The Electron Microscopy of the 'Golgi Apparatus' in the Purkinje Cells of Owls

By S. K. MALHOTRA *and* G. A. MEEK

(From the Cytological Laboratory, Department of Zoology, Oxford University; and Department of Human Anatomy, Oxford University)

With four plates (figs. 1–4)

SUMMARY

The classical site of the 'Golgi apparatus', the Purkinje cells of the cerebellum of owls, has been examined by electron microscopy. The greater part of the cytoplasm consists of aggregates of closely packed granular membranes of endoplasmic reticulum. The objects described by Dalton and Felix in electron micrographs and called by them the Golgi apparatus are rarely seen in these preparations. It seems likely that the 'Golgi apparatus' of this cell as seen in the optical microscope is formed by the deposition of silver or osmium on the membranes of the endoplasmic reticulum, which form a network throughout the cytoplasm of this cell.

INTRODUCTION

IT was shown by one of us (Malhotra, 1959*a*) that the classical 'Golgi apparatus' of the perikaryon of the neurone of vertebrates was a deposit of silver or of osmium on a reticulum, which could be seen in the living cell by interference microscopy. This reticulum was identified as the basiphil reticulum or *Netz*, described by Nissl (1894), in these cells. These conclusions were also supported by the study of the Golgi apparatus in its classical site, that is to say, the Purkinje cells of the cerebellum of owls (Malhotra, 1960). The evidence suggested that the bulk of the 'Golgi apparatus' of these cells was a deposit of silver or of osmium on the endoplasmic reticulum, with which small basiphil granules are associated (Palade, 1955*a*, 1958; Palay, 1956, 1958; Palay and Palade, 1955).

It has become customary among electron microscopists (Dalton and Felix, 1954; Sjöstrand, 1956; Lacy and Challice, 1957; Pollister and Pollister, 1957; Palay, 1958) to describe as Golgi apparatus in various cells a system of vacuoles, small vesicles, and closely packed parallel membranes, characteristically devoid of the ribonucleoprotein granules of Palade (1955*b*, 1958) that are found in association with the granular endoplasmic reticulum. This implies that the Golgi 'apparatus' or 'network', as originally described by Golgi and as seen in typical Golgi preparations under the light microscope, is always composed of such elements. The purpose of this investigation was to find out what structures seen in the electron microscope might correspond with the typical network seen in the classical site described by Golgi himself in his original publication (Golgi, 1898), namely, the Purkinje cells of the owl.

[Quarterly Journal of Microscopical Science, Vol. 101, part 4, pp. 389–94, 1960.]

Material and Methods

Living owls are very difficult to obtain. We were fortunate at the beginning of this investigation in obtaining from Mr. Robert Jackson of Hale, Cheshire, two live tawny owls, *Strix aluco*. The greater part of the cerebellar tissue from these two birds was used in making vital observations, in following Golgi's original method of silver impregnation, and in carrying out histochemical studies. Very little was available for electron microscopy. The small amount of material from one bird was fixed in Palade's osmium tetroxide fixative and embedded in methacrylate. The preservation of the Purkinje cells in these preparations was considered poor by modern standards, and a search was made for more tawny owls. These proved to be unobtainable, but one live spotted owl, *Bubo africanus*, was obtained from the Regent Pet Stores, Camden Town, London. The whole of the cerebellum of this bird was used to prepare more material for electron microscopy. Fixations were made in both the standard Palade medium and in buffered potassium permanganate; araldite was used for embedding.

Strix aluco. The owl was killed by chloroform, and the cerebellum was dissected out immediately after death. Pieces of tissue, about 1-mm cubes, were cut out, fixed in ice-cold 1% buffered osmium tetroxide solution at pH 7·2 for 2 h (Palade, 1952), dehydrated in graded alcohols, and embedded in *n*-butyl methacrylate.

