OBSERVATIONS ON THE PLACENTAL GIANT CELLS OF THE RAT

By A. D. DICKSON and D. BULMER

Department of Anatomy, University of Aberdeen

The trophoblastic giant cells of the rat placenta have been divided into two groups by Alden (1948). They differ in position and time of appearance, rather than in nature. The first set appears during the antimesometrial implantation and is derived from the ab-embryonic trophoblast. Alden called the cells of this set the primary giant cells, though it may be better to call them the implantation giant cells. They will later be related to the decidua capsularis. The cells of the other set, with which alone this paper deals, first become numerous at the 8th day (Alden), are derived from the ectoplacental cone and are applied to the mesometrial decidua over the whole area of the chorio-allantoic placenta. Alden termed these the placental giant cells. Though his study was mainly concerned with the implantation, or primary, giant cells, he ascribed phagocytic properties to the placental giant cells. These cells have also been described as phagocytic in the mouse (Snell, 1956) and in the hamster (Orsini, 1954). Bridgman (1948*a*, *b*) made a study of them in the rat. She found that they ingest trypan blue injected into the mother and that, at the 9th day, they appear to contain ingested red cells. Further, she states that they are involved at the 11th day in symplasma formation, ingesting or absorbing the cytoplasm of decidual cells and leaving masses of nuclei, which are later ingested.

In a study of the carbohydrate materials in the rat placenta, Bulmer & Dickson (1960) described PAS positive diastase fast granules, which gave a positive coupled tetrazonium reaction for protein, in the placental giant cells. These granules range in size up to approximately the diameter of an erythrocyte and they can so resemble erythrocytes in, for example, a trichrome stain that the conclusion is attractive that these granules represent the basis for suggestions of phagocytosis of red cells. Phagocytosis, either of red cells or of decidua basalis, by placental giant cells was not a striking feature of the material utilized in the study referred to. Evidence of it was sought, therefore, with the results described below.

The placental giant cells are of interest, not only because of their alleged phagocytosis of red cells and decidua but also because of their very gigantism, their possible polyploid condition and their apparently complete failure to multiply by mitotic division. Certainly Bridgman found no mitotic figure in a giant cell in her study of the rat placenta, even in colchicine-treated specimens. The finding of Orsini for the golden hamster was similar. Yet the layer of placental giant cells undergoes a manifold increase in area as the placenta grows. If the giant cells cannot increase in number by their own division, it would seem that the increase in area of the layer can come about by the sliding of the cells over one another, the layer increasing in area at the expense of its thickness, or by an addition to the number of giant cells from elsewhere during the period of growth of the placenta. In the former case,

one might expect to find that not only does the giant cell layer become thinner as it increases in area but also that its individual cells become attenuated. In the investigation to be described, the thickness of the giant cell layer has been noted at various stages and measurements have been made in an attempt to discover whether the cells become altered in form. Since the form of the giant cell, while it is in general fusiform, is somewhat variable and since, moreover, the outline of the individual cells is not always distinct, the nuclei have been taken as indicators, as they were by Orsini in the hamster. It is probable that a minor attenuation of the cell would leave the nucleus unaltered and that, conversely, an attenuation of the nucleus would indicate a major alteration of the form of the cell.

MATERIAL AND METHODS

The material utilized was part of that used for the study of the carbohydrate material in the rat placenta (Bulmer & Dickson, 1960). Evidence of phagocytosis was sought in sets of trichrome-stained 5μ or 7μ sections extending through 10-, 12-, 14- and 17-day placentae, which had been fixed in 10 % formalin and embedded in paraffin. Cytoplasmic basiphilia was investigated by staining in toluidine blue, azur A or thionin. Ribonucleic acid was identified by digestion in a 0·1 % solution of crystalline ribonuclease (L. Light and Co.). With further sections, the ribonucleic acid was removed by a 15 min. hydrolysis in normal hydrochloric acid at 60°C.

The measurements of giant cell nuclei were made on trichrome-stained material. It was accepted that measurements of nuclei which may reach 60μ in length could not be of a high order of accuracy in 5μ sections. These measurements were regarded, therefore, as being in the nature of an attempt to indicate objectively something which was obvious subjectively, namely, that the giant cells and their nuclei increase in size during the period under study. It is likely that, in a 5μ section, only an occasional nucleus will show anything like its maximum dimensions. Accordingly, the thirty largest sections of giant cell nuclei were measured in a number of slides so spaced that no nucleus could be measured twice. The length and breadth of the nuclei were measured and their means taken as indicative of the size of the nuclei at the various stages.

