantoic stalk; *Ch*, chorion; *V*, yolk sac. (Minot.)

well fitted for the support of a delicate embryo. In the sauropsida and mammalia, on the contrary, development takes place within a rigid shell or the uterus; the development, in these classes, of an amniotic sac filled with fluid in which the delicate embryo is immersed might be regarded as a useful modification by which the advantages of a fluid medium supporting the embryo are secured; or as a contrivance by which the primitive phylogenetic condition of existence in an aqueous environment is retained. The development of an amnion in insects, many of whose ova also develop out of the water, is a fact of perhaps similar significance. The common occurrence of an amniotic membrane in two groups so widely separated as the arthropoda and vertebrata seems to indicate the operation of some deep-seated principle.

As already stated, the amnion (in vertebrates) probably first developed in a type just emerging from the amphibian group. A detailed theory of the course of the evolution of the amnion is given by Hubrecht in the work cited above. This authority suggests that the phylogenetic beginning of amnion development may have appeared in a viviparous amphibian, in which an accumulation of fluid occurred in the embryo, forming a vesicle. He regards the mode of amnion formation in the hedgehog (see above) as approximating the primitive and original type, consisting in a splitting off of a layer of the *Deckschicht* over the embryo, and he traces out the various modes of amnion formation in the other amniota as modifications of this type. Some of the placental mammals, according to this view, therefore exhibit the oldest and most primitive method of amnion formation, while the process in the sauropsida and the large-yolked ova of the latter are secondary and more divergent modifications from the original type.

The evolution of the allantois simultaneously with that of the amnion is to be taken into account, and is an obscure and puzzling point. It will be sufficient here to suggest that with the splitting off of the true amnion from the "false amnion" or chorion, this latter outer envelope would remain without any connection with the embryo, and hence would be a useless structure that would soon degenerate. But with the development of an allantois to bring the chorion into vascular and vital connection with the embryo, the chorion would become a useful nutritional structure. Hence the allantois may be regarded as a development complementary or vicarious to the amnion, which saves and puts to useful purposes the chorion.

ANATOMY AND HISTOLOGY OF THE HUMAN AMNION.—The amnion in the fully developed afterbirth is a thin, smooth, translucent membrane lining the inner or fœtal surface of the placenta and membranes. It rests upon the chorion, to which it is loosely attached—so loosely that it has some play on the chorion and can be easily stripped off. At the placental insertion of the umbilical cord the amnion merges into the integumentary covering of the cord, which, while corresponding to the amnion, differs from the latter in some important particulars.

The amnion is made up of two layers: (1) a superficial epiblastic epithelial layer, and (2) a deeper mesoblastic connective-tissue layer.

1. The inner free surface of the amnion, that directed toward the fœtus, is lined by a single layer of epithelial cells of epiblastic or ectodermic origin. These cells at an early period are thin, but later become thicker, low columnar or cuboidal in form. At times, however, in the mature state they appear thin and squamous. The measurements of the dimensions of these cells (diameters or diagonals) given by various observers vary from 0.008 to 0.012 mm. (Dohrn), 0.011 to 0.019 mm. (Kölliker), 0.011 to 0.014 mm. (Lange), 0.011 to 0.033 mm. (Nichols). The varying sizes of these cells, as stated by different observers, probably depend, partly at least, upon the degree to which the membrane is stretched in the process of preparation for microscopical examination. When hardened by the usual reagents without taking any precautions, the membrane is apt to contract or shrink in superficial extent and at the same time to become thicker, thus giving these cells an appearance of greater thickness and less superficial area; while when stretched on a cork and so hardened, the cells may be unduly stretched and thinned.

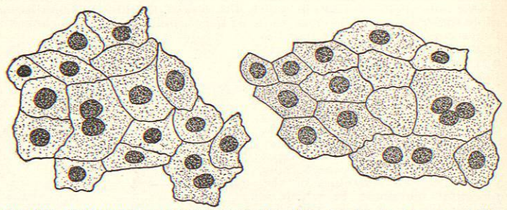

FIG. 105.—Epithelial Cells Lining Inner Surface of the Amnion. Surface view. Silver nitrate and hæmatoxylin. × 1,000.

Viewed from the surface, as after treatment by the silver-nitrate method to bring out the cell boundaries, these cells present the appearance of pavement epithelium, uniting in a single layer edge to edge, with often slightly wavy margins, and being in shape polygonal (often pentagonal and hexagonal), elongated, or irregular (Fig. 105).