Bubo africanus. The owl was lightly anaesthetized with ether and the cerebellum quickly removed in two pieces. One piece was placed in Palade's fixative and one in 1% potassium permanganate solution buffered at pH 7·3 by veronal/acetate (Luft, 1956). Each piece was at once cut into 1-mm cubes with a sharp razor-blade. Material was fixed in Palade's medium for ½, 1, and 2 h; in Luft's medium for 15 min, ½, and 1 h. The fixative was kept ice-cold in each case. The tissue blocks were then dehydrated in graded alcohols and embedded in araldite (Glauert and Glauert, 1958).

Blocks were in all cases sectioned on a Porter-Blum microtome. Methacrylate sections were mounted on carbon-filmed Smethurst Highlight grids; araldite sections were examined on unfilmed grids. Only sections showing silver or grey interference colours were used. The sections were examined in a modified Siemens Elmiskop I (Meek, 1960), with the double condenser (400-μ aperture), a beam current of 10-μA, and an objective aperture of 30-μ diameter.

Results

The electron micrographs of the Purkinje cells of the first preparation of the cerebellum of *S. aluco* showed very widely dilated cisternae of the endoplasmic reticulum. These apparent spaces were filled with featureless

---

Fig. 1 (plate). Electron micrograph of a Purkinje cell of *B. africanus* (from tissue fixed in Palade's fluid and embedded in araldite). *er*, endoplasmic reticulum; *l*, lipid globules; *n*, nucleus.

