THE CYTOLOGICAL STRUCTURE OF THE HUMAN CHORIONIC VILLUS AND DECIDUA PARIETALIS

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FOUR PLATES (THIRTY FIGURES)

A large number of investigators have studied the general histology of the chorionic villus and decidua parietalis but few of them have been particularly concerned with the cellular components which may be associated with the process of secretion. In 1916, de Kervily published two papers in which he described in considerable detail the cytology of the human chorionic villus. Many of his observations, especially those relating to secretion by the trophoblast, have failed to receive general acceptance. Since that date, little attention has been given to the finer structure of the placenta. More extensive information concerning the cytology of the placenta is desirable in view of the current interest in the physiology of this organ. It has been shown to play an important rôle in the regulation of the transfer of substances between the maternal and fetal blood streams and much accumulated evidence suggests that the placenta also acts as a gland of internal secretion. Hence, during the past several years we have carried on a cytological investigation of the human placenta, seeking to find structures within the villi and nearby decidua parietalis to which we might attribute an endocrine function.

MATERIAL AND METHODS

The source of the material studied is listed in table 1. The reasons for the interruption of pregnancy included tuberculosis, brain tumor, mammary carcinoma, uterine fibroma, glau-

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coma, anemia, mechanical injury to the fetus and threatened spontaneous abortion. This pathology was considered in evaluating the cytological picture of each case and those specimens which showed evidence of infection or degeneration were used only as reference material. The operations in cases 1–34 (except nos. 14 and 25) were performed under general anesthesia.

Although the tissue obtained from therapeutic abortions was extremely disorganized, the zona compacta of the decidua parietalis could be differentiated easily from fetal fragments and villi by its pale color, compactness and glistening epithelial surface. Some of the zona spongiosa usually remained attached to it. When hysterectomies were performed, samples of the decidua were obtained about 2 cm. from the edge of the placenta. The villi and deciduae were handled separately, small pieces of each being placed into the following fixatives: Bouin's fluid, picric-alcohol-formol, Zenker-formol followed by chromation in 3% potassium dichromate, and in Champy's fluid followed by osmication and chromation according to the Severinghaus pituitary gland methods. In many cases, the tissues were handed directly to one of the authors by the surgeon so that not more than 1 or 2 minutes transpired between the time of removal from the patient and fixation. It was these specimens which exhibited the most delicate cytological detail in the trophoblast. In only a few cases did more than 5 minutes elapse before fixation. Zenker-formol-fixed and Champy-fixed material was double embedded in celloidin and 60–62° C. paraffin. Best's carmine stain with and without hematoxylin was employed for the demonstration of glycogen after fixation in picric-alcohol-formol and was controlled by saliva digested preparations. A trichrome stain, described by Severinghaus ('39), was used after the other fixatives. Some Bouin-fixed tissues were impregnated with silver according to the Bielschowsky technique (McClung). The preparations in the University of Michigan Embryological Collection were stained with hematoxylin and eosin or Congo red.

TABLE 1

Specimens studied.

CASE	TYPE OF OPERATION	DECIDUA (D) VILLUS (V)	MENSTRUAL AGE (WKS.)	EST. MENST. AGE (WKS.)[1]
1	Therapeutic abortion	D	6–8	
2	Hysterectomy	D V	11	10.7 ?
3	Hysterectomy	D V	20	
4	Therapeutic abortion	D V	12.5	
5	Hysterectomy	D V	17 ?	15.7
6	Therapeutic abortion	D V	7	
7	Therapeutic abortion[2]	V	13	14 ?
8	Spontaneous abortion	D	14.2	14 ?
9	Spontaneous abortion	V	13	14 ?
10	(Tubal pregnancy)	D V	13	
11	Spontaneous abortion	D V	10	14 ?
12	Hysterectomy	D V	15.1	13.5
13	Hysterotomy	D V		14.1
14	Subtotal hysterectomy	D V	14.2	13.5
15	(Ectopic pregnancy)	D V		12.4
16	Therapeutic abortion[2]	D V		15.5
17	Hysterectomy	D V	10	9.7
18	Therapeutic abortion	D V	8	
19	Delivery	V	full term	
20	Delivery	V	full term	
21	Delivery	V	full term	
22	Delivery	V	full term	
23	Therapeutic abortion	D V		
24	Therapeutic abortion[3]	D V		8 (Dr.)
25	Therapeutic abortion	D		6 (Dr.)
26	Therapeutic abortion	D V	12.7	
27	Therapeutic abortion	D V	7	
28	Therapeutic abortion	V	15 ?	14.7
29	Therapeutic abortion		13	13.2
30	Therapeutic abortion	V	6–9	
31	Therapeutic abortion	V	12	
32	Therapeutic abortion	V	8	
33	Delivery	V	29	
34	(Hydatidiform mole)	V		
EH 47[4]	Hysterectomy	D V		10.4
EH 78	?	V		9.6
EH 82	?	V		11.1
EH 102	Hysterectomy	V		9.0
EH 138	Hysterectomy	V		8.4
EH 147	Hysterectomy	V	7	9.3
EH 173	Hysterotomy	D		13.3
EH 181	Spontaneous abortion	V		15.8
EH 265	(Tubal pregnancy)	V		11.1

[1] Except when estimated by the doctor during the operation, "(Dr.)", the menstrual age of the fetus was determined from the data of Mall on the basis of the crown-rump length, Mall ('18) and Keibel and Mall ('10), p. 199.

[2] Following mechanical injury to fetus.

[3] Because of threatened spontaneous abortion.

[4] The specimens designated by E H are from the University of Michigan Embryological Collection.

OBSERVATIONS

The chorionic villus (6–14 weeks)

Cytotrophoblast. The cells of the cytotrophoblast varied considerably in size, the larger ones appearing more vesicular after fixation in Champy's fluid than in the other fixing solutions. This agrees with a previous observation by Langhans ('01) who used osmic acid fixatives also. The cytotrophoblast cells showed frequent variations in shape, being somewhat cylindrical when arranged in a continuous row (fig. 1), and more ovoid with a convex syncytial surface when they were separated from each other by syncytium reaching to the basement membrane as was usually the case (fig. 3). The nucleus possessed a distinct membrane which was usually folded and which enclosed palely-stained nucleoplasm and one or more irregularly-shaped acidophilic nucleoli (figs. 2 and 6). If cytoplasmic structure was visible, it tended to be lightly stained and somewhat granular. The cytoplasm of the large, vesicular cells was most dense in the region of the Golgi apparatus from where finer strands extended toward the periphery (fig. 1).

The Golgi apparatus was found almost invariably on the side of the nucleus toward the syncytium (fig. 7). If the cytotrophoblast consisted of a continuous layer of polygonal cells, this organelle was definitely polarized toward the surface of the villus. When the cells were separated by projections of the syncytium reaching to the basement membrane, especially in later stages, the Golgi apparatus was frequently located lateral to the nucleus. It was almost never found between the nucleus and the thin but distinct reticular tissue basement membrane which supported the trophoblast. Usually the Golgi apparatus consisted of a meshwork, with or without vesicles (fig. 8), the looseness of arrangement varying directly with the size of the cell. In proximity to it, were found small granules and less regularly shaped material which stained with aniline blue in post-chromated Champy-fixed preparations (fig. 1). These bodies occurred in no other parts of the cell and could not be demonstrated in all speci-

mens. Mitochondria were present throughout the cytoplasm but tended to be most concentrated at the edges of the Golgi body (figs. 1 and 3). In confirmation of de Kervily ('16) the mitochondria consisted chiefly of short rods or beads arranged in the form of chains of varying lengths. Some cells contained small quantities of glycogen.

The cytotrophoblast showed rather intense mitotic activity which, in localized areas, was responsible for the formation of cell columns and cell islands.

Cell columns. The cell columns of the cytotrophoblast were covered by syncytium and occurred at the decidual ends of anchoring villi uniting the chorion to the decidua basalis (fig. 22). They have long been regarded as derivatives of the cytotrophoblast (v. Lenhossék, '02; Rossi Doria, '05) and since mitotic figures were most numerous at the fetal end of the cell column this origin can hardly be questioned. Similar cell groups were found at the unattached ends of the branches of the villi. Since only very small areas of the placenta were shown in our cytological preparations, it was not always possible to determine the exact location of such proliferations. The cells of the cell columns and of the cell groups at the ends of villous branches differed from the cytotrophoblast layer in the following particulars, these differences being most pronounced in those cells farthest removed from the point of continuity with the cytotrophoblast layer. In preparations which did not preserve glycogen, the cells appeared to enlarge and to contain less stainable cytoplasm as they were pushed away from the site of origin by the continued multiplication of cells in the cytotrophoblast layer of the villus (fig. 22). The empty cytoplasmic spaces in these cells is accounted for by the removal of glycogen since we, in agreement with Driessen ('07), found them to contain large amounts of this substance. The mitochondria were fewer in number and more variable in size (fig. 21) and shape although tending to be globular or vesicular. They were less frequently arranged in beaded chains. Rarely could a distinct Golgi apparatus be discerned.

