A STUDY OF SILVER DEGENERATION METHODS IN THE CENTRAL NERVOUS SYSTEM

By D. H. L. EVANS and L. H. HAMLYN

Department of Anatomy, University College London

INTRODUCTION

A number of reduced silver methods have been used for the demonstration of degenerating axons and their terminations within the central nervous system. The present study is an attempt to evaluate the relative potentialities of the two methods which the authors have found most useful, namely the Gless and Nauta techniques.

Much of the literature dealing with silver impregnation methods, as applied to the termination of central nervous axons, is concerned with investigations into the structure of the synaptic region. Studies on the appearances of boutons terminaux, both normal and degenerating, have been reviewed by Gibson (1937), and recently by Glees & Nauta (1955). Work by Glees & Le Gros Clark (1941) and Glees (1941, 1942, 1944) has indicated that reduced silver techniques are especially useful for the investigation of neuro-anatomical connexions in experimental material. It is with this aspect that the present paper is concerned.

Following the use of several modifications of the Bielschowsky–Gros technique, Glees developed a reduced silver method that is particularly suitable for the investigation of degenerating material (Glees, 1946). However, this technique also stains normal fibres, with the result that degenerating axons that are scattered through a matrix of normal fibres are not easily seen. This disadvantage has been overcome by a method designed to stain the degenerating axons while suppressing the normal fibre pattern (Nauta & Ryan, 1952), and this technique was later modified to give greater consistency in staining (Nauta & Gygax, 1954).

Although several authors have investigated the degeneration cycle of boutons terminaux in their earlier stages (Hoff, 1932; Foerster, Gagel & Sheehan, 1933; Gibson, 1937), there appears to be little data on the time of persistence of the degeneration material derived from the axonal terminal aborizations or from degenerating axons of passage within the central nervous system. Furthermore, the appearance of degeneration produced by the Nauta technique differs from that given by the Glees method, and in some situations has a different time course.

In the present work the two methods have been used at different sites within the central nervous system, with a view to determining their relative advantages, and to comparing the appearances produced by each. The time course of the degeneration process has been studied with particular reference to the time of persistence of the degeneration material.

It was found that degeneration products stained with the Nauta method persisted for a much longer period than those stained by the Glees method. Additional experiments were therefore carried out in an attempt to investigate the nature of

the degeneration products demonstrated by the two methods. In view of the delayed absorption of the Nauta degeneration material it was thought that it may consist, at least in part, of lipid compounds. The effects of various methods of fat extraction both alone and preceded by fat fixatives were therefore investigated.

For the purpose of this work sites of two sorts have been chosen, some that permit a massive degeneration to be caused, and others where relatively few fibres out of the total become degenerated. Massive degeneration of axonal terminal aborizations occurs in the avian optic tectum following section of the contralateral optic nerve, and in the mammalian gracile and cuneate nuclei after hemisection of the spinal cord. Relatively sparse degeneration is seen in the mammalian cerebral cortex following a lesion of the contralateral hemisphere. In addition to these situations the process of degeneration has been studied in the tractus solitarius and its nucleus following section of the vagus nerve above the nodose ganglion.

MATERIAL AND METHODS

Operative techniques

Avian optic tectum. Adult chickens of various breeds and both sexes were used. For the anaesthetic pentobarbitone sodium (Nembutal) was used as described by Cragg, Evans & Hamlyn (1954), and in addition the orbit was infiltrated with 2 % procaine. The eye was then enucleated using an aseptic technique, and survival times allowed of 3, 5, 9, 11, 13, 15, 19, 28 and 32 days. Following pentobarbitone sodium anaesthesia the brain was removed and the two optic tecta were fixed by immersion in 10 % neutral formol saline. After a fixation time of not less than 7 days frozen sections were cut in the coronal plane at 20 μ and stored in 10 % neutral formol saline. In view of the complete decussation in the chiasma in birds, the tectum of the ipsilateral side of the lesion was used as the control.

Mammalian material. Adult rabbits of various breeds and both sexes were used. All operative procedures were carried out under pentobarbitone sodium anaesthesia (30 mg./kg. body weight intravenously), supplemented when necessary by open ether. After survival periods ranging from 3 to 170 days the animal was anaesthetized and the whole brain fixed *in situ* by perfusion with 10 % neutral formol saline. The brain was then removed and stored in the same fixative for at least 7 days. All materials were sectioned at 20 μ thickness with the freezing microtome and the sections stored in the fixative.

Cerebral cortex. The cranium was exposed and trephined over the parietal cortex and an area of 5 mm. diameter of the full thickness of the cortex removed. Survival periods of 5–6 days were allowed. The hemisphere on the side opposite to the lesion was sectioned parasagittally using the procedure described. Control material was obtained from a normal animal at the same time.

The central connexions of the vagus nerve

Degeneration of the sensory fibres of the vagus in the medulla was produced by unilateral excision of the nodose ganglion. This was performed in six rabbits with survival periods ranging from 3 to 170 days.