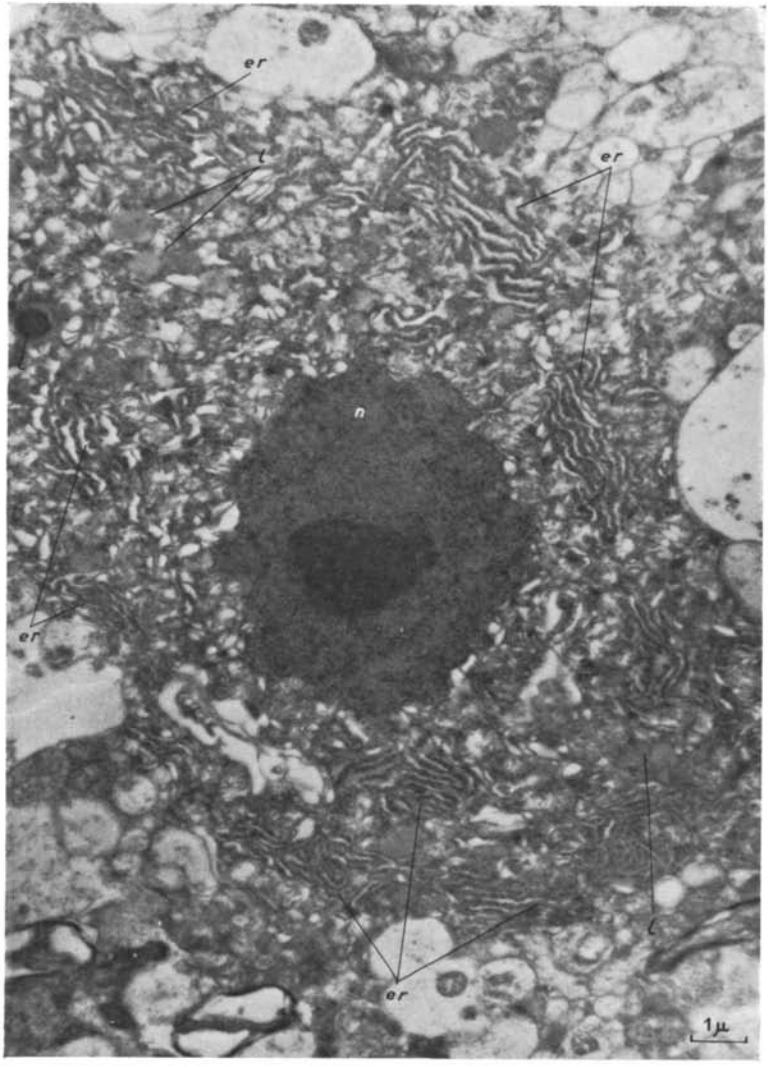

FIG. 1
S. K. MALHOTRA *and* G. A. MEEK

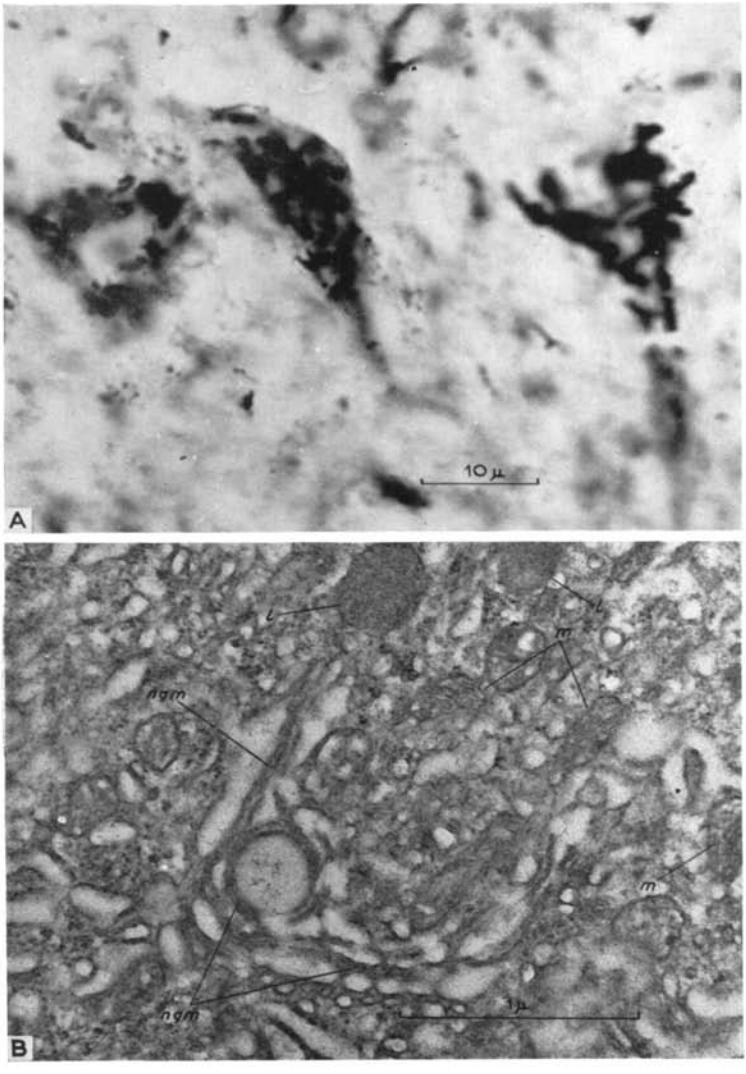

FIG. 2

S. K. MALHOTRA *and* G. A. MEEK

embedding material. It was thought that these spaces might be artifacts of fixation or embedding. Pieces of the cerebellum of *B. africanus* were prepared with the greatest care and embedded in araldite. The cisternae of the endoplasmic reticulum of the Purkinje cells of this owl were seen to be almost as widely dilated as in the first preparation. It was therefore concluded that these spaces were probably not artificial. No significant difference could be seen between the cytoplasmic inclusions of the Purkinje cells of the two species of owl, and the following description applies equally to both.

Four kinds of cytoplasmic inclusions can be clearly recognized in the electron micrographs. These are:

(1) endoplasmic reticulum with associated particles of Palade (1955*a*, 1958);
(2) non-granular membranes or γ-cytomembranes associated with vacuolar structures (the 'agranular reticulum' of Palay and Palade, 1955) often described as the 'Golgi apparatus' (Dalton and Felix, 1954; Hess, 1955; Sjöstrand, 1956; Lacy and Challice, 1957; Pollister and Pollister, 1957; Palay, 1958; Oberling, 1959);
(3) rounded bodies, presumably sections of spherical or ovoid lipid inclusions;
(4) mitochondria.

