OBSERVATIONS ON HUMAN CHORIONIC VILLI USING THE ELECTRON MICROSCOPE

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The acquisition recently of a very fresh human chorionic sac containing a 6 mm. C.R. length embryo (H. 543), through the good services of Mr O. Lloyd, F.R.C.S., of Addenbrooke's Hospital, Cambridge, has enabled us to make some observations on the structure of the chorionic villi using the electron microscope.

METHODS

For electron microscopy chorionic villi were fixed in 2% osmic acid for 24 hr., washed in several changes of distilled water, and dehydrated. In previous observations on the electron microscopy of embryonic material (Hughes, unpublished) no difference in the quality of fixation has been observed between tissue fixed in buffered osmic acid (Palade, 1952) and in the unbuffered solution. From absolute alcohol the villi were transferred to a mixture of half absolute alcohol and half butyl methacrylate monomer, and after 24 hr. to the pure monomer. A day later portions of the material were placed in gelatin capsules, together with the monomer to which was added a small quantity of benzoyl peroxide, as catalyst for the polymerization of the methacrylate. The capsules were then placed in an incubator at approximately 45° C. for several days, after which the gelatin envelope was dissolved in hot water and the polymerized block mounted on a Cambridge Rocker microtome adapted for fine section cutting. Sections were cut as thin as possible with glass knives and were mounted on copper grids coated with nitrocellulose. They were examined under the Siemens electron microscope in the Cavendish Laboratory, thanks to the courtesy of Dr V. E. Cosslett and the staff of the Electron Microscope Group. The thickness of the best sections was judged to be of the order of 0·03 μ. The initial magnifications of the negatives were either $\times 4500$ or $\times 8000$. Subsequent enlargements were made at a final magnification of 10,000–26,000 diameters.

In addition to the sections for electron microscopy, series of sections of 4, 6 and 10 μ in thickness, and stained with haematoxylin and eosin, Heidenhain's iron haematoxylin, Mallory, and Azan were available for observation under light microscopy.

OBSERVATIONS

The syncytiotrophoblast, the cytotrophoblast, the mesodermal core and the capillaries of the villi have been studied both by light and electron microscopy.

Syncytiotrophoblast. As is well known the syncytium covering the chorionic villi in the central mass of the developing placenta shows certain differences from that which covers the trophoblastic cell columns and lines the trophoblastic shell. Our material prepared for electron microscopy was taken from villi in a region about midway between the differentiating basal and chorionic plates. It is, therefore, villous (or 'resorptive', Grosser, 1927) syncytium with which our observations are principally

concerned. We are not able to comment effectively on the differences alleged by such investigators as Grosser (1927) and Florian (1928) to exist between such syncytium and that related to the cytotrophoblastic shell and which has been called 'implantation' or 'proliferative' syncytium, or plasmodium. From a study of the sections prepared for light microscopy, however, there do not seem, in fact, to be sufficient grounds for the division of the syncytium into these two types, a conclusion similar to that which was reached by Wislocki & Bennett (1943).