Terminations of fibres of the gracile and cuneate fasciculi in their respective nuclei

Degeneration in these nuclei was studied in one cat in which the spinal cord had been hemisected at the level of the atlas vertebra 5 days previously.

Staining techniques

Three methods were used: The Bielschowsky–Gros with slight modification, the Glees (1946) and the Nauta & Gygax (1954). As far as the Glees and Bielschowsky methods are concerned, the histological picture produced was the same. The Glees method, however, proved more reliable, and gave a much more even impregnation of the section.

(1) For the Bielschowsky–Gros preparations, the procedure is as follows:

(*a*) Store the sections in 10 % neutral formol saline and wash in three dishes of distilled water.

(*b*) Impregnate in 10 % silver nitrate for 15–25 min.

(*c*) Pass through four baths of 10 % formalin with tap water plus six drops of pyridine per 25 ml. (10 ml. May and Baker 40 % formaldehyde solution and 90 ml. of tap water) until no further white precipitate appears. The time in the formalin baths is critical. The best results are obtained when the sections have become greyish brown in colour. This takes about 15–30 min. The first formalin bath should contain a large volume of fluid (about 200 ml.); trail the sections through this with a glass rod so as to leave most of the precipitate in this bath.

(*d*) For this stage and until after reduction sections are handled individually. Blot the section and put it into an ammoniacal silver solution for 30–60 sec., made up as follows: 10 % silver nitrate, 5 ml.; 0·880 ammonia drop by drop until no further precipitate appears. Then add two drops excess ammonia.

(*e*) Wash sections briefly in distilled water.

(*f*) Reduce in 10 % neutral formol in distilled water.

(*g*) Wash.

(*h*) Fix in 5 % sodium thiosulphate for 2 min.

(*i*) Wash thoroughly.

(*j*) Dehydrate, clear in creosote and mount in neutral Canada balsam.

(2) The Glees method (1946) was used without modification, except for a quick rinse in distilled water between the ammoniacal silver bath and the reducer.

(3) In general, for the Nauta method, the technique of Nauta & Gygax (1954) was used as described by those authors, except that the preliminary treatment with 15 % ethyl-alcohol was usually left out. This appeared to make no difference to the result.

(4) *Controls.* In the case of the tractus solitarius and nucleus and the gracile and cuneate nuclei, the corresponding normal structures on the opposite side served as a control. For the optic tectum and the rabbit cortex, the controls were separate sections. The experimental sections and the controls were therefore stained at the same time and with the same reagents in two series of dishes until the ammoniacal silver bath was reached. At this stage sections have to be handled individually. The sections were therefore passed through the ammoniacal silver bath and reducer

alternately from the experimental and control series, and then for washing fixation, etc., were again taken into separate dishes.

(5) *Lipid fixation and extraction.* The sites used for this investigation were the tractus solitarius and nucleus and the funiculus cuneatus and nucleus; both Glees and Nauta methods were applied to these. The Glees method was also applied to the avian optic tectum following fat extraction.

For lipid extraction frozen sections were first placed in 50 % alcohol for 12 hr., followed by dehydration in ascending alcohols. The sections were then placed in one of the following mixtures for 90 min.: alcohol–chloroform, 1 : 1; ether–chloroform, 1 : 1; dioxan–iso-propyl alcohol, 1 : 1.

The sections were then brought to water and stained with the methods described.

RESULTS

The general appearances seen with the Glees and Nauta methods

When fully developed the Glees preparations showed a characteristic degeneration appearance against the normal fibre background. The most prominent feature of this was the progressive thickening and fragmentation of the terminal axonal aborizations. Many club and ring-like forms were seen on and near the nerve cell surfaces (Pl. 1, figs. 1, 2). These were absent in the control tectum (Pl. 1, fig. 3). In many instances a fine fibre could be seen in continuity with one of these forms. Whether these endings represent degeneration of the ultimate synaptic structures could not be decided on the evidence available. Massive degeneration of the larger fibre tracts was readily observed in the Glees preparations, but axons in small bundles or in those running individually could not be seen owing to the many normal fibres present.

In critically stained sections the Nauta technique allowed suppression of the staining of the normal fibres (Pl. 1, fig. 5). Consequently, those that were degenerating stood out clearly against a yellow background (Pl. 1, fig. 4). Characteristic of the degeneration was the drop-like disintegration of the fibres described by Nauta & Ryan (1952). Although in the Nauta preparations degeneration was seen in the immediate pericellular region the formations appeared to differ from those of the Glees preparations in that evidence of continuity could be seen. By this is meant that either a stained fragment of the continuing fibre could be observed or its 'ghost-like' outline (Pl. 1, fig. 6).

Time course of degeneration in different sites

Avian optic tectum. In this situation both Glees and Nauta degenerations were delayed in appearance compared with the other sites studied. The Glees preparations showed only increased affinity for silver staining of the interrupted optic axons after 5 days, and the fully established picture of degenerating terminal aborizations was observed only after a 9 to 11-day interval. From 15 days onwards the degenerating material was decreased in quantity, and by 28 days only a little granular debris and some fragments of thicker fibres were visible.