Endoplasmic reticulum. The most obvious and extensive structure seen in the cytoplasm is the granular endoplasmic reticulum. The elements of this system mostly form aggregates of varying sizes (fig. 1). These correspond to the Nissl bodies of preparations stained for light microscopy. The membrane-bounded cisternae of this reticulum are arranged in a more orderly way than in the Purkinje cells of other vertebrates (compare Palay and Palade, 1955). Very often they are placed more or less in parallel rows like those seen in the dorsal root ganglion cells (Hess, 1955; Palay and Palade, 1955). When this is so, the rows are more or less equidistant. A considerable variation in the width (reaching up to about 100 mμ) of the cavities of the endoplasmic reticulum is seen in the neurones fixed in buffered osmium tetroxide solution embedded in methacrylate (fig. 3) or araldite (fig. 4, A). Palay and Palade (1955) give 30–50 mμ as the common figures for the diameter of this lumen in the neurones of the rat. It is difficult to be certain whether the cisternae of the endoplasmic reticulum of the Purkinje cells of the owl are very wide in life. Living neurones of vertebrates show certain 'canalicular spaces' of very low refractive index in the cytoplasm; these are bigger in the Purkinje cells of the owl (Malhotra, 1960) than in the neurones of the mouse (Malhotra,

---

FIG. 2 (plate). A, light micrograph of an Aoyama preparation, showing 3 Purkinje cells of *S. aluco* with reticular apparatus of Golgi.

B, electron micrograph of part of the cytoplasm of a Purkinje cell of *S. aluco* (from material fixed in Palade's fluid and embedded in methacrylate). *l*, lipid globules; *m*, mitochondria; *ngm*, non-granular membranes.

1959*a*). These canalicular spaces may be the dilated cisternae of the endoplasmic reticulum seen in electron micrographs.

A very different appearance is presented by the cytoplasm of the Purkinje cells fixed in buffered potassium permanganate solution. The cisternae of the endoplasmic reticulum have disappeared, leaving well-preserved, closely apposed pairs of membranes (fig. 4, B). The rosettes of granules which are so prominent in the ground cytoplasm surrounding the cisternae in the osmium-fixed cytoplasm (fig. 4, A) are no longer present in the permanganate-fixed material. This is in accordance with the findings of Bradbury and Meek (1960) in exocrine pancreas cells. In view of the fact that permanganate has been shown to remove many of the cell components, it is probable that fixation by buffered osmium tetroxide gives a closer representation of the structures found in the living cell. It may be mentioned that the nuclear membrane is particularly prominent after permanganate fixation, and shows well-defined pores (fig. 4, B).

Occasionally, within an aggregate of the endoplasmic reticulum, an appearance resembling non-granular membranes is seen (fig. 3, A; arrows). The general morphology of these structures differs from the typical non-granular membrane system (part of the Golgi apparatus of Dalton and Felix, 1954), which is very rarely seen in the electron micrographs of this cell (fig. 2, B; *ngm*). In the typical non-granular membrane system, many membranes are closely packed against one another. In fig. 3, A the arrows show only two membranes in apposition, and these appear to be the membranes of two different cisternae. The space between them is very small, and there is scarcely room for the small granules of Palade. Thus the two membranes in apposition give, here and there, the appearance of 'non-granular membranes'. If they are indeed non-granular, they provide the best illustration yet of the continuity of granular and non-granular endoplasmic reticulum. However, close scrutiny of the narrow space between these membranes occasionally reveals particles of about the same size as Palade's granules (fig. 3, B, C). This appearance may, therefore, have been caused by artificial swelling of the cisternae of the endoplasmic reticulum resulting in a close apposition of the granular membranes. We are inclined to believe that this is the correct interpretation.

Elements of the endoplasmic reticulum are also scattered in the cytoplasm between the highly organized aggregates. They presumably correspond to the basiphil strands seen in Nissl preparations, connecting the aggregates into a reticulum. The aggregates of endoplasmic reticulum are seen in the dendrites of the cell, but not in the axons.

FIG. 3 (plate). A, electron micrograph of a typical aggregate of endoplasmic reticulum. *S. aluco*; fixed in Palade's fluid and embedded in methacrylate. Arrows indicate closely apposed membranes continuous with the membranes of the endoplasmic reticulum.