Cell islands. The intervillous cell islands are attached to villi (Keibel and Mall, '10) and have been differentiated from the cell columns on the basis of their position between villi and lack of a continuous syncytial covering (fig. 25). In our preparations, the cell islands showed variable degrees of degeneration. This process was most advanced centrally, possibly due to the failure of the cell islands to become vascularized. Degeneration was accompanied, first, by the appearance of small, irregularly-shaped formations of fibrinoid (fig. 25). Later, these seemed to unite to form larger, denser masses (fig. 13) which spread toward the periphery, ultimately overtaking the cellular constituents. The fibrinoid showed a strong affinity for eosin and a variable reaction to the acid fuchsin-aniline blue stain, appearing light brown in many instances. In the region of fibrinoid and less frequently elsewhere, shrunken cells with pycnotic nuclei were observed in process of degeneration. Elsewhere, many cells possessed a lobated nucleus, osmiophilic nuclear membrane, small Golgi apparatus, few mitochondria and little cytoplasm. However, cells which seemed to be more active were observed, especially near the periphery of the islands next to the maternal blood. Both the cells and their nuclei were larger, the latter tending to be spherical. The Golgi apparatus was hypertrophied and encompassed dense cytoplasm and small blue granules, the latter increasing in size toward the periphery of the cell (fig. 13). Striking accumulations of mitochondria occurred in the Golgi region (fig. 19). A separation of the cells at the edge of the island was quite evident since maternal erythrocytes were frequently observed between them. These cells with their numerous blue granules then appeared to be sloughed off into the blood of the intervillous space. Ghosts of these cells, depleted of granules, were observed (case 13) in the intervillous space a short distance from the islands. A few mitotic figures and large amounts of glycogen (fig. 16) were present in the islands.

Syncytium. The nuclei of the syncytium differed rather sharply from those of the cytotrophoblast after Bouin fixation

by virtue of their smaller size, darkly-stained and wrinkled membrane and coarse chromatin masses (figs. 2, 3 and 6). The cytoplasm was sometimes homogeneous and structureless, but frequently it had a granular or reticular appearance. This variability even within the same villus may be attributed to the promptness of fixation, the fixing fluid used, and possibly to the age of the area of trophoblast in question. Staining of Champy-fixed material by the Severinghaus technique differentiated mitochondria, lipid droplets and granules, these being colored fuchsin, brown, and blue, respectively. The free surface of the syncytium was modified to form a brush border after fixation in Champy's fluid (fig. 5), Bouin's solution (fig. 6) or Zenker-formol. In some areas it reached a height of about 1.6 μ. In general, the brush border consisted of fine, vertical, closely-approximated projections which appeared coarser and more distinct after fixation in Bouin's fluid. The brush border varied in height and in distinctness and regularity of arrangement of its individual elements in different parts of the same villus. Our techniques did not reveal definite basal granules such as those described by v. Lenhossék ('02). However, a fuchsinophilic line which may have been composed of very minute granules was sometimes seen beneath a regular brush border.

The Golgi apparatus of the syncytium was generally located in the subnuclear and nuclear zones but in thick syncytium, it occasionally extended into the area immediately above the nucleus. The Golgi apparatus consisted of threads of variable thickness as is well illustrated by a 16-week-old placenta (fig. 20). Most commonly they were curved to form loops and, in some locations, seemed to be united to form a network. The negative image of this structure was seen clearly in non-osmicated Champy and Bouin-fixed preparations as clear, curved channels similar in width and shape to the blackened threads of the osmicated Golgi body (fig. 2). In some instances we have observed areas of condensed cytoplasm in association with this negative image.

Nassonov ('23) and Bowen ('29) first reported that the Golgi apparatus plays an important rôle in the formation of secretory products in glandular cells. Their contention has been supported by subsequent investigators who have studied a wide variety of glandular tissues. If this is true, it is significant that minute blue granules, singly and in clusters, were observed in the subnuclear zone of the syncytium (fig. 2). We could not demonstrate the relationship of the granules to the Golgi apparatus in osmicated preparations, for in these the granules could not be stained satisfactorily. However, in non-osmicated Champy-fixed preparations the granules were occasionally surrounded by a light zone which may have been the negative image of Golgi material. These granules stained similarly to the blue granules already described in the nearby Golgi region of the cytotrophoblast. Towards the free surface of the syncytium they increased in size, and become more perfectly spherical in the supranuclear zone and exhibited a homogeneous texture (fig. 2). Occasionally, large bodies as wide as 3.6 μ and apparently composed of the same substance, were observed in the region of, or adjacent to the nucleus. These granules were preserved by Champy's fluid, Zenker-formol and, at least the larger ones, by Bouin's fluid. Large numbers of granules were found more commonly in thick than in thin syncytium and in these areas the general cytoplasm tended to stain more intensely with aniline blue.

Liquefaction of the syncytical granules was suggested by their intimate relationship to clear superficial vacuoles which occurred most regularly immediately subjacent to the brush border. The largest granules measured about 1.8 μ in diameter and were comparable with the average size of the superficial vacuoles. Some granules were surrounded by a clear areola (figs. 2 and 14); others appeared as a minute granule in the center of a clear vacuole. The composition and affinity for aniline blue of other granules varied from a homogeneous, dark blue sphere to a delicate, non-stained coagulant or clear fluid (fig. 4) within a vacuole. In parts of

some villi, especially where the vacuoles were most numerous, large, and clear, the brush border was obscure and the outermost boundary of the syncytium undulated (fig. 18), suggesting that the vacuoles had ruptured through the brush border leaving the innermost wall of the vacuoles as the outer boundary of the syncytium. Occasionally, the elements of the brush border were separated by clear vesicular spaces without disruption of the subjacent cytoplasm (fig. 18). However, the vacuoles were found most frequently beneath an even and regular brush border. Blue granules occurred in the absence of any vacuoles but could never be found in the brush border or intervillous space.

The mitochondria of the syncytium possessed the shape of short, granular filaments or rods. Generally in form and size they were somewhat similar to those of the cytotrophoblast; in a few cases, they were definitely finer and more delicate. They could be found throughout the syncytium and were not oriented in respect to the surface. Individual mitochrondria were not smaller in the basal zone nor did they accumulate around the lipid droplets as described by de Kervily ('16). However, accumulations of mitochondria occurred frequently in certain locations, the chief one being the supranuclear zone (fig. 2). These aggregations were most frequently seen in association with numerous blue granules and superficial vacuoles but either could be found in the absence of the other (fig. 3). We observed concentrations of mitochondria in the nuclear and subnuclear regions and in some instances these have seemed to be arranged about the negative image of the Golgi apparatus (fig. 2).

The lipid droplets, which have been described by many workers, appeared chiefly in the supranuclear and nuclear zones (figs. 5 and 14) but were found at other depths. They were readily differentiated from the Golgi apparatus in osmicated preparations by their form and brown color. The lipid droplets were present following Champy fixation without post-osmication in all cases including one of hydatidiform mole (no. 34). We observed the clear central space and ec-

centric shape of the droplets described by de Kervily ('16) in only one specimen but in this case the adequacy of fixation was open to question.

The syncytial buds did not present noticeable variations in cytological structure as compared with the adjacent syncytial layer (fig. 12). A Golgi apparatus (fig. 17), blue granules and superficial vacuoles were demonstrable in them. The numerous mitochondria sometimes appeared to be less discrete. The brush border on at least the sides of the buds possessed a regular form but the terminal ends usually contained numerous superficial vacuoles and, over them, the brush border was frequently disrupted.

Origin of syncytium. We frequently observed cells adjacent to the basement membrane which seemed to be in the process of transformation from cytotrophoblast to syncytium. The cytoplasm in parts or all of such cells had become dark and indistinguishable from that of the syncytium (figs. 4 and 11). The nuclear membrane was wrinkled and, with the chromatin material, stained intensely, similar to that of the syncytial nuclei. Most significantly, the cell membrane was still clearly distinguishable throughout all or part of its extent (fig. 11). The mitochondria seemed to be rather variable in these cells, some being larger and intensely stained; others, faintly stained and clumped.

Connective tissue. The connective tissue in the branches of the villous tree consisted of loosely arranged connective tissue cells associated with delicate fibers which stained with aniline blue and blackened with silver. Some of these fibers blended into the basement membrane of the trophoblast. Numerous Hofbauer cells were scattered throughout the connective tissue. They possessed small, eccentrically-placed nuclei, vacuoles of varied sizes, granules which stained with aniline blue, granular or rod-shaped mitochondria and a small Golgi apparatus located close to the nucleus. Some of these characteristics have been described by de Kervily ('16). It seems that the Hofbauer cells should be classed as macrophages since they have been shown to take up supravital dyes

(Lewis, '24) and to increase in number in syphilitic infections (Hofbauer, '25). The connective tissue was denser and Hofbauer cells reduced in number in the trunks of the villous tree, as well as throughout the villus in late pregnancy. At term the basement membrane of the trophoblast was considerably thickened except over the blood-filled, subepithelial blood vessels.

The decidua parietalis (7–17 weeks)

In the following description attention will be directed chiefly to the cellular elements of the stroma [1]. The glands will be omitted since their epithelium has been reported to show only minor changes during early pregnancy (Bartelmez and Bensley, '32).

Large decidual cell. The large decidual cell was the largest of the cellular elements of the decidua, being found at all levels but reaching its greatest size in the zona compacta. When the large decidual cells were densely packed they tended to be spherical in shape but in areas of loosely arranged connective tissue, such as occurred frequently beneath the surface epithelium, pseudopod-like processes of variable length extended out from the cell body. The nucleus was large and contained pale nucleoplasm, a fine chromatin network and one or more irregularly shaped acidophilic nucleoli (fig. 28). Some cells possessed multiple nuclei. The cytoplasm appeared finely granular following Zenker-formol fixation and delicately reticular or granular after fixation in Bouin's or Champy's fluids. Ulesko-Stroganoff ('08) found the nucleus to lie in an uncolored field surrounded by darker cytoplasm in deciduae fixed in Flemming's fluid and stained with safranin. In our material, if any portion of the cytoplasm stained more deeply, it was that area encompassed by the Golgi apparatus.

