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Abstract

1. In the stage later than the middle stage of pregnancy, morphological differences appear between the amniotic epithelial cells of placenta and those of the free part and the majority of cases the amniotic epithelial cells of placenta present more marked columnar shape than those in the surrounding area of ruptured orifice or those in the vicinity of placenta. However, there still remains a question whether or not such a phenomena is directly related to the secretory function of the placenta amniotic epithelium. 2. It seems that amniotic epithelial cells divide and multiply themselves by mitosis at least in the early and middle stages when their functions are at height. 3. Even in the stage later than the middle stage generally the amniotic epithelium of placenta is consisted of a single layer of columnar epithelial cells, and therefore, the author cannot agree to Forssell's theory. 4. In glycogen and lipid stainings, the amniotic epithelial cell layer shows more striking changes with the progress of gestational month when compared with those cells in other layers. 5. Glycogen in the amniotic epithelial cell layer is abundant in the early and middle stages of pregnancy, and it rapidly decreases near the late stage. Lipid granules on the contrary are less in the early stage, and start to appear in the middle stage, increasing rapidly towards the late stage. In general, the regressive degeneration picture of the late stage is not distinct histologically, but assuming glycogen to represent the cell activity and the lipid deposit to mean just the reverse, the amniotic epithelium functionally seems to fall into regressive degeneration from the middle stage. Other layers of fetal membranes likewise undergo fatty degeneration as the pregnancy progresses from the middle stage to the late stage. 6. There still remain problems to be solved on the question what role this regressive degeneration of the amniotic epithelial cell layer plays in de Watteville's theory, "Labor originates from the fetal membranes". However, granular PAS-positive substances in the amniotic epithelium are glycogen, and it seems difficult to connect simply the existence or non-existence of PAS-positive granules or Sudan-positive granules directly with the continuation or interruption of pregnancy.

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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON HUMAN FETAL MEMBRANES

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In the research field of obstetrics morphological studies[1,2] of the fetal membrane have been done mainly correlating with the production of the amniotic fluid and the rupture of the membrane at the terminal stage of pregnancy, but the mechanism of the production of amniotic fluid is still now one of the important problems to be settled, though a number of investigations concerning the problem have been reported in the past presenting several theoretical views. Among them the "Secretion theory of amniotic epithelium" proposed by POLANO[3], BONDI[4] and MANDEL et al.[5] is now generally accepted and supported by the histological and histochemical studies by Forsell[6] SCHMIDT[7] and others[8,9,10,11].

Concerning the rupture of fetal membrane de WATTEVILLE[12,13,16] and STAMM[12] proposed recently a new opinion on the mechanism of the initiation of the labor and the rupture of fetal membrane from their investigations on abortus by injecting hypertonic saline solution into the amniotic cavity, the method of which is reported first by ABUREL[14] in 1938. They claim, as BORTH[15] contends, that the labor and the rupture of the fetal membrane are caused by the activation of Eutocine-like substance in the gravida with the cessation of the secretion of some inhibitory substance which is suposed to be produced in amniotic membrane and acts as to surpress the contraction of uterine muscle, opposing the theory of "Regressed secretion of progesteron or of the disturbance of estrogen-progesteron balance", which is generally accepted. The opinion of Watteville and Stamm seems to be accepted only on the basis of the degenerative or functional change of amniotic membrane at the last trimester pregnancy, by which the cessation of the secretion of the inhibitory factor for the muscle contraction may be terminated, and the investigations of the membrane support this view revealing the loss of PAS positive granules and fatty degeneration of the amniotic epithelium after the initiation of

the labor, whereas the amniotic epithelial cells obtained during the pregnancy are rich in PAS positive granules and poor in lipoid droplets. These observations seem to suggest that PAS possitive granules may closely be correlated with the production of the substance supposed to be secreted from the amniotic memdrane and act as to suppress the muscle contraction. In this connection, the precise histochemical and histological observations on the amniotic membrane done before and after the labor are presented in this paper.

MATERIALS AND METHODS

1. *Materials :* Materials used for the present investigation were fresh amniobic membranes obtained from 142 cases treated in the Department of Obstetrics and Gynecology, Okayama University Medical School, Okayama Lying-in Hospital and other affiliated hospitals. The duration of pregnancy, the onset of labor and the method for taking the specimens are shown in Tables 1 to 3,

Of the cases in Tables 2 and 3, those without complications were given artificial abortion in accordance with Japanese law, "Eugenic Protection Law".

A. *Sites and methods*

A strip of membrane 1.5 cm × 3 cm is taken from the amniotic membrane immediately after delivery or operation at the central portion of placenta, from the fetal membrane of the marginal portion but 1 cm apart from the edge of placenta, and from the fetal membrane around the ruptured part (from the rather thin portion somewhat opposite to placenta in the case of Caesarean section), so as to see any variability on the locations. These strips are made into spiral specimens with hairpins and tied with No. 4 silk suture, and the sites of resection are made distinguishable by the way how the suture is cut and by the number of the ligature. They are put as soon as possible into the fixatives consistent to the purpose of the different stainings as soon as possible. In the case amnion and chorion are split into two, as can often be observed either around the ruptured part or the part in the margin of placenta, these leaves are kept as much in original form as possible and then the specimen strip is resected. Besides these, in the case of the amnion of early gestational month the membrane is stretched on a slide glass avoiding crease and the edges are held tightly with smallcrips, and they are put into fixative as soon as possible. However, in the case of the amniotic membrane at the middle or late stage of pregnancy, it is rather too thick

and therefore, it is somewhat unsuitable for obtaining histological and histochemical findings only for amniotic epithelial cell layer. Therefore, in such a case after wiping the surface of the fetal membrane clean with cotton sponge, the author scraped the surface with a razor blade, and after obtaining a piece of amniotic epithelium specimen, it is quickly fixed and stained. With a little experience it is easy to obtain a relatively good small stretched specimen of amniotic epithelium. In the case of the material from the early stage of pregnancy, especially taken upon curettage, often the site may become indistinct due to the method, but it is not so difficult to prepare a specimen as reported by HAGIWARA[17].