B, C, electron micrographs of *S. aluco* (Palade/methacrylate) at higher magnification than A, showing particles of Palade (*g*) in the ground cytoplasm between one cisterna (*c*) and another. In some places the cisternae are closely apposed to one another, leaving only a small intervening space in which the particles are visible here and there.

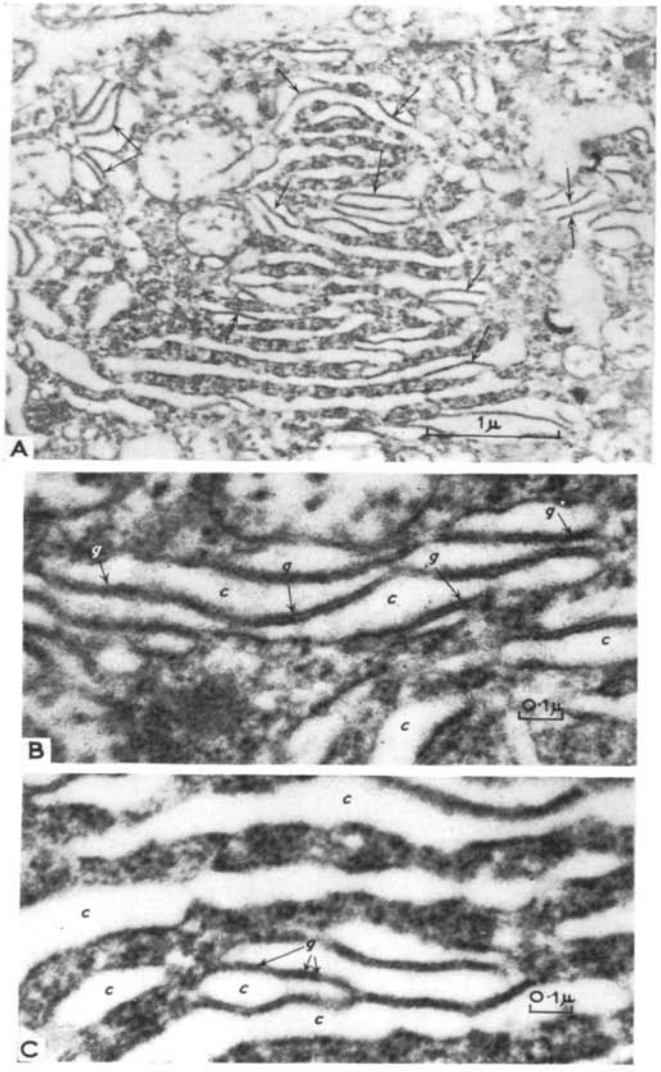

FIG. 3

S. K. MALHOTRA *and* G. A. MEEK

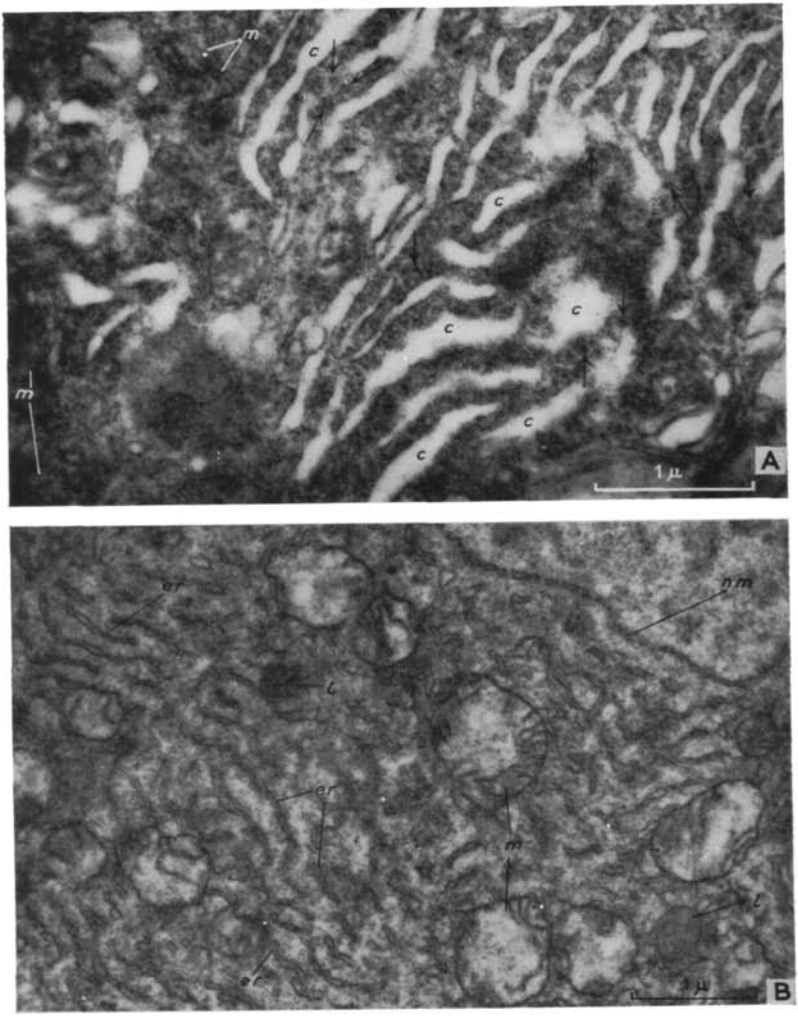

FIG. 4

S. K. MALHOTRA *and* G. A. MEEK

Non-granular membranes. The second structure is very rarely seen in the electron micrographs (fig. 2, B). It is composed of non-granular membranes associated with round or elongate vacuoles of very low electron density. These membranes are more closely packed than the elements of the granular endoplasmic reticulum. They are very scanty in the electron micrographs of the Purkinje cells of the owls.

Rounded bodies regarded as lipid globules. Most of the spherical or almost spherical bodies seen in the micrographs are bounded by a thin membrane (figs. 1; 2, B; 4, B). These bodies have a finely granular appearance and are electron-dense: the largest is a little less than 1 μ in diameter. Some of these inclusions show vacuoles of lower electron-density in them.

Mitochondria. These show an outer limiting membrane and the characteristic cristae (figs. 2, B; 4) (Palade, 1953; Hess, 1955; Palay and Palade, 1955; Andrew, 1956; Sjöstrand, 1956). They are spherical or elongate, narrow bodies. The longest seen in the electron micrographs measure about 3 μ.

Discussion