In the stained sections the syncytium can be seen to possess a cytoplasm darker than that of the cytotrophoblastic cells; its nuclei are smaller, more irregular and usually have a greater affinity for haematoxylin. In our sections we have not noticed any sign of the shadows of former cell boundaries such as have been reported by Hamilton & Gladstone (1942) in the syncytium of the chorionic villi in a much younger embryo. In the syncytial cytoplasm the 'foamy' appearance described by many workers and stressed by Wislocki & Bennett (1943) can frequently be seen. In certain regions, mainly on the smaller villi, less frequently in the covering of the larger villous stems and rarely on the chorionic plate more or less marked cytoplasmic vacuolization can be seen in the syncytium. In many regions, too, a 'brush border' (*Bürstenbesatz*) can be identified. The brush border is very variable. So far as observation with ordinary microscopy can take one (for the dimensions go beyond the limits of resolution) there appear to be areas of the syncytial surface with no sign of a brush border. Adjacent areas, however, may possess either a well-defined one or a complicated and variable series of protoplasmic processes most readily studied in thin (3–5 μ) sections, which are not well enough ordered, or regular enough, to justify the use of the term brush border. These last regions undoubtedly correspond to the areas 'which exhibit pale, ill-defined streamers or delicate fronds of stippled cytoplasm' in the syncytial surface of the placental villi in the rhesus monkey and in the human which have been described and discussed by Wislocki & Bennett (1943). Indeed, careful examination of the syncytial surface bordering the inter-villous space leads to the conclusion that the surface is never completely smooth if fixation has been early and adequate, and that a whole spectrum of appearances can be presented from a mere shagginess to a tall brush border of up to 2 μ in height. To what extent the differences fluctuate in life is a problem that cannot be decided from a study of fixed material, but there is no appearance of a fixed structure in the syncytial surface that could prevent any part of it from producing processes or a brush border. The brush border and the irregular processes seem to stain, in haematoxylin and eosin sections, less deeply than the general cytoplasm of the syncytium, but thin paraffin sections suggest that this difference is not due to any histochemical distinction between the two.

Under the electron microscope also the appearance of the syncytiotrophoblast varies in different regions, both at the surface and within. Where the brush border is not present (Pl. 2, fig. 2) the cytoplasm consists mainly of densely packed vesicles about 0·3 μ in diameter. This vesicular texture extends to the surface of the syncytiotrophoblast, in a marginal zone beyond a surface membrane. This is the zone which in other regions may be occupied by the brush border. The latter, where present, is made up of irregular filaments 40–100 mμ in diameter and extending in places up to 3 μ beyond the surface membrane. Some of these filaments terminate in globular

expansions (Pl. 2, figs. 4, 5) which may be as much as 0·3 μ across. Scattered along the course of the filaments are tiny granules near the limits of resolution of the microscope. Some of these seem to project beyond the general surface of the filaments. There are no signs of basal granules in the region of attachment to the surface membrane.

When the surface of the syncytiotrophoblast has a brush border, the cytoplasm just beneath is much vacuolated (Pl. 1, fig. 1). These vacuoles vary much in size; the largest are about 3–4 μ across. Small vacuoles in this position are still seen underneath areas where the border has an intermediate type of structure (Pl. 2, fig. 3). Appearances suggest that vacuoles may be formed by the dilation of the protoplasmic vesicles.

Two other types of inclusion are also seen within the cytoplasm. Of these, one consists of abundant ovoidal bodies 0·1–0·4 μ across. These clearly correspond to mitochondria. The other type is made up of fewer and larger granules which are densely impregnated with osmium and are 1 μ or more in diameter. These larger osmiophil granules correspond to the lipoid droplets which Wislocki & Bennett (1943) regard as consisting of placental steroid hormones.

The nuclei of the syncytiotrophoblast are vesicular in form, with most of their contents aggregated either beneath the nuclear membrane or in central clumps. They are clearly distinguishable in texture from those of the cytotrophoblast.

Cytotrophoblast. Our electron microscopic observations on the cytotrophoblast were made almost exclusively on the Langhan's cells of the villi. Consequently, no descriptions will be given of the appearance in the material prepared for light microscopy of the trophoblastic shell or the cytotrophoblastic cell columns. It should perhaps be mentioned, however, that, in fortunate sections, the Langhan's cells of the established villi can be traced towards the cytotrophoblastic cell columns and can be seen gradually to take on the characteristics of the latter. As the chorionic sac had been separated from the decidua we can make little effective comment on the cells of the trophoblastic shell. In Langhan's layer only rare mitoses can be seen. Such evidence of cell division is more frequent in the cytotrophoblastic cell columns. In our specimen the cells of Langhan's layer of the cytotrophoblast are usually so arranged that they form a continuous lining to the overlying syncytium. There are, however, regions in which the Langhan's cells are discontinuous and the underlying mesodermal core of the villus is separated from the inter-villous space only by syncytium. This process of gradual disappearance or, at least, marked diminution in the number of the cytotrophoblastic cells, is one that progresses rapidly in stages older than that with which we are concerned. Other material available to us (Hamilton & Boyd, unpublished) suggests that there is little or no degeneration of the cells of Langhan's layer; most of the cells seem eventually to become transformed into syncytium and possibly into cells of the mesodermal core. A few, however, persist in isolation and can still be identified in full-term placentae.