Table 1. Classification by duration of pregnancy and onset or non-onset of labor

gravid months / cases	II	III	IV	V	VI	VII	XIII	IX	X
before	7	12	9	4	0	0	1	0	6
after	0	0	1	5	7	4	5	9	72
total	7	12	10	9	7	4	6	9	78

Note : 'before and after' means specimens extracted before or after the onset of labor.

Table 2. Complications. (Fetal membranes taken before the onset of labor)

gravid months	material taken by	cases	main complications
X mo.	caesarean section	3	(eclampsia, syphilis) (contracted pelvis) (breech presentation, hydramnion)
	fractionated injection of oxytocin before caesarean section	3	(contracted pelvis, kidney of pregnancy) (elderly primpara, premature rupture) (elderly primpara, breech presentation)
VIII mo.	Okabayashi's operation	1	cervical cancer, stage 1
V mo.	abdominal caesarean section	2	none
	Okabayachi's operation	1	cervical cancer, stage 1
	Porro's operation	1	uterine myoma
	curettage	9	none
IV mo.	Subtotal hysterectomy	2	uterine myoma
III mo.	curettage	10	none
II mo.	curettage	7	none

Table 3. Complications. (Fetal membranes taken after the onset of labor)

gravid months.	material taken by	nos. of cases	main complications
X mo.	normal delivery	37	no complication during pregnancy nor at delivery, on pathological findings in placenta and fetal membranes
	drip injection of 5% glucose normal delivery	15	cases given drip injection of 500 cc 5% glucose between the 1st and 2nd stages of labor
	quininine bougie	15	10—20 days past ; C. D. C. no other complication, artificially induced labor by bougienage, quinine
	caesarean section	5	(elderly primpara, breech presentation, cervical canal myoma) (elderly primpara, breech present) 2, (inert labor pain contracted pelvis) 2,
IX mo.	spontaneous premature delivery	8	premature rupture of membranes 5, with no other complication
	caesarean section	1	placenta praevia, transverse presentation
VIII mo.	spontaneous premature delivery	3	premature rupture, 1, no other complication
	bougie quinine	2	insufficiency cardiac pre-eclampsia
VII mo.	spontaneous abortion	2	none
	bougie quinine	2	none
VI mo.	spontaneous abortion	2	premature rupture of membrane, 2,
	bougie quinine	5	none
V mo.	spontaneous abortion	2	one of them had miscarriage with normal fetal membranes containing amniotic fluid and fetus.
	rivanol	3	none
IV mo.	spontaneous abortion	1	after bleeding and pain D. & C, were done.

B. *Preparation of tissue specimens :*

1. *Hamatoxylin-eosin staining :* The material is fixed with 10% formalin solution and after embedding in paraffin, it is cut into thin slices 4—5 μ thick and stained by Böhner's H-E staining.

2. *Azan staining :* After fixing the material in 10% formalin solution and embedding in paraffin, it is sliced into pieces 4—5 μ thick and stained with Azan stain in the routine manner.

3. *Periodic Acid Schiff's Staining :* After fixing in absolute

alcohol (for some Carnoy's solution is used) and embedding in paraffin, the material is sliced into pieces 4—5 μ thick, and during deparaffinization, they are immersed in the mixture of absolute alcohol and ether added with celloidin at the rate of 2% and stained with PAS. In the PAS staining the differentiation of mucopolysacchorides was performed. In addition, in a few cases the stainability of celloidin embedded specimens is compared with that of paraffin embedded ones.

4. *Sudan black-B staining :* After fixing the sample in 10% formalin solution and embedding it in water soluble polyvinyl alcohol it is sliced and stained with Sudan black-B.

5. In the case of stretched specimens and amniotic epithelial fragment specimens, they are appropriately stained immediately after fixation.

C. *Symbols representing the varying degree of the reaction*

Symbols representing varying degree of the reaction of polysaccharides and fat demonstrated histochemically are as follows ; (−) stands for almost none or a trace of it ; (±) for slight anount ; (+) for a small amount ; (++) for a moderate amount ; and (+++) for a large quentity, but if the amount seen in the same tissue differs by the site, it is represented by an approximate average amount.

D. *Distinction of pregnant stage :*

For the convenience of descerption the pregnant period is divided into the following three stages ; namely, (1) the early stage of pregnancy, from the second to fourth gestational ; (2) the middle stage, from the fifth to seventh gestational month ; and (3) the late stage, from the eighth to the tenth gestational month.

RESULTS

1. *General findings*

A. Amniotic epithlial cell layer :

(1) Morphology : The shape of amniotic epithelial cells differs by the stage of pregnancy and by the sites such as placenta and free part from where the samples are resected.