The general morphology of the endoplasmic reticulum seen in the electron micrographs of the Purkinje cells of *S. aluco* and *B. africanus* corresponds with the Golgi preparations of this cell (see fig. 2, A). It also resembles the illustration of the 'reticular apparatus' of Golgi (1898) in the Purkinje cells of *S. flammea.* It therefore seems likely that the Golgi apparatus seen in this cell by light microscopy is a deposit of silver or of osmium or of compounds of these metals on the endoplasmic reticulum. This finding is in accordance with the conclusions already reached by one of us (Malhotra, 1959*a*, 1960). The elements of the non-granular membranous system seen in the electron micrographs of the Purkinje cells of the owl are not nearly abundant enough to form the massive apparatus of Golgi. In view of these conclusions the use of the term 'Golgi apparatus' by Hess (1955), Palay (1958), and Oberling (1959) for the non-granular membranes of the neurones of vertebrates does not seem to be appropriate.

It has previously been shown that a reticulum, which corresponds in distribution to the endoplasmic reticulum, can be seen in the living Purkinje cell of the owl (Malhotra, 1960). This reticulum has an unusually great affinity for silver. The evidence suggested either that the endoplasmic reticulum of this particular cell contained more phospholipid than usual, or that its phospholipid component was more easily unmasked.

Fig. 4 (plate). A, electron micrograph of a typical aggregate of endoplasmic reticulum. *B. africanus*; Palade/araldite; compare with fig. 3. The arrows indicate rosettes of particles of Palade. *c*, cisternae; *m*, mitochondria.