Sections taken from the same block were also stained by the Nauta method. After

5 days' degeneration this technique showed only a few fibres, not yet in process of disintegration in the optic fibre layer only (Pl. 2, fig. 7). As the control sections were completely negative (Pl. 4, fig. 20) staining of these fibres was presumably due to the increased argyrophilia already noted in the early Glees preparations. At 9 days evidence of fragmentation and drop-like disintegration was apparent and by 28 days (Pl. 2, fig. 8) full Nauta degeneration was established and reached the same depth from the tectal surface as that seen in the Glees preparation. Control sections were at all times completely negative and there was no staining of normal fibres.

Mammalian preparations

The Nauta staining was consistent in the sites studied. Degeneration was seen as early as 3 days, following the lesion and was fully established by 5 days (Pl. 1, fig. 4). In the case of the Glees preparations positive results were obtained only in the cuneate and lateral cuneate nuclei.

In the cerebral cortex, the dense felt-work of the normal neuropil masked any sign of degeneration with the Glees method and even with the Nauta slides as a guide, no evidence of degeneration could be found (Pl. 3, fig. 18).

Repeated attempts were made to obtain evidence of degeneration in the tractus solitarius and nucleus with the Glees method. Although the site could be exactly localized by means of Nauta preparations from the same block, no appearances of degeneration were observed in the Glees material at the times examined (3, 4, 5, 17 and 45 days).

Time of persistence of Nauta degeneration material

The process of disappearance of degeneration products has been studied in the tractus solitarius and its nucleus following supranodose (extracranial) vagotomy. Survival periods ranged from 3 to 170 days. The quantity of degeneration material, which was maximal at 5 days, showed little change up to 45 days (Pl. 2, fig. 9). It was diminished at 112 days (Pl. 2, fig. 10), and by 170 days it had completely disappeared (Pl. 4, fig. 21). It was interesting to note that the degeneration was most persistent in the nucleus, whereas it was absent in the tract at an earlier stage (112 days).

The effect of fat extraction on the staining techniques

In view of the observed differences in the morphology and time course of the two types of degeneration material, it was thought that the chemical processes involved might be different. The long persistence of the degeneration in the case of the Nauta method suggested that lipid elements were being stained.

To investigate this possibility, treatment with fat solvents was carried out as already described, the sites concerned being the avian optic tectum and cuneate nuclear complex. The results were very different in the Nauta preparations compared with the Glees. Of the fat solvents used, the alcohol-chloroform mixture was found to be the most effective. In the case of the Glees preparations, preliminary treatment with this mixture resulted in an overall improvement in staining compared with the non-treated controls (Pl. 4, figs. 23, 24). The background was clearer,

and the neuropil and fibre tracts more uniformly stained. The characteristic degeneration forms were easily observed and undiminished in density.

The effect of using the same solvent prior to carrying out the Nauta process was to abolish completely the staining of degenerated fibres (Pl. 2, fig. 11), whereas these were seen in abundance in the non-treated controls (Pl. 2, fig. 12).

The ether-chloroform and dioxan-isopropyl alcohol mixtures produced similar results but were less effective, the Nauta staining being only incompletely abolished.

These results indicate that the Nauta technique stains altered lipid substances in the degenerating nerve fibres. At the same time the persistence of the Glees degeneration products following fat extraction shows that the chemical processes are different and do not involve the staining of lipid substances.

An attempt to fix the lipids before fat extraction and Nauta staining was made by preliminary mordanting of the sections in 5 % potassium bichromate for 2 days. This enabled the Nauta degeneration material to be stained in spite of the fat extraction (Pl. 3, fig. 13). But in contrast to the homogeneous yellow background normally found, additional elements were stained in non-degenerated regions. In tracts where large fibres were seen in transverse section (medial lemniscus, normal tractus cuneatus), examination under low power showed black rings surrounding the nerve fibres. At higher magnifications the rings were seen to be crenated and only incompletely enclosed the light brown axon. On examination in optical section, it was seen that the distance between the inner margin of the ring and the axon varied, suggesting that the ring was part of a reticulated structure (Pl. 3, fig. 14). This was confirmed by examining fibres in the nucleus cuneatus which were running in the plane of the section. A coarse and wide-meshed reticulum was seen surrounding the axon, apparently occupying the region of the myelin sheath (Pl. 3, fig. 15; Pl. 4, fig. 22), although the latter was not stained.

The appearances of the degenerating tractus cuneatus were different in that as well as the reticulum which was found in the normal regions of the section, the axonal degeneration characteristic of the Nauta stain was also present.

Closer examination showed that the reticulum in this degenerating tract was in the process of disintegration. In transverse section it could frequently be seen that the reticular ring surrounded a darkly staining axon, itself in process of degeneration (Pl. 3, fig. 16).