OBSERVATIONS

At 10 days, a layer of giant cells, six or seven cells thick and containing maternal blood spaces, is applied to the decidua basalis. The interface between the giant cell layer and the decidua is smooth and featureless, there being no indications of invasiveness in the form of extension of giant cells, or even of cytoplasmic processes of giant cells, among the decidual cells.

There is no evidence, in the form of foreign nuclei or recognizable fragments of foreign cytoplasm, that the placental giant cells are ingesting decidua basalis cells. It is not possible to state categorically that no placental giant cell contains an ingested red cell, for every giant cell, it would appear, has at least one maternal blood space related to it and, if the interface lies obliquely to the plane of section, it may be exceedingly difficult or impossible to be certain on which side of the interface a red cell lies. Since evidence of phagocytosis to be acceptable should be unequivocal

and since even equivocal evidence is very infrequent, it is best to conclude that, if it occurs, ingestion of decidua basalis cells or of maternal red cells by the placental giant cells is not of major importance.

The cytoplasm of the majority of giant cells contains numerous glycoprotein granules. The cytoplasm exhibits marked basiphilia, which is removed by ribonuclease. The nucleoli are extremely dense. Though there is considerable mitotic activity in the cells of the adjacent junctional (spongy) zone trophoblast, no mitotic figure is seen in a placental giant cell.

The mean dimensions of thirty of the largest sections of giant cell nuclei were: length 33 μ, and breadth 15 μ.

At 12 days the area of the interface between the giant cell layer and the decidua basalis is many times greater than at 10 days, but the layer remains six or seven cells thick. It is thus evident that the number of giant cells must be greatly increased. Not only have they increased in number, but they also appear to have increased in size, as indicated by the mean dimensions of the thirty largest nuclei observed: length 43 μ, and breadth 18 μ.

The cytoplasmic basiphilia due to ribonucleic acid appears to be as dense as at 10 days (Pl. 1, figs. 1, 2). The content of glycoprotein granules is, in general, still high, though there is now some individual variation in this respect, some giant cells containing very few or even no granules. It is interesting to note that these granules have now appeared in occasional junctional (spongy) zone trophoblast cells. These cells are striking in their pleomorphism. Some resemble giant cells in their size and form, but it is not necessarily these which contain cytoplasmic glycoprotein granules. Frequently the junctional zone cells which contain these granules appear to be otherwise indistinguishable from the majority of the other cells in the zone. They are scattered, apparently at random, through the thickness of the zone. All types of junctional zone cells show frequent mitoses. Mitoses in cells containing granules are not difficult to find, but have been noted only in the periphery of the junctional zone, immediately under the giant cell layer.

At 12 days, no unequivocal evidence was observed of phagocytosis, either of decidual cells or of maternal red cells.

The features of the placenta of 14 days distinguishing it from those of earlier stages are the very large vascular channels in the decidua basalis and the numerous polymorphonuclear leucocytes in these channels as well as in the maternal vessels of the giant cell layer. Since the latter are often obliterated by the shrinkage of fixation, the contained leucocytes frequently appear to a casual glance to constitute phagocytosed material contained in the cytoplasm of giant cells.

At 17 days, a radical alteration is noted in the placenta as a whole, for, while at 12 and 14 days there is a wide interval between the giant cell layer and the 'capsule' (Bulmer & Dickson, 1960) which is occupied by decidua, at 17 days the giant cell layer is applied to the capsule. The decidua basalis has effectively disappeared (Holmes & Davies, 1948).

The thickness of the giant cell layer now shows considerable variation from place to place around the periphery of the placenta, being four cells thick in a few places and only one cell thick or even absent in others. There does not appear to be any order or arrangement in this variability. The mean dimensions of the largest

sections of nuclei at this stage are: length 63μ, and breadth 18μ. No evidence of phagocytic activity has been noted. The content of glycoprotein granules is greatly diminished, only occasional giant cells possessing any at all. It is noteworthy that a high content is present in some junctional zone trophoblast cells. There is now no evidence of mitotic activity in this zone. The ribonucleic acid content of the giant cells may be slightly reduced at this stage.

Many of the giant cell nuclei at this stage display an interesting effect (Schiebler, 1958), which varies in its degree from one nucleus to another. In its least developed form, it is evident as minute depressions of the surface of the nucleus. In more developed form, the depressions are deeper, giving the sectioned nucleus the appearance of containing a number of vacuoles (Pl. 1, fig. 3). These 'vacuoles' sometimes look like nucleoli in a trichrome preparation. However, the contents of the vacuoles show the finely fibrous structure of fixed cytoplasm, whereas the nucleoli are homogeneous. Moreover, in thionin preparations, for example, digestion with ribonuclease for just sufficient time to remove cytoplasmic basiphilia removes also the basiphilia of the contents of the 'vacuoles' but is insufficient to remove the basiphilia of the nucleoli.

