J. Anat., Lond. (1962), 96, 3, pp. 309–316
With 5 plates and 1 text-figure
Printed in Great Britain

Electron microscopy of experimental degeneration in the avian optic tectum

By E. G. GRAY and L. H. HAMLYN
Department of Anatomy, University College London

INTRODUCTION

One of the major problems of electron microscopy of the central nervous system is to locate with precision the region involved in the degenerative process in grey or white matter. For this reason, the avian optic tectum was chosen to study the effects of degeneration of axons and their terminals. Axon section is accomplished simply by unilateral removal of the eye so that the optic nerve afferent fibres to the contralateral hemisphere undergo degeneration (Evans & Hamlyn, 1956). Their trunks are easily located by electron microscopy since they enter the tectum as a superficial layer of myelinated fibres and the terminal ramifications and presynaptic processes are also easily located by reference to a discrete double layer of neuronal perikarya marking the deepest limits of their distribution (Evans & Hamlyn, unpublished; Cowan, Adamson & Powell, 1961).

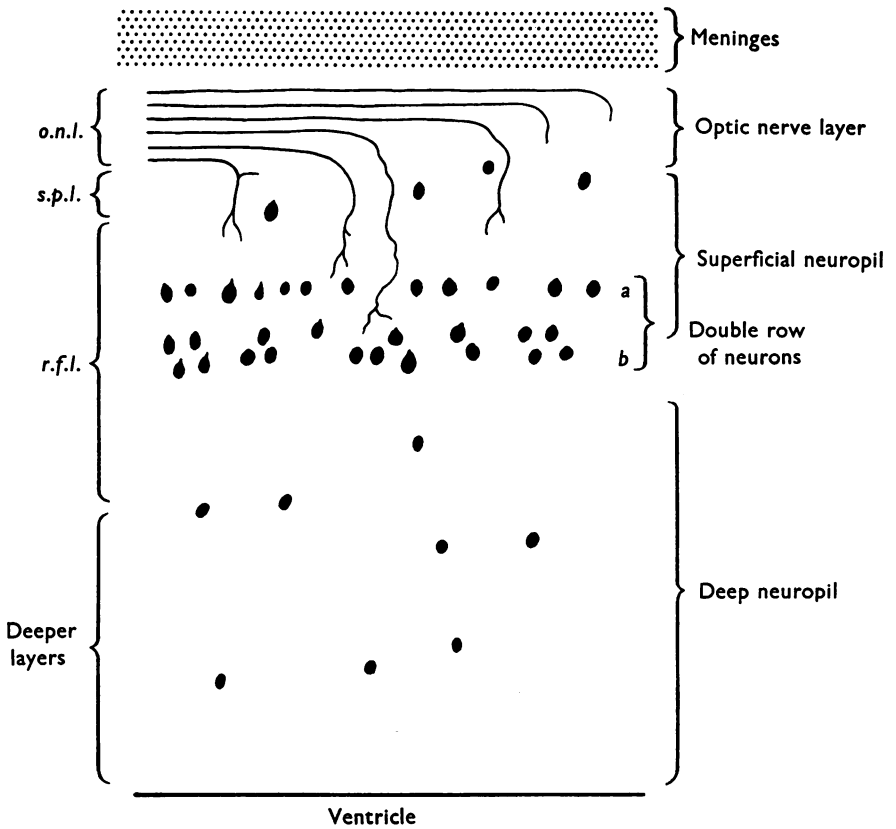
This present work follows naturally from the light-microscopic degeneration studies of Evans & Hamlyn (1956) with the Glees (1946) and Nauta–Gygax (1954) methods. The time courses of the two methods are quite distinct, the Glees method showing rings and clubs, absent from normal tectum, at the 7–11-day stage. At 28–30 days the Glees method is negative, but the Nauta–Gygax picture is fully established. Here electron microscopy has been used to follow these changes in the axons and their presynaptic processes in order to relate them to the different mechanisms of the Glees and Nauta techniques.

Few attempts have been made so far to study central nervous degeneration with the electron microscope. De Robertis (1956) has described experimental changes in the ventral acoustic nucleus. Bunge, Bunge & Ris (1960, 1961) have reported on experimental demyelination and remyelination in the spinal cord, the axons remaining apparently unaffected. In this account the simplified classification of the tectal layers used by Cragg, Evans & Hamlyn (1954) modified from van Gehuchten (1892) will be used.

METHODS

Adult chickens were lightly anaesthetized with sodium pentobarbital (Nembutal) followed by local infiltration of the orbit with 2% procaine and the eye was then enucleated using an aseptic technique. At intervals of 3, 6, 7 and 30 days the contralateral optic tectum was removed under anaesthesia and placed on filter-paper moistened with saline. A tangential slice, about 1 mm. thick, was quickly cut off with a razor blade and placed in 1% osmium tetroxide in saline, at pH 7·4. Vertical slices, about 0·3 mm. thick, were then cut from the slice and placed in fresh fixative for 3 hr. They were then dehydrated in ethanol, stained in alcoholic PTA and

embedded in Araldite for sectioning (see Gray (1959) for details). In addition to the operated animals, the optic tecta of three normal chickens were treated and examined in the same way. In all cases thick hand sections (see Gray, 1961) were first cut from the blocks and examined by phase-contrast microscopy. The pial surface with its conspicuous underlying zone of optic nerve fibres was identified (Text-fig. 1) and the block was trimmed to include the region between this and the double layer of neurons (*a* and *b*) crossing the middle of the radial fibre layer. In this way one can be certain of locating the region covering the entire course of the optic fibres (normal or degenerating) in the tectum in a single section in the electron microscope.