DISCUSSION

Time course of the degeneration process

The time of appearance of degeneration of axons and their terminal arborizations as studied by reduced silver methods varies to some extent between different species, and more particularly between different classes of animals. In mammals Glees & Nauta (1955) point out that corticofugal fibres will degenerate to the stage of 'drop-like' disintegration within 5 days in the rat and cat, but in the monkey require upwards of 7 days to reach the same stage. Armstrong (1950), in a study of the visual pathways in reptile, noted that after eye enucleation no changes were observed in the axons of the optic tract earlier than 7 days following operation, and that this was followed by a period of 6 days, during which the only change was a progressive increase in the affinity of the axons for silver. Evidence, in the form of

fine rings, of the degeneration of the terminal aborizations within the lateral geniculate nucleus was first seen on the sixth day, and from this time onwards thickening and fragmentation of the aborizations was progressive. The degeneration material had mainly disappeared by 25 days and completely at 10 weeks. The time, course and appearances of this degeneration cycle correspond closely to that described in the present paper for the avian optic tectum, in preparations made with the Glees method. A further point of similarity is that both in the reptile and bird it was not possible to stain the ring- or club-like forms in normal material, and in the case of the avian tectum, the terminal aborizations themselves failed to appear in the normal specimens. However, in this connexion, it is of interest that signs of degeneration of terminal boutons have been described in the stellate ganglion of cephalopods as early as 13 hr. after interruptions of the nerve fibres (Sereni & Young, 1932). As these animals are poikilothermous such unexpectedly early changes may be attributable to the relatively high temperature of the sea water of their environment (around 25° C.).

When the Nauta method was used on avian material the appearances of degeneration were not fully established until about 28 days following the operation. This delay was not experienced in any of the mammalian experiments, and it has not been possible to trace any reference to it in the literature. Such a considerable variation in the time of appearance of the degeneration material in the two methods suggests that different chemical processes are involved. This possibility is reinforced by the long time of persistence of the Nauta degeneration. In the nucleus of the tractus solitarius following supra-nodose vagal section, much fragmented axonal material was still to be seen even at 112 days after the operation.

Appearances and nature of the degeneration material stained

Preliminary treatment of the sections with fat solvents emphasizes that there is a difference in the chemical processes involved in the two staining procedures. It was first thought that this finding could be best explained by assuming that the Nauta method was staining only the degenerating myelin sheath. Examination of sites containing large degenerating fibres was consistent with this view, but where finer fibres are also present (e.g. Pl. 3, fig. 17) there can be no doubt that the degenerating axons are also involved in the staining process. Similar evidence is seen in sites where finer axons are to be expected, such as in pericellular aborizations (Pl. 2, fig. 12). It seems then that the Nauta method stains both degenerating axons and myelin.

Even when fully established, the Nauta degeneration picture appeared different from that of the Glees. 'Drop-like' disintegration of the axons with fragmentation and vacuole formation were characteristic (Pl. 1, fig. 6) of the former method. The club- and ring-like forms and finer ramifications of the aborization, which were a prominent feature in the Glees preparations of 5–15 days (Pl. 1, fig. 2), were at no stage to be seen in Nauta material. In fact, the impression was formed that the Nauta method showed the disintegration of the main axons and their branches, but did not stain their finest ramifications.

This could be due to two factors. One of these is that if the myelin sheath is not present on the final aborizations no contribution to the degeneration material can

be expected from this source. However, recent work using the electron microscope indicates that even the finest so-called non-medullated fibres possess a sheath which may represent a single myelin lamella (Gasser, 1952; Fernandez-Moran, 1952). It may be that the absolute amount of lipid substances in the finest fibres is so small that it appears as scattered granules and therefore only gives an amorphous appearance.

The involvement of lipid materials in the Nauta staining process also invites comparison with the Marchi technique. For tracing degenerating myelinated tracts, the latter has the advantage that serial sections can be used. However, in situations where the degenerating fibres are diffused, interpretive difficulties are experienced, particularly in view of the relative ease with which artefacts are produced with the Marchi method. The Nauta method is more reliable and gives as a result of its axonal component a clearer picture of the degenerating pathway. In addition, this technique demonstrates the degenerating fibres as far as their pericellular ramifications and because the nerve cells are also stained, interpretation is easier.

The applications of the Glees and Nauta methods to investigation of neuro-anatomical pathways

With regard to the relative usefulness of the two methods in tracing neural pathways, it was found that the Glees method was very suitable, when successful, for demonstrating the exact site of the terminal arborization. The expression 'terminal degeneration' has been avoided, as it was not always certain that the ring- and club-like forms seen were terminal structures in the synaptic sense. Armstrong (1950), in his study of the reptile visual pathways, also experienced this difficulty. The main limitation of the Glees technique was in tracing axons of passage. This was due to the staining of the normal neuropil, which frequently masked the appearances of degeneration, as may be seen in the case of the cerebral cortex (Pl. 3, fig. 18), which should be compared with the Nauta preparation from the same block (Pl. 3, fig. 19).

