OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA

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There is a considerable body of previous work, both histological and histochemical, on the rat placenta. The histochemical descriptions are, however, in some respects incomplete and much of the work was carried out with techniques which are not now considered entirely satisfactory. Since the histochemistry of the placenta is clearly relevant to many problems of placental function, it was felt that the previous findings should be checked, and, where possible, amplified. The present paper describes a series of studies on the carbohydrate material of the rat placenta between 10 and 17 days, when most of the elements of the placenta become fully formed. It is hoped to amplify this information in later publications.

MATERIALS AND METHODS

Rat placentae (hooded Rowett strain) of between 10 and 17 days were submitted to fixation in 10 % formalin, Bouin's fluid, Rossman's fluid, Carnoy's fluid, acetic alcohol formalin (Lillie, 1954), acetone, or by the method of Lison & Vokaer (1949) in Gendre's fluid. In order to compare the efficacy of different fixatives for the preservation of carbohydrate material a series of 12-day placentae were submitted to the following procedure. Each placenta was bisected. One half was fixed in 10 % formalin and the other in Carnoy's fluid, Rossman's fluid, Bouin's fluid or acetic alcohol formalin. This procedure was carried out both at room temperature and, with the duration of fixation doubled, at 4° C. With another specimen, half was fixed in 10 % formalin at room temperature and half in 10 % formalin at 4° C. for twice as long. With yet another specimen, half was fixed in 10 % formalin at room temperature and the other half in Gendre's fluid at −73° C. by the method of Lison and Vokaer.

All the specimens were embedded in paraffin and sectioned throughout the entire placental region. With some the complete series was mounted. With others every twenty-fifth or every fiftieth section was mounted throughout the whole placenta to make a set of slides for staining by the periodic acid-Schiff technique, and intermediate sections were mounted to make at least four similar sets for staining by other methods. Each specimen was, therefore, investigated throughout the whole placenta and the findings are not dependent upon a few isolated sections.

Carbohydrates were investigated by the PAS technique. Glycogen was identified by digestion for one hour at room temperature in a 1 % solution of diastase in phosphate buffer at pH 6·5. Diastase digestion was always carried out on a section immediately adjacent to one stained by the PAS technique without digestion. In addition, glycogen was demonstrated by using a short dimedone blockade in combination with the PAS technique (Bulmer, 1959). This too was controlled by diastase digestion.

Glycoproteins, mucoproteins and mucopolysaccharides were further studied by staining in alcian blue, used as a 1 % aqueous solution (Steedman, 1950) or as a 1 % solution in ½ % acetic acid in the method of Lison (1954). Some sections were stained in azur A (Kramer & Windrum, 1955). This was also carried out on sections which had been sulphated in an equivolume mixture of sulphuric acid and glacial acetic acid at 4° C. (Moore & Schoenberg, 1957). The methylene blue extinction points of some of the carbohydrate materials were ascertained by the method described by Pearse (1953), and the presence of a protein component in many of the PAS-positive materials was confirmed by the coupled tetrazonium reaction (Danielli, 1953), using tetrazotized *o*-dianisidine and *β*-naphthol. In addition, attempts were made to stain carbohydrate materials by Gomori's aldehyde fuchsin technique (Pearse, 1953), without prior oxidation, and by the method for mucoproteins described by Leach (1947).

Occasional sections were stained for reticulin fibres by the method of Long (1948) and for alkaline phosphatase activity by the calcium–cobalt and azo-dye methods described by Gomori (1952).

The general topography of the placenta was studied in sections stained by a trichrome technique, using *ponceau de xylidene* as the plasma stain and aniline blue as the fibre stain.

OBSERVATIONS

It may first be advisable to refer briefly to the morphology of the rat placenta during the 10–17 day period, as we have found it in our trichrome preparations. Fuller and more detailed accounts may be found in the works of Duval (1891) and Bridgman (1948).

At 10 days the mesometrial decidua, which forms the maternal side of the allantoic placenta, is well developed, and may roughly be divided into three main zones. The outer zone lies immediately internal to a layer of unchanged stroma just beneath the uterine muscle. Histologically it resembles the unchanged stroma itself, except for a large content of the granular cells of the metrial gland (Selye & McKeown, 1935) principally distributed around the blood vessels. Beneath this, the middle zone consists of large cells with an evenly staining, rather basophil cytoplasm. Some granulated metrial-gland cells also occur in this zone. The inner zone, which abuts against the foetal tissues, contains large areas of cells with completely unstained cytoplasm. Between these areas are cells with evenly staining cytoplasm, resembling those of the middle zone.

The antimesometrial decidua is a fairly thick layer and the new uterine lumen is present on each side, though it does not extend to the antimesometrial pole. The antimesometrial decidua is surrounded by a yellow or yellowish brown-staining capsule, which appears to correspond to the 'fibrous capsule' described by Bridgman (1948). It extends round as far as the junction with the mesometrial decidua and contains many small nuclei. The antimesometrial decidua itself contains large cells with a moderately basophil cytoplasm, rather resembling the cells of the middle zone of the mesometrial decidua. Between these cells is a fairly thick network of yellow-staining material, which resembles that of the 'capsule' and which also contains many small nuclei.

At the 12-day stage the antimesometrial decidua is much thinner and forms the decidua capsularis. It consists of a mass of 'symplasma' (Bridgman, 1948) in which nuclei of varying shapes and sizes are distributed. It is surrounded by the yellowish-staining 'capsular' layer, which extends for a short distance into the mesometrial decidua between the peripheral parts of the middle and outer zones. The uterine cavity is continuous around the antimesometrial pole. The arrangement of the foetal portion of the placenta is shown in Pl. 1, fig. 1.

By the 17-day stage Reichert's membrane has ruptured and recoiled to the margin of the allantoic placenta, where a small remnant of the decidua capsularis, now devoid of nuclei, is still attached. The 'capsular' layer starts at the periphery of the allantoic placenta from this remnant of decidua capsularis and extends mesometrially around the outside of the placenta for some distance, disappearing before it reaches the mesometrial pole. Here, in the region where the 'capsular' layer is deficient, a mass of tissue projects from the placenta, forming a bulge into the mesometrium and stretching the uterine muscle, which is an attenuated and often interrupted layer. This mass of tissue consists mainly of two types of cell—granulated metrial gland cells and large cells with completely unstained cytoplasm. Areas of cells similar to those of the latter type occur also amongst the cytotrophoblast of the junctional zone. It is often difficult to distinguish between the clear-staining cells of the decidua and those of the junctional zone.