Text-fig. 1. Diagrammatic radial section of avian optic tectum based on Nissl preparations (for details see Cragg *et al.* 1954). The simplified nomenclature on the right has been used in this present work.

RESULTS

Normal tectum. A detailed description of the structure seen by light microscopy (Text-fig. 1) together with relevant references has been given by Cragg *et al.* (1954). By electron microscopy sections of the numerous myelinated axons from the retina could be observed forming the superficial optic fibre layer, their axoplasm containing the usual mitochondria and neurofilaments. Few if any unmyelinated axons are

present, although one cannot be certain for it is easy to confuse an unmyelinated axon containing neurofilaments with a process of a fibrous astrocyte.

The superficial neuropil lying immediately below this layer (Text-fig. 1) appears as a vast complex of profiles of neuronal and glial cell bodies and their processes. The optic fibre axons descend through this layer, where they form a proportion of the numerous axo-dendritic synaptic contacts that are present. The presynaptic processes range from about 0·4–2 μ in diameter and contain the usual synaptic vesicles, mitochondria and dense post-synaptic thickenings. No neurofilaments have ever been observed in the presynaptic bags of normal material from this region (Pl. 1, fig. 1). Both Type 1 and Type 2 contacts have been observed and the contact is either directly on the dendritic trunk or with a spine, which however in this situation contains no spine apparatus (see Gray, 1959). The deep neuropil (Text-fig. 1) has not been studied with the electron microscope since degeneration does not occur in this region.

EXPERIMENTAL MATERIAL

At the outset it should be mentioned that even in the 30-day stage the majority of presynaptic processes remain apparently unaltered and so are presumably not the terminals of the optic axons but of axons arriving from the deeper layers or from cells within the neuropil.

Three-day stage. At this stage no obvious changes were apparent in the myelinated axons of the optic nerve layer (Pl. 3, fig. 8). No high-resolution studies on the arrangement and spacing of the myelin lamellae have been attempted here, however, although this might well prove profitable in what is clearly an excellent experimental situation. The myelin sheaths remained intact and the axoplasm still contained mitochondria and neurofilaments.

A distinct change was observed in a few of the axon terminals in the superficial neuropil in that in addition to the synaptic vesicles and mitochondria, some now contained small bundles of neurofilaments (100 Å. in diameter). Pl. 1, fig. 2, shows such a presynaptic process. In contrast to a normal presynaptic process (Pl. 1, fig. 1) it contains neurofilaments which, in this case, are orientated in the form of a ring.

Six- and seven-day stage. Clear changes in the structure of the myelinated axons could be observed at this time. The general contours of the myelin sheaths appeared crinkled, and 'pale' fissures could be seen between the lamellae, which appeared less distinct than normal. Clear zones were also apparent between the innermost layer of the sheath and surface membrane of the axon. The general tubular arrangement of the myelin, however, remained normal. In general the axoplasm appeared little changed, although dense patches could be seen scattered within the groups of neurofilaments. Some of the mitochondria appeared pale, with apparent loss of internal structure.

Pronounced changes were observed in many of the larger axon terminals. The presynaptic cytoplasm becomes occupied with neurofilaments (Pl. 1, fig. 3) at the expense of the synaptic vesicles and mitochondria (compare normal structure, Pl. 1, fig. 1). Pl. 1, fig. 4, shows a less advanced stage, where synaptic vesicles and mitochondria are still present and neurofilaments are beginning to appear. In

Pl. 2, fig 5, very few synaptic vesicles remain and some of the mitochondria (m_1 and m_2) show clear zones; the clear vesicles (v) might represent later stages of mitochondrial degeneration. Pl. 2, fig. 7, shows an '*en passant*' contact, and again the presynaptic process is packed with neurofilaments.

A small proportion of the degenerating presynaptic processes did not show a proliferation of neurofilaments (Pl. 2, fig. 6). Instead the vesicles were aggregated into a dense mass in the presynaptic cytoplasm.

Thirty-day stage. Drastic changes could be observed in practically all the myelinated axons of the optic nerve layer at this stage. The sheaths appeared irregular in outline with globules or complicated foldings and with electron-transparent gaps in the lamellae. In many cases (Pl. 4, fig. 10) no axoplasmic material could be detected within the myelin sheath, only a very electron-transparent zone. In other examples, dense granular material, presumably derived from axoplasm, was still present at this stage. Frequently, reactive neuroglial cells occurred within the myelin sheaths (Pl. 4, fig. 9).

It will be recalled that by 8 days many boutons showed a depletion or absence of synaptic vesicles and mitochondria and the cytoplasm contained instead numerous neurofilaments. The surface membrane and synaptic thickening appeared normal however. At 30 days these endings packed with neurofilaments had almost completely disappeared. In a few cases a presynaptic process (Pl. 4, fig. 11) consisting of irregular dense masses (x) (degenerating mitochondrial material?) related to a cup-shaped clear process, presumably glial (gl) was observed. The surface membrane of the presynaptic process is not distinguishable, although the post-synaptic membrane and thickening remain apparently normal.