These changes are displayed, usually, by giant cells which are applied to the capsule. Some giant cells are incorporated in the capsule and these display further degenerative changes. First, the density of the nucleolar material diminishes until it matches that of the cytoplasm, and, secondly, in some cases at least, the nucleus becomes fragmented, possibly by the growth and coalescence of cytoplasmic pockets (Pl. 1, fig. 4). The fragments later disappear. It would be interesting to know what controls this process, which is presumably degenerative, and whether it is the nucleus or the cytoplasm which is the active agent. On the face of it, the whole process looks like a cytoplasmic activity which has escaped from nuclear domination or which has been taken over by some other control.

DISCUSSION

The number of placental giant cells increases at least until the 12th day. Evidence is lacking that they increase by mitotic division. It would appear, therefore, that, like the original placental giant cells derived from the ectoplacental cone, they have heteromorphic precursors. The junctional zone trophoblast cells would seem likely to be these precursors, for they show evidence of active multiplication, the mitosing cells frequently contain the glycoprotein granules which the giant cells contain and they are themselves derived from the ectoplacental cone which produces the first generation of placental giant cells. It is of interest to note that the junctional zone cells can divide even when they contain a considerable amount of stored non-cytoplasmic protein, if it is legitimate so to refer to the glycoprotein granules. While it is well known that a cell with a heavy content of accessory material cannot divide, it has been shown that cultured fibroblasts can divide when the amount is not too great (Bensch *et al.* 1959).

Binucleate placental giant cells are occasionally seen (Bridgman, 1948*a*, *b*) and are not easy to account for if mitosis of giant cells does not occur, for the most economical hypothesis concerning their origin would be that they result from failure

of cytokinesis in a giant cell mitosis. They could, however, result from failure of cytokinesis of a junctional zone mitosis, followed by growth of the resulting binucleate cell to giant cell size. In whatever manner they arise they could represent a mechanism by which giant cells become polyploid. This sort of mechanism was described by Beams & King (1942) in regenerating liver cells and by Fell & Hughes (1949) in cultures of infant mouse tissues.

During the period under study the layer of giant cells diminishes in thickness. At 10 days it is six or seven cells thick, while at 17 days it varies from nothing up to, in a few places, four cells thick. There appear to be two mechanisms involved in this decrease. In the first place, the production of giant cells comes to an end, as evidenced by the lack of mitotic figures in the junctional zone, and, in the second, giant cells are incorporated in the capsule. One cannot say whether the decrease is also partly due to the cells sliding relative to one another to maintain the integrity of the layer as it increases in area with placental growth. One can say that cellular attenuation is not involved to such an extent as to affect nuclear form, for the breadth of the nucleus appears to remain unaltered.

The length of the giant cell nucleus increases from about 33 μ at 10 days to about 65 μ at 17 days. The size of the whole cell is presumably increased proportionately, or more than proportionately, with the increase of the nucleus. This increase would entail the synthesis of a considerable amount of protein. The giant cells are also apparently producing protein-containing granules. It must be borne in mind that these granules may, on the other hand, represent the accumulation of material taken into the cells by pinocytosis. The giant cells, and also the cyto- and syncytiotrophoblast of the placenta, are ideally situated for exercising pinocytic properties, their plasma membranes being bathed in maternal blood. Until evidence of such activity is found, it would seem best to assume that the granules are actually synthetized by the giant cells. It may be, then, that the granular synthesis and the cytoplasmic increase with the growth of the cells are sufficient to account for their ribonucleic acid content. It would seem unnecessary to postulate, as did Wislocki, Deane & Dempsey (1946), that the cytoplasmic basiphilia of the giant cells, like that of the junctional zone trophoblast, is evidence of their synthetizing blood proteins for the embryo. The placental giant cells and the junctional zone trophoblast are remote from foetal vessels, which do not penetrate beyond the labyrinth. It is difficult to see how materials synthetized in either region could reach the embryo. One would have to postulate, as Bridgman did for glycogen, a handing-on of the material from cell to cell until an embryonic vessel was reached. Such a process would appear to be uneconomic in respect of energy requirements. It seems unnecessary, in any case, to postulate protein synthesis in the placenta on behalf of the embryo, for the multiplication and differentiation of its cells are evidence of its ability to synthetize protein for itself. Furthermore, Brambell & Hemmings (1954) showed that, in the rabbit, certain proteins—antibodies—reach the foetus by way of the yolk-sac placenta and not by the chorio-allantoic placenta. Cyto-chemical evidence of activity in placental cells is not necessarily evidence of a mechanism of transfer to the embryo. It might indicate no more than a metabolic activity of the cells or it might indicate the production of a substance, not for the embryo but, through an action on the maternal system, for the benefit of the embryo. One is