Histochemical findings

The results of the differing methods of fixation on the members of the series of 12-day placentae were first investigated. No obvious differences could be detected in the amounts of carbohydrate demonstrable after the various fixatives. Each method of fixation preserved glycogen and diastase-fast PAS-positive materials in the same tissues and the intensity of the PAS reaction was approximately constant. There was, as might be expected, a varying degree of glycogen 'flow' with differing fixatives. It would seem that all the fixatives which we used are equally satisfactory for the preservation of PAS-positive materials in the rat placenta. It is interesting to note that Davies (1956) considered placental glycogen in the rabbit to be extremely stable to histological fixation.

The distribution of the various PAS-positive materials in the different parts of the placenta is described below.

Decidua

In the 10–14-day placentae there are large amounts of glycogen in the inner zone of the mesometrial decidua, occupying the cytoplasm of the large clear-staining cells which were identified in the trichrome preparations. The aggregations of these cells constitute the 'glycogen areas' of the decidua (Pl. 1, figs. 2, 3). The individual cells of the glycogen areas are separated from one another by intercellular material which gives a strongly positive diastase-fast PAS reaction (Pl. 1, fig. 3). The middle and outer zones of the mesometrial decidua contain only small amounts of glycogen, mainly in the cytoplasm of the granulated cells of the metrial gland. These latter cells Wislocki, Weiss, Burgos & Ellis (1957) found to be characterized by the strong

diastase-fast PAS reaction of their acidophil granules. It is, of course, difficult to make certain that all the acidophil granules are PAS-positive. However, in a section which has been submitted to the PAS technique it is not possible, by using acidic stains, to demonstrate any granules which are not already coloured by Schiff's reagent. The acidophilia of the granules is not destroyed by the oxidation, as one might suspect from the work of Dempsey, Singer & Wislocki (1950), since it can be demonstrated in preparations which have been exposed to periodic acid. The cytoplasm of the basophil cells of the middle zone gives a diffuse diastase-fast PAS reaction. There is little glycogen in the antimesometrial decidua, though small patches do occur. The basophil cells of the antimesometrial decidua of the 10-day placenta give a similar PAS reaction to that of the basophil cells of the middle zone of the mesometrial decidua, and the capsular material, yellowish-staining in the trichrome preparations, is also PAS-positive and diastase-fast. This reaction, however, is rather patchily distributed and more intense in some regions than in others.

In the 17-day placenta both types of cell in the mesometrial decidua contain glycogen. The metrial gland cells, as in the earlier stages, contain glycogen and diastase-fast granules. The other cells contain glycogen (Pl. 1, fig. 4) and are separated from each other by diastase-fast intercellular material. The remnant of decidua capsularis, which contains no glycogen, gives, like the capsular layer around the periphery of the allantoic placenta, a diastase-fast reaction.

In all our specimens the uterine contents and the distal borders of the uterine epithelial cells give diastase-fast PAS reactions. In neither of these situations were we able to demonstrate glycogen.

A feature of considerable interest, though doubtful significance, is the occurrence of a positive reaction with the dimedone-PAS technique in some of the maternal vessels of 12–17-day placentae. This is diffusely distributed, and is detected in every section in two or three of the vessels of the inner layer of the mesometrial decidua. It has not been observed in sections which have been pre-digested with diastase. In ordinary PAS preparations it is difficult to be certain of the presence of diastase-soluble material in the maternal vessels, because of the diastase-fast reaction of the fibrin. Nor do preparations stained with Best's carmine give a convincingly positive reaction in the vessels. It is, however, possible that a substance giving some of the histochemical reactions of glycogen occurs in the maternal vessels. Wolman & Feingold (1953) pointed out that diastase-soluble materials other than glycogen may be PAS-positive. Glycogen in the maternal vessels was described by Goldmann (1912), while Bridgman found considerable numbers of metrial gland cells lying free in the maternal vessels. She suggested that these might be a possible source of intravascular glycogen. In our material, while we have seen them, metrial gland cells in this position have been uncommon.

Giant cells

The distribution of glycogen in the giant cells is patchy, and in any one section glycogen is demonstrated in only a very small proportion of the giant cells (Pl. 1, fig. 5). This proportion is usually higher in the giant cells which lie against the inner zone of the mesometrial decidua than in those which lie peripherally, around the

outside of Reichert's membrane. At the 17-day stage the glycogen content of the giant cells appears to be reduced.

A striking feature of the giant cells is the presence of cytoplasmic granules of diastase-fast PAS-positive material. These vary in size from very fine granules to large globules of the size of a red blood cell (Pl. 1, fig. 6). They occur in every stage which we have examined, but appear to be less numerous at 17 days than at 10–14 days.

Trophoblast

There is an abundance of glycogen in the 'glycogen cells' of the spongy or junctional zone trophoblast. At the 10–14-day stage the cells of the junctional zone have a deeply basophil cytoplasm. The glycogen cells occur in clumps, usually separated from the maternal vessels by other cytotrophoblast cells, and only a small proportion of them have cytoplasmic vacuoles. Most of their glycogen is diffusely distributed throughout the basophil cytoplasm, and this is particularly evident in the specimens fixed by the method of Lison and Vokaer (Pl. 2, fig. 7). In the 17-day placenta the glycogen areas of the junctional zone consist of cells with a cytoplasm which no longer takes the basic stain in a trichrome preparation, but which contains large granules of glycogen (Pl. 2, fig. 8). Individually, as Bridgman pointed out, these cells are indistinguishable at this period from the glycogen cells of the decidua.

Diastase-fast intercellular material separates the cells of the junctional zone at 10–14 days, but tends to be rather indistinct. At the 17-day stage it is extremely well marked, and clearly indicates that the junctional zone trophoblast is not a syncytium. Most cell areas contain only one nucleus, but some are binucleate. Around the larger maternal vessels of the junctional zone the PAS reaction of the intercellular material is particularly intense. Intercellular material can be identified between the glycogen cells, but only discontinuous segments of this give a diastase-fast PAS reaction.

In the 10-day placenta the laminae, and in the 12-day placenta the labryrinth to which they give rise, contain no PAS-positive material. At 14 days fine granules of glycogen are distributed throughout the labyrinth (Pl. 2, fig. 9). At 17 days the labyrinth contains rather less glycogen than at 14 days, and there is a roughly equal distribution of fine granules of PAS-positive diastase-fast material. The boundaries of the maternal blood spaces of the trophoblast give a rather faint diastase-fast PAS reaction, and this is more marked at the 14-day stage than at either 12 or 17 days (Pl. 2, fig. 10).