The cytoplasm of the Langhan's cells is distinctly less basophilic than that of the syncytium, and consequently the cells are readily distinguishable in sections by their pale-staining. In general, too, the cytoplasm of the cytotrophoblast is less vacuolated than the syncytium, though the vacuolation is most variable. The nuclei of Langhan's cells are distinctly larger than those of the syncytium, and are also more

palely staining with haematoxylin, though, as with the vacuolation in the cytoplasm, there is some variability.

Under the electron microscope the cytotrophoblast is clearly distinguishable from the syncytiotrophoblast by certain features in addition to the presence of sharply demarcated cell boundaries. Thus the cytoplasm is of a more even texture, the mitochondria have a more definite boundary layer (Pl. 3, fig. 6), and the nucleoplasm is much more uniform (Pl. 3, fig. 7). The nuclei of the cytotrophoblast correspond closely in appearance with other embryonic nuclei which we have observed in electron micrographs.

Mesodermal core of villi and capillaries. The stroma of the villi contains a loose mesenchymatous connective tissue embedded in which are thin-walled capillaries, possessing diameters of 5–25 μ. At rare intervals, larger blood vessels can be found. With suitable staining the collagenous fibres of the connective tissue stroma apparently form a very loosely woven network. At the boundary between the stroma and Langhan's layer collagen fibres can be found to terminate in intimate relations with the cytotrophoblastic cells and occasionally it appears as if the collagen surrounds those cells.

Our studies on the mesodermal core in electron micrographs are at present limited to observations on the collagen fibres within the tenuous mesenchyme.

The collagen at this stage is mainly in the form of single macromolecular fibrils, in each of which the repeat pattern is seen as a row of dots (Pl. 3, fig. 9). The dots are separated by distances of the order of 600 Å, which correspond to the spacing within adult rather than embryonic collagen fibres (Randall, Frazer, Jackson, Martin & Worth, 1952). The fibrils are often arranged in parallel bundles, in which the single filaments are distinct. At the edge of the mesodermal core, these fibrils are seen to enter the substance of the cell walls of the cytotrophoblastic cells, and form a relatively dense network within the cell margin. This same arrangement is seen in the endothelial cells of a capillary within the mesodermal core (Pl. 3, fig. 8).

DISCUSSION

The most striking feature of the electron micrographs of the placental villi is, perhaps, the brush border of the syncytium. The possession by the syncytium of this brush border, or *Bürstenbesatz*, has frequently been recorded. Kastschenko (1885), in his description of the syncytium, writes that 'der freie Rand ist gewöhnlich von Wimpern besetzt'. As Grosser (1927) states, this appears to be the first description of the brush border, and Amoroso (1953) is mistaken in attributing the first observations to Minot. There are many later references to the brush border (Bonnet, 1903; v. Lenhossek, 1902; Marchand, 1903; Hofbauer, 1905; Jung, 1908; Herzog, 1909; Johnstone, 1914; von Möllendorff, 1921; Stieve, 1926; Greenhill, 1927). The observations up to this last date were summarized by Grosser who wrote 'An der äusseren Oberfläche trägt das Syncytium einen Besatz aus starren Stäbchen oder Härchen (Stereocilien) auch als Bürstenbesatz bezeichnet...der Besatz is nicht immer nachweisbar, und seine Erhaltung ist nicht an besonders gute Fixierung gebunden'. More recently, Wislocki & Bennett (1943) have given a much fuller account of the surface of the syncytium than was previously available. From their own studies, and from a survey of the literature, these investigators consider that the so-called brush