In most cases thickening of the post-synaptic membrane had disappeared, suggesting a transneuronal change. Normal post-synaptic thickenings were observed in only two cases (Pl. 5, fig. 12—enlarged in Pl. 5, fig. 13; Pl. 5, fig. 14) in which the presynaptic process was completely replaced by a neuroglial cell recognisable by its nucleus (*nuc.g.*). The cytoplasm of the cell contained clusters of small granules, presumably ribosomes.

DISCUSSION

The appearance of rings, clubs or reticulated boutons during the 8 days after eye removal in Glees preparations (Evans & Hamlyn, 1956) is clearly correlated with the appearance in electron micrographs of neurofilaments in the presynaptic cytoplasm, replacing the synaptic vesicles and mitochondria. These neurofilaments are presumably 'solid' protein fibrils, 100 Å in diameter, and they clearly form a basis for the staining of neurofibrillae by reduced silver methods of light microscopy (see Palay & Palade, 1955; Boycott, Gray & Guillery, 1960, 1961; Gray & Guillery, 1961). Using the Glees method it is clear that the neurofilaments in normal axon trunks stain, and normal boutons stain when the presynaptic process contains neurofilaments. In the normal tectum, however, boutons cannot be stained by the Glees method, because they contain no neurofilaments. At present it is not known whether the filaments make their appearance because they are newly synthesized from amino acids, or are merely precipitated from a previously soluble form of protein in the cytoplasm (Guillery, unpublished). Precipitation might result from slight

changes of pH for example (see Maxfield & Hartley, 1957) in the degenerating cytoplasm. If this second interpretation is the case, then the soluble form is apparently not argyrophilic, since no boutons appear in the unoperated tectum. It is of interest that neurofibrillae appear in the cytoplasm of chromatolytic neuron perikarya (Cajal, 1928; Young, 1932) and a corresponding increase in their electron microscope counterpart, the neurofilaments, can also be seen in chromatolysis (Evans & Gray, 1961). In both structures the proliferation is an injury reaction, but in the tectum it occurs in the distal stump, deprived of continuity with the cell body. Can protein synthesis take place in this isolated state?

It has long been known that degeneration in the cerebral cortex and certain other regions does not result in the appearance of ring-shaped, clubbed or reticulated boutons (see Evans & Hamlyn, 1956). It can now be concluded that the reason is that presynaptic processes in these regions do not produce neurofilaments during the degenerative cycle of their cytoplasm. It could be that, in these regions, the rate of enzymic or phagocytic destruction is so fast that the degenerating endings never exist long enough to reach a neurofilamentous phase. Alternatively, there might be fundamental differences in metabolism in the endings that do and those that do not produce neurofilaments. Experiments are in progress to elucidate this point. It must be remembered that at present it is not understood why only a proportion of apparently normal boutons contain neurofilaments (see Boycott *et al.* 1960, 1961; Gray & Guillery, 1961).

The staining reaction of the Nauta–Gygax method (Nauta & Gygax, 1954) is much more difficult to correlate with the electron microscopic observations. Clearly, since the Nauta–Gygax staining is ineffective at the time when the Glees method is staining fibres in the normal tectum and degenerating fibres and their boutons in operated animals (Evans & Hamlyn, 1956), then the Nauta–Gygax method, unlike the Glees, does not stain neurofilaments at these stages. At 30 days after enucleation, the Glees method shows practically nothing, but the Nauta–Gygax method has a maximum effect, showing fine threads and droplets (Evans & Hamlyn, 1956; Cowan *et al.* 1961). At this stage, with the electron microscope the myelin of the optic fibres is still present, though clearly in a degenerated condition. The tubular structure is still often recognizable, although 'spherical' masses of fragmenting myelin are also a common feature. Degenerating axoplasm is also still present at this time, and neurofilaments may occasionally still be recognizable, or dense granular material may be present in the axoplasm. Although many of the degenerating myelin sheaths appear to have a completely structureless lumen, it cannot be assumed that in these cases the axoplasmic material has entirely disappeared. It could be then, that both degenerating myelin and axoplasm are involved with the Nauta–Gygax method as suggested by Evans & Hamlyn (1956).

In 30-day preparations it was surprising to find an almost complete disappearance of the optic axon terminals that were plentiful and packed with neurofilaments on the eighth day. No phagocytosed masses that might correspond with these structures could be detected in glial cytoplasm. It could be, therefore, that degenerating axoplasm in this situation is removed by extracellular enzymes. Myelin debris is probably ultimately engulfed by phagocytic glial cells (see Bunge *et al.* 1960, 1961). Some evidence for this could be seen in the 30-day preparations but investigations

over a longer period are necessary to determine more precisely the ultimate fate of the myelin.

The fate of the post-synaptic thickening is not yet understood. In two instances the post-synaptic thickening remained apparently unchanged at thirty days although it was contacted by a glial cell, which had completely replaced the presynaptic axonal process. However, since all the neurofilament-filled boutons have disappeared at 30 days, it was surprising that their post-synaptic thickenings could not still be detected. It seems that either the post-synaptic thickening is lost and the membrane returns to an unspecialized state (unless contacted by a glial cell), representing a transneuronal change, or at 30 days, sprouts have grown in and the junctions have become re-innervated (cf. Gutmann & Young, 1944). These synapses would not be distinguishable from the numerous normal ones that remain apparently unaltered throughout. These new connexions would of course be abnormal since the sprouts would not be derived from the optic fibres, which at this stage are in an advanced state of degeneration.