(i) Morphological changes by the stage of pregnancy :

Judging from spiral specimens, the epithelial cells in the early stage of pregnancy, lacking in cytoplasma and arranged in a single layer, present apparently flat and slender endothelial cells. The earlier the stage is, the more sparse and irregular are their arrangement. Nuclei are located in the center of the cell and are relatively large as compared with

the cytoplasma, and they are either elliptical or oval in shape and are projecting markedly toward amniotic space. Even by the fifth gestational month this endothelial-like shape remains unchanged, but cell arrangement grows more dense and quite orderly (Plate No. 1). Median height of the amniotic epthelial cell in the early stage of pregnancy is about 4—6 μ. Looking at the stretched specimen from the front, the amniotic epithelial cells in the second to third gestational month have an irregular polygonal shape, varying in their shape and they are arranged densely in a place while sparsely in another place, but generally they are arranged less densely and present a premature shape. Nuclei are situated in the middle of the cell and are either elliptical or oval in shape. The nucleus is stained relatively light with hematoxylin as compared with that in the late stage, and in some distinct chromosomic reticulum, showing one or several nucleoli. Moreover, mitotic figure can often be encountered. There is no marked change in the size of nuclei in the early stage of pregnancy and the late stage. By the fifth gestational month epithelial cells for the first time become dense and also are arranged quite regularly, and their size becomes about equal to one another with slightly round pentagonal or hexagonal shape, and thereafter their general appearance approaches more and more to that in the late stage. In the late stage the epithelial cells have rather uniformly pentagonal or hexagonal shape, and they are arranged in a regular order closely but with fixed intercellular spaces, having ovoid or elliptical nuclei in the center of the cell.

After the sixth gestational month these epithelial cells, increasing in height and growing into a cubic or a low columnar shape, gradually present a marked regional difference between the amniotic epithelial cells in placenta and those in the free amnion.

(ii) Regional difference : From the middle stage of pregnancy amniotic epithelial cells generally grow taller and taller from the margin of the ruptured orifice to the surrounding of placenta, and in placenta they become markedly tall columnar cells.

Such a regional difference becomes more marked closer to the late stage. In the amnion at the tenth gestational month epithelial cells of placenta even in the case undergone caesarean section before the onset of labor often present clear-cut columnar shape as compared with those in other region, and they are generally 15—25 μ in height. The manner how specimens are sliced may influence the shape of cells, but some epithelial cells as tall as 36—48 μ have been observed. Generally, these cells are arranged in a single layer, and there are cells of rod-like shape with its terminal end swollen of slender rectangular shape or conversely with its

terminal end pin-pointed are arranted compactly. Their nuclei are either elliptical or oval, stained deep with hematoxylin, as big as 4—6 μ, and are situated either at apical end or in the center and rarely found at the base of the cell (Plate No. 2). Moreover, there can frequently by recognized proliferative picture of epithelial cells, namely, the proliferation in 2—3 or more lines, multi-layer flat epithelial cell or transitional epithelial cell proliferation (Plate Nos. 3, 4). In the amniotic epithelium of placenta when epithelial cells are taller columnar shape, individual cells are often separated from each other or disarranged as if grass is blown by wind, while on the other hand, in the free amniotic epithelium no such finding can be observed (Plate No. 5).

The surrounding area of placenta even in the late stage of pregnancy is consisted of numerous cuboidal or low columnar cells with an average height of 9 μ, and in this region the proliferative picture of epithelial cells as observable in placenta can hardly be seen.

Epithelial cells in the vicinity of the ruptured orifice are flat or cubic and about 6—9 μ in size, and often they reveal the degenerative picture from pyknosis to karyorrhexis, or of further karyolysis. In H-E stained specimens the cell boundary seems to have disappeared and the cells appear to be a slender band of single layer. This may be caused mainly by the pressure due to the descend of fetus and to the expansion by amniotic bag formation during the lador.

However, in some cases even in the late stage, in a rare instance even in the tenth gestational month, amniotic epithelial cells of placenta still maintain their cubic or low columnar shape, showing no morphological difference with the amniotic epithelial cells in the vicinity of placenta.

(2) *Intercellular connection of amniotic epithelial cells and their attachment to subepithelial connective tissue*

In the arrow-shaped cut sections of the amnion at the late stage there can be recognized numerous fine intercellular bridges running more or less in straight line, but no such bridges are observable in the amnion at the early and middle stages. Moreover, even in the amnion at the late stage these bridges are more distinct when the epithelial cells are beginning to separate from one another ; but when the entire surface of the cell is closely attached to one another, it is difficult to observe such intercellular bridges. Amniotic epthelial cells and sub-epithelial homogenous layer seem to be closely connected with each other by fine saw-teeth-like projections, without showing any interspace. Consequently, it is difficult to separate the epithelium from the subepithelial homogenous layer, and

the separation occurs between the homogenous layer and deeper connective tissue. It is believed, however, that the details of the intercellular connection of epithelial cells and the way how the epithelium and the homogenous layer are connected with each other in the early, middle, and late stages when there are not any contraction or extension among epithelial cells must hereafter be explained under electron microscope rather than by light microscopic examination.

(3) *On the free border of the amniotic epithelial cells :*

The free border of amniotic epithelial cells in every stage of pregnancy seems to be of a very fine saw-teeth-like form, but not any peculiar shape like the brushborder can at all be encountered in any of them.

(4) *The mitotic pattern of amniotic epithelial cells :*

In the stretched amnion specimens of the early and middle stages of pregnancy numerous mitotic pictures of epithelial cells can be observed rather readily (Plate No. 6). However, in the amniotic epithelium in the tenth gestational month, despite the detailed observations, no mitotic figure can be seen.

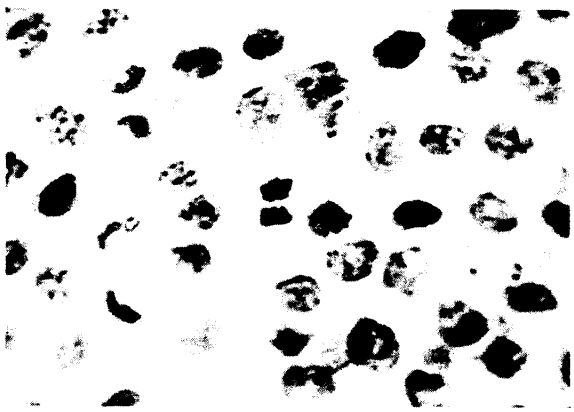

Plate No. 6.