border is very variable in structure and that it is inconstant in its occurrence and distribution within any given placenta. They consider that there is some justification to assume that the syncytium is unstable and that its contours must be subject to fairly constant modification, and they suggest that the syncytium, temporarily at least, may be less mobile where the brush border is most apparent and complete. On the other hand, in those regions where fronds and streamers of the syncytial surface occur the cytoplasm may be in a greater state of flux. From considerations such as this Wislocki & Bennett suggest that the various surface irregularities of the syncytium are implicated in the taking up of fluid and nutriment from the blood in the intervillous space.

Recent studies by means of the electron microscope have shown that more than one type of structure in various tissues has been included under the heading of a brush border. In the cells of the intestinal epithelium, as shown by Bretschneider's study (1949) of *Ascaris*, the brush border consists of a dense pile of parallel filaments; in the kidney tubules (Sjöstrand & Rhodin, 1953) the brush border consists of densely arranged cylindrical 'ducts' which are closed towards the tubular lumen. In human chorionic villi, the present work demonstrates that the brush border is made up of individual filaments, but in an irregular arrangement with little or no tendency towards alignment in parallel tufts.

In the chick chorio-allantoic membrane, Murphy & Bang (1952) have shown that from the surface fine filaments ('microvilli') project which hypertrophy in material infected with Newcastle virus (Bang, 1952), when they often develop 'balloons' at the tip. These microvilli are reminiscent of the corresponding structures in human chorionic villi, though in the chick membrane they are more sparsely distributed. Again, somewhat similar projections are seen in the yolk-sac of the guinea-pig (Dempsey, 1953). These are spherical in early stages, but later become cylindrical. This author considers these structures to be absorptive in function. The correlation between the distribution of superficial vacuoles and the presence of these fully developed microvilli in the human syncytiotrophoblast strongly suggests the same conclusion.

The evidence that the surface of the syncytioblast is pinocytic (Lewis, 1931) has been already discussed by Wislocki & Bennett (1943). To this may now be added our observations that the presence of relatively small vacuoles close beneath the surface of the syncytiotrophoblast is always correlated with a fully developed brush border.

Presumably the distribution of microvilli in limited areas is due to their cyclic formation and disappearance; this interpretation is supported by the appearance of areas of an intermediate type such as is shown in Pl. 2, fig. 3, though whether this represents a stage in the development or the degeneration of microvilli cannot yet be decided.

It is clear, however, that the brush border of the chorionic villi is a less elaborate and less permanent structure than that of intestinal and renal epithelia, and we suggest that the term be abandoned for the syncytiotrophoblastic surface fringe. The individual elements of this, in those regions where order and structure are apparent, are adequately described as microvilli, the term introduced for comparable structures in other embryonic membranes by Murphy & Bang (1952).

SUMMARY

1. Observations on the structure of human chorionic villi, using electron microscopy, are reported.

2. In areas possessing the so-called 'brush border' the surface of the syncytium of the villi shows filaments (microvilli) of up to 3μ in height and with diameters of 40–100 mμ. The microvilli may terminate in globular expansions which may be as much as 0·3μ across.

3. The cytoplasm of the syncytium has a vesicular structure in which vacuoles, mitochondria and large osmiophil granules can be identified. The presence of many vacuoles beneath the syncytial surface can be correlated with the presence of microvilli, and it is suggested that these areas are regions of absorption, possibly of a pinocytic nature. The osmiophil granules correspond to the lipoid droplets of the syncytium.

4. Differences between the nuclei of the syncytium and of the cytotrophoblast are noted.

5. The early collagen fibrils of the mesodermal core of the villi are described.

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EXPLANATION OF PLATES

Figs. 1–9 are electron micrographs of areas in sections of villi from a human chorionic sac which contained an embryo of 6 mm. C.R. length. The initial magnifications of the prints were from 11,000–26,000 diameters. The distance corresponding to a micron in the section is given for each figure.