Some observers have seen intercellular bridges uniting these cells (Fig. 106).

Viewed in vertical sections, the amniotic epithelial cells appear as low columnar, cuboidal, or thinner cells; the nuclei are often situated near the free ends of the cells, leaving a clearer protoplasmic zone in the deeper portions of the cells (Figs. 107, 110, 111).

The nuclei of these cells are rounded or spherical, about 0.004 mm. in diameter. Most of the cells contain a single nucleus each, but cells containing two, three, or even four nuclei are common; these multinucleated cells are of larger size than the uninucleated. In the latter part of pregnancy the epithelial cells sometimes undergo a certain degree of degeneration. Among these cells are occasionally observed round, clear spaces or objects, which have been variously interpreted as stomata, vesicles, or cells that have undergone mucinous degeneration and burst.

2. Beneath the superficial epithelial layer is a connective-tissue stratum of mesoblastic origin. This stratum

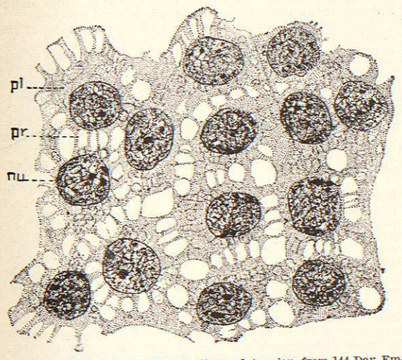

FIG. 106.—Surface View of Epithelium of Amnion from 144-Day Embryo, Showing Intercellular Bridges or Processes (*pr*). *pl*, Protoplasm (cytoplasm); *nu*, nuclei. × 1,225. (Minot.)

can be divided into two layers: (*a*) a thick connective-tissue layer (mesenchymatous), and (*b*) a thin endothelioid (mesothelial) layer lining, partially at least, the outer surface of the amnion, that directed toward the chorion.

(*a*) The connective-tissue layer of the amnion, underlying the epithelial layer, makes up the larger part of the thickness of the membrane, and corresponds to that portion of the mesoblast which has been termed the mesenchyma. This layer is somewhat embryonic in character, and consists of connective-tissue cells embedded in an

FIG. 107.—Section of Placental Portion of Amnion of Two-Months' Embryo. *Ec*, Epiblastic or epithelial layer; *Mes*, mesenchymal (mesoblastic) connective-tissue layer; *Msth*, mesothelial or endothelioid layer. × 250. (Minot.)

abundant matrix. The cells for the most part occupy the deepest plane of the amnion, often leaving in the upper portion of this connective-tissue layer, immediately beneath the epithelial layer, a zone that is free from cells (Fig. 110). The nuclei of these cells are at first rounded and oval, but later become irregular in form and size. The cells are flat and thin, arranged flatwise with the surface. The shapes of these cells, especially in the mature amnion, have not been well made out; one specimen from a mature afterbirth in which the amnion had

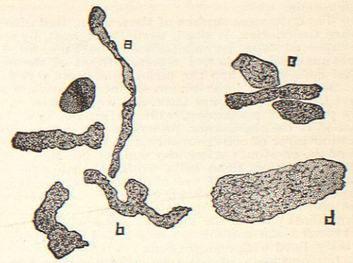

FIG. 108.—Surface View of Nuclei of Mesoblastic Cells of Amnion from Five-Months' Fœtus. × 1,225. (Minot.)

remained permanently separate from the chorion, presenting unusually favorable conditions for observation, has been examined by the writer (Fig. 109). In this instance the connective-tissue cells were mostly large flat cells, very irregular in form, giving off irregular processes and branches, some broad, some fine and filamentary. The processes of neighboring cells were often directly continuous with one another. The general outlines of the smaller of these cells were often roughly rounded; of the larger, polygonal or altogether irregular. These cells ranged in size from 0.025 to 0.100 mm. in extreme dimensions, measuring between the extremities of the processes (perhaps the specimen in which these measurements were made was somewhat overstretched). Mingled with these larger cells were smaller rounded or oval cells, not so well provided with processes and ranging in size from about 0.008 to 0.016 mm.

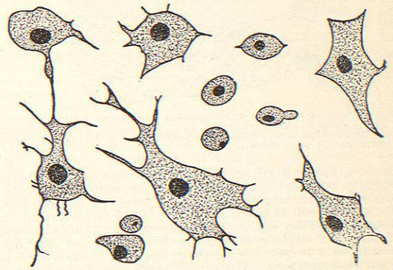

FIG. 109.—Connective-Tissue Cells from Mesenchymatous Layer of the Amnion. Silver nitrate and hæmatoxylin. × 500.