In conclusion it is clear that much remains to be done. Intervals between 8 and 30 days need close study with the electron microscope to follow the complete degenerative cycle and final disappearance of the optic fibre boutons, and the fate of the post-synaptic thickening needs closer investigation. Also stages later than 30 days should show the final fate of the myelin. There is no doubt, however, that this is a very useful situation for the study of degeneration in the central nervous system.

SUMMARY

1. Changes in the axons, their presynaptic processes and neuroglia of chicken optic tectum that result from section of the contralateral optic nerve, have been studied by electron microscopy. Observations have been correlated with those of light microscopy.

2. During the first 8 days the synaptic vesicles in the presynaptic processes disappear, the mitochondria become disorganized and the presynaptic cytoplasm becomes crammed with neurofilaments.

3. At 30 days the majority of these presynaptic processes have completely disappeared. Occasionally a glial cell could be observed occupying a 'presynaptic' position.

4. At 30 days axoplasm and myelin sheaths of the optic fibres can still be observed, though in various stages of degeneration.

5. The problems of correlating these observations with the degenerative changes observed in the Glees & Nauta methods by light microscopy are discussed.

We are indebted to Professor J. Z. Young, F.R.S., and Dr R. W. Guillery for discussion and advice; to Miss J. de Vere for Text-fig. 1 and to Miss J. Spillman for photography.

REFERENCES

BOYCOTT, B. B., GRAY, E. G. & GUILLERY, R. W. (1960). A theory to account for the absence of boutons in silver preparations of the cerebral cortex, based on a study of axon terminals by light and electron microscopy. *J. Physiol.* **152**, 3–5*P*.

BOYCOTT, B. B., GRAY, E. G. & GUILLERY, R. W. (1961). Synaptic structure and its alteration with environmental temperature: a study by light and electron microscopy of the central nervous system of lizards. *Proc Roy. Soc.* B, **154**, 151–172.

BUNGE, M. B., BUNGE, R. P. & RIS, H. (1960). Electron microscopic study of demyelination in an experimentally induced lesion in adult cat spinal cord. *J. biophys. biochem. Cytol.* **7**, 685–696.

BUNGE, M. B., BUNGE, R. P. & RIS, H. (1961). Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. *J. biophys. biochem. Cytol.* **10**, 67–94.

CAJAL, S. R. (1928). *Degeneration and Regeneration of the Nervous System*, vol. II. New York: Haffner.

COWAN, W. M., ADAMSON, L. & POWELL, T. P. S. (1961). An experimental study of the avian visual system. *J. Anat., Lond.*, **95**, 545–562.

CRAGG, B. C., EVANS, D. H. L. & HAMLYN, L. H. (1954). The optic tectum of *Gallus domesticus*: a correlation of the electrical responses with the histological structure. *J. Anat., Lond.*, **88**, 292–305.

DE ROBERTIS, E. (1956). Submicroscopic changes of the synapse after nerve section in the acoustic ganglion of the guinea pig. *J. biophys. biochem. Cytol.* **2**, 503–512.

EVANS, D. H. L. & GRAY, E. G. (1961). Changes in the fine structure of ganglion cells during chromatolysis. In *Cytology of Nervous Tissue.* London: Taylor and Francis.

EVANS, D. H. L. & HAMLYN, L. H. (1956). A study of silver degeneration methods in the central nervous system. *J. Anat., Lond.*, **90**, 193–202.

GEHUCHTEN, A. VAN (1892). La structure des lobes optiques chez l'embryon de poulet. *Cellule*, **8**, 1re fascicule.

GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath.* **5**, 54–59.

GRAY, E. G. (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat., Lond.*, **93**, 420–433.

GRAY, E. G. (1961). Accurate localization in ultrathin sections by direct observation of the block for trimming. *Stain Tech.* **36**, 42–44.

GRAY, E. G. & GUILLERY, R. W. (1961). The basis for silver staining of synapses of the mammalian spinal cord: a light and electron microscope study. *J. Physiol.* **157**, 581–588.

GUTMANN, E. & YOUNG, J. Z. (1944). The re-innervation of muscle after various periods of atrophy. *J. Anat., Lond.*, **78**, 15–43.

MAXFIELD, M. & HARTLEY, R. (1957). Dissociation of the fibrous protein of nerve. *Biochim. biophys. acta*, **24**, 83–87.

NAUTA, W. J. H. & GYGAX, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system: a modified technique. *Stain Tech.* **29**, 91–93.

PALAY, S. L. & PALADE, S. E. (1955). The fine structure of neurons. *J. biophys. biochem. Cytol.* **1**, 68–88.

YOUNG, J. Z. (1932). On the cytology of the neurons of cephalopods. *Quart. J. Micr. Sci.* **75**,1–47.

LIST OF ABBREVIATIONS

cyt neuroglial cell cytoplasm
gf neuroglial fibrils
gl neuroglial process
m mitochondrion
my myelin
nf neurofilaments of axoplasm
nuc g neuroglial cell nucleus
onl optic nerve layer
post post-synaptic membrane thickening
rib ribosomes (RNP granules)
rfl radial fibre layer
rnf ring of neurofilaments
spl superficial plexiform layer
sv synaptic vesicles
v clear vesicle
x dense material in degenerating presynaptic cytoplasm

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Synapse of optic tectum with normal structure.