PLATE 1

Fig. 1. Tip of a villus, showing well-developed microvilli at the surface, with numerous vacuoles within the syncytium. One syncytial nucleus is shown near the lower margin of the figure.

PLATE 2

Fig. 2. Edge of a villus with amorphous cytoplasm at the margin. Mitochondria and lipoid granules are seen within the syncytium. No vacuoles are present.

Fig. 3. Edge of a villus where the syncytial margin is intermediate in form. Some small vacuoles are present beneath the border.

Figs. 4, 5. Microvilli at higher magnification. They are irregular in arrangement and some terminate in globular expansions.

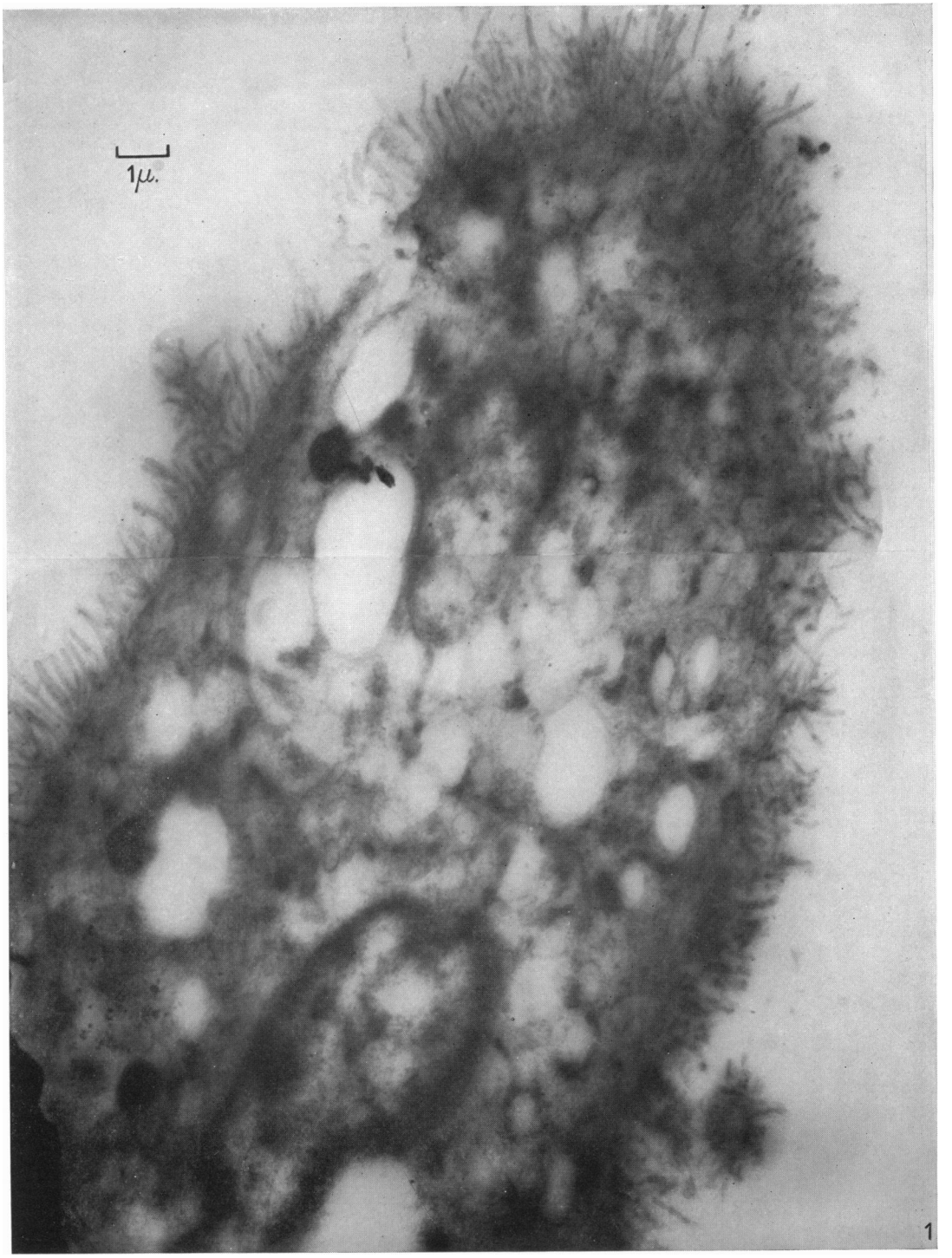
PLATE 3

Fig. 6. Section through a villus extending from the syncytium (top left) through a cytotrophoblastic cell into the mesodermal core (bottom