The intercellular matrix in which the connective-tissue cells of this layer are embedded is a homogeneous ground substance said to be of gelatinous or mucinous character. At times, however, toward the close of pregnancy, the deeper portion of the layer (where the cells are mostly situated) becomes markedly fibrous in character (Fig. 110), the outer subepithelial non-cellular stratum still retaining its homogeneous mucinous

nature. The amnion of man is a non-vascular structure and contains no blood-vessels; the presence of an extensive system of lymph channels has not been definitely demonstrated, though such vessels may be present. The amnion of the chick is contractile, and is said to contain muscle cells.

(*b*) The outermost surface of the amnion, that directed toward the chorion, is lined, partially at least, by a single layer of thin, flat endothelioid cells (Figs. 107, 111). These are descendants and representatives of the mesothelial cells which line the cœlom and from which the endothelial cells of the pleura and peritoneum are also derived. These cells are naturally well marked in the early period of pregnancy, while the amnion is still unattached to the chorion and presents a free outer surface. A similar layer of cells probably lines the innermost surface of the chorion. After the amnion becomes united with the chorion, these cells would probably be suppressed at the points of union of the two membranes, though even at full term such cells have been seen at a plane corresponding to the deepest part of the amnion or innermost part of the chorion, perhaps lining spaces left between the membranes similar to lymph spaces in the body lined with endothelium.

In the specimen of afterbirth above referred to, in which the amnion remained permanently separate from the chorion and presented a free outer surface, this layer of cells was nicely demonstrated by the silver-nitrate method (Fig. 112). On surface view these cells were mostly of hexagonal shape (some pentagonal and heptagonal), quite uniform and regular in shape and size, with slightly rounded angles. They were united to one another by their edges, which were straight, not sinuous. Their size was small, measuring 0.0055 to 0.007 mm. in diameter. They did not form a complete lining over the entire outer surface of the amnion, or at least they appeared only in patches; perhaps many of them were lost from degeneration. No nuclei were visible in them—possibly another degenerative sign; if present, they did not take the nuclear stains employed. Patches of precisely similar cells were also observed on the inner surface of the chorion in this case.

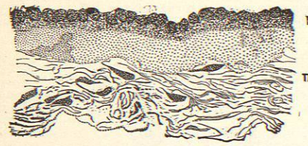

FIG. 110.—Section of Placental Amnion from Eight-Months' Embryo. *ect*, Epiblastic epithelial layer; *mes*, mesoblastic connective-tissue layer, showing non-cellular subepithelial stratum and deeper fibrous stratum. × 340. (Minot.)

The covering of the *umbilical cord*, which is continuous at the placental end with the amnion and at the fœtal end with the skin, differs in some marked characters from the amnion elsewhere. This covering consists of a superficial layer of epithelium, which rests directly upon the mucofibrous tissue composing the chief part of the cord. The integument of the cord is therefore intimately adherent to, or an integral part of, the cord, and cannot be stripped off as can the amnion elsewhere. The epithelial covering is composed at first of a single layer of cells, but later becomes stratified squamous in character, consisting of two to four layers of lenticular-shaped cells.

Union of Amnion and Chorion.—In its origin and early period the amnion is distinct from the chorion and separated from it by a space, which is the extra-embryonic part of the cœlom, and is homologous and at first continuous with the pleural and peritoneal cavities. After about the third month of pregnancy, in man, the amnion comes into contact with the chorion, and the two membranes grow loosely together. The precise character of the histological connection between the amnion and chorion has not been well made out.

ABNORMALITIES OF THE AMNION.—Very rarely is the amnion the seat of abnormal or pathological conditions.

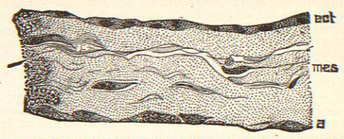

FIG. 111.—Section of Placental Amnion, at Term. *ect*, Epiblastic epithelial layer; *mes*, mesoblastic (mesenchymatous) connective-tissue layer; *a*, mesothelial endothelioid layer. × 340. (Minot.)

Such abnormalities may arise in two ways: from anomalies of development, or from pathological processes.