Yolk sac

The visceral layer of the yolk sac epithelium contains no glycogen until 14 days, when it can be demonstrated, usually basally, in some of the yolk sac cells. At the 17-day stage large quantities of glycogen occur in most cells, distributed throughout the cytoplasm (Pl. 2, fig. 11). Diastase-fast PAS-positive material is found in the visceral yolk sac epithelium in all our specimens, towards the distal poles of the cells (Pl. 2, fig. 12). Supranuclear granules of varying size, but which seem to be larger and more numerous at 14 days than in the earlier or later stages, can be

distinguished from the sharply stained distal border of the cell, which Wislocki & Dempsey (1955), by electron microscopy, confirmed to be a brush border. Though our findings, except for our demonstration of the absence of glycogen before the 14-day stage, largely agree with those of Wislocki & Padykula (1953), our material does not always confirm their observation of a clear zone between the PAS-positive brush border and the PAS-positive supranuclear granules. This can occasionally be seen in specimens fixed in alcoholic fluids, but does not occur after fixation in formalin or by the method of Lison and Vokaer (Pl. 2, fig. 12). It is most obvious in a specimen fixed in ice-cold acetone. Nor do we find any appreciable differences in the intensity of the PAS reaction of the supranuclear granules at the various stages we have examined. Our findings on the reactions of Reichert's membrane, the parietal layer of the yolk sac epithelium, the 'visceral' and 'serosal' basement membranes and the vitelline pockets of the chorio-allantoic placenta confirm those of Wislocki & Padykula.

The endovascular plasmodium

The thickening of the lining of many of the vessels of the mesometrial decidua is apparent in the 10-day placenta. The cells are rather small, with a basophil cytoplasm. In the 12-day placenta the lining cells are large, and of similar size to those of the junctional zone trophoblast. Their intensely basophil cytoplasm makes them resemble trophoblast cells, and they appear to be in continuity with the junctional zone trophoblast. Only rarely is it possible to demonstrate glycogen in any of these cells, but one does see granulated metrial gland cells, containing glycogen, in close association with them. A striking feature is the presence of a basement membrane around the outside of the endovascular plasmodium, separating it from the decidua. This membrane, which is sometimes incomplete, gives an intense diastase-fast PAS reaction (Pl. 3, fig. 13), and is also stained by Long's method. Cell boundaries can be detected in the endovascular plasmodium, but they are only faintly PAS positive. At the 17-day stage the endovascular plasmodium retains its intense cytoplasmic basophilia, and the basement membrane which surrounds it is now complete. Again, glycogen is only rarely found in any of these cells, but the metrial gland cells and glycogen cells of the decidua are closely related to them.

Further investigations of diastase-fast PAS-positive materials

Since none of the diastase-fast PAS-positive materials which we demonstrated is sudanophilic, and with the coupled tetrazonium technique all give positive reactions (Pl. 3, fig. 14) which are abolished or greatly reduced by benzoylation, it is likely that they all consist of mucoprotein or glycoprotein. However, none stained significantly with Leach's Bismarck brown technique, and the only sites to give appreciable staining with aldehyde fuchsin were Reichert's membrane, the distal brush borders of the visceral yolk sac cells and the capsular layer of the decidua. With alcian blue, staining occurred only in the distal brush border of the visceral yolk sac epithelium, and even this was marked only in specimens which had been fixed in formalin. This may presumably be associated with the increased tissue acidity produced by formalin fixation (Dempsey & Wislocki, 1946).

Staining with azur A produced alcohol-fast metachromasia in some of the supra-

nuclear granules of the visceral yolk sac cells. We were unable to demonstrate any alcohol-fast metachromasia in the metrial gland cell granules. With aqueous azur A, without dehydration, there was metachromatic staining in the intercellular material of the decidua, and, fairly faintly, in the metrial gland cell granules. The diastase-fast inclusions in the giant cells were not metachromatic, staining a greenish colour similar to that shown by fibrin and red blood cells.

After sulphation, staining in azur A produced a very different picture. With the exception of fibrin in the maternal vessels all the PAS-positive materials exhibited metachromatic basophilia. The staining of the diastase-fast materials was seen more clearly in sections which had been digested with diastase, since the glycogen appeared to be diffused by sulphation and tended to overlie the other materials. The diastase-fast granules and globules in the giant cells were extremely well demonstrated after sulphation, and many of the giant cells were seen to have a ring of fine granules immediately around the nucleus (Pl. 3, fig. 15). These perinuclear granules are only very faintly PAS-positive. Prolonged digestion with diastase or ribonuclease before sulphation does not alter the metachromatic basophilia induced in these structures in the giant cell cytoplasm, and the staining is therefore unlikely to be due to cytoplasmic nucleic acid. Moreover, the pattern of staining in the giant cell cytoplasm after sulphation, with the granules and globules of varying sizes, is quite different from the rather reticular pattern, due to ribonucleic acid, seen in a section stained in azur A without previous sulphation.

The boundary line between the maternal blood spaces and the trophoblast in contact with them is very well marked after sulphation (Pl. 3, fig. 16), and it is interesting that this boundary line also contains alkaline phosphatase. The distribution of alkaline phosphatase in this site has been described by Wislocki, Deane & Dempsey (1946), Pritchard (1947) and Padykula (1958), all of them using calcium–cobalt techniques. In our azo-dye preparations we have obtained a very precise linear distribution of alkaline phosphatase activity (Pl. 3, fig. 17). Borghese (1957) believed that activity in this site, between the maternal blood and foetal tissues, may be associated with the transfer of materials across the placental barrier.

An interesting feature is the failure of sulphation to induce metachromatic staining in the fibrin of the blood vessels. An attempt to produce metachromatic basophilia in the PAS-positive materials by prolonged chromic acid oxidation (Lison, 1953; Burkl, 1953) was unsatisfactory and successful only with the glycogen.