B, electron micrograph of part of a Purkinje cell of *B. africanus*. Buffered potassium permanganate / araldite. Note the high contrast of the membrane structures of the endoplasmic reticulum (*er*), mitochondria (*m*), and nuclear membrane (*nm*) with well-defined pores. The membranes of the endoplasmic reticulum are closely apposed and not separated by cisternae as in material fixed in buffered osmium tetroxide (fig. 4, A). *l*, lipid globules.

It was stated earlier (Malhotra, 1959*a*) that the endoplasmic reticulum is not by any means always blackened by the Golgi methods. In the exocrine cell of the pancreas, where it exists in its closely packed form (Palade, 1955*a*, 1958; Sjöstrand, 1956; Haguenau, 1958), it was known as 'ergastoplasm' long before the use of the electron microscope in biology. The 'ergastoplasm' of the pancreatic cell and the 'Nissl substance' of the neurone of vertebrates are similar in their ultrastructure, but they react very differently to acid dyes. The ergastoplasm (besides being intensely basiphil) is also remarkably acidophil (Baker, 1959), whereas the Nissl substance shows no affinity for acid dyes (Malhotra, 1959*b*). In view of this striking difference in response to dyes, it is perhaps not surprising that the two objects react differently to osmium tetroxide and silver nitrate.

It is a pleasure to thank Dr. J. R. Baker, F.R.S., and Dr. R. Barer for their valuable criticism throughout the course of this work. This work was made possible by the generous loan of a Siemens electron microscope by the Wellcome Trust to the Department of Human Anatomy, Oxford. One of us (S. K. M.) is a Postdoctoral Research Fellow of the Panjab University (India), and received a Travel Grant under the Commonwealth University Interchange Scheme of the British Council.

REFERENCES

ANDREW, W., 1956. Internat. Rev. Cytol., **5**, 147.
BAKER, J. R., 1959. Unpublished observations.
BRADBURY, S., and MEEK, G. A., 1960. Quart. J. micr. Sci., **101**, 241.
DALTON, A. J., and FELIX, M. D., 1954. Amer. J. Anat., **94**, 171.
GLAUERT, A. M., and GLAUERT, R. H., 1958. J. biophys. biochem. Cytol., **4**, 191.
GOLGI, C., 1898. Arch. ital. Biol., **30**, 60.
HAGUENAU, F., 1958. Internat. Rev. Cytol., **7**, 425.
HESS, A., 1955. Anat. Rec., **123**, 399.
LACY, D., and CHALLICE, C. E., 1957. Symp. Soc. exp. Biol., **10**, 62.
LUFT, J. H., 1956. J. biophys. biochem. Cytol., **2**, 799.
MALHOTRA, S. K., 1959*a*. Quart. J. micr. Sci., **100**, 339.
—— 1959*b*. Unpublished observations.
—— 1960. Quart. J. micr. Sci., **101**, 69.
MEEK, G. A., 1960. IV. Internat. Kong. Elektmikr., Berlin.
NISSL, F., 1894. Neur. Centralbl., **13**, 676.
OBERLING, C., 1959. Internat. Rev. Cytol., **8**, 1.
PALADE, G. E., 1952. J. exp. Med., **95**, 285.
—— 1953. J. Histochem. Cytochem., **1**, 188.
—— 1955*a*. J. biophys. biochem. Cytol., **1**, 567.
—— 1955*b*. Ibid., **1**, 59.
—— 1958. *Frontiers in cytology*. New Haven (Yale University Press).
PALAY, S. L., 1956. *Neurochemistry*. London (Cassell).
—— 1958. *Frontiers in cytology*. New Haven (Yale University Press).
—— and PALADE, G. E., 1955. J. biophys. biochem. Cytol., **1**, 69.
POLLISTER, A. W., and POLLISTER, P. F., 1957. Internat. Rev. Cytol., **6**, 85.
SJÖSTRAND, F. S., 1956. Ibid., **5**, 455.