tempted to take this view in the case of the placental giant cells, related as they are to maternal, and not to embryonic, blood. If their glycoprotein content represents a secretion, it would seem more likely that this secretion acts on the mother than on the embryo. It may be that the glycoprotein represents the substance having the luteotrophic activity demonstrated by Astwood & Greep (1938) and further investigated by Mayer & Canivenc (1950) and Ray, Averill, Lyons & Johnson (1955). This substance might, however, be expected to be PAS negative, for the representative of luteotrophic hormone in the rat pituitary gland is apparently PAS negative (Purves & Griesbach, 1951). The question of the nature of the glycoprotein remains open, then, and requires investigation by the parallel to Barrnett's work (1958) on the anterior pituitary, using cytochemistry and extraction procedures, with control by biological assay.

SUMMARY

The number of placental giant cells increases until the 12th day of gestation in the rat, apparently by production from the junctional (spongy) zone trophoblast. Production stops about the 12th day, after which their number diminishes. At least partially responsible for this is their incorporation in the 'capsule', during which their nuclei undergo a peculiar process of penetration by cytoplasmic ingrowths followed by fragmentation.

Satisfactory evidence of phagocytosis between 10 and 17 days is not forthcoming. It is suggested that one function of the placental giant cells may be the secretion of a glyco-protein hormone into the maternal circulation.

Our thanks are due to Mr A. Cain, A.R.P.S., who made the prints from our negatives.

REFERENCES

ALDEN, R. H. (1948). Implantation of the rat egg. III. Origin and development of primary trophoblast giant cells. *Amer. J. Anat.* **83**, 143–181.

ASTWOOD, E. B. & GREEP, R. (1938). A corpus luteum stimulating substance in the rat placenta. *Proc. Soc. exp. Biol., N.Y.*, **38**, 713–716.

BARRNETT, R. J. (1958). Some aspects of protein histochemistry, with special reference to protein hormones. In *Frontiers of Cytology* (ed. S. L. Palay) New Haven: Yale University Press.

BEAMS, H. W. & KING, R. L. (1942). The origin of binucleate and large mononucleate cells in the liver of the rat. *Anat. Rec.* **83**, 281–298.

BENSCH, K. G., SIMBONIS, S., HILL, R. B. & KING, D. W. (1959). Phagocytosis and cell division. *Nature, Lond.*, **183**, 476–477.

BRAMBELL, F. W. R. & HEMMINGS, W. A. (1954). Active transport through embryonic membranes. *Symp. Soc. exp. Biol.* **8**, 476–489.

BRIDGMAN, J. (1948*a*). 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.

BRIDGMAN, J. (1948*b*). A morphological study of the development of the placenta of the rat. 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. & DICKSON, A. D. (1960). Observations on carbohydrate materials in the rat placenta. *J. Anat., Lond.* **94**, 46–58.

FELL, H. B. & HUGHES, A. F. W. (1949). Mitosis in the mouse: a study of living and fixed cells in tissue cultures. *Quart. J. micr. Sci.* **90**, 355–380.

HOLMES, R. P. & DAVIES, D. V. (1948). The vascular pattern of the placenta and its development in the rat. *J. Obstet. Gynaec. Brit. Emp.* **55**, 583–607.

MAYER, G. & CANIVENC, R. (1950). Autogreffes de placenta chez la rate. *C.R. Soc. Biol., Paris*, **144**, 410–412.

ORSINI, M. W. (1954). The trophoblastic giant cells and endovascular cells associated with pregnancy in the hamster, *Cricetus auratus*. *Amer. J. Anat.* **94**, 275–331.

PURVES, H. D. & GRIESBACH, W. E. (1951). The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by the McManus-Hotchkiss staining for glycoprotein. *Endocrinology*. **49**, 244–264.

RAY, E. W., AVERILL, S. C., LYONS, W. R. & JOHNSON, R. E. (1955). Rat placental hormonal activities corresponding to those of pituitary mammotrophin. *Endocrinology*, **56**, 359–373.

SCHIEBLER, T. H. (1958). Mitteilungen über die Riesenzellen der Nagetierplacenta. *Verh. Anat. Ges., Erganz. Anat. Anz.* **104**, 318–323.

SNELL, G. D. (1956). The early embryology of the mouse. In *Biology of the Laboratory Mouse*, (ed. G. D. Snell). New York: Dover Publications.