An attempt was made to differentiate between the diastase-fast PAS-positive materials in the various parts of the placenta by comparing their methylene blue extinction points. All these tests were carried out on formalin-fixed material, and the pH values of the methylene blue solutions were checked with a pH meter. Staining of the diastase-fast granules and globules of the giant cell cytoplasm, the red cells, the fibrin of the maternal vessels, Reichert's membrane and the contents of the uterine and yolk sac cavities became extinct between pH 6·6 and pH 5·6. At pH 5·6 the distal borders of the visceral yolk sac cells and most of their supra-nuclear granules continued to stain, though there was some variation from cell to cell. In addition, most of the diastase-fast granules of the metrial gland cells were still stained. The intercellular material between the glycogen cells of the decidua was strongly stained. At pH 4·8 a small proportion of metrial gland cell granules, the

distal borders of some of the yolk sac cells and occasional supranuclear granules continued to stain. The intercellular material between the glycogen cells was faintly stained. At pH 3·75 the only PAS-positive structure to stain with methylene blue was the capsular layer which surrounds the decidua capsularis and extends mesometrially between the middle and outer layers of the mesometrial decidua (Pl. 3, fig. 18).

DISCUSSION

While there have been many histochemical investigations of the rat placenta, there has been no comprehensive study of the PAS reactions throughout the whole placenta. The only publications describing the results of the PAS technique have been those of Wislocki & Padykula (1953) on the yolk sac and Wislocki, Weiss, Burgos & Ellis (1957) on the granular cells of the metrial gland. Previous workers have described the distribution of glycogen in the placenta, using either Best's carmine (e.g. Bridgman, 1948) or the Bauer-Feulgen technique (Wislocki, Deane & Dempsey, 1946). Only Wislocki & Padykula and Wislocki, Weiss, Burgos & Ellis make it clear that digestion controls were used.

Our findings on the distribution of glycogen in the metrial gland cells and in the yolk sac cells essentially confirm those of Bridgman; Wislocki, Weiss, Burgos & Ellis and Wislocki & Padykula. We show that there is no glycogen in the visceral yolk sac epithelium at 12 days—a stage at which Wislocki & Padykula did not use digestion controls. We confirm the presence of fine flecks of glycogen in the labyrinthine syncytium at 14–17 days, as Goldmann (1912) and Bridgman described. Wislocki, Deane & Dempsey were unable to demonstrate glycogen in this situation with the Bauer-Feulgen technique.

Our account of the glycogen cells of the junctional zone trophoblast corresponds essentially with that of Bridgman. There has been considerable controversy about the origin of these cells since their first description by Duval (1891), who considered them to be of maternal origin. This view has been corroborated by recent workers, and Pritchard (1947) considered them to be 'morphologically and histochemically identical' with metrial gland cells. On the other hand, Jenkinson (1902) described the foetal origin of the trophoblastic glycogen cells in the mouse. He found that the trophoblast cells, as they came to acquire their glycogen, became increasingly vacuolated. There seems no evidence to suggest that the junctional zone glycogen cells of the rat have a maternal origin. We are unable to agree with Pritchard's view that they are identical with metrial gland cells. While many of the trophoblastic glycogen cells and many of the metrial gland cells are binucleate, the presence of the acidophil and diastase-fast PAS-positive granules in the metrial gland cells and the intense cytoplasmic basophilia of the glycogen cells of the junctional zone in the earlier stages, which in trichrome preparations often makes them indistinguishable from other trophoblast cells of this region, demonstrates that there are considerable differences between the two types of cells. Their common absence of alkaline phosphatase activity, cited by Pritchard, does not distinguish the glycogen cells from other trophoblast cells of the junctional zone or the metrial gland cells from other decidual cells (Padykula, 1958). There seems, therefore, no reason to suggest that the glycogen cells of the junctional zone are anything other than derivatives of the

cytotrophoblast. We find no evidence to support the view of Szendi (1933) that the glycogen areas of the trophoblast are lymphatic spaces containing glycogen cells which are migrating from the maternal side of the placenta to the foetal side.

The possibility of the existence of glycogen in the maternal vessels is of considerable interest, since Boyd (1957) has described glycogen in the maternal and foetal vessels of the human placenta. Like him, we are not satisfied that the intravascular diastase-soluble material is glycogen, or that it is not a fixation artefact. Using Best's carmine, Goldmann described glycogen in the maternal vessels of the rat placenta. He also pointed out the similarity between the distribution of glycogen particles in the syncytium and the distribution of vitally ingested particles of Trypan blue. Bridgman, referring to Goldmann's views, extended them by indicating the similarity between the patchy distribution of glycogen in the giant cells and the patchy distribution of vitally ingested Trypan blue. She suggested that the glycogen of the giant cells may actually be ingested as glycogen.

The significance of the placental glycogen has been the subject of discussion since Bernard (1859) suggested that it constitutes a part of the glycogen store for the embryo during the period before its liver assumes that function. After the lapse of a hundred years it is still impossible to do more than speculate. In the rat placenta the principal sites of glycogen deposition are the inner zone of the decidua and the junctional zone trophoblast. Selye & McKeown (1935) suggested that glycogen is transferred as such from the decidua to the foetal tissues by the passage of metrial gland cells along the maternal vessels. Bridgman adduced evidence in support of this view. Apart from any other consideration, the infrequency with which we have found metrial gland cells in this situation makes us doubt whether this is a significant mechanism.

Few of the PAS-positive diastase-fast materials in the rat placenta have been reported previously. The granules of the metrial gland cells were investigated histochemically by Wislocki, Weiss, Burgos and Ellis, who suggested that they may be the source of the relaxin which is known to be formed in the rat placenta (Zarrow, 1956). We have obtained a methylene blue extinction point for these granules lower than that described by Wislocki and his colleagues, but this may be explained by the use of different fixatives. The metachromasia demonstrated by Wislocki *et al.* (1957) and by Asplund, Borell & Holmgren (1940), after lead acetate fixation, was poorly marked in our specimens, and was removed by dehydration in alcohol. It is clear that the granules consist of glycoprotein material, and because of their close association with the maternal vessels it is tempting to suggest that they represent a site of secretion of placental hormone. The evidence that they are relaxin, however, is inconclusive, particularly since relaxin appears to be a simple protein (Frieden & Hisaw, 1953).

The PAS-positive brush border and the PAS reaction of the supranuclear granules of the visceral yolk sac epithelium were described by Wislocki and Padykula, who suggested that they might indicate absorption from the PAS-positive contents of the yolk sac cavity and the uterine lumen. The experiments of Gerard (1925) on the mouse and the rabbit indicate that such absorption may occur, and this evidence seems more significant than a common PAS reaction of the various materials con-

cerned. Moreover, these materials do not appear to be identical. The distal borders of the yolk sac cells and the supranuclear granules show a lower methylene blue extinction point than the yolk sac or uterine contents, and the distal borders are the only placental structures to stain significantly with alcian blue.