Among conceivable anomalies of development of the amnion might be: complete absence of the amnion; incomplete development of the amnion from failure of one of the amnion folds to grow; failure of the edges of the amnion folds to unite, leaving a hiatus in the amnion and chorion; persistence of a cord or connection of tissue between the amnion and chorion (the "amniotic cord"), such as normally occurs in ruminants; incomplete expansion of amnion after closure, compressing the fœtus. Some such anomalies of development have been occasionally observed in some animals, but in man they are exceedingly rare.

A couple of human cases are recorded (Hamard) in which there was a separate small amniotic pouch around the abdominal insertion of the umbilical cord. The reporter of one of these cases attributed the condition to a rupture of the amnion (the chorion remaining intact) with retraction of the amniotic membrane. Hamard, who reported the other case, considered the condition to be due in both cases to an early anomaly in the development of the amnion.

It happens, rarely, that the primitive separation of the amnion and chorion persists, in man, throughout pregnancy, so that the fœtus to the time of birth is enveloped in two separate sacs, the amnion internally and the chorion (united to the decidua) externally. This constitutes a rare anomaly of the human afterbirth, of which the writer has reported one case and cited seven other cases found recorded.

Small nodules or caruncles have been observed in the human amnion, scattered about in considerable numbers, some flat and sessile, some more or less pediculated, and ranging in size from that of a pinhead to that of a pea. Structurally, these are of two kinds, one composed of epithelium, the other of connective tissue. The epithelial nodules are commoner and have little or no pathological significance; they are small aggregations of epithelial cells. The connective-tissue nodules are composed of tissue like that of the mesoblastic portion of the amnion; they are very rare, and have been observed in connection with early fœtal death.

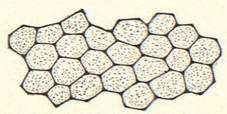

FIG. 112.—Endothelioid Cells of Outer Surface of Amnion (and Inner Surface of Chorion). Silver nitrate. × 1,000.

Adhesions of the amnion to various parts of the fœtus, with resulting deformities of the latter, have been observed. These adhesions have apparently been due to inflammatory action.

Deficiency or excess in the quantity of the amniotic fluid, with the resulting pathological consequences, are considered in other articles.

AMNIOTIC FLUID.—The amniotic sac is filled with a serous fluid, the amniotic fluid or liquor amnii, in which the fœtus is immersed.

In quantity the amniotic fluid at full term in the human female may vary greatly, but ordinarily ranges from about 500 to 1,000 c.c., averaging from 600 to 800 c.c. Abnormally there may be a deficiency (oligohydramnios) or an excess (polyhydramnios) of amniotic fluid, both conditions giving rise to certain pathological conditions and dangers. The differences in quantity at different periods of pregnancy are not well determined; it is quite possible that the fluid increases in amount during the earlier portion of pregnancy, and diminishes in the later portion.

The liquor amnii is a serous or watery fluid, containing in solution a small proportion of proteid, organic, and mineral substances. It is normally clear, limpid, and transparent, colorless, alkaline in reaction, and has a specific gravity of about 1.007 or 1.008. It contains from one to two per cent. of dry solids, besides a small amount of adventitious epithelial cells, hairs, vernix caseosa, and occasionally leucocytes. Proteids (albumin, globulin, mucin, etc.) are present in the early part of pregnancy in large amount (10.77 per cent. at four months, 7.67 per cent. at five months, 6.67 per cent. at six months), but undergo a great decrease toward the end of pregnancy, when there is only a small proportion present (0.82 per cent.). The inorganic salts present are those usually found in serous fluids, chiefly salts of sodium, potassium, ammonium, and calcium. Urea is present in slight proportion; the amount is less early in pregnancy and gradually increases, 0.03 or 0.045 per cent. being present at the ninth and tenth months.

Marked abnormalities in the physical and chemical characteristics of the amniotic fluid have been rarely encountered.

As to the source from which the amniotic fluid originates, there have been two opposing views: one that it is derived (in mammals at least) from the maternal tissues by transudation from the decidua through the chorion and amnion; the other that it is derived from the fœtus, being the excretory products of the urinary or sweat glands of the latter. The view that the liquor amnii is of fœtal origin has long been held; but in opposition thereto and in support of its maternal origin it has been urged by Minot that the fluid in its composition does not resemble urine, but is more of the nature of a serous fluid transuded from the blood-vessels; that the fluid appears before the urinary or other excretory glands of the embryo are developed and while the urethral outlet of the male is still imperforate; and that substances experimentally administered to the mother have afterward been found in the liquor amnii but not in the fœtal tissues. On the contrary, the fluid occurs in sauropsidan embryos which have lost their connection with the misternal tissues; and as to the finding of drugs administered to the mother in the liquor amnii but not in the fœtus, it is possible that the substances may have been entirely excreted and eliminated from the fœtus and discharged into the amniotic fluid. Possibly in mammals the fluid is derived from both the fœtus and the mother—from the mother at first and later from the urine of the fœtus.