It is in this respect that the Nauta method is complementary to the Glees. The complete suppression of the staining of normal fibres permits easier recognition of the criteria of degeneration as described by Glees & Nauta (1955). In addition, the suppression of the normal fibres allows the use of much thicker sections (up to 30 μ in some situations) than would otherwise be possible. This is a considerable advantage in tracing the course of fibres over long distances. However, it must be emphasized that when using either the Glees or the Nauta methods the careful use of control material is essential.

SUMMARY

1. A comparison has been made between the Glees and Nauta silver degeneration methods in the central nervous system. This has been carried out in relation to the morphological appearances produced, the time course of the degeneration cycle and the effect of lipid solvents on the staining.

2. In Glees preparations the characteristic appearances showing club- and ring-like forms, as described by previous authors, were confirmed. The Nauta method

demonstrated clearly, with complete suppression of the normal fibre background, the course of degenerating axons and their branches. However, in our experience the finest degenerating ramifications, shown by the Glees technique, were not stained in Nauta preparations. This interpretation is partly based on the apparent terminal appearance of the Glees degeneration forms compared with the probable derivation from axonal and myelin fragments as seen in the Nauta.

3. The time course of the degeneration cycle has been studied in the avian optic tectum and in the tractus solitarius and its nucleus in the rabbit. In the former the time of establishment of the Glees degeneration appearances was delayed to 7–11 days following operation, compared with 3 days for mammalian material. In the tectum the Glees degeneration products had virtually disappeared by 28 days. In the same site the Nauta degeneration picture became fully established only by 28 days. With regard to the time of persistence of Nauta degeneration material in the tractus solitarius and nucleus, this was still present at 112 days but had disappeared by 164 days.

4. The effect of preliminary treatment of the frozen sections with fat solvents was to abolish the Nauta staining, whereas that of Glees preparations was unimpaired or even improved. Taking into account the variation in morphological appearance and time course in the two methods, combined with the effect of fat solvents, it is considered that the underlying chemical processes involved in the two methods must be different.

5. It is concluded that the Glees method is satisfactory for studying the exact site of termination of axonal aborizations in appropriate regions. It is at a disadvantage in situations where scanty degeneration is present amongst a mass of normal fibres. The Nauta method is most useful where fewer degenerating fibres must be traced through a matrix of normal fibres as the staining of the latter is suppressed, but it fails to demonstrate degeneration in the finest branches.

We wish to thank Professor J. Z. Young and Mr K. C. Richardson for valuable advice and criticism and Mr J. A. Armstrong and Miss Thelma Marchment for help with the photomicrography.

REFERENCES

ARMSTRONG, J. A. (1950). An experimental study of the visual pathways in a reptile (*Lacerta vivipara*). *J. Anat., Lond.*, **84**, 146–167.

CRAGG, B. G., 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**, 292–306.

FERNANDEZ-MORAN, H. (1952). The submicroscopic organization of vertebrate nerve fibres. *Exp. cell Res.* **3**, 282–359.

FOERSTER, O., GAGEL, O. & SHEEHAN, D. (1933). Veränderungen an den Endösen im Rückenmark des Affen nach Hinterwurzeldurchschneidung. *Z. ges. Anat.* 1 *Z. Anat. EntwGesch.* **101**, 553–565.

GASSER, H. S. (1952). *Cold Spr. Harb. Symp. quant. Biol.* **17**, 32–36.

GIBSON, W. C. (1937). Degeneration of the boutons terminaux in the Spinal Cord. *Arch. Neurol. Psychiat., Chicago.*, **38**, 1145–1157.

GLEES, P. (1941). Termination of optic fibres in the lateral geniculate body of the cat. *J. Anat., Lond.*, **75**, 434–440.

GLEES, P. (1942). The termination of optic fibres in the lateral geniculate body of the rabbit. *J. Anat., Lond.*, **76**, 313–318.

GLEES, P. (1944). The anatomical basis of cortico-striate connections. *J. Anat., Lond.*, **78**, 47–51.

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

GLEES, P. & LE GROS CLARK, W. E. (1941). Termination of optic fibres in the lateral geniculate body of the monkey. *J. Anat., Lond.*, **75**, 295–308.

GLEES, P. & NAUTA, W. J. H. (1955). A critical review of studies on axonal and terminal degeneration. *Mschr. Psychiat. Neurol.* **129**, 74–91.

HOFF, E. C. (1932). Degeneration of the boutons terminaux in the spinal cord. *J. Physiol.* **74**, 4–5*P*.

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.

NAUTA, W. J. H. & RYAN, L. F. (1952). Selective silver impregnation of degenerating axons in the central nervous system. *Stain. Tech.* **27**, 175–179.

SERENI, ENRICO & YOUNG, J. Z. (1932). Nervous degeneration and regeneration in cephalopods. *Pubbl. Staz. zool. Napoli*, **12**, 1–36.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Avian optic tectum, 11 days following contralateral optic nerve section. Appearances of degeneration of the terminal aborizations of the optic nerve afferents are seen throughout the field. Glees method. × 552.