right). Notice fine collagen fibres inserted within the marginal zone of the cytotrophoblastic cell and the mitochondria within this cell.

Fig. 7. Section through a cytotrophoblastic cell showing the nucleus and nucleolus. Notice the uniform texture of the nucleoplasm in contrast to that of the syncytial nuclei at this stage (Figs. 1 and 3).

Fig. 8. Section through a blood vessel within the mesodermal core of a villus. On the outside the collagen fibrils of the core are inserted within the marginal zone of the endothelial cells.

Fig. 9. Collagen fibrils within the mesodermal core. The dots within each fibril are separated by about 600Å. In the original negatives these dots are very clear indeed.



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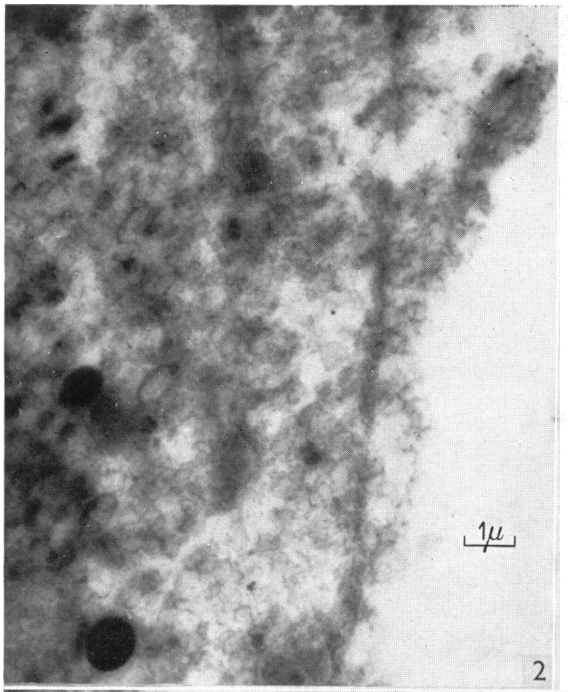