The function of the amniotic fluid is largely to afford protection to the fœtus in utero, by equalizing the pressure on all parts of the fœtal body and preventing undue direct pressure of the uterine walls on particular parts of the fœtus. By maintaining a symmetrical shape of the uterus, and protecting the umbilical cord and uterine walls from excessive and unequal local pressure, it obviates interference with the umbilical, placental, and uterine circulation. The amniotic fluid also permits the movement of the fœtus in the uterus, and prevents adhesions of the fœtus to the amnion or of parts of the fœtus with one another from taking place. The symmetrical distention of the womb by it facilitates and assists in the dilatation of the os uteri during labor. It has been also asserted that the amniotic fluid serves as a source of water for the fœtus; as the fluid contains only a small proportion of solids, it could have little nutritive value except as supplying water. It is well settled that both mammalian and bird embryos swallow amniotic fluid; but whether this is done as a reflex act or for nutritive purposes, or whether the placental circulation is incapable of furnishing sufficient water to the fœtus, is not known.

J. B. Nichols.

AMNION, PATHOLOGY OF.—The amnion is the innermost of the membranes inclosing the fœtus. It is continuous with the fœtal epidermis at the umbilicus and forms a sheath about the umbilical cord. The exact manner of the development of the human amnion is as yet unknown, for in the earliest embryos examined it forms a complete sac about the embryo. Morphologically, it is a part of the body wall. It consists of two layers: an epithelial one continuous with the ectoderm, and a layer of embryonic connective tissue continuous with the somatic mesoderm. The epithelial layer is on the inside of the membrane, toward the fœtus; the connective-tissue layer on the outside, next to the chorion and uterus wall.

The membrane is thin and translucent, containing no blood-vessels, but is rich in large lymph spaces, forming lacunæ in which the mesodermic cells lie. These spaces are connected by a system of very fine lymphatics. In the earliest stage the tissue of the amnion consists of but two layers of cells (ectodermal and mesodermal), between which lies a distinct space. By the second month these layers have become united, and the mesodermal portion has increased greatly in thickness so that it is capable of being divided into two parts, a thin mesothelial layer covering the chorionic surface of the membrane, and the mesenchyma, which makes up the greater part of the fully developed amnion. The tissues of the amnion do not normally develop beyond an early embryonic stage; the ectoderm preserves its one-layered structure, and the mesodermal tissue remains embryonic in character. No blood-vessels or nerves have been found in the human amnion. In the later months of pregnancy, physiological degenerative changes occur in both mesodermal and ectodermal nuclei.

The amniotic fluid (liquor amnii) is most probably, for the greater part, a secretion of the amnion, but the manner of this secretion or the source of supply to the amnion is still unknown. In the later months of pregnancy some portion of the fluid is undoubtedly derived from the fœtus. It is probable that the fluid is secreted by the capillaries of the chorionic villi next to the amnion, and is passed on through the amnion by means of the activity of its cells. The fluid serves as a source of water supply to the fœtus; and, as a mechanical protection against blows, shocks, pressure, etc., it assists in maintaining a uniform temperature, allows room for fœtal movements, and aids in delivery.

During the first two months there is a definite space between the amnion and chorion, but in the third month the amnion is gradually pressed against the chorion, until an agglutination takes place between the two membranes through the formation of a homogeneous fluid or gelatinous matrix containing few cells. This union is always very slight, as the amnion in all normal cases can be readily stripped from the chorion. In the first three weeks the membrane is somewhat removed from the embryo; in the fourth week the rapid growth of the latter almost entirely fills the amniotic cavity. During the second month the membrane enlarges more rapidly, forming a larger space for the amniotic fluid, but after the fourth month it fits more closely about the fœtus, from which it is kept separated by the fluid.

The structure of the amnion is analogous to that of the serous membranes, and there is consequently a close analogy between the general pathology of the fœtal membrane and that of the latter. The tendency toward plastic exudations with the formation of more or less extensive adhesions, changes in the amount and character of the secretion, etc., occur here as upon other serous surfaces. The peculiar function of the amnion and its