Fig. 2. 3-day stage. Bouton contains a ring of neurofilaments.

Fig. 3. 6–7-day stage. The presynaptic cytoplasm is crammed with neurofilaments.

Fig. 4. 6–7-day stage. Neurofilaments in presynaptic cytoplasm. Some synaptic vesicles and mitochondria are still present.

PLATE 2

Fig. 5. 6–7-day stage. Terminal containing neurofilaments, mitochondria (degenerating?) and large vesicles.

Fig. 6. 6–7-day stage. Dense and tightly packed synaptic vesicles in presynaptic process.

Fig. 7. 6–7-day stage. *En passant* synaptic contact crammed with neurofilaments.

PLATE 3

Fig. 8. 3-day stage. Region of the optic fibre layer.

PLATE 4

Fig. 9. 30-day stage. Myelin sheath containing reactive neuroglial cell.

Fig. 10. 30-day stage. Myelin sheaths with structureless lumina.

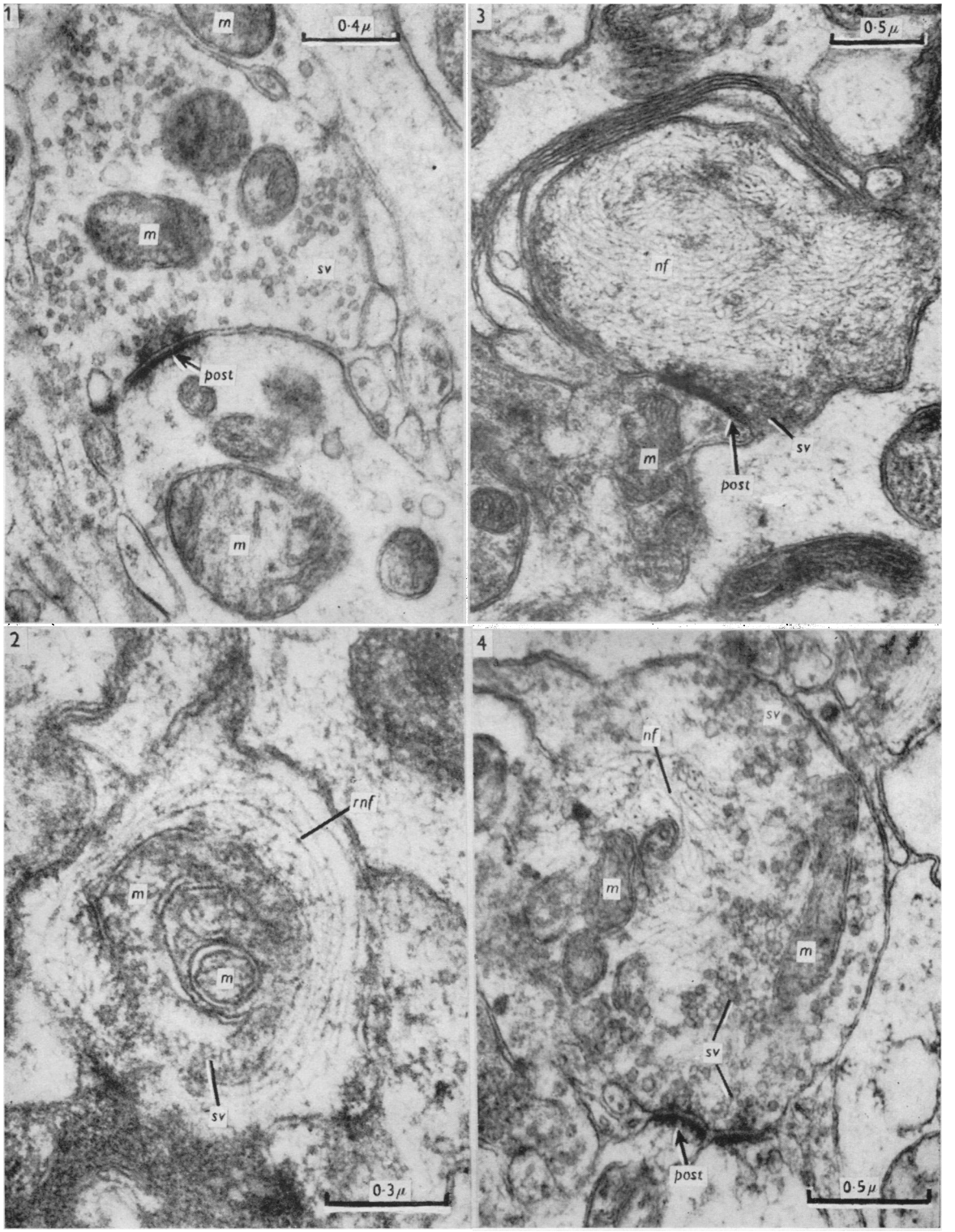
Fig. 11. 30-day stage. Dense masses (x) are apparently the debris from synaptic vesicles and mitochondria of a degenerating presynaptic process.