[1] General histological descriptions of the decidua parietalis may be found in the works of Marchand ('04), Ulesko-Stroganoff ('08), Stieve ('26) and Schroeder ('30).

The Golgi apparatus was a large, loose meshwork of spherical to oval shape and measured as much as 11.2 μ in its greatest diameter (fig. 24). Vesicles occurred frequently within its component parts. Generally, the nucleus fitted into an indentation at one side of the network. The negative image of this organelle was clearly defined after fixation in Zenker-formol, Bouin's or Champy's fluids. Without entering into the discussion concerning the possible lipoproteid character of the Golgi apparatus (Kirkman and Severinghaus, '38) it should be noted that we consistently observed dense cytoplasm alongside these clear channels which, in most preparations, definitely outlined the Golgi apparatus without need of osmication. Mitochondria were sparsely scattered, only occasionally seeming to be more numerous in the region of the Golgi body (fig. 27). They consisted of faintly stained, delicate filaments. Clusters of small lipid droplets and sharply-outlined vesicles were infrequently present outside of the Golgi region. Variable quantities of glycogen were demonstrated.

The large decidual cell was usually surrounded by a dense connective tissue covering which was continuous with similar structures about other cells. It stained with aniline blue and reduced silver (fig. 30). Although frequently exhibiting a homogeneous appearance, in most cases its fundamental fibrous nature was clearly discernible. Many cells appeared to contain homogeneous or fibrillar masses of connective tissue material which exhibited the staining properties of reticular fibers (fig. 26). However, they were demonstrated regularly to be continuous with the extra-cellular connective tissue and seemed to have been secondarily enfolded by swelling or possibly movement of the processes of the cell. Therefore, they cannot be designated true intra-cytoplasmic structures. Mitochondria-laden processes of some cells partially surrounded marked condensations of the peri-cellular covering apparently preparatory to more completely enfolding this material (fig. 27). Occasional fine argyrophilic

fibers also appeared to be located within the cell but this position could not be verified definitely.

Small decidual cell. This type was scattered throughout the zona compacta and spongiosa frequently seeming to be rather numerous beneath the basement membrane of glands. Bouin fixation revealed an intensely fuchsinophilic nuclear membrane enclosing prominent chromatin masses, in rare instances the picture approaching one of pycnosis (fig. 28). In some instances the nucleus was definitely lobated while in others, it was drawn out into a dumb-bell shape which has been taken as an indication of rapid amitosis (Ulesko-Stroganoff, '08). In our material occasional cells contained mitotic figures. Glycogen filled most of the cytoplasmic space (fig. 29) so that after removal of this substance by the usual fixatives the cell appeared empty except for a dense mass of granulated cytoplasm usually found in an indentation at one side of the nucleus and from which fine strands extended toward the periphery of the cell. The granules reached a rather large size, stained with either acid fuchsin or aniline blue in Champy preparations and were still present after fixation in Bouin's fluid (fig. 28). Those which stained with acid fuchsin after Champy fixation frequently possessed a deep red rim and a pale center. The mitochondria were concentrated in this region and varied in shape from short curved rods to granules. The Golgi apparatus was not always demonstrable tending to be a small net (fig. 23) or fragmented. Post-osmication brought out black droplets in many of these cells, some of which possessed a clear center. It was thought that these were the fuchsinophilic granules previously described. Bleaching of these osmium deposits proved to be extremely difficult.

Histiocytes, lymphocytes, neutrophiles and eosinophiles were also present in the decidua. The histiocytes contained blue granules, small vacuoles, rod-shaped mitochondria and little glycogen. These cells were spherical in loose connective tissue but also assumed shapes which suggested ameboid motion. Their nuclear structure was somewhat similar to that

of the small decidual cell and lymphocyte. Although a specific stain was not used in this study we have never observed cells with the cytoplasmic characteristics of plasma cells. Plasma cells have been described in the decidua (Wederhake, '06) but Ulesko-Stroganoff ('08) employed Unna's stain and found few of them.

DISCUSSION

Trophoblast. In attempting to interpret the cytological variations in the trophoblast one is soon impressed by the probability that other factors than the state of secretory activity influence the appearance of this epithelium. Regions of thick and thin trophoblast were commonly continuous with each other on the same villus. Fluctuations in intra-villous pressure might account for this thinning of the epithelium but such areas were frequently continuous with a thick syncytium covering polygonal cytotrophblast cells. These variations may be more satisfactorily explained on the basis of ameboid movement by the trophoblast, a deduction previously presented by Hofbauer ('03) and which is further supported by the occurrence of syncytial buds on the villi and syncytial giant cells in the myometrium. Failure of the cellular layer to pile up under these buds may indicate that syncytial movement, if present, occurs over the outer surface of the cytotrophoblast and is, to some extent, independent of that layer. Actual phagocytosis of maternal erythrocytes by the trophoblast has been reported in the human placenta by Boerma ('13), Brewer ('37) and de Kervily ('16), the latter describing a preliminary active engulfment of the erythrocytes by the elements of the brush border. In our material red blood corpuscles seemed to have been held against the brush border in life by some viscous material (fig. 14). In agreement with v. Cauwenberghe ('07) we did not observe erythrocytes in a position which would suggest that they were passing through the brush border. They did exhibit indications of histolysis and varied staining reactions when in the intervillous space which might indicate that they were in some way

affected by a substance released from the trophoblast. It seems possible that the trophoblast may phagocytize erythrocytes more actively during the period of active invasion of the endometrium than during the later stages which we have studied.

Some disagreement exists concerning the cytoplasmic constituents of the cytotrophoblast. Small grains of fat have been described by de Kervily ('16) and Hofbauer ('05) after impregnation with osmium but according to Bondi ('11) neutral fat is not present in the cytotrophoblast. In our material all intra-cellular structures which were blackened by osmic acid could be classified as parts of the Golgi apparatus or mitochondria except in one case (case no. 14, fig. 8), in which brown bodies, possibly a fatty pigment, were present in some of these cells after fixation in Champy's fluid. Wienbeck ('36) was not able to produce a blackening of granules in the cytotrophoblast of full-term placentae by means of the Da Fano technique. The finding of small quantities of glycogen in the cytotrophoblast agrees with the observations of Todyo ('12). Driessen ('07) observed insignificant amounts.

The function of the cell columns and cell islands is not entirely clear. Obviously, the former play a part in fixation of the villi to the decidua basalis. Both cell columns and cell islands are characteristic of early pregnancy, the former having been reported to disappear by the end of the second month (Schroeder, '30) or the fifth month (Terasaki, '27). In our material they were observed as late as 3½ months. The changes described in the mitochondria and Golgi apparatus of the cell columns were suggestive of loss of vitality. The large amount of glycogen in these cells might be given a similar interpretation since metabolic depression has been used to explain the accumulation of this material in the renal cells following obstruction of the renal vessels (Gierke, '05) and in the vaginal epithelium of the guinea pig (Tribby, '43). A few cells in the cell islands showed some indications of secretory activity, but for the most part, they were degenerating to form fibrinoid. In fact, these islands have been

thought to be an important source of the fibrinoid which occurs commonly in the human placenta (Keibel and Mall, '10; Terasaki, '27).

Many origins have been postulated for the syncytium (Schroeder, '30) but most workers are now agreed that it arises from the cytotrophoblast, although little cytological evidence for such a change has been presented. Our observations support the view that the syncytium arises from the cytotrophoblast. We doubt that the dense cytoplasm of the "cytocentrum" of cytotrophoblast cells necessarily represents a stage in this transformation as has been described by Florian ('28); in our material the cytoplasm was typically most dense in the Golgi region. V. Lenhossék ('02), Rossi Doria ('05) and v. Cauwenberghe ('07) have reported amitotic division of the syncytial nuclei and this is suggesetd by the frequent elongation of the nuclei and their accumulation in the syncytial buds. However, these formations are equally well explained on the basis of ameboid movement by the syncytium. Until more concrete evidence is supplied, the presence of amitosis in the syncytium must be viewed with skepticism. Neuweiler ('27) and Lewis ('24) have failed to observe proliferation of the syncytium in tissue culture.

The irregular appearance of the brush border raises the possibility that it might be a fixation artefact. Numerous workers have been impressed by the apparent lability of this structure. There can be no question that prompt fixation is prerequisite to satisfactory demonstration of it. Our findings indicated that in some locations, rupture of the superficial vacuoles accounted for the lack of a brush border, while in other places its absence could not be explained on this basis. Nevertheless, it is difficult to understand how the act of fixation could induce the appearance of projections of such height and regularity of form as are shown in figure 6. In fact, v. Lenhossék ('02) reported their presence on fresh, non-fixed villi and called them "stereocilia."

We interpret the blue granules within the syncytium to be a formed product of cytoplasmic activity and a probable

source of secretion. Judging from the illustrations of Brewer ('37) there is no certain indication of identity between these granules and those which he described in the syncytium as phagocytized cells and which he homologized with those structures previously referred to by other workers as secretory products. V. Cauwenberghe ('07) observed an areola around some granules during late pregnancy and believed that the "basophilic granules" liquefy prior to secretion. He took the position that the syncytium is a gland and later received the support of de Kervily ('16) who further suggested that this secretion might be of a hormonal nature. The superficial vacuoles have been observed by these workers and illustrated but not described by Florian ('28) and Runge and Hartman ('29), yet their relationship to the blue granules has never been clearly set forth. Our observations strongly suggest that the superficial vacuoles are formed by liquefaction of the blue granules. Bonnet ('03) hinted at this finding but was probably considering larger, deep syncytial vacuoles in the following statement: "Ausserdem finde ich in den grossen Vacuolen der Proliferationsknoten oft einem mehr oder minder färbbaren wolkigen Inhalt, Gerinnsel einer noch unbekannten Flüssigkeit oder homogene, glänzende, rundliche Körper von ebenfalls unbekannter Bedeutung."