Fig. 2. Higher power of the same preparation as fig. 1. Club- and ring-like forms of various sizes are seen. × 1320.

Fig. 3. Control of figs. 1 and 2. This is the contralateral optic tectum in which the clubs and rings are absent. Glees method. × 552.

Fig. 4. Nucleus of the tractus solitarius, 5 days after supra-nodose section of the vagus nerve. Numerous nerve fibres are in process of degeneration. Nauta method. × 552.

Fig. 5. Control of fig. 4. The contralateral nucleus of the tractus solitarius in the same section. In this normal field no nerve fibres are stained. Nauta method. × 552.

Fig. 6. High power of the lateral cuneate nucleus 5 days after ipsilateral hemisection of the spinal cord at the level of C. 1. The droplets and vacuoles formed along the course of the degenerating axons are seen. Nauta method. × 1320.

PLATE 2

Fig. 7. Avian optic tectum, 5 days' degeneration. At this stage the staining of nerve fibres does not extend deep to the optic fibre layer (*o.f.l.*). Nauta method. × 552.

Fig. 8. Avian optic tectum, 28 days' degeneration. The optic fibre layer is now packed with degenerating nerve fibres which extend into the deeper layer. × 552.

Fig. 9. Nucleus of the tractus solitarius, 45 days after supra-nodose vagotomy. There is slight diminution of the degeneration material compared with fig. 4. × 552.

Fig. 10. Nucleus of the tractus solitarius 112 days after supra-nodose vagotomy. Considerable diminution in the quantity of the degeneration material is now apparent. Nauta method. × 552.

Fig. 11. Cuneate nucleus 5 days after ipsilateral hemisection of the spinal cord at the level of C. 1. Following treatment of the sections with fat solvents no degenerating fibres are seen. Nauta method. × 552.

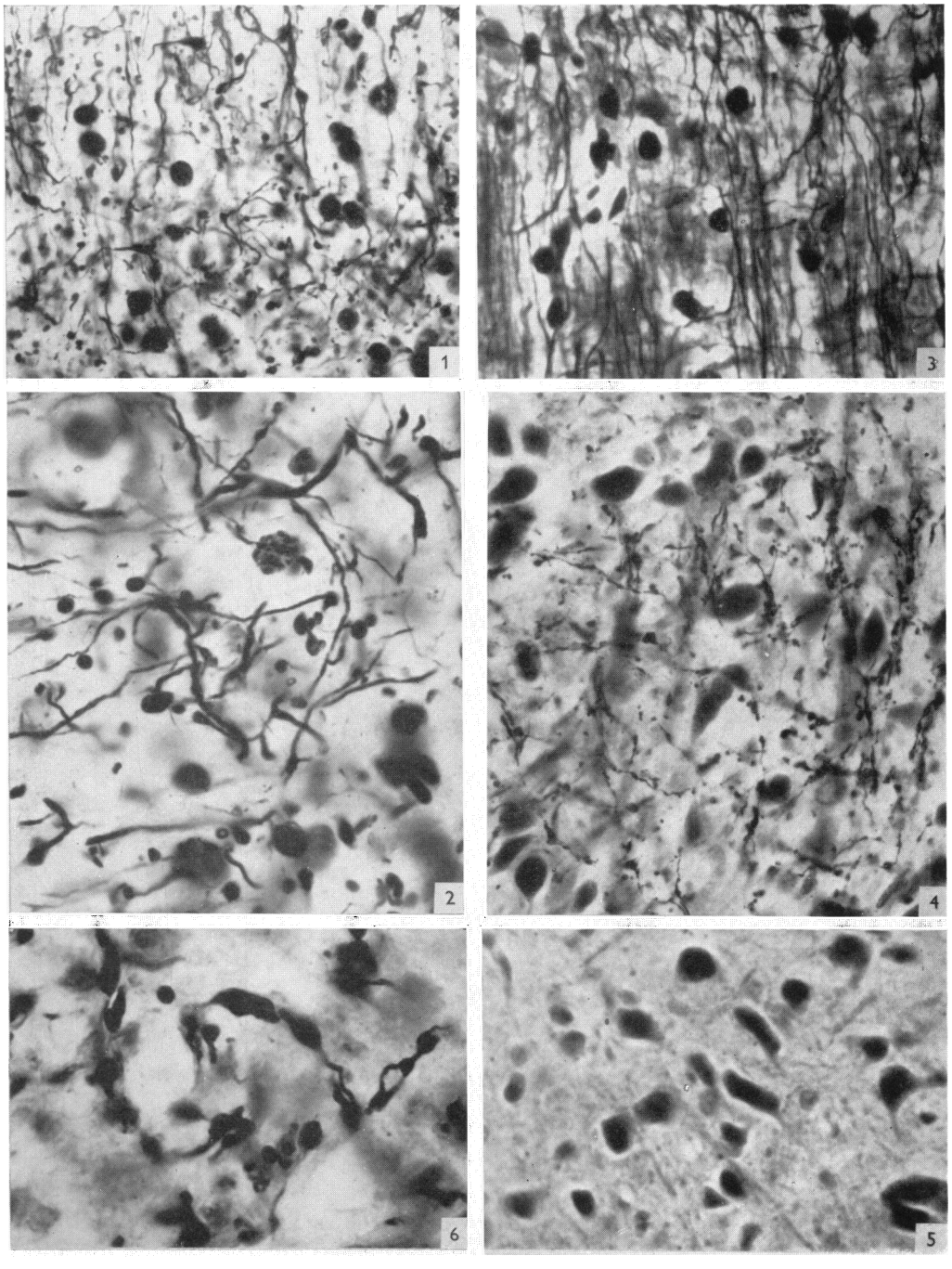
Fig. 12. Control of fig. 11. Many degenerating fibres are seen in this field. Nauta method without fat extraction. × 552.