B. *Subepithelial connective tissue of the anmion*

In consideration of the purpose and method of the present experiment various layers of fetal membranes below the amniotic epithelium are discussed mainly centering around the spiral arrow-shaped sections prepared from the cases with normal delivery.

The amniotic subepithelial connective tissue in the late stage is about 20—40 μ thick and immediately under the epithelial layer there can be recognized a homogenous layer of about 15 μ in thickness, and what appears to be structureless and containing no cellular components. Under

this layer strands of connective tissue fibers are drawing a slow curve and running approximately parallel to one another. In between these strands are slender spindle-shaped connective tissue cells distributed evenly. The connection between these strands are relatively tight, but the connection between the chorionic connective tissue and the transitional area is somewhat loose. However, there are no histological changes and the boundary line is not distinct. Connective tissue cells in the arrow-shaped section are star-shaped, irregular triangular or spindle-shaped and of various size, and they are spread in the plane parallel to the epithelium. These cells possess oval or elliptical nucleus about the center of the cell (Plate No. 7).

C. *Connective tissue of chorionic membrane*

This tissue is far thicker than subepithelial connective tissue of amnion, reaching the thickness of 40—80 μ. The connection between the connective tissue strands is rather coarse, but otherwise they differ not so much from the subepithelial connective tissue.

D. *The epithelial layer of chorionic membrane*

Embryologically, this layer is derived from the cytotrophoblast, and is consisted of 4—8 layers, reaching the thickness of 60—100 μ. The cells in this layer are relatively large, and intercellular spaces can be clearly distinguished by Azan staining, and cytoplasma is very brilliant with large and oval nuclei generally lacking in chromatin.

E. *Middle layer*

In the majority of cases between the epithelial layer of chorionic membrane and the decidual cell layer there can be recognized a thin band-like layer that is structureless and lacking in cell components but necrotic, and sometimes a marked fibrinoid degeneration. Embryologically this layer is consisted of degenerated material of the chorion leave and basal ectoderm, residual substances of decidua capsularis and fibrinoid. (Plate Nos. 8, 9).

F. *The decidual cell layer*

Decidual cells are large elliptical or spindle-shaped cells with large oval nucleus, lacking in chromatin and lightly stained. Glandular lumen becomes slender and narrow paralleled to amnictic epithelial layer, and glandular cells have become markedly flat endothelioid cells. Since observation had been carried on the placenta after the delivery, being limited to the decidual tissue attached to the fetal membrane, it was impossible to get clear findings on the layers below the spongiosa layer of the endometrium.

HISTOCHEMICAL FINDINGS

A. *Amniotic epithelial cell layer*

In the histochemical observation of polysaccharides and lipids in the amniotic epithelial cell layer in every stage of pregnancy, the regional difference in the quantities appearing in the region of placenta and in the free part could not be made distinctly clear.

(1) *Findings of PAS staining :* Those substances in the amniotic epithelium that are positive to PAS reaction are generally stained reddish purple, and those fine to large granules or in a larger amount are fused into an irregular mass or band and the cytoplasma is stained wholly and evenly. In the stretched amnion specimens with the exception of the nucleus, these granules appear diffusely in the cytoplasma, and also there can be observed an eccentric picture of a mass of these granules congregating on one side of the cell. In some extreme case, these granules are present in one pole of the cell, sometimes presenting a crescent form. Since these granular or massive substances taking PAS positive reaction can be completely obliterated by diastase digestion test, these substances are confirmed to be glycogen. On the other hand, the substances positive to PAS reaction and staining uniformly reddish purple are not in any way affected by diastase, these seem to be acid muco-polysaccharides, glycoproteins, mucoprotein, and glycolipids. Moreover, these substances show no special difference by the stage of pregnancy, site from where the specimens are taken, or by the onset of labor pain or before the onset of labor pain. However, in the case of glycogen granules, although regional difference could not distinctly be recognized by the stage of pregancy and before and after the onset of labor, the following changes was observed.

(i) *On the amniotic epithelium obtained before the onset of labor ;* Findings of glycogen granules (hereafter to be abbreviated to G-granules) contained in the amniotic epithelial cells of the specimens obtained before the onset of labor in different stage of pregnancy are summarized in Table 4. Namely, G-granules can be seen in the greatest amount in the early stage of pregnancy (Plate No. 10).

Although a moderate amount of G-granules can be found even in the middle stage, the amount decreases markedly from the middle stage to the late stage, and in the tenth gestational month one can detect only a trace or almost none. It is worthy of noting that in the tenth gestational month four cases out of six show not any PAS-positive granules.

(ii) *On the amniotic epithelium after the onset of labor :* Findings on the appearance of G-granules contained in the amniotic epithelial cells

Table 4. Results of PAS staining in 39 specimens of fetal membranes obtained before the onset of labor

gravid month	material	cases	total	amniotic epith. layer		subepith. connective tissue		connective tissue of chorionic membranes		epith. layer of chorionic membranes		ducidua vera	
X mo.	caesarean section before the onset of labor.	3	6	4(−)	(±)	1(±)	(+)	1(±)	(+)	2(+)	(++)	1(−)	(±)
	fractionated injection of oxytoxin before caesarean section	3		2(+)		4(+) 1(++)		3(+) 2(++)		1(++) 3(+++)		2(±) 3(+)	
VIII mo.	Okabayashi's operation	1	1	1(+)	(+)	1(++)	(++)	1(++)	(++)	1(+++)	(+++)	1(+)	(+)
V mo.	abdominal caesarean section	2	4	2(+++)	(++)	3(++)	(++)	3(++)	(++)	2(+++)	(++)	3(++)	(++)
	Okabayashi's operation	1											
	Porro's operation	1		2(+++)		1(+)		1(+)		2(++)		1(+)	
IV mo.	curettage	9	9	6(+++) 3(++)	(+++)	3(+++) 6(++)	(++)						
III mo.	subtotal hysterectomy	2	12	9(+++) 3(++)	(+++)	7(+++) 5(++)	(+++)						
	curettage	10											
II mo.	curettage	7	7	5(+++) 2(++)	(+++)	2(+) 3(++) 2(+++)	(++)						