It is probable that the contents of the vacuoles reach the maternal blood most frequently by diffusion. Some evidence of rupture was observed but since the act of fixation could conceivably cause an engorged vacuole to burst, a final conclusion in regard to the mode of emptying cannot be drawn from fixed material. The vesicular spaces within the brush border, a formation previously observed by v. Cauwenberghe ('07) and Hedenberg and Strindberg ('16) and regarded by the former as evidence for the rapid interchange of substances, may have resulted from a sudden emptying of the vacuoles.

Vacuoles have been described in the syncytium of the monkey trophoblast by Wislocki and Streeter ('38) and related by them to pinocytosis. It seems to be more likely that

the superficial vacuoles of the syncytium in man are concerned with a secretory rather than an absorptive process. The cytological evidence for this deduction is supported by the failure of Lewis ('24) to find a concentration of neutral red in the superficial zone after supra-vital staining with this dye. Also, these vacuoles are associated with the thick syncytium of early pregnancy and are markedly reduced in the thin syncytium of late pregnancy. They are, then, least numerous at a time when the most rapid transfer of materials would occur as indicated by the finding of Flexner and Gellhorn ('42) that the rate of transfer of radioactive sodium from mother to fetus parallels the rate of fetal growth and is indirectly related to the thickness of the trophoblast. We wish to emphasize that other clear vacuoles of varied shapes and sizes occurred less frequently during early pregnancy at all levels of the syncytium and that their presence could be due to pinocytosis. This may have been the origin of the large syncytial vacuoles of young embryos (13½ days to 4 weeks) reported by Friolet ('05) and Stieve ('26) to be as large as 50 μ in diameter. Some of comparable size were observed occasionally in our specimens, especially in syncytical buds (fig. 25). Indeed, the general syncytial cytoplasm has been described by some investigators to be vacuolated. There are other factors, however, which may explain this appearance. Fixation in Bouin's fluid, for example, emphasizes the vacuolated appearance of the nuclear and supranuclear zones by removing fat. Also, delayed fixation may account for the fine vacuoles reported by Herzog ('09). Since we, like Driessen ('07), have been unable to detect significant amounts of glycogen in the syncytium, vacuoles could not result from its removal.

De Kervily ('16) pointed out, on the basis of the disposition of mitochondria and granules, that the syncytium is polarized toward the surface and added that the cytotrophoblast does not share in this orientation. Our observations on the distribution of superficial vacuoles and their relationship to the granules of the syncytium confirm his contention in regard to

this layer. Although the position of the Golgi body cannot be taken as an indubitable indication of the direction of secretion (Kirkman and Severinghaus, '38), the rather regular location of this structure on the syncytial side of the cytotrophoblast nucleus in association with mitochondria and occasional differentially-stained granules, might indicate that the cytotrophoblast also is physiologically as well as anatomically polarized toward the maternal blood stream. The presence of differentially-stained granules with similar tinctorial properties in both the cytotrophoblast and syncytium, viewed in the light of the general cytological arrangement of both layers might also suggest that the syncytium merely continues the elaboration of a substance the formation of which is already begun in the cytotrophoblast. These cytological features are believed to be strong evidence that the trophoblast of early pregnancy performs a significant secretory function.

The decidua parietalis. It is well known that the connective tissue of the non-gravid uterine endometrium contains abundant argyrophilic fibers which are associated with cells of an embryonic type. In the opinion of Hitschman and Adler ('08) the decidual picture is to be regarded as an intensification of structural changes begun 6 to 7 days prior to the first missed menstrual period. The association of the large decidual cells with reticular fibers is, then, suggestive evidence of their origin from the embryonic type of connective tissue cell.

Costero ('31) observed the dense covering of the large decidual cells following use of the Rio-Hortega technique and designated it a "membranoid peri-cellular condensation." As this author has indicated, it appears to have been formed chiefly by compression of the inter-cellular stroma due to enlargement of the decidual cells. However, the presence of accumulated, brightly-stained mitochondria in cytoplasmic processes which are adjacent to areas of dense connective tissue (fig. 27) might indicate that the large decidual cells continue to play an active rôle in the deposition

of the matrix. The enfolding of homogeneous plaques of this material by these cells has lead to some confusion. They are probably identical to the "intra-cellular erythrocytes" and the cytoplasmic "discs" which Ulesko-Stroganoff ('08) described in decidual cells. These bodies with their peripherally projecting fibers also correspond to the star-shaped argyrophilic network which Terasaki ('27) observed extending out from the center of decidual cells. However, the latter author recognized the possibility that they were located externally to the cell membrane.

In view of their size, the amount of glycogen contained by the large decidual cells was not great and not demonstrable in all cells. Driessen ('07), who used similar techniques, found the cells of the decidua parietalis positive in only six of eleven cases. It may be pertinent that most samples of deciduae were obtained from patients under general anesthesia.

The small decidual cell of our classification seems to correspond roughly with that described by Marchand ('04) and Ulesko-Stroganoff ('08) although the latter author further subdivided the small cells with hyperchromatic nuclei and granules. Our observations indicated that all of the small cells with dark nuclei, granules and large amounts of glycogen fall within the same category although the quantity of these cytoplasmic constituents varied considerably. The origin of these cells seems obscure. Both Marchand and Ulesko-Stroganoff observed transitional forms between small and large decidual cells. There were indications in our material that they may have a common origin, namely, the embryonic type of connective tissue cell. The characteristics of the small decidual cell could be construed as being indicative of degeneration.

Endocrine significance. Browne and Venning ('36), Evans, Kohl and Wonder ('37), and Smith and Smith ('37) have performed careful assays of human urine of pregnancy in order to determine the quantities of gonadotropin excreted at various times during the gestation period. From their data it is clear that the curve of excretion reaches a high peak at

about the sixtieth day counting from the last menstrual period and then descends by about the eighty-fifth day to a low level which is maintained until parturition. The data of Hamburger ('33) and of Smith and Smith ('37) showed that, in some cases, a high level of excretion might occur at, or be continued into, the third month. This period of increased excretion corresponds rather well with the secretory phase of the trophoblast as shown by our specimens up to about the fourteenth week of pregnancy. It was not possible to observe a marked cytological difference between the villi at 7 to 9 weeks and those at about 14 weeks, although we did gain the impression that the cytophoblast cells from earlier cases contained more stainable cytoplasm and granules. At 16 weeks (no. 5) although the syncytial granules and mitochondria were still quite prominent, the latter were evenly distributed throughout the syncytium and rarely could a clear superficial vacuole be observed. The 20-week specimen showed a further reduction in secretory activity as evidenced by a thinner syncytium, low and irregular brush border, rarely a superficial vacuole, fewer and smaller mitochondria (fig. 15). This regression was still more marked at term when few granules and no vacuoles of a definitely secretory nature could be observed (fig. 10). Also, from about the fourteenth week on, the cytotrophoblast cells became continually more widely separated from each other.

Direct evidence for the production of gonatotropin by the placenta has been supplied by the implantation experiments of Phillipp and Huber ('36) which point more to the villi than to the decidua as its source. This view is supported by the induction of a positive Aschheim-Zondek test by injection of the medium from placental cultures (Gey et al., '38; Seegar Jones et al., '43). These findings show that the cytotrophoblast may be the orginal source of the hormone since the cytotrophoblast but not the syncytium proliferates in tissue culture (Lewis, '24; Neuweiler, '27; Gey et al., '38). We have offered suggestive cytological evidence of secretion in these cells and possibly also in those of the related cell

islands which are most prominent during early pregnancy. If this is true one should expect that the cytotrophoblast would be present throughout gestation since gonadotropin is usually excreted in significant quantities until parturition. Cytotrophoblast cells were found at term and possessed a demonstrable Golgi apparatus (fig. 9) and mitochondria (fig. 10). On an average they were possibly larger than during early pregnancy, although fewer in number in a given length of trophoblast. The syncytium was frequently extremely thin over these cells (fig. 10).

In spite of this correlation one cannot yet conclude that the syncytial granules are a hormonal precursor. Kennedy ('33) assayed human blood of pregnancy and found the content in gonadotropin to increase steadily until the thirtieth week which, if confirmed, would show that rate of excretion is not an index of secretion. However, Kennedy's findings are contradicted by the evidence submitted by Smith and Smith ('37) who assayed both the blood serum and urine of twelve normal pregnant women for gonadotropin, and found the greatest concentration of this substance to occur contemporaneously in these fluids. These latter findings tend to strengthen the correlation which we have made between excretion of gonadotropin and trophoblast cytology. A further cause for hesitancy in assuming that the blue syncytial granules are a precursor of gonadotropin is the fact that only the tissue culture studies of Gey et al. ('38) and Seegar Jones et al. ('43) offer evidence that the trophoblast is capable of producing gonadotropin in the absence of the hypophysis. Indeed, the cytological picture of the hypophysis of pregnancy is one of intense activity (Severinghaus, '39) which suggests that this gland might participate, at least, in the production of gonadotropin. Two of the theories which have been postulated to explain the manner in which the urinary gonadotropin may stimulate the ovaries during pregnancy, involve the participation of the anterior hypophysis (Evans, Meyer and Simpson, '32; Hamburger, '33). It is also possible that the syncytical granules represent material in transit

from the fetal to the maternal circulation or an histiolytic enzyme, although in the opinion of Wislocki and Streeter ('38) the importance of the secretion of such a substance by the trophoblast has been over-emphasized.