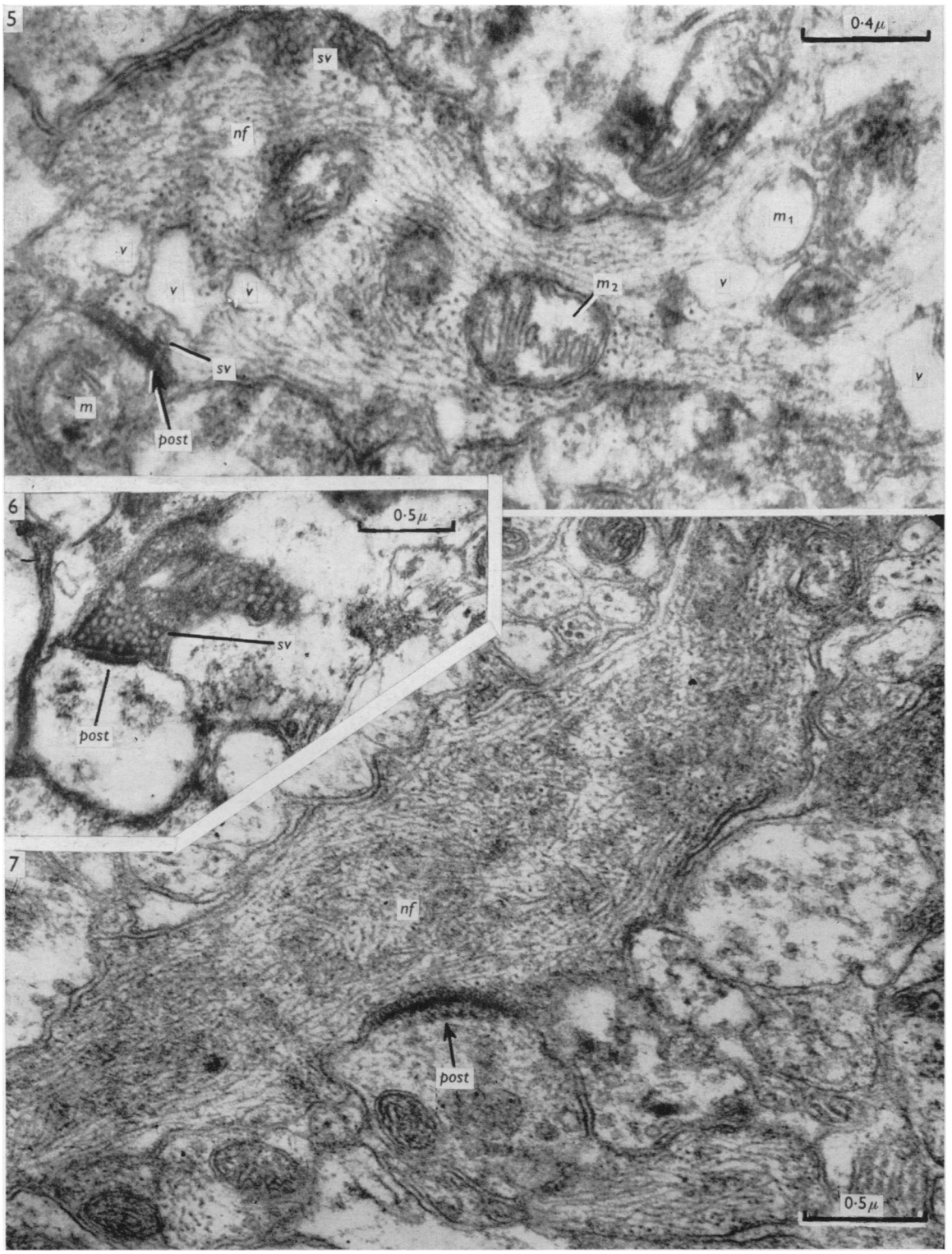
PLATE 5

Fig. 12. 30-day stage. Neuroglial cell has replaced a presynaptic process.