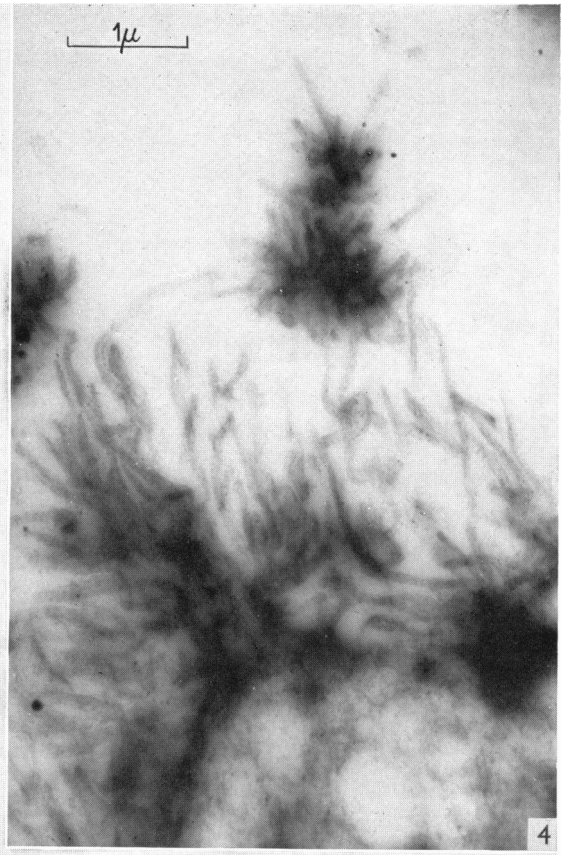

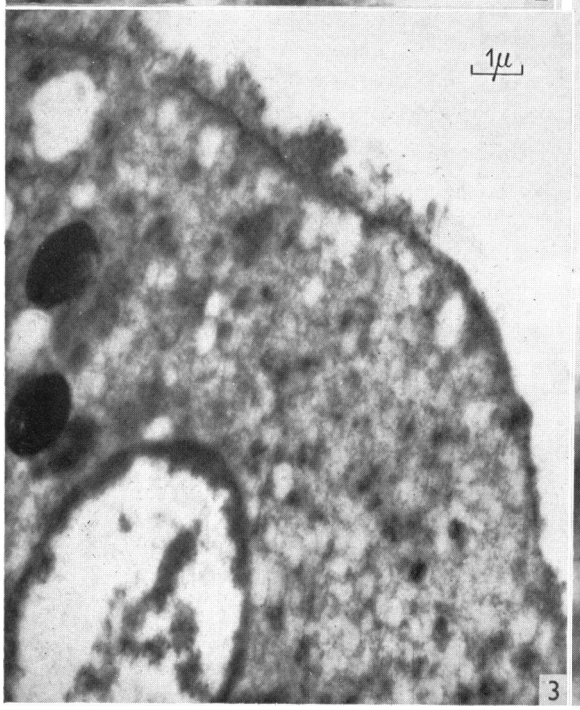

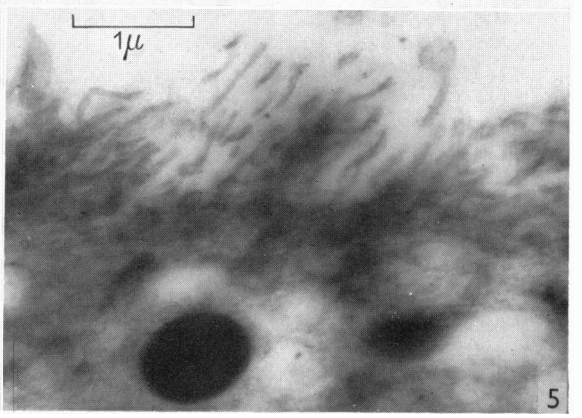

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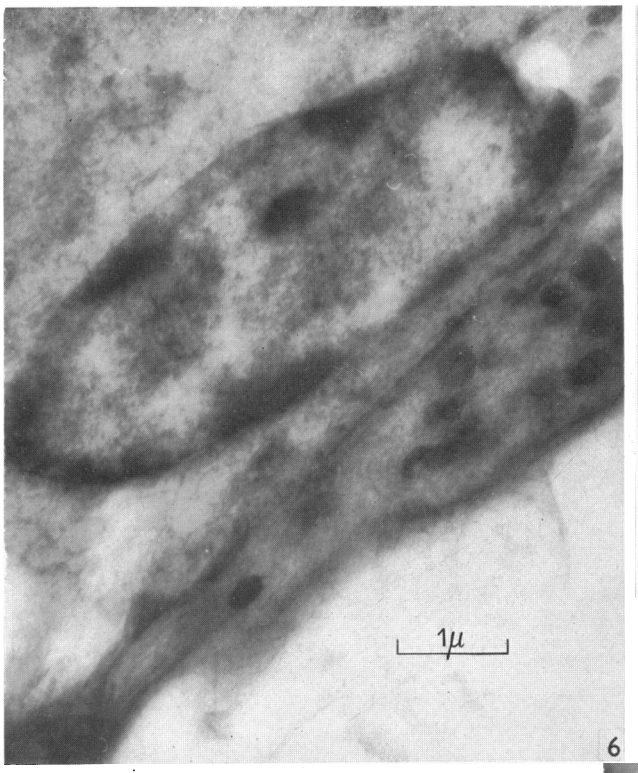