An extra-ovarian source for estrogen and progestin during pregnancy seems to be established (Newton, '39) and the placenta has been thought to be this source. Bennett and Wislocki ('42) have made the significant discovery that the syncytium but not the cytotrophoblast renders a positive reaction to treatment with phenylhydrazine hydrochloride suggesting that the syncytium produces steroid substances. In view of the reported failure of the syncytium to proliferate, it is, then, of interest that Seegar Jones et al., ('43) failed to detect estrogens or progestin in assays of the media of placental cultures. The excretion of total estrogens (Cohen et al., '35; Marrian et al., '35; Smith et al., '37) and of pregnanediol glucuronide (Browne et al., '37; Smith et al., '41) reaches a peak within the 2 months prior to parturition. If one relates the syncytial lipid droplets to this period of rapid excretion, it is important that they have been found to be least abundant during the latter part of pregnancy by Bondi ('11), Bennett and Wislocki ('42) and by us.

It appears to be doubtful that the decidua parietalis performs an endocrine function. The vascular pattern is one which does not suggest this activity since an intimate relationship between the capillaries and the large decidual cells is non-existant. Thus, any substance produced by these cells would have to diffuse some distance through connective tissue before reaching the blood stream. The large decidual cells were without differentially stained granules and the mitochondria, which are commonly related to the liberation of secretion, were usually not prominent in staining capacity, number, or size. Ulesko-Stroganoff ('08) reported cells similar in structure to our small decidual cells to be more common around the blood vessels but the significance of their granules is at present not clear. The non-endocrine nature of the decidua parietalis is further indicated by the failure of artifi-

cially induced deciduomata to prolong pseudo-pregnancy in the rat (Long and Evans, '22) or to disturb the estrous cycle of guinea pigs (Dempsey, '37).

SUMMARY

Pieces of the chorionic villus and decidua parietalis were obtained from various stages of pregnancy (forty-three cases) and studied by a variety of cytological techniques. Evidence was presented which indicated that the trophoblast of early pregnancy performs a significant secretory function, this period of activity being roughly contemporaneous with the time of greatest excretion of gonadotropin. Both cytotrophoblast and syncytium were polarized toward the surface of the villus. In some cases differentially-stained granules were present in the region of the Golgi apparatus of the cytotrophoblast cells. Similar minute granules occurred in the subnuclear and nuclear zones of the syncytium in association with the Golgi apparatus. These enlarged toward the surface of the syncytium assuming a spherical shape. The granules then appeared to liquefy forming vacuoles which liberated their contents through the brush border. Mitochondria were prominent about the Golgi apparatus of the cytotrophoblast cells and throughout the syncytium, frequently accumulating in the superficial zone of the latter layer. In late pregnancy, the cytotrophoblast cells were more sparsely scattered; the syncytium became thinner and possessed a low irregular brush border, fewer mitochondria and lipid droplets, and practically no blue granules or superficial vacuoles. These changes were interpreted to be indicative of reduced secretory activity. No convincing evidence of secretion was found in the small and large decidual cells of the decidua parietalis.

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LITERATURE CITED

BARTELMEZ, G. W., AND C. M. BENSLEY 1932 Human uterine gland cells. Special Cytology, vol. 3, edited by E. V. Cowdry, Paul Hoeber Inc., New York.

BENNETT, H. S., AND G. B. WISLOCKI 1942 The nature of the lipoid droplets in the syncytial trophoblast. Anat. Rec., vol. 82, suppl., p. 65.

BOERMA, N. J. 1913 Beitrag zur Kenntnis der Einbettung des menschlichen Eies. Monats. f. Geb. u. Gynäk., vol. 37, p. 723.

BONDI, J. 1911 Ueber das Fett in der Placenta. Arch. f. Gynäk., vol. 93, p. 189.

BONNET, R. 1903 Ueber Syncytien, Plasmodien und Symplasma in der Placenta der Säugetiere und des Menschen. Monats. f. Geb. u. Gynäk., vol. 18, p. 1.

BOWEN, R. H. 1929 The cytology of glandular secretion. Quart. Rev. Biol., vol. 4, p. 484.

BREWER, J. I. 1937 A normal human ovum in a stage preceding the primitive streak. (The Edwards-Jones-Brewer ovum). Am. J. Anat, vol. 61, p. 429.

BROWNE, J. S. L., AND E. M. VENNING 1936 Excretion of gonadotropic substances in the urine during pregnancy. Lancet, vol. 231, p. 1507.

BROWNE, J. S. L., J. S. HENRY AND E. M. VENNING 1937 The corpus luteum hormone in pregnancy. J. Clin. Invest., vol. 16, p. 678.

COHEN, S. L., G. F. MARRIAN AND M. WATSON 1935 Excretion of oestrin during pregnancy. Lancet, vol. 1, p. 674.

COSTERO, I. 1931 Histologische Untersuchungen über die feine Struktur der Placenta und des schwangeren Uterus. I. Mitteilung: Über die Uterus-placentäre Gitterfaserstruktur. Zeits. Anat. u. Entwick., vol. 96, p. 766.

DE KERVILY, M. 1916 La villosité du placenta. Arch. mens. d'Obstet. et Gyn., vol. 8, pp. 93, 296.

DRIESSEN, L. F. 1907 Ueber Glykogen in der Placenta. Arch. f. Gynäk., vol. 82, p. 278.

DEMPSEY, E. W. 1937 The relation of deciduomata to the reproductive cycle in the guinea pig. Anat. Rec., vol. 70. suppl. 1, p. 119.

EVANS, H. M., K. MEYER AND M. E. SIMPSON 1932 Relation of Prolan to the anterior hypophyseal hormones. Am. J. Physiol., vol. 100, p. 141.

EVANS, H. M., C. L. KOHLS AND D. H. WONDER 1937 Gonadotropic hormone in the blood and urine of early pregnancy; the normal occurrence of transient extremely high levels. J. Am. Med. Assn., vol. 108, p. 287.

FLEXNER, L. B., AND A. GELLHORN 1942 A comparative study of placenta permeability using radioactive sodium. Anat. Rec., vol. 82, suppl., p. 19.

FLORIAN, J. 1928 Ueber das Syncytium im Trophoblast junger menschlichen Embryonen. Verhandl. d. anat. Gesellsch., vol. 66, suppl., p. 211.

FRIOLET, H. 1905 Beitrag zum Studium der menschlichen Placentation. Beitr. z. Geb. u. Gynäk., vol. 9, p. 1.

GEY, G. O., G. E. SEEGAR AND L. M. HELLMANN 1938 Production of gonadotropic substance (prolan) by placental cells in tissue culture. Science, vol. 88, p. 306.

GIERKE, E. 1905 Das Glykogen in der Morphologie des Zellstoffwechsels. Beitr. z. path. Anat. u. z. allgem. Path., vol. 37, p. 502.

HAMBURGER, C. 1933 Studies on gonadotropic hormones from the hypophysis and chorionic tissue with special reference to their differences. Acta Path. et Microb. Scand., suppl. 17.

HEDENBERG, M., AND L. STRINDBERG 1916 Beitrag zur Kenntnis der Anatomie und der Funktion der menschlichen Plazenta. Anat. Anz., vol. 49, p. 41.

HERZOG, M. 1909 A contribution to our knowledge of the earliest known stages of placentation and embryonic development in man. Am. J. Anat., vol. 9, p. 361.

HITSCHMANN, F., AND L. ADLER 1908 Der Bau der Uterusschleimhaut des geschlechtsreifen Weibes mit besonderer Berücksichtigung der Menstruation. Monats. f. Geb. u. Gynäk., vol. 27, p .1.

HOFBAUER, J. 1903 Die Fettresorption der Chorionzotte. Ein Beitrag zur normalen Anatomie und Physiologie der menschlichen Placenta. Sitzungsber. d. kaiserl. Akad. d. Wissensch. vol. 112, part 3, p. 204.

———— 1905 Grundzüge einer Biologie der menschlichen Plazenta. Leipzig.

———— 1925 The function of the Hofbauer cells of the chorionic villus particularly in relation to acute infection and syphilis. Am. J. Obst. and Gyn., vol 10, p. 1.

KEIBEL, F., AND F. P. MALL 1910 Human Embryology, vol. 1, J. B. Lippincott Co., Philadelphia and London.

KENNEDY, W. 1933 Quantitative variation of the anterior pituitary hormone, "APH-B", in the blood during pregnancy. Quart. J. Exp. Physiol., vol. 23, p. 367.

KIRKMAN, H., AND A. E. SEVERINGHAUS 1938 A review of the Golgi apparatus. Parts I and II. Anat. Rec., vol. 70, pp. 413, 557.

LANGHANS, T. 1901 Syncytium und Zellschicht Placentarreste nach Aborten. Chorionepitheliome. Hydatidenmole. Beitr. z. Geb. u. Gynäk., vol. 5, p. 1.

LEWIS, W. H. 1924 Hofbauer cells (clasmatocytes) of the human chorionic villus. Bull. Johns Hopkins Hosp., vol. 35, p. 183.

LONG, J. A., AND H. M. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif., vol. 6, p. 1.

MALL, F. P. 1918 On the age of human embryos. Am. J. Anat., vol. 23, p. 397.

MARCHAND, F. 1904 Beitrag zur Kenntniss der normalen und pathologischen Histologie der Decidua. Arch. f. Gynäk., vol. 72, p. 155.

MARRIAN, G. F., S. L. COHEN AND M. WATSON 1935 Observations on the excretion of estrin during pregnancy. J. Biol. Chem., vol. 109, p. lix.