The PAS-positive inclusions in the giant cells have not been reported previously. They are more numerous at 10 days than at 17 days, but the significance of their origin and decrease is at present completely obscure. Many workers have ascribed phagocytic properties to the giant cells. For example, Bridgman stated that at the ninth day they appear to contain ingested red cells. In our trichrome preparations the larger PAS-positive globules very much resemble red cells, and it is possible that they have been so interpreted by earlier workers. In the mouse, however, Jenkinson described granular inclusions in the giant cells, and his illustration of these suggests that they correspond with the diastase-fast PAS-positive granules which we describe in the rat.

The PAS-positive intercellular substance is present in large amounts in both foetal and maternal tissues. Davies (1956), working on the rabbit, suggested that the intercellular material of the decidua constitutes a barrier to invasion by the foetal tissues. While this might be correct, little is known about the mechanics of invasiveness of foetal tissues. The reactions of the 'capsule', which encloses the decidua capsularis and extends into the mesometrial decidua, are interesting. Its yellowish stain in the trichrome preparations, with its low methylene blue extinction point, suggest that Bridgman's description of it as a fibrous capsule may be incorrect. Amoroso (1952) mentions the presence of muscle fibres in the decidua capsularis. We find no evidence of this; nor would it be expected if Sabotta's description (1903) of the origin of the new uterine lumen from the remaining basal segments of the uterine glands is correct. The intercellular material of the foetal tissues demonstrates the persistence of cell boundaries in the junctional zone at 17 days. Duval described the complete syncytialization of all the cytotrophoblast, but Bridgman pointed out that if the glycogen cells of the junctional zone were of trophoblastic origin this was clearly incorrect. Moreover she found that cell boundaries did persist to some extent in the rest of the junctional zone trophoblast. Some of the 'intercellular walls' at 17 days are quite thick and stain a pale blue colour in our trichrome preparations. Sometimes binucleate cells are seen, but usually the PAS-positive intercellular material separates cells with single nuclei.

The endovascular plasmodium of the rat placenta, first described as such by Duval, has been a further feature of controversy. Duval considered that the large cells lining many of the maternal vessels of the decidua were derivatives of the cytotrophoblast, and Bridgman supported this view. Jenkinson denied the existence of an endovascular plasmodium in the mouse. The intense cytoplasmic basophilia of the endovascular plasmodium of the rat placenta and its frequent apparent continuity with the junctional zone trophoblast suggest the conclusion that it is derived from the trophoblast. More evidence is needed, however, before this can be regarded as proven. The PAS-positive basement membrane which separates the endovascular plasmodium from the surrounding decidua is an interesting feature. Cell boundaries can be detected between the cells of the endovascular plasmodium but these are only very faintly PAS-positive. We find no evidence that the endovascular

plasmodium is a syncytium, and a similar view was expressed by Orsini (1954) for the hamster.

Our findings have largely confirmed those of Bridgman (1948), Wislocki & Padykula (1953), and Wislocki *et al.* (1957). In addition, we report the occurrence of many PAS-positive and diastase-fast materials in the rat placenta, but we feel that speculation on the significance of many of these is both premature and inadvisable. The solution of such problems requires the correlation of histochemical findings with those of physiology and biochemistry. Otherwise, hypotheses concerning the significance of the occurrence and distribution of the various materials are based on inadequate evidence.

SUMMARY

1. The carbohydrate material of the rat placenta has been studied histochemically.

2. Our results confirm that glycogen occurs in the decidua, the junctional zone trophoblast and the giant cells, and later in the visceral layer of the yolk sac and the labyrinthine syncytium.

3. PAS-positive diastase-fast materials, apparently of a glycoprotein nature, are widely distributed. Among those which have not been reported previously are large numbers of granules in the cytoplasm of the giant cells and, at 17 days, sparsely distributed fine granules in the labyrinthine syncytium.

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REFERENCES

AMOROSO, E. C. (1952). Placentation. In Marshall's *Physiology of Reproduction*, 3rd edition, Vol. II, edited by A. S. Parkes. London, New York and Toronto: Longmans, Green and Co.

APSLUND, J., BORELL, U. & HOLMGREN, Hj. (1940). In der Uteruswand während der Gravidität auftretende metachromatisch granulierte Zellverbande und ihre Stellung zur 'Glandula myometrialis'. *Z. mikr.-anat. Forsch.* **48**, 478–528.

BERNARD, C. (1859). De la matière glycogène considerée comme condition de développement de certains tissus, chez le foetus, avant l'apparition de la fonction glycogénique du foie. *C.R. Acad. Sci., Paris*, **48**, 673–684.

BORGHESE, E. (1957). Recent histochemical results of studies on embryos of some birds and mammals. *Int. Rev. Cytol.* **6**, 289–341.

BOYD, J. D. (1957). Observations on glycogen in relation to the blood vessels of the human placenta and uterus. *J. Anat., Lond.*, **91**, 605.

BRIDGMAN, J. (1948). A morphological study of the development of the placenta of the rat. I. An outline of the development of the placenta of the white rat. *J. Morph.* **83**, 61–85. II. An histological and cytological study of the development of the chorio-allantoic placenta of the white rat. *J. Morph.* **83**, 195–223.

BULMER, D. (1959). Dimedone as an aldehyde blocking reagent to facilitate the histochemical demonstration of glycogen. *Stain Tech.* **34**, 95–98.

BURKL, W. (1953). Zur Klassifizierung der Schleimdrüsensekrete in der Histologie. *Z. Zellforsch.* **39**, 74–84.

DANIELLI, J. F. (1953). *Cytochemistry. A Critical Approach.* New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd.

DAVIES, J. (1956). Histochemistry of the rabbit placenta. *J. Anat., Lond.*, **90**, 135–142.

DEMPSEY, E. W. & WISLOCKI, G. B. (1946). Histochemical contributions to physiology. *Physiol. Rev.* **26**, 1–28.

DEMPSEY, E. W., SINGER, M. & WISLOCKI, G. B. (1950). The increased basophilia of tissue proteins after oxidation with periodic acid. *Stain Tech.* **25**, 73–80.

DUVAL, M. (1891). Le placenta des rongeurs. III. Le placenta de la souris et du rat. *J. Anat., Paris*, **27**, 24–96, 344–395, 515–612.