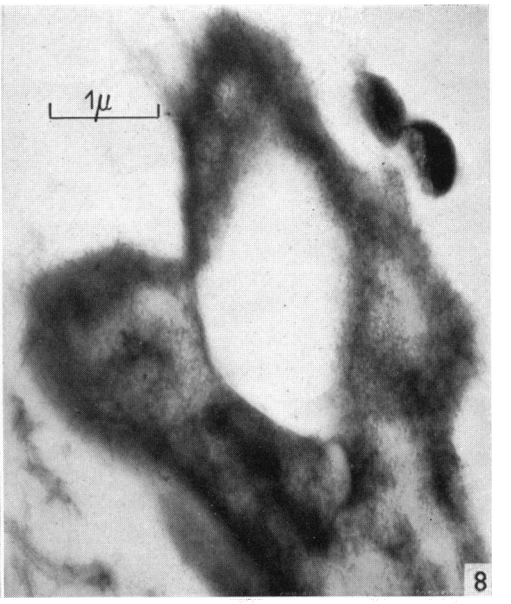

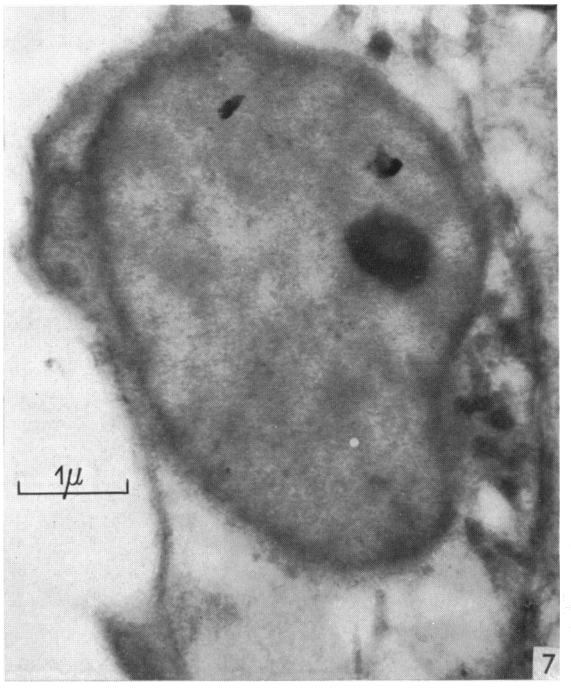

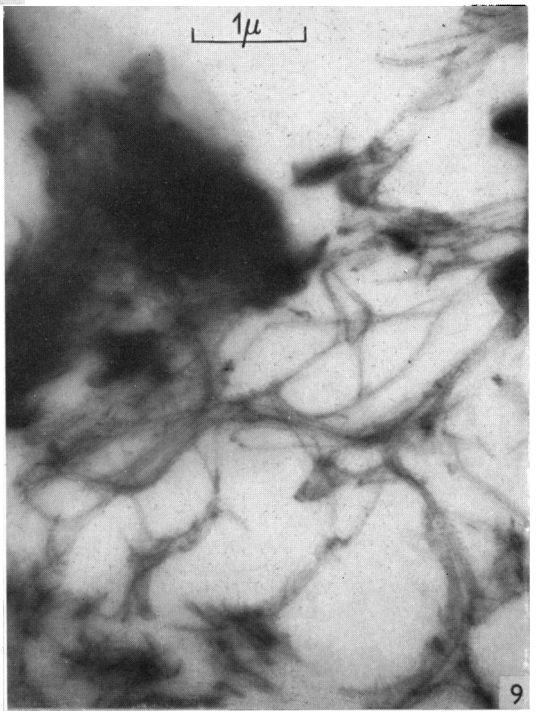

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