NASSONOV, D. 1923 Das Golgische Binnennetz und seine Beziehungen zu der Sekretion. Untersuchungen über einige Amphibiendrüsen. Arch. f. mikr. Anat., vol. 97, p. 136.

NEUWEILER, W. 1927 Ueber Explantationsversuche menschlicher Plazenta. Monats. f. Geb. u. Gyn., vol. 77. p. 437.

NEWTON, W. H. 1939 Some problems of endocrine function in pregnancy. Sex and Internal Secretions, second edition, edited by E. Allen, C. H. Danforth and E. A. Doisy, Williams and Wilkins Co., Baltimore.

PHILLIP, E., AND H. HUBER 1936 Die Hormonale Rolle der Decidua. Zentr. f. Gynäk., vol. 60, p. 2706.

ROSSI DORIA, T. 1905 Ueber die Einbettung des menschlichen Eies, studirt an einem kleinen Eie der zweiten Woche. Arch. f. Gynäk., vol. 76, p. 433.

RUNGE, H., AND H. HARTMANN 1929 Beitrag zur Histologie der menschlichen Placenta. Arch. f. Gynäk., vol. 139, p. 51.

SCHROEDER, R. 1930 Weibliche Genitalorgane. v. Mollendorf's Handb. der mikr. Anat. des Menschen, vol. 7, part 1, Julius Springer, Berlin.

SEEGAR JONES, G. E., G. O. GEY AND M. K. GEY 1943 Hormone production by placental cells maintained in continuous culture. Bull. Johns Hopkins Hosp., vol. 72, p. 26.

SEVERINGHAUS, A. E. 1939 Anterior hypophyseal cytology in relation to the reproductive hormones. Sex and Internal Secretions, second edition, edited by E. Allen, C. H. Danforth and E. A. Doisy, Williams and Wilkins Co., Baltimore.

SEVERINGHAUS, A. E., AND K. W. THOMPSON 1939 Cytological changes induced in the hypophysis by the prolonged administration of pituitary extract. Am. J. Path., vol. 15, p. 391.

SMITH, G. V., O. W. SMITH AND G. W. PINCUS 1937 Total urinary estrogen, estrone and estriol during a menstrual cycle and a pregnancy. Am. J. Physiol., vol. 121, p. 98.

SMITH, O. W., AND G. V. S. SMITH 1937 Prolan and estrin in the serum and urine of diabetic and non-diabetic women during pregnancy, with especial reference to late pregnancy toxemia. Am. J. Obst. and Gyn., vol 33, p. 365.

SMITH, O. W., G. V. S. SMITH AND S. SCHILLER 1941 Estrogen and progesterone metabolism in pregnancy. I. Spontaneous and induced labor. J. Clin. Endocr., vol. 1, p. 461.

STIEVE, H. 1926 Ein 13½ Tage altes, in der Gebärmutter erhaltenes und durch Eingriff gewonnenes menschliches Ei. Zeits. f. mikr.-anat. Forsch., vol. 7, p. 294.

TERASAKI, O. 1927 Über die Gitterfaserstrukturen in der menschlichen Plazenta. Zeits, f. Geb. u. Gynäk., vol. 92, p. 94.

TODYO, R. 1912 Ein junges menschliches Ei. Arch. f. Gynäk., vol. 95, p. 425.

TRIBBY, C. L. 1943 The intracellular lipin, mucoid, and glycogen of the vaginal epithelium of the guinea pig. Anat. Rec., vol. 86, p. 425.

ULESKO-STROGANOFF, K. 1908 Zur Frage von dem feinsten Bau des Deciduagewebes, seiner Histogenese, Bedeutung und dem Orte seiner Entwickelung im Genitalapparat der Frau. Arch. f. Gynäk., vol. 86, p. 542.

VAN CAUWENBERGHE, A. 1907 Recherches sur le rôle du syncytium dans la nutrition embryonnaire chez la femme. Arch. de biol., vol. 23, p. 13.

VON LENHOSSÉK, M. 1902 Ueber das Chorionepithel. Rev. in Zentrbl. f. Gynäk., 1904, vol. 28, p. 229.

WEDERHAKE, K. J. 1906 Über Plasma- und Deciduazellen. Monats. f. Geb. u. Gynäk., vol. 24, p. 316.

WIENBECK, J. 1936 Beitrag zur Histologie und Physiologie der Placentarzotte. Zeits f. mikr.-anat. Forsch., vol. 39, p. 135.

WISLOCKI, G. B., AND G. L. STREETER 1938 On the placentation of the macaque (Macaca mulatta), from the time of implantation until the formation of the definitive placenta. Carnegie Contr. to Embry., vol. 27, p. 3.

PLATE 1

EXPLANATION OF FIGURES

Unless otherwise stated, the figures are from 3 μ sections of Champy-fixed material stained by the Severinghaus Altmann-Masson technique; Golgi preparations were osmicated by the Severinghaus (Nassonov-Kolatschev) method before staining.

1 Drawing of a cytotrophoblast cell showing two granules in the Golgi region with associated mitochondria (black). A large vacuole is present at the right of the syncytial nucleus. Case 6. About $\times$ 4300.

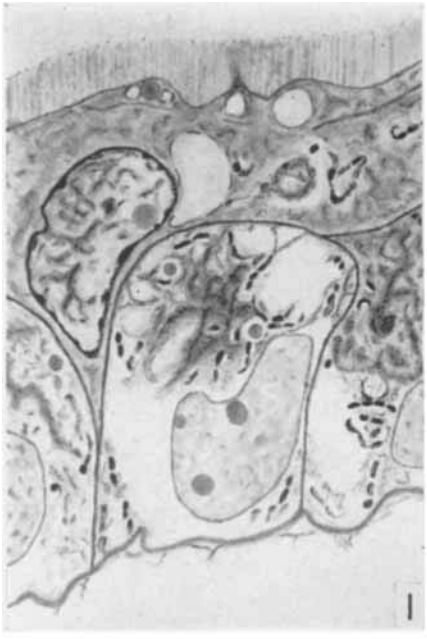
2 Drawing of the syncytium showing minute granules in the subnuclear zone in association with clear curved channels, the negative image of the Golgi apparatus. The granules increase in size towards the surface of the syncytium and show varied degrees of affinity for aniline blue. The majority are homogeneous; one large granule at upper right shows definite structure. Superficial vacuoles at upper left. Mitochondria have accumulated in the supranuclear zone and about the negative image of the Golgi apparatus. Case 12. About $\times$ 3200.

3 Accumulation of mitochondria in the superficial zone of the syncytium in the absence of vacuoles and granules. Mitochondria (black) are shown in the cytotrophoblast cell, especially in the Golgi region at the left of the nucleus. The syncytial nuclei are more chromatic than those of the cytotrophoblast. Case 12. $\times$ 1160.

4 Secretory granules in the superficial zone apparently in various stages of liquefaction, with some accumulated mitochondria. A cytotrophoblast cell (arrow) appears above the capillary probably in process of transformation to syncytium. The cytoplasm is dark, and the mitochondria at left of nucleus, large and intensely stained. Contrast cytoplasm to that of cytotrophoblast cells in figures 3 and 5. Case 12. $\times$ 1160.

5 Regular brush border and even distribution of mitochondria throughout syncytium. Some lipid droplets (black) in the syncytium. Chain of mitochondria above nucleus of middle cytotrophoblast cell. Case 12. $\times$ 1160.

6 Brush border after Bouin fixation. Case 13. $\times$ 1160.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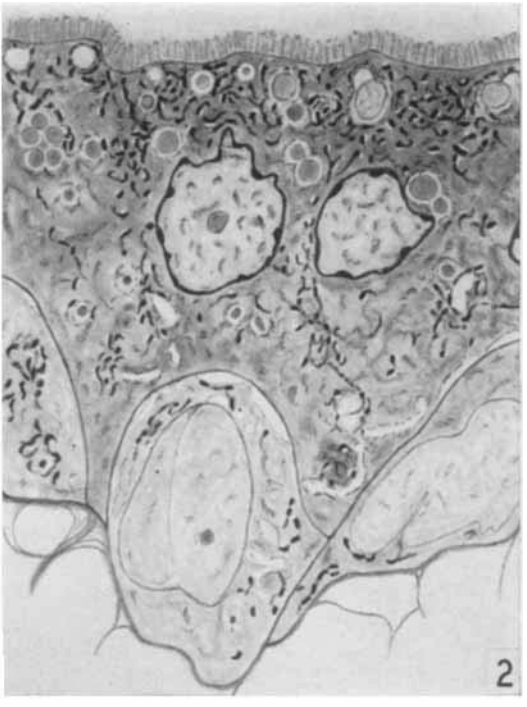
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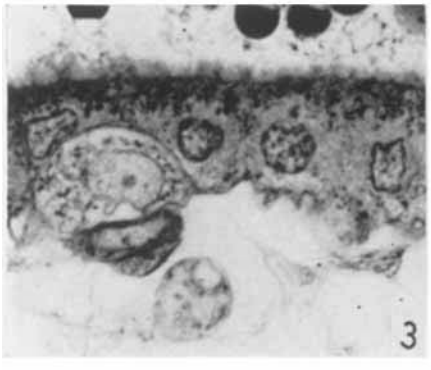
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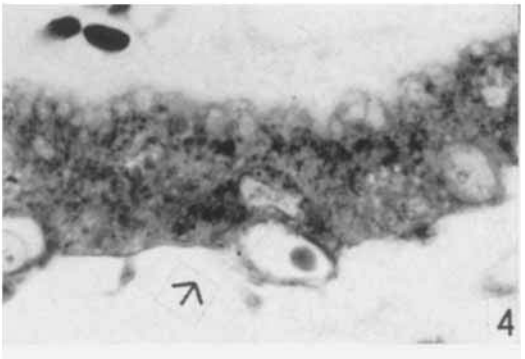
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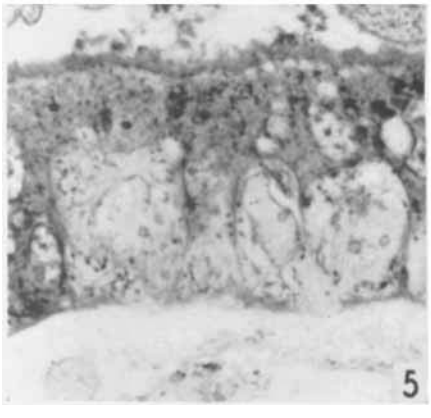
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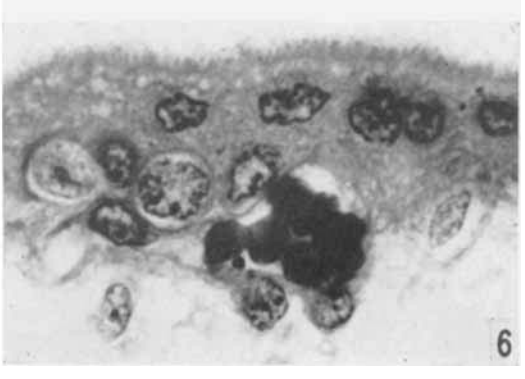
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PLATE 2