FRIEDEN, E. H. & HISAW, F. L. (1953). The biochemistry of relaxin. In *Recent Progress in Hormone Research*, Vol. VIII, edited by G. Pincus. New York: Academic Press.

GERARD, P. (1925). Recherches morphologiques et expérimentales sur la vésicule ombilicale des rongeurs à feuillets inversés. *Arch. Biol., Paris*, **35**, 269–293.

GOLDMANN, E. E. (1912). Die aüssere und innere Sekretion des gesunden und kranken Organismus im Lichte der 'Vitalen Farbung', Teil II. *Beitr. Klin. Chir.* **78**, 1–108.

GOMORI, G. (1952). *Microscopic Histochemistry.* University of Chicago Press.

JENKINSON, J. W. (1902). Observations on the histology and physiology of the placenta of the mouse. *Tijdschr. ned. dierk. Ver.*, 2nd series, **7**, 124–198.

KRAMER, H. & WINDRUM, G. M. (1955). The metachromatic staining reaction. *J. Histochem. Cytochem.* **3**, 227–237.

LEACH, E. H. (1947). Bismarck brown as a stain for mucoproteins. *Stain Tech.* **22**, 73–76.

LILLIE, R. D. (1954). *Histopathologic Technic and Practical Histochemistry.* New York and Toronto: The Blakiston Company Inc.

LISON, L. (1953). *Histochimie et cytochimie animales.* Paris: Gauthier-Villars.

LISON, L. (1954). Alcian blue 8 G with chlorantine fast red 5 B. A technic for selective staining of mucopolysaccharides. *Stain Tech.* **29**, 131–138.

LISON, L. & VOKAER, R. (1949). Sur la détection histochimique du glycogène des cellules vaginales chez la femme. *Ann. Endocr. Paris*, **10**, 66–72.

LONG, M. E. (1948). Differentiation of myofibrillae, reticular and collagenous fibrils in vertebrates. *Stain Tech.* **23**, 69–75.

MOORE, R. D. & SCHOENBERG, M. D. (1957). Low temperature sulfation of tissues and the demonstration of metachromasy. *Stain Tech.* **32**, 245–247.

ORSINI, M. W. (1954). The trophoblastic giant cells and endovascular cells associated with pregnancy in the hamster, *Cricetus auratus*. *Amer. J. Anat.* **94**, 273–331.

PADYKULA, H. (1958). A histochemical and quantitative study of the enzymes of the rat's placenta. *J. Anat., Lond.*, **92**, 118–129.

PEARSE, A. G. E. (1953). *Histochemistry, Theoretical and Applied.* London: J. and A. Churchill, Ltd.

PRITCHARD, J. J. (1947). The distribution of alkaline phosphatase in the pregnant uterus of the rat. *J. Anat., Lond.*, **81**, 352–364.

SABOTTA, J. (1903). Die Entwicklung des Eies der Maus vom Schlusse der Furchungsperiode bis zum Auftreten der Amniosfalten. *Arch. mikr. Anat.* **61**, 274–330.

SELYE, H. & MCKEOWN, J. (1935). Studies on the physiology of the maternal placenta of the rat. *Proc. roy. Soc.* B, **119**, 1–31.

STEEDMAN, H. F. (1950). Alcian blue 8 GS: A New Stain for Mucin. *Quart. J. micr. Sci.* **91**, 477–479.

SZENDI, B. (1933). Die Wege des Glykogens durch die hämochoriale Placenta. *Z. Anat. Entw-Gesch.* **101**, 791–798.

WISLOCKI, G. B., DEANE, H. W. & DEMPSEY, E. W. (1946). The histochemistry of the rodent's placenta. *Amer. J. Anat.* **78**, 281–345.

WISLOCKI, G. B. & PADYKULA, H. (1953). Reichert's membrane and the yolk sac investigated by histochemical means. *Amer. J. Anat.* **92**, 117–151.

WISLOCKI, G. B. & DEMPSEY, E. W. (1955). Electron microscopy of the placenta of the rat. *Anat. Rec.* **123**, 133–167.

WISLOCKI, G. B., WEISS, L. P., BURGOS, M. H. & ELLIS, R. A. (1957). The cytology, histochemistry and electron microscopy of the granular cells of the metrial gland of the gravid rat. *J. Anat., Lond.*, **91**, 130–140.

WOLMAN, M. & FEINGOLD, D. (1953). The histochemical tests for glycogen and their specificity. *Acta med. orient.* **12**, 218–222.

ZARROW, M. X. (1956). Maternal hormones in pregnancy. In Transcript of *3rd Conference on Gestation.* Princeton, New Jersey: J. Macy Jr. Foundation.

EXPLANATION OF PLATES

Plate 1

Fig. 1. A 12-day placenta showing the 'capsule' (1), the middle and inner zones of the decidua (2), the giant cell layer (3), the junctional zone (4), the labyrinth (5), the yolk sac (6) and the allantoic mesoderm (7). Trichrome. × 25.
Fig. 2. A decidual glycogen area in a 12-day placenta. PAS. × 650.
Fig. 3. The same area as shown in fig. 2 in an adjacent section. Diastase-PAS. × 650.
Fig. 4. A decidual glycogen area in a 17-day placenta. PAS. × 650.
Fig. 5. Glycogen in trophoblastic giant cells of a 12-day placenta. Dimedone-PAS. × 650.
Fig. 6. Cytoplasmic granules in trophoblastic giant cells. Diastase-PAS. × 650.