of the specimens prepared at various stage of pregnancy after the onset of labor are presented in Table 5. In the amniotic epithelium delivered either spontaneously or by artificial induction, the amount of G-granules is trivial or almost none in all stage of pregnancy (Plate No. 11). However, in two out of 72 cases in the tenth gestational month, though the number is small, a moderate amount of G-granules has been detected, and in consideration of de Watteville's opinion this seems to be profoundly significant. In addition, one case of spontaneous abortion on the fourth gestational month had lower abdominal pains like labor for 2 or 3 days and had bloody discharge with clot as big as a hen's egg, and was given curettage after being diagnosed as abortion (Plate No. 12). Another case

Table 5. Results of PAS staining in 103 specimens

gravid months	material	cases	total	amniotic epithel. layer	
X mo.	natural delivery	37	72	27(−) 1(+) 9 (±)	(−)
	drip inj. of 500cc 5% glucose nat. delivery	15		9(−) 2(+) 3(±) 1(卄)	(−)
	bougienage quinine	15		9(−) 1(+) 5(±)	(−)
	caesarian section after onset of labor	5		2(−) 1(卄) 2(+)	(+)
IX mo.	spontaneous premature delivery	8	9	4(−) 1(+) 3(±)	(−)
	caesarian section after onset of labor	1		1(±)	(±)
VIII mo.	spontaneous premature delivery	3	5	1(−) 2(±)	(±)
	bougienage quinine	2		2(−)	(−)
VII mo.	spontaneous abortion	2	4	2(−)	(−)
	bougienage quinine	2		1(−) 1(+)	(±)
VI mo.	spontaneous abortion	2	7	1(+) 1(−)	(±)
	bougienage quinine	5		3(−) 2(±)	(−)
V mo.	spontaneous abortion	2	5	1(+) 1(卄)	(卄)
	rivanol	3		3(−)	(−)
IV mo.	spontaneous abortion	1	1	1(卄)	(卄)

of the fifth gestational month had aborted with both amniotic fluid and fetus contained in the fetal sac at the time of examination (Plate No. 13). It is quite interesting that a large amount of G-granules was demonstrated in the amniotic epithelium in these two cases.

On 15 cases of full-term normal delivery, given as much food as permissible in the beginning of labor and administered drip injection of 500cc 5% glucose during the period from the end of the first stage of labor to the second stage, and on the examination of the changes in the amounts

of fetal membranes obtained after the onset of labor

subepithel. connective tissue of amnion		connective tissue of chorionic membrane		chorionic membrane		decidua vera	
6( ) 19(+) 11(±) 1(卄)	(+)	6(−) 17(+) 9(±) 4(卄) 1(卅)	(+)	5(±) 14(卄) 13(+) 5(卅)	(卄)	5(−) 10(+) 22(±)	(±)
4(−) 3(+) 8(±)	(±)	5(−) 4(+) 4(±) 2(卄)	(+)	4(±) 5(卄) 5(+) 1(卅)	(卄)	5(−) 3(+) 5(±) 2(卄)	(±)
5(−) 3(+) 7(±)	(±)	3(−) 8(+) 4(±)	(+)	3(±) 4(卄) 5(+) 3(卅)	(卄)	3(−) 5(+) 6(±) 1(卄)	(+)
1(±) 2(卄) 2(+)	(卄)	1(±) 2(卄) 2(+)	(卄)	1(±) 2(卅) 2(卄)	(卅)	1(−) 2(卅) 2(+)	(卄)
2(−) 3(+) 2(±) 1(卄)	(+)	2(−) 2(+) 3(卄) 1(卅)	(卄)	1(±) 3(卄) 2(+) 2(卅)	(卄)	2(±) 3(卄) 2(+) 1(卅)	(卄)
1(+)	(+)	1(卄)	(卄)	1(卅)	(卅)	1(+)	(+)
2(+) 1(卄)	(+)	2(+) 1(卄)	(+)	1(+) 1(卅) 1(卄)	(卄)	1(±) 2(卄)	(卄)
2(±)	(±)	1(±) 1(+)	(±)	2(卄)	(卄)	2(±)	(±)
1(±) 1(卄)	(+)	1(±) 1(+)	(+)	1(±) 1(卄)	(+)	1(±) 1(+)	(+)
1(±) 1(+)	(+)	1(+) 1(卄)	(+)	1(卄) 1(卅)	(卄)	1(±) 1(+)	(+)
1(+) 1(卄)	(卄)	2(+)	(+)	1(+) 1(卄)	(+)	2(卄)	(卄)
2(±) 3(+)	(+)	1(±) 1(卄) 3(+)	(+)	1(+) 2(卅) 2(卄)	(卅)	2(±) 3(+)	(+)
2(+)	(+)	2(+)	(+)	2(卅)	(卅)	2(卄)	(卄)
2(−) 1(±)	(−)	2(−) 1(±)	(−)	2(±) 1(−)	(±)	2(−) 1(±)	(−)
1(卅)							

of G-granules appearing in the amniotic epithelium after delivery, there can be found no difference worthy of mentioning.