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

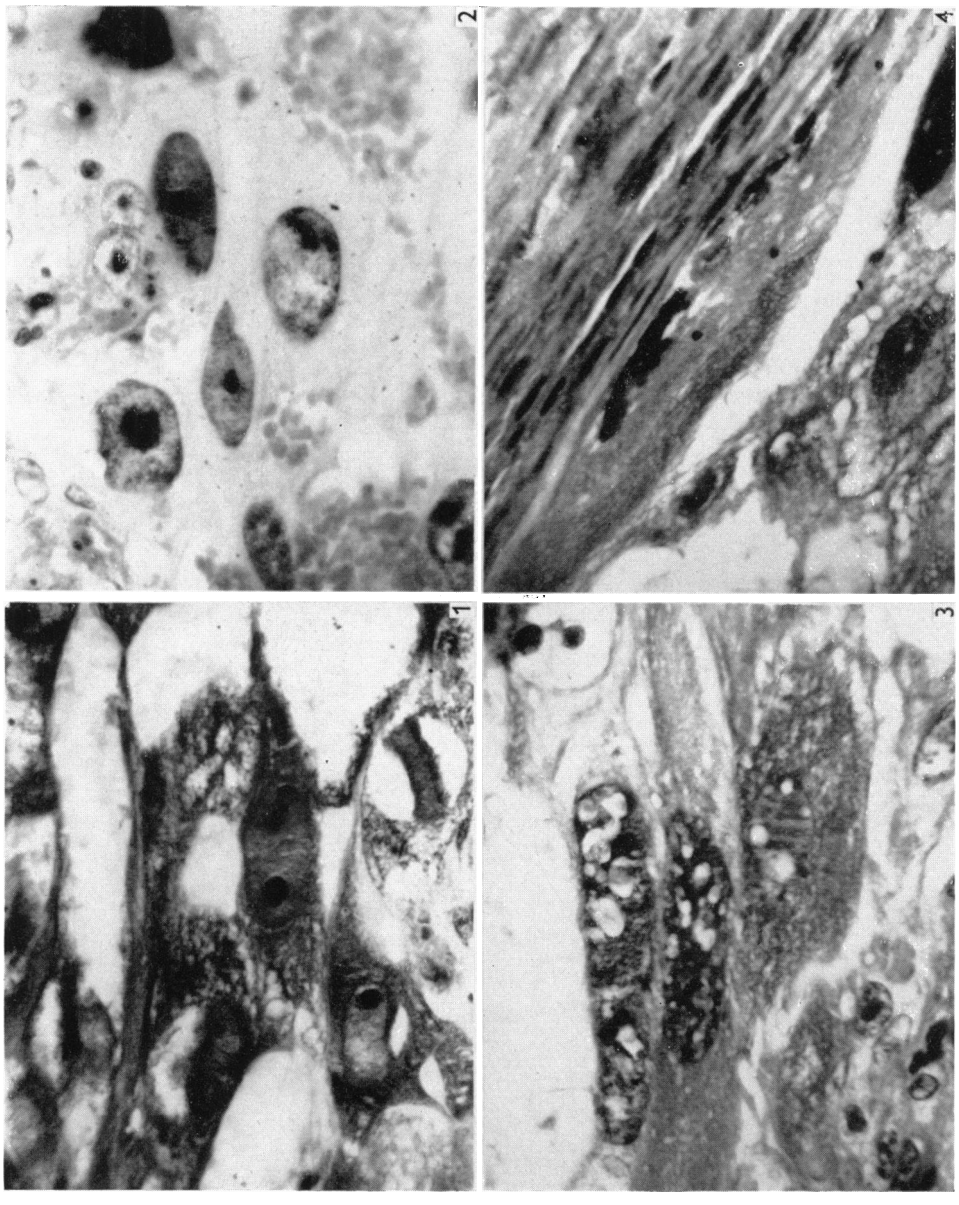
EXPLANATION OF PLATE

Fig. 1. Placental giant cells of a 12-day placenta. Toluidine blue, ×200.

Fig. 2. The same area as shown in fig. 1, in an adjacent section. Toluidine blue after ribonuclease digestion, ×200.

Fig. 3. Vacuoles in giant cell nuclei in a 17-day placenta. Trichrome, ×200.

Fig. 4. A fragmented nucleus of a giant cell undergoing incorporation in the capsule of a 17-day placenta. Trichrome, ×200.



DICKSON AND BULMER—OBSERVATIONS ON THE PLACENTAL GIANT CELLS OF THE RAT

(*Facing p.* 424)