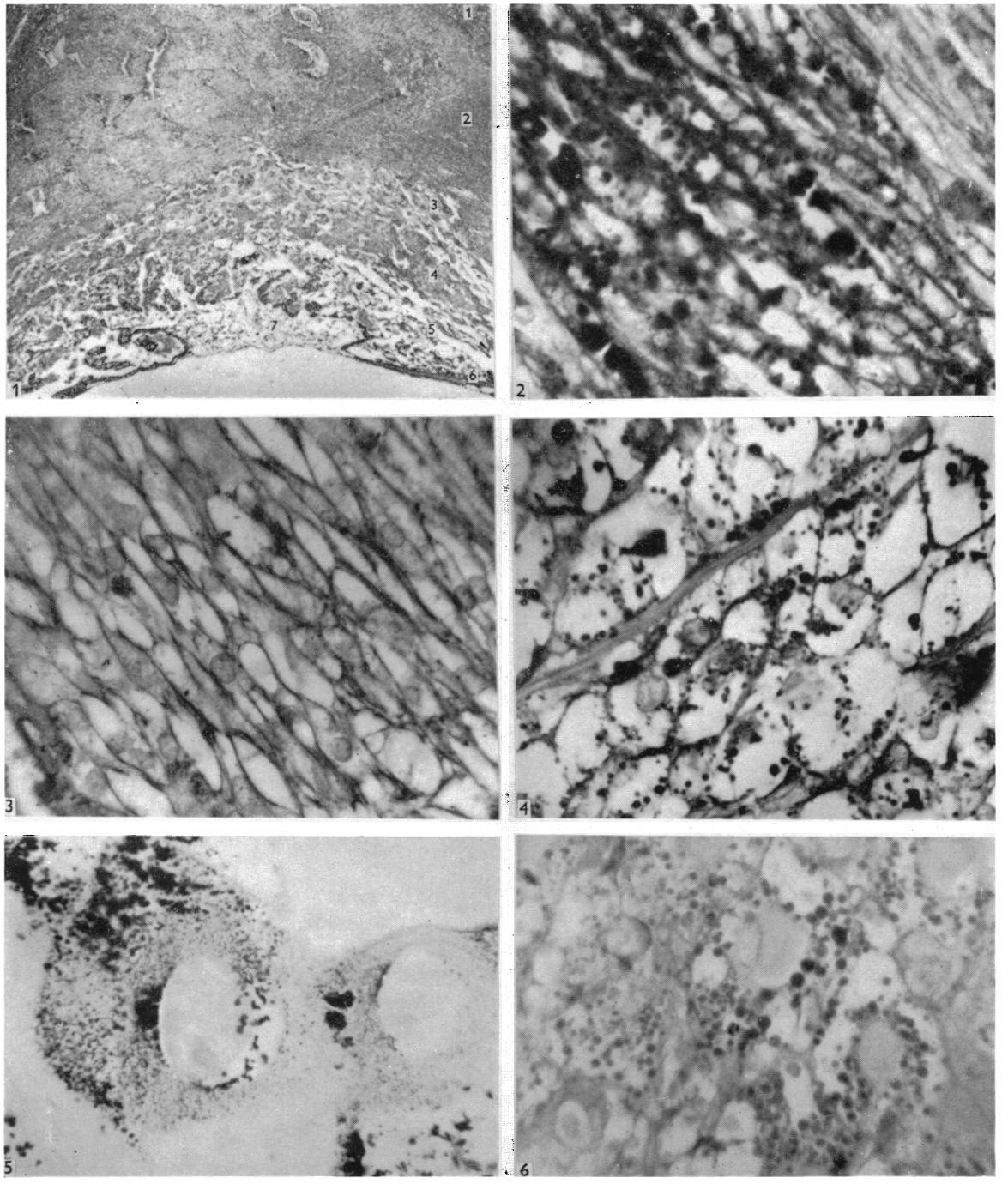
Plate 2

Fig. 7. Glycogen in the junctional zone trophoblast of a 12-day placenta. Dimedone-PAS. × 650.
Fig. 8. A junctional zone glycogen area at 17 days. PAS. × 650.
Fig. 9. Glycogen in the labyrinth of a 14-day placenta. Dimedone-PAS. × 1200.
Fig. 10. The labyrinth of a 14-day placenta. Diastase-PAS. × 650.
Fig. 11. Glycogen in yolk sac villi at 17 days. Dimedone-PAS. × 650.
Fig. 12. The visceral yolk sac epithelium at 12 days. PAS. × 650.

Plate 3

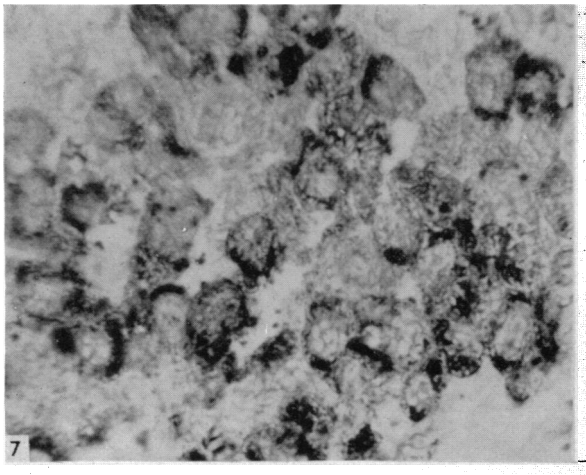
Fig. 13. The endovascular plasmodium in a 12-day placenta. Several granulated metrial gland cells can also be seen. Diastase-PAS. × 650.
Fig. 14. Cytoplasmic granules in trophoblastic giant cells. Coupled tetrazonium. × 850.
Fig. 15. Perinuclear granules of a 12-day trophoblastic giant cell. Sulphation-azur A. × 1500.
Fig. 16. The labyrinth of a 17-day placenta. Sulphation-azur A. × 650.
Fig. 17. The labyrinth of a 14-day placenta. Azo-dye method for alkaline phosphatase. × 750.
Fig. 18. The capsule situated between middle and outer zones of mesometrial decidua. Methylene blue at pH 3·75. × 650.

All the PAS preparations which are illustrated are lightly counter-stained with celestin blue.