Recapitulating the results in (i) and (ii), it seems that PAS positive granules of the amniotic epithelial cell layer, the G-granules, show the changes most distinctly during the pregnancy; appearing most abundantly in the early stage and beginning to decrease in the middlle stage; and almost all of them disappear late in the pregnancy. Moreover, it is believed that G-granules of the amniotic epithelial cell layer are readily

Table 6. Results of 39 cases of the fetal membranes obtained before the onset of labor and stained with Sudan black-B

gravid month	material used	cases	subtotal	amniotic epithel. cell layer		amniotic subepithel connective tissue		connective tissue of chorionic membrane		epithel. layer of chorionic membrane		decidua vera	
X mo.	caesarian sect. before onset of labor	3	6	2(+) 3(卄) 1(卅)	(卄)	2(+) 4(卄)	(卄)	3(+) 3(卄)	(卄)	4(+) 2(卄)	(+)	2(+) 3(卄) 1(卅)	(卄)
	fractionated inject. of oxytocin bef. caes. sect.	3											
VIII mo.	Okabayashi's operation	1	1	1(+)	(+)	1(+)	(+)	1(+)	(+)	1(+)	(+)	1(卄)	(卄)
V mo.	abdominal caesarian section	2	4	2(−) 1(±) 1(+)	(±)	2(−) 3(±)	(±)	2(−) 3(±)	(±)	1(±) 4(+)	(+)	4(+)	(+)
	Okabayashi's operation	1											
	Porro's operation	1											
IIII mo.	curettage	9	9	4(−) 5(±)	(±)	4(−) 5(±)	(±)						
III mo.	subtotal hysterectomy	2	12	12(−)	(−)	12(−)	(−)						
	curettage	10											
II mo.	curettage	7	7	7(−)	(−)	7(−)	(−)						

disintegrated and obliterated during the delivery.

(2) *Findings of Sudan black-B staining :* By Sudan black-B staining lipids can be recognized as round black granules. The amounts of lipids appearing in the amniotic epithelium taken both before and after the onset of labor and stained with Sudan black-B are shown in Tables 6 and 7.

As is clear from Tables, it is difficult to find essentially any difference between the results of staining and the onset of labor, but there can be recognized a definite relationship between the gestational month and the amount of lipoid granules stained with Sudan black-B. In other words, the amniotic epithelium of the early stage of pregrancy is hardly stained by Sudan black-B, and no deposit of pigment within epithelial cells can be recognized. It is only by the fourth gestational month that fine round

black granules for the first time appear slightly in a few of epithelial cells. These black granules gradually increase in their size and quantity from the middle stage of pregnancy, and in the late stage cytoplasma of almost all the cells seems to be filled up with black granules of various size (Plate Nos. 14, 15).

B. *Amniotic subepithelial connective tissue and the connective tissue of chorionic membrance :*

In the homogenous layer immediately under amniotic epithelium G-granules or lipid granules can hardly be detected. In the connective tissue under this homogenous layer G-granules are predominant in the early stage of pregnancy, and although they tend to decrease from the middle stage, still a small or a moderate amount of them can be recognized even in the late stage. As for the site of the apperance, in the interspaces of connective tissue, the granules appear in the form of a line or a belt and also they appear in connective tissue cells excepting in nuclei diffusely and in granular form (Plate No. 7). It is especially interesting that in the case of spontaneous full term delivery no G-granules whatsoever can be detected in the amniotic epithelium, while on the contrary, markedly distinct G-granules appear in band formation in subepithelial connective tissues (Plate No. 11). Although any lipid granules can hardly be detected in the early stage of pregnancy, by the fifth gestational month the pigment gradually begins to be deposited, and thereafter the amount of lipid granules gradually increases as it approaches nearer to the full term. The site where lipid granules appear is mainly in the connective tissue cells. Histologically no special difference can be observed between the amniotic subepithelial connective tissue and the connective tissue of the chorionic membrane.

C. *Epithelial cell layer of the chorionic membrane.*

In the examination of the epithelial cell layer of the chorionic membrane in the stage later than the middle stage, extremely abundant amount of G-granules can be detected regardless before or after the onset of labor, filling up cytoplasma in a band form and often presenting an eccentric picture. Even in the case where G-granules are found in an extremely less amount in other layers of the fetal membranes, a moderate to a large amount of G-granules can be detected in the epithelial layer of the chorionic membrane. (Plate No. 16).

Lipid granules of various size appearing in a round granular shape, though far less coarse than G-granules, tend to increase considerably towards the late stage.

Table 7. Results of 55 cases of fetal membranes taken after the

gravid month	material used	cases	subtotal	amniotic epithel. cell layer	
X mo.	normal delivery	15	28	9(卅) 6(廾)	(卅)
	bougie introduction quinine course	8		5(卅) 3(廾)	(卅)
	caes. sect. aft. onset of labor	5		2(廾) 3(卅)	(卅)
IX mo.	spontaneous premature delivery	5	5	1(卅) 2(廾) 2(+)	(廾)
VIII mo.	spontaneous premature delivery	3	5	2(廾) 1(+)	(廾)
	bougie introduction quinine	2		2(廾)	(廾)
VII mo.	spontaneous abortion	2	4	1(+) 1(±)	(+)
	bougie introduction quinine	2		2(+)	(+)
VI mo.	spontaneous abortion	2	7	2(+)	(+)
	bougienage quinine	5		4(+) 1(±)	(+)
V mo.	spontaneous abortion	2	5	2(+)	(+)
	rivanol (by Aburel's)	3		2(+) 1(±)	(+)
IV mo.	spontaneous abortion	1	1	1(−)	(−)

D. *Decidual cell layer*

The glycogen content in the decidual cell layer in the stage later than the middle stage is generally poor, but it shows somewhat a gradually decreasing tendency towards the full term. On the other hand, even in the same decidual cell layer of the same specimen the observable glycogen content varies from site to site. As the pregnant stage advances from the middle stage to the late, the presence of lipid granules clearly increases in all individual cells.