PLATE 3

Fig. 13. Cuneate tract, 5 days' degeneration. Preliminary treatment with potassium bichromate has preserved the staining of the degeneration material in spite of the use of fat solvents. Nauta method. × 552.

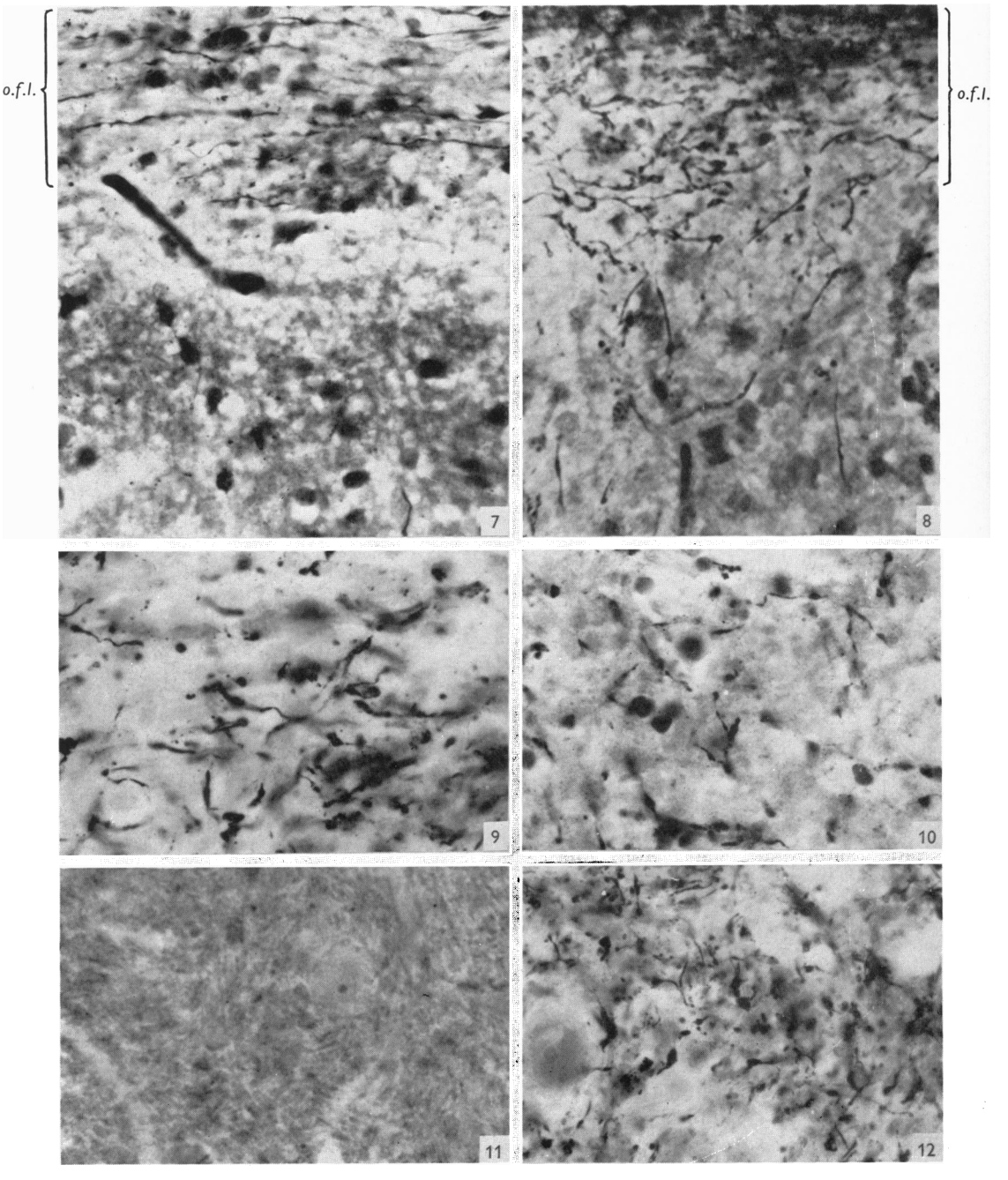
Fig. 14. Normal cuneate tract after the same treatment as fig. 13. An incomplete ring of darkly stained material is seen surrounding the faintly stained axons. × 1080.