BULMER AND DICKSON—OBSERVATIONS ON CARBOHYDRATE MATERIALS IN THE RAT PLACENTA

(*Facing p.* 58)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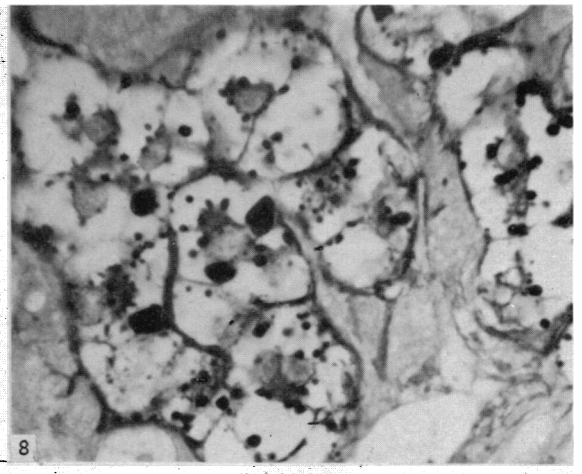
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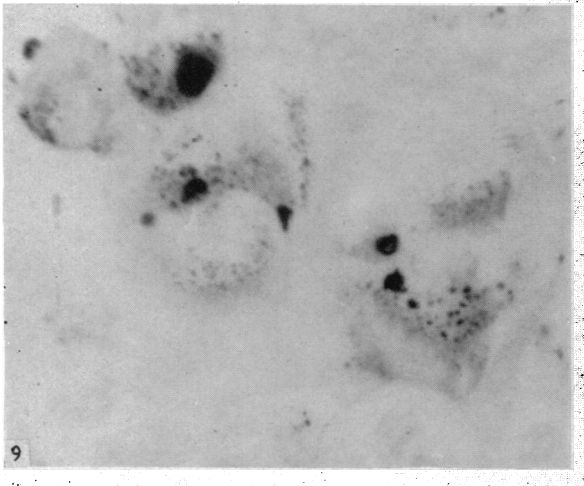
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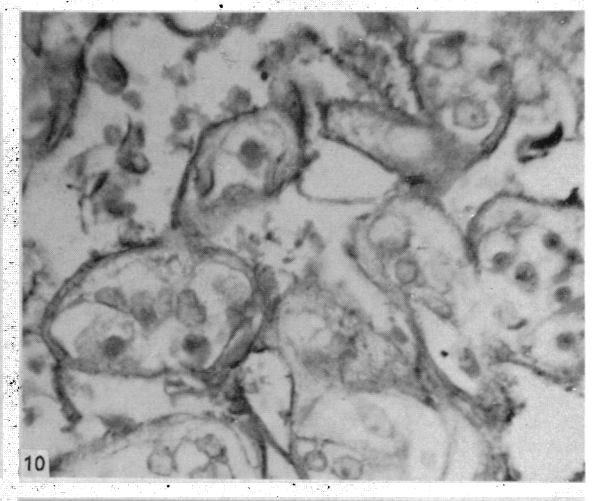
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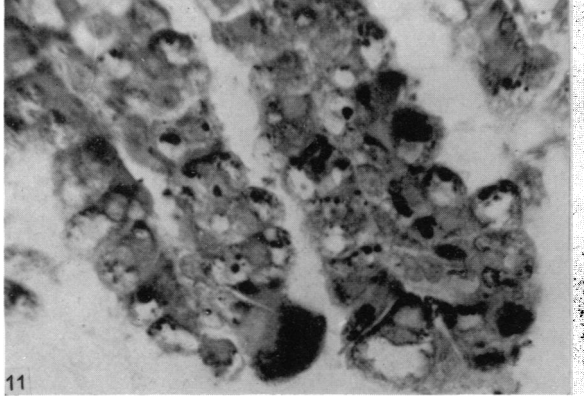
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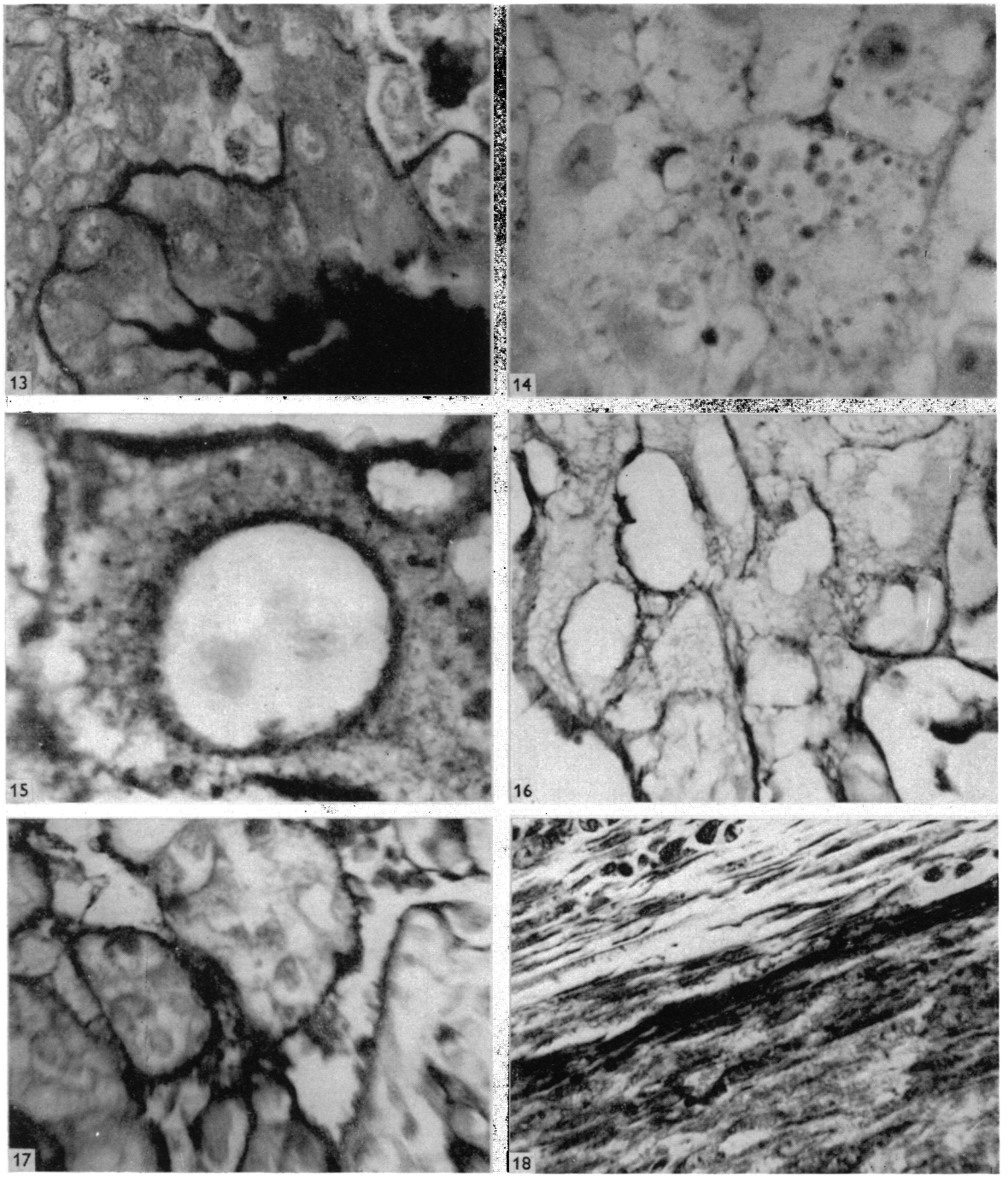

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