EXPLANATION OF FIGURES

7 Loose Golgi apparatus of a large cytotrophoblast cell. Case 5. × 1160.

8 Compact Golgi apparatus containing vesicles in a small cytotrophoblast cell. At left of nucleus are brown lipid bodies. Not stained. Case 14. × 1740.

9 Golgi apparatus of a full-term cytotrophoblast cell. Case 22. × 1160.

10 Full-term cytotrophoblast cell (arrow) with condensed cytoplasm in the Golgi region containing a few mitochondria. Thin syncytium covers this cell and on right side of villus above figure number is seen the low, scrubby brush border. The basement membrane is markedly thickened. Case 22. × 1160.

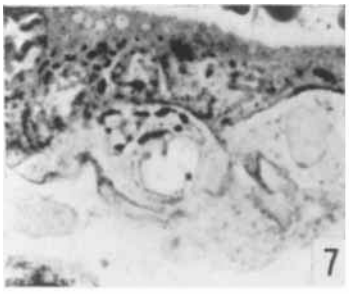
11 On the right (arrow) a cytotrophoblast cell in process of transformation to syncytium in which the cytoplasm is indistinguishable from that of the syncytium, but the cell membrane is clearly visible above the nucleus. Mitochondria are clumped and brightly stained. The syncytium shows some granules and superficial vacuoles with surrounding accumulation of mitochondria. Case 12. × 1400.

12 A syncytial bud with many mitochondria and a few small granules at the right of the most distal nucleus. Case 12. × 1160.

13 An area from the edge of a cell island showing homogeneous fibrinoid at the top and a large granule-packed cell (arrow) with hypertrophied Golgi body near the bottom. Separation of the cells is indicated by the maternal erythrocytes scattered among them. Case 13. × 1160.

14 Seemingly viscous material on the surface of the villus holding maternal erythrocytes. Many of the latter stained partly red and partly yellow, or various shades of blue. A highly active syncytium containing, in the supranuclear zone, many lipid droplets (black) and granules (gray), the latter appearing to be in various stages of liquefaction. Case 12. × 1160.

15 Thin, inactive syncytium of 5 months, containing only one or two minute granules at the left. Case 3. × 1160.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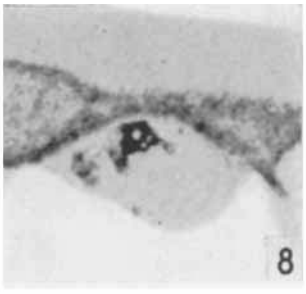
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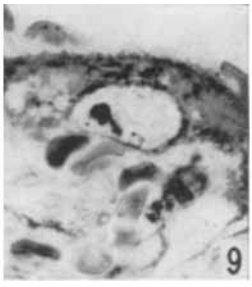
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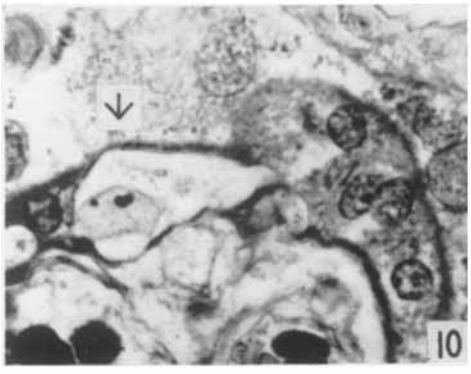
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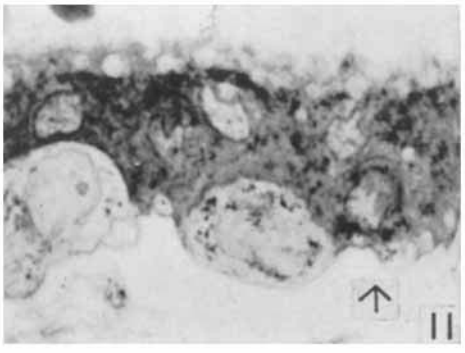
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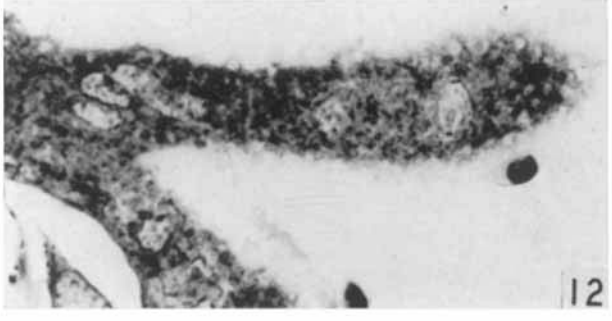
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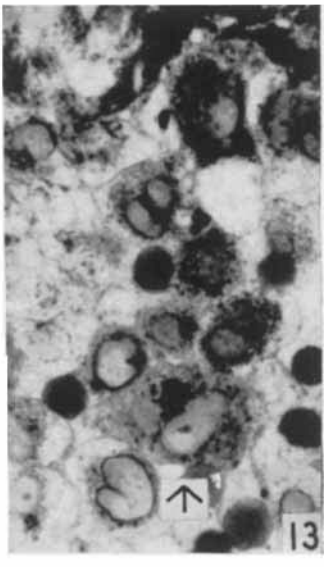
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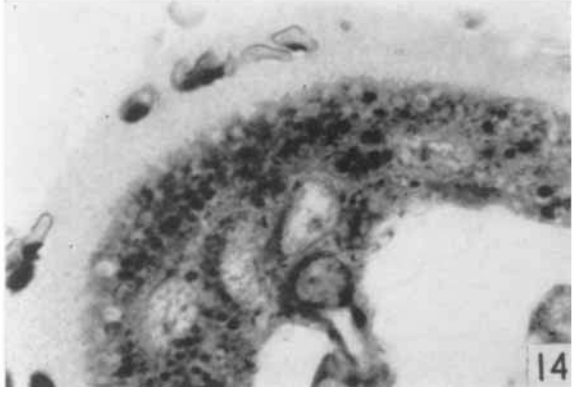
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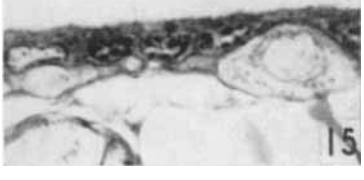
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PLATE 3

EXPLANATION OF FIGURES

16 Glycogen in a cell island and none in the syncytium at the lower right. Picric-alcohol-formol. Best's carmine. 5 μ. Case 14. $\times$ 320.

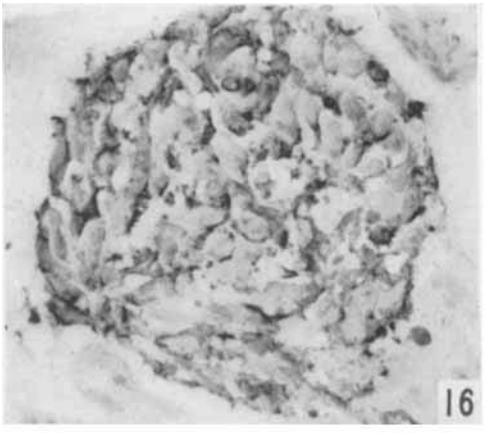
17 Golgi apparatus in a syncytial bud. Case 18. $\times$ 1160.

18 The lower villus shows a vesiculated brush border, and accumulated mitochondria in the basal zone of the syncytium. The brush border of the upper villus is more completely disorganized by the presence of superficial vacuoles. Case 12. $\times$ 1160.

19 The edge of a cell island showing several cells with mitochondrial and granular accumulations in the Golgi region. Case 13. $\times$ 1280.

20 An oblique section through the trophoblast showing the extensive Golgi apparatus of the subnuclear and nuclear zones of the syncytium. Case 5. $\times$ 1160.