DISCUSSION

1. *On the general findings*

POLANO et al[3]. contended that by about the fifth gestational month there appears one of the morphologically striking localized differences

onset of labor and stained with Sudan black-B

amniotic subepithel. connective tissue		connective tissue of chorionic membrane		epithel. layer of chorionic membrane		decidua vera	
3(卅) 7(艹) 5(+)	(艹)	3(卅) 8(艹) 4(+)	(艹)	4(卅) 6(艹) 5(+)	(艹)	5(卅) 7(艹) 3(+)	(艹)
1(卅) 5(艹) 2(+)	(艹)	2(卅) 4(艹) 2(+)	(艹)	1(卅) 4(艹) 3(+)	(艹)	2(卅) 6(艹)	(艹)
2(艹) 3(+)	(+)	2(艹) 3(+)	(+)	3(艹) 2(+)	(艹)	1(卅) 2(艹) 2(+)	(艹)
1(卅) 3(艹) 1(+)	(艹)	1(卅) 3(艹) 1(+)	(艹)	1(艹) 3(+) 1(±)	(+)	2(艹) 1(卅) 2(+)	(艹)
1(±) 2(+)	(+)	1(±) 2(+)	(+)	2(艹) 1(+)	(艹)	2(+) 1(艹)	(+)
2(+)	(+)	2(+)	(+)	2(+)	(+)	2(+)	(+)
2(+)	(+)	1(艹) 1(+)	(+)	1(卅) 1(艹)	(艹)	1(+) 1(±)	(±)
2(+)	(+)	2(+)	(+)	1(艹) 1(+)	(艹)	1(+) 1(±)	(±)
2(±)	(±)	2(±)	(±)	2(+)	(+)	2(+)	(+)
2(+) 3(±)	(±)	1(+) 4(±)	(±)	1(艹) 3(+) 1(±)	(+)	3(+) 2(±)	(+)
2(±)	(±)	2(±)	(±)	2(+)	(+)	2(+)	(+)
1(−) 2(±)	(±)	1(−) 2(±)	(±)	3(±)	(±)	2(−) 2(±)	(±)
1(−)	(−)						

between amniotic epithelial cells of the placent region and those in the free part, and in the former region epithelial cells grow up into columnar shape while in the latter part they stay in a cubic shape and that the amniotic epthelium of the placental region plays a special role in the amniotic fluid secretion.

Recently BAUTZMANN[24] claimed that the tall columnar shape of amniotic epithelial cells of plancenta is a passive mechanical transformation due to a special contracting action such as the contraction of uterine muscles or loss of blood in intervillous spaces during the course of labor, amniotic fluid outflow and delivery of placenta, and he raised a strong question to the fact reported by POLANO et al. that the epithelium consisted of rod-like columnar cells having their apical ends swollen and nuclei located in the swollen part near the surface, has a directly close connection with the secre-

tory function of the epithelial cells. As the basis for his contention: (1) he points out that when the epithelium forms an undulating wave in the arrow-shape section specimens, epithelial cells can maintain their plane or tall columna shape respectively at the peak or at the bottom of the wave; (2) according to the original descriptive figure of intercellular bridge in POLANO's report of 1922, intercellular bridge can be recognized only a little over one half the height of the cell and beyond that each cell is completely free from one another. The epithelial cells appear to be quite closely connected by relatively short intercellular bridge at the basal part. This seems to suggest that the flat cells possessing intercellular bridge are transformed into columnar shape by the mechanical prsessure; (3) on the whole, real secretory columnar epithelial cells have nuclei in the basal part of the cell and the secreted substance fills up the apical part with generally uniform-sized cells, and the surface of the epithelium is flat; and (4) in the placental amniotic epithelium, epithelial cells are greatly inclined to left or right and are often observed in a disorderly arrangement. These are the bases of contentions of BAUTZMANN[24]. From author's own results, the author agrees with Bautzmann's observations of (1) and (4). These observations and the regional characteristics of tall columnar shape of placental amniotic epithelial cells, as already mentioned, are more marked towards the late stage of pregnancy and in rare instances such a characteristic may not be observable even in the placental amniotic epithelium of the tenth gestational month, and also that according to the studies of GIL VERNET et al.[25] the secretory function of amniotic epithelium commences in the third week, reaching its maximum in the seventh gestational month and it decreases in the late stage. Jundging from these, the question of doubt against the opinions of POLANO and FORSSELL[6] raised by BUATZMANN[24] seems to require a further carefull investigation in future.

According to FORSSELL[6], morphological changes appear in the amniotic epithelial cells of placenta and the free part from the middle stage of pregnancy, and the placental amniotic epithelium commonly is of a double line, of which one is consisted of tall columnar cells rich in granules and having narrow base and the other is consisted of low cells lacking in granules with wide base. These columnar cells possess small nuclei rich in chromation in the part which is pointing towards the amniotic cavity, showing characteristic brushborder and bubble-like secretion, but the basal cells, not reaching the surface of epithelium, are a kind of reserve cells, possessing big nuclei rich in chromatin and often showing amitosis but no brushborder or bubble-like serection. No such changes occur in the free amniotic epithelium which maintains a cubic single-layer epithelium. In

author's own observations even in the stage later than the middle stage the placental amniotic epithelium is generally of a single layer and findings similar to Forssel's contention can be recognized only in a portion.

PETRY[26], HATA[27], and HAGIWARA[17] contend that amniotic epithelial cells multiply themselves by amitosis, while against this, BAUTZMANN[24], detecting 71 mitotic pictures in the area of 35 square millimeters of the amniotic epithelium obtained from the fetus of 10 cm in sitting height, contends that these epithelial cells, at least in the early stage of growth, multiply by mitosis.

The author likewise detected a relatively large number of mitotic pictures in the amniotic epithelium of the early and middle stages but it was difficult to detect such pictures in the amniotic epithelium of the tenth gestational month. In view of the fact that histologically amniotic pictures can often be encountered in degenerated cells, senile cells and in other pathological cells and it is rarely detectable in any normal cells, amniotic epithelial cells, at least in the early and middle stages when their functions are at height, seem to multiply themselves by mitosis.