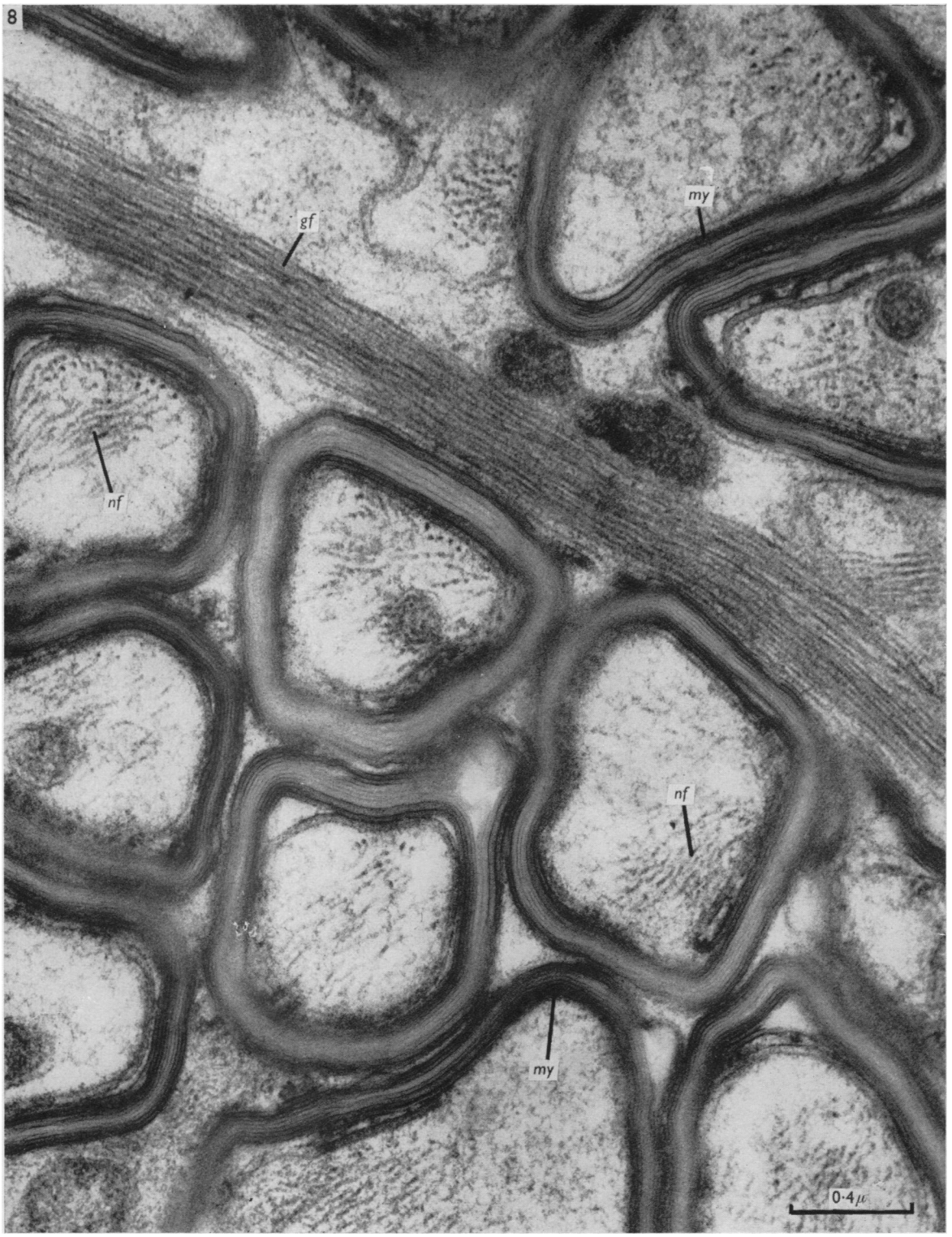
Fig. 13. 30-day stage. Neuroglial process has replaced presynaptic process.

Fig. 14. 30-day stage. Enlargement of contact region in fig. 12.