Fig. 15. Material from the same specimen as fig. 14 but sectioned longitudinally. The dark staining material is seen to form a reticulum surrounding the axon. Nauta method preceded by mordanting and fat solvents. × 1080.

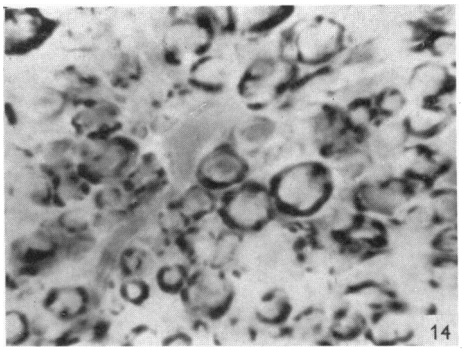


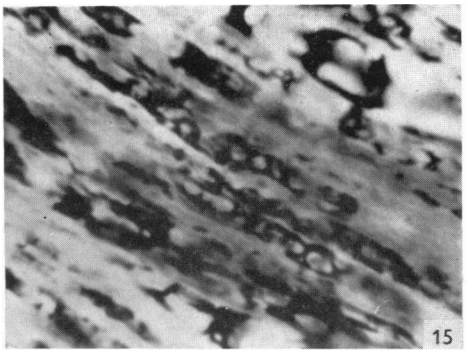
EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM

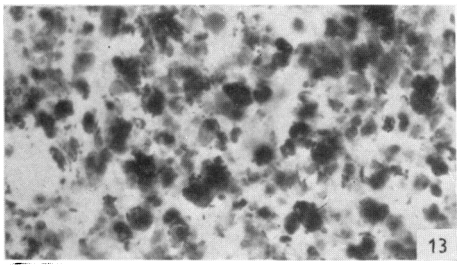
(*Facing p.* 202)

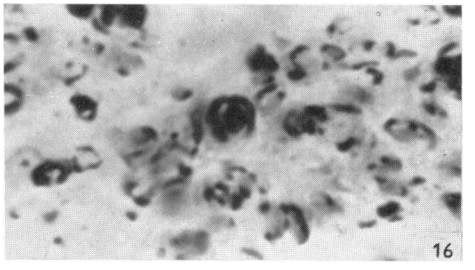


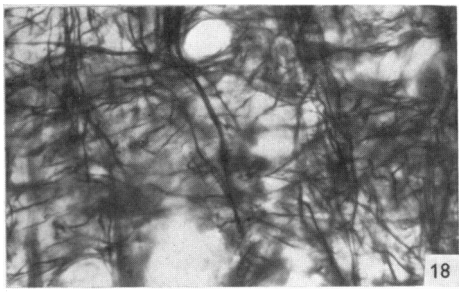
EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM

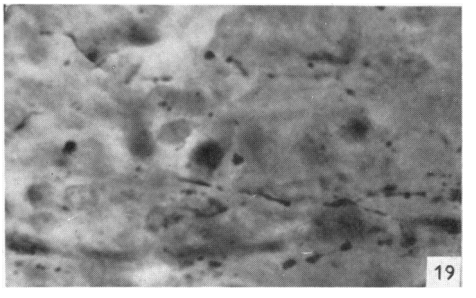


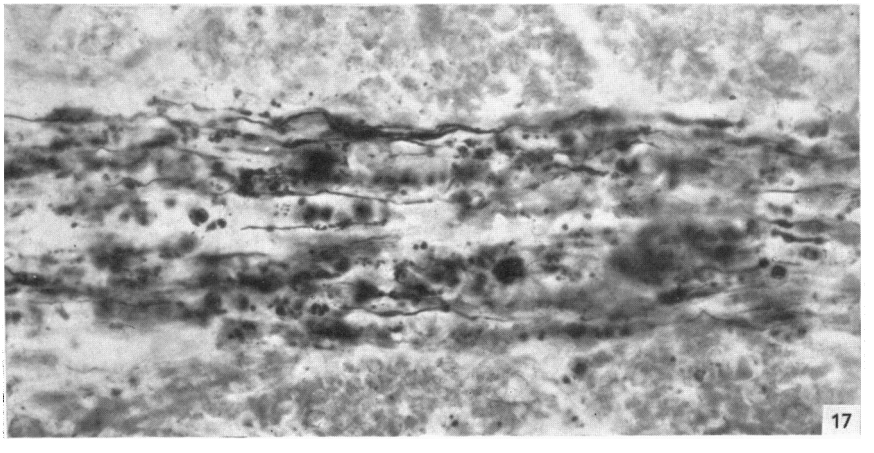