21 A proliferation of the cytotrophoblast at the end of a villus. Basement membrane at lower left. Point of continuity with cytotrophoblast layer at lower right. Some reduction in number of mitochondria. Fibrinoid degeneration of the syncytium at the right. Case 12. $\times$ 880.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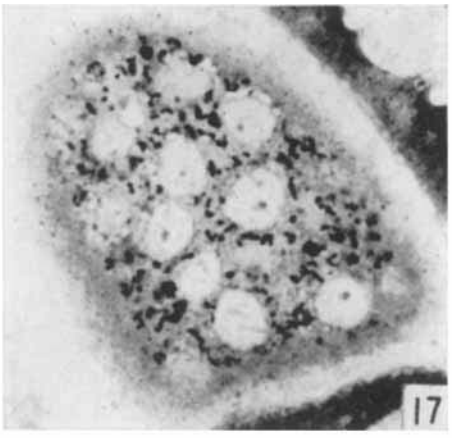
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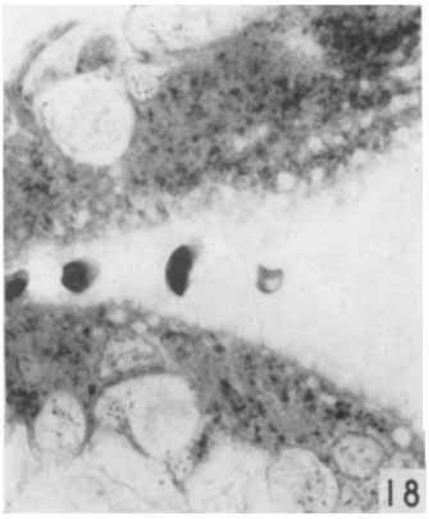
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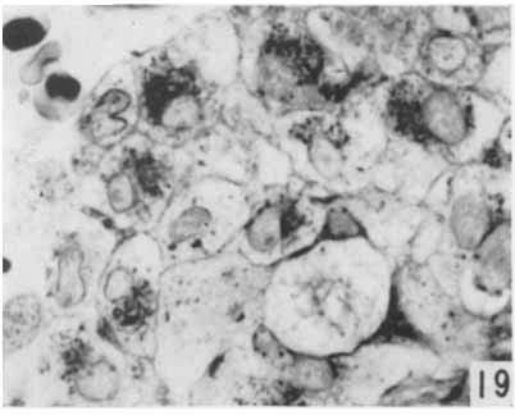
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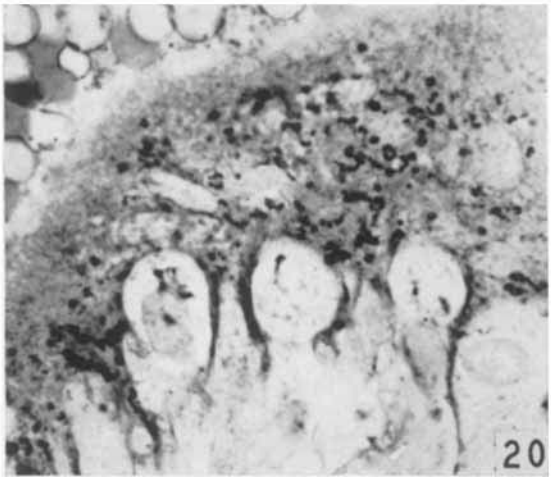
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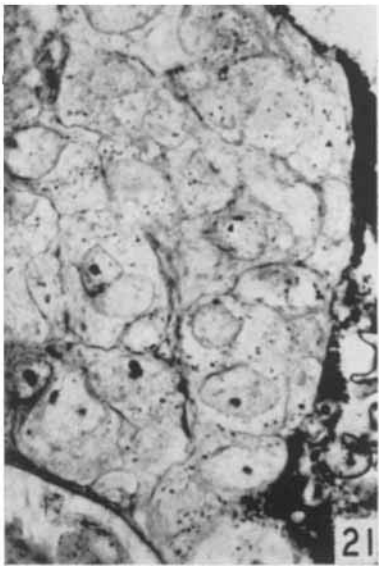
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PLATE 4

EXPLANATION OF FIGURES

22 Cell column connecting a villus at left to wall of Fallopian tube out of field at right. Three mitoses are present near cytotrophoblast of villus. Cells farthest removed from villus are larger and enclose empty cytoplasmic spaces from which glycogen has been removed. EH 265. 10 μ. Hematoxylin and eosin. $\times$ 230.

23 Golgi apparatus and lobated nucleus of a small decidual cell at lower left. Case 14. $\times$ 1360.

24 Golgi apparatus in four large decidual cells. Case 14. $\times$ 960.

25 A cell island near two villi, the trophoblast of which is seen at lower right and at the left. The island is not covered with syncytium and shows an early stage of degeneration at the center with loss of cell structure and appearance of small masses of fibrinoid. At left of island is a syncytial bud containing large vacuoles. The small black dots in the syncytium and bud are lipid droplets Case 13. $\times$ 240.

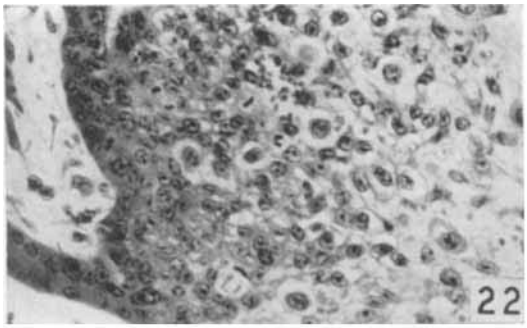
26 Connective tissue material enfolded by a large decidual cell and continuous with the peri-cellular condensation. Bouin. Severinghaus Altmann-Masson. 5 μ. Case 5. $\times$ 1160.

27 Large decidual cell with two mitochondria-laden processes partially surrounding dense connective tissue matrix. Mitochondria also accumulated in the Golgi region above the nucleus. Case 2. $\times$ 1160.

28 Three large decidual cells and three small decidual cells, the latter with intensely chromatic nuclei and empty cytoplasmic space. The one at the right shows a clump of blue granules. Bouin. Severinghaus Altmann-Masson. 5 μ. Case 17. $\times$ 960.

29 Glycogen deposits in three small decidual cells. Large decidual cell at upper right with some glycogen at its upper border. Picric-alcohol-formol. Best's carmine. 5 μ Case 5. $\times$ 960.

30 Peri-cellular condensations around large decidual cells of zone compacta. Bouin. Bielschowsky and Harris' hematoxylin. Case 12. $\times$ 176.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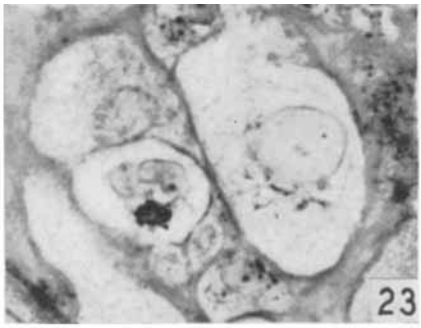
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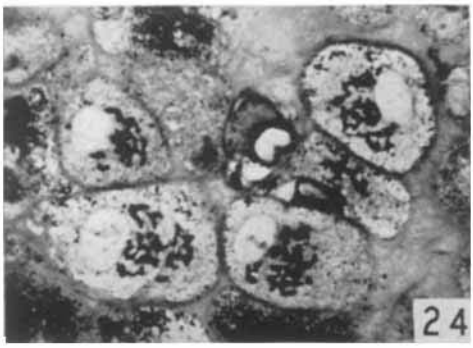
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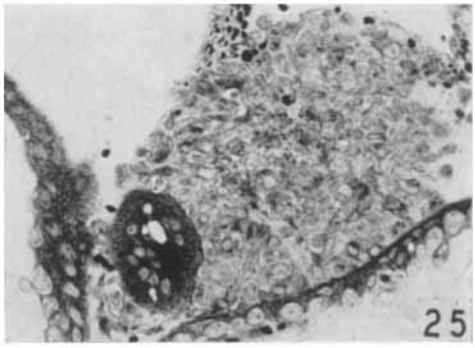
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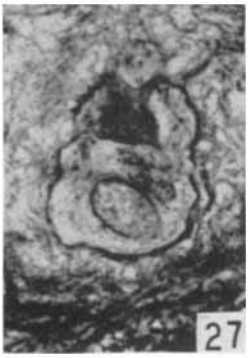
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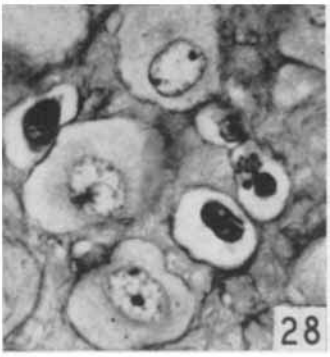
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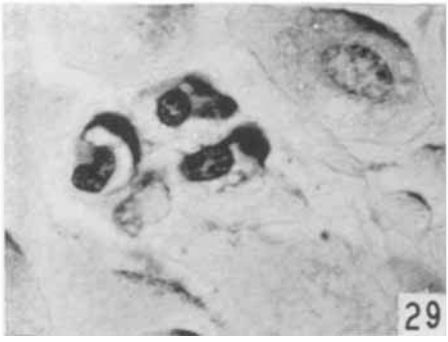
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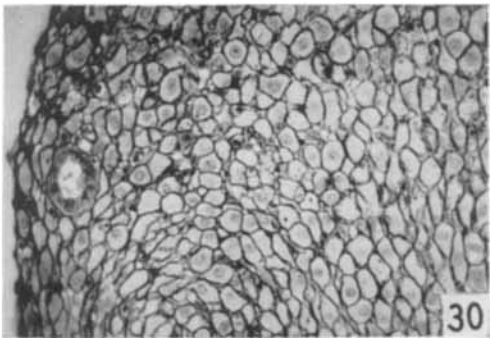
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