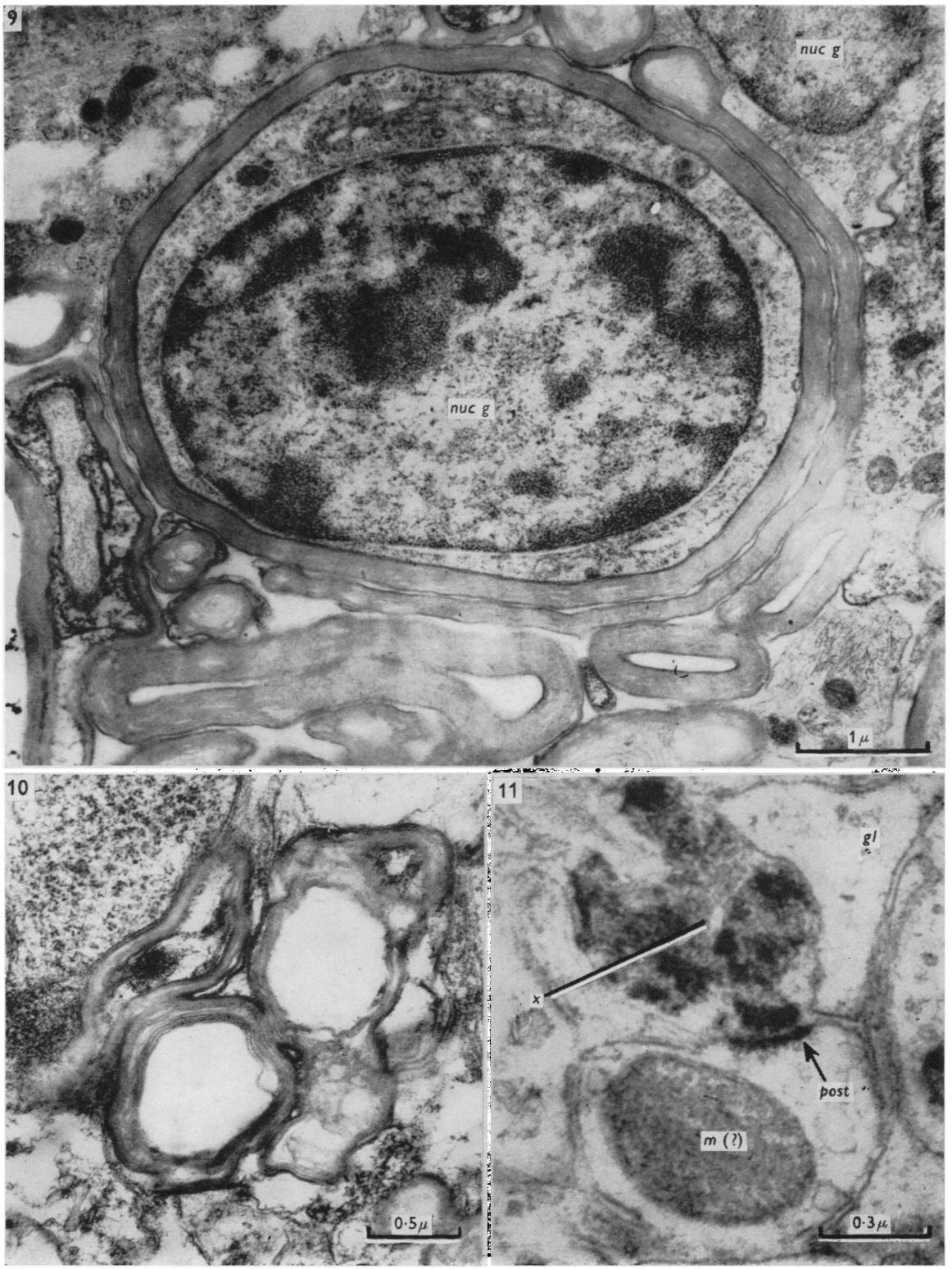

1
m
0·4 μ
m
sv
post
m
3
0·5 μ
nf
m
sv
post
2
rnf
m
m
sv
0·3 μ
4
sv
nf
m
m
sv
post
0·5 μ

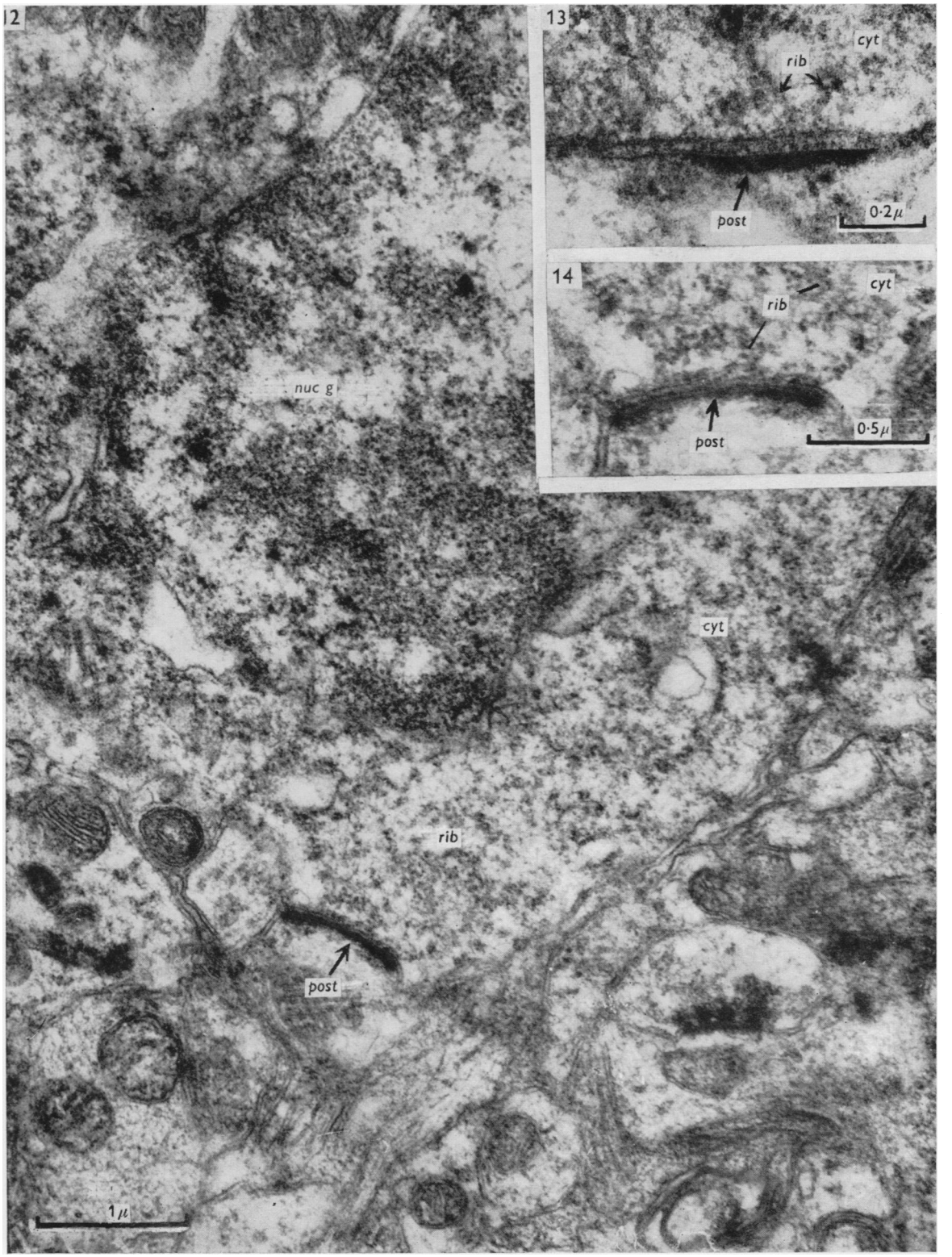


E. G. GRAY AND L. H. HAMLYN



E. G. GRAY AND L. H. HAMLYN


9
nuc g
nuc g
1 μ
10
0·5 μ
11
gl
x
post
m (?)
0·3 μ


12
nuc g
cyt
rib
post
1μ
13
cyt
rib
post
0·2μ
14
cyt
rib
post
0·5μ