In Japan, there is an extensive histochemical study on glycogen reported by KIMURA and TANAKA[28-32].

It is considered that granular substance in amniotic epithelial cells, stained reddish purple with PAS stain and often presenting an eccentric picture and being completely lost by diastase digestion test, is not glycoprotein but glycogen. From the PAS-staining reaction of the amnion taken both before and after the onset of labor (at various stages of pregnancy), it seems that glycogen in amniotic epithelial cells is generally abundant in the early and middle stages and it is trivial or has disappeared so completely as to be unable to detect it in the late stage of pregnancy. In addition, glycogen granules (G-granules) seem to be lost relatively easily during the course of labor, but from author's own findings, it seems difficult to connect the onset of labor, in other words, the continuation of pregnancy, directly with existence or non- existence of G-granules.

On the other hand, the lipid granules in amniotic epitheilial cells made detectable by Sudan black-B show no particular difference before and after the onset of labor, but in contrast to G-granules they cannot be detected in the early stage of pregnancy but they begin to appear in the middle stage and increase rapidly towards the late stage.

HATA[27] stated that this is simply an absorptive lipid deposit due to an acceleration of the fat metabolism but not the regressive degeneration. However, it seems still difficult to designate it as the progressive fatty degeneration that absorbs fat actively and forms fat, and therefore,

the author can not agree with him. Generally, regressive degeneration findings are not histologically, so distinct but provided that glycogen in the epithelial cells indicates the cell activity and the lipid deposit, on the contrary, indicates regressive degeneration, it can be assumed that the amniotic epithelial cell layer starts to regress from the middle stage of pregnancy at least functionally and finally degenerates. As for the role played by this regressive degeneration of the amniotic epithelium in de Watteville's theory "Labor commences from fetal membrane", it is seems to require further study.

CONCLUSION

1. In the stage later than the middle stage of pregnancy, morphological differences appear between the amniotic epithelial cells of placenta and those of the free part and the majority of cases the amniotic epithelial cells of placenta present more marked columnar shape than those in the surrounding area of ruptured orifice or those in the vicinity of placenta. However, there still remains a question whether or not such a phenomena is directly related to the secretory function of the placenta amniotic epithelium.

2. It seems that amniotic epithelial cells divide and multiply themselves by mitosis at least in the early and middle stages when their functions are at height.

3. Even in the stage later than the middle stage generally the amniotic epithelium of placenta is consisted of a single layer of columnar epithelial cells, and therefore, the author cannot agree to Forssell's theory.

4. In glycogen and lipid stainings, the amniotic epithelial cell layer shows more striking changes with the progress of gestational month when compared with those cells in other layers.

5. Glycogen in the amniotic epithelial cell layer is abundant in the early and middle stages of pregnancy, and it rapidly decreases near the late stage. Lipid granules on the contrary are less in the early stage, and start to appear in the middle stage, increasing rapidly towards the late stage. In general, the regressive degeneration picture of the late stage is not distinct histologically, but assuming glycogen to represent the cell activity and the lipid deposit to mean just the reverse, the amniotic epithelium functionally seems to fall into regressive degeneration from the middle stage. Other layers of fetal membranes likewise undergo fatty degeneration as the pregnancy progresses from the middle stage to the late stage.

6. There still remain problems to be solved on the question what role this regressive degeneration of the amniotic epithelial cell layer plays in de Watteville's theory, "Labor originates from the fetal membranes". However, granular PAS-positive substances in the amniotic epithelium are glycogen, and it seems difficult to connect simply the existence or non-existence of PAS-positive granules or Sudan-positive granules directly with the continuation or interruption of pregnancy.

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EXPLANATION OF PLATES

Plate No. 1. Fifth gravid month, vaginal caesarian section, placenta, hematoxylin-eosin staining.

Plate No. 2. Tenth gravid month, natural delivery, placenta, H-E staining.

Plate No. 3. Tenth gravid month, natural delivery, placenta, H-E staining.

Plate No. 4. Tenth gravid month, natural delivery, placenta, Azan staining.

Plate No. 5. Tenth gravid month, natural delivery, placenta, H-E staining.

Plate No. 6. Fifth gravid month, vaginal caesarian section, free amniotic epithelium, H-E staining, mitosis at anaphase.

Plate No. 7. Tenth gravid month, natural delivery, showing fine PAS-positive granules in the connective tissue cells of the amniotic subepithelial connective tissue.

Plate No. 8. Tenth gravid month, natural delivery, the vicinity of placenta, showing the intermediate layer and its fibrosis, Azan staining.

Plate No. 9. Tenth gravid month, caesarian section, the vicinity of placenta, Azan staining.

Plate No. 10. Third gravid month, curettage, PAS staining

Plate No. 11. Tenth gravid month, natural delivery, the vicinity of placenta, PAS staining.

Plate No. 12. Fourth gravid month, spontaneous abortion, PAS staining.

Plate No. 13. Fifth gravid month, spontaneous abortion, PAS staining.

Plate No. 14. Tenth gravid month, natural delivery, the surrounding of the ruptured orifice, Sudan black-B staining

Plate No. 15. Fifth gravid month, vaginal caesarian section, placenta, Sudan black-B staining.

Plate No. 16. Tenth gravid month, natural delivery, the surronding of the ruptured orifice, PAS staining.

No. 1

No. 2

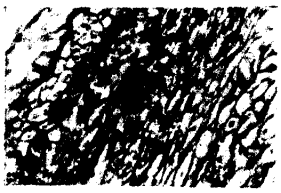
No. 3

No. 4

No. 5

No. 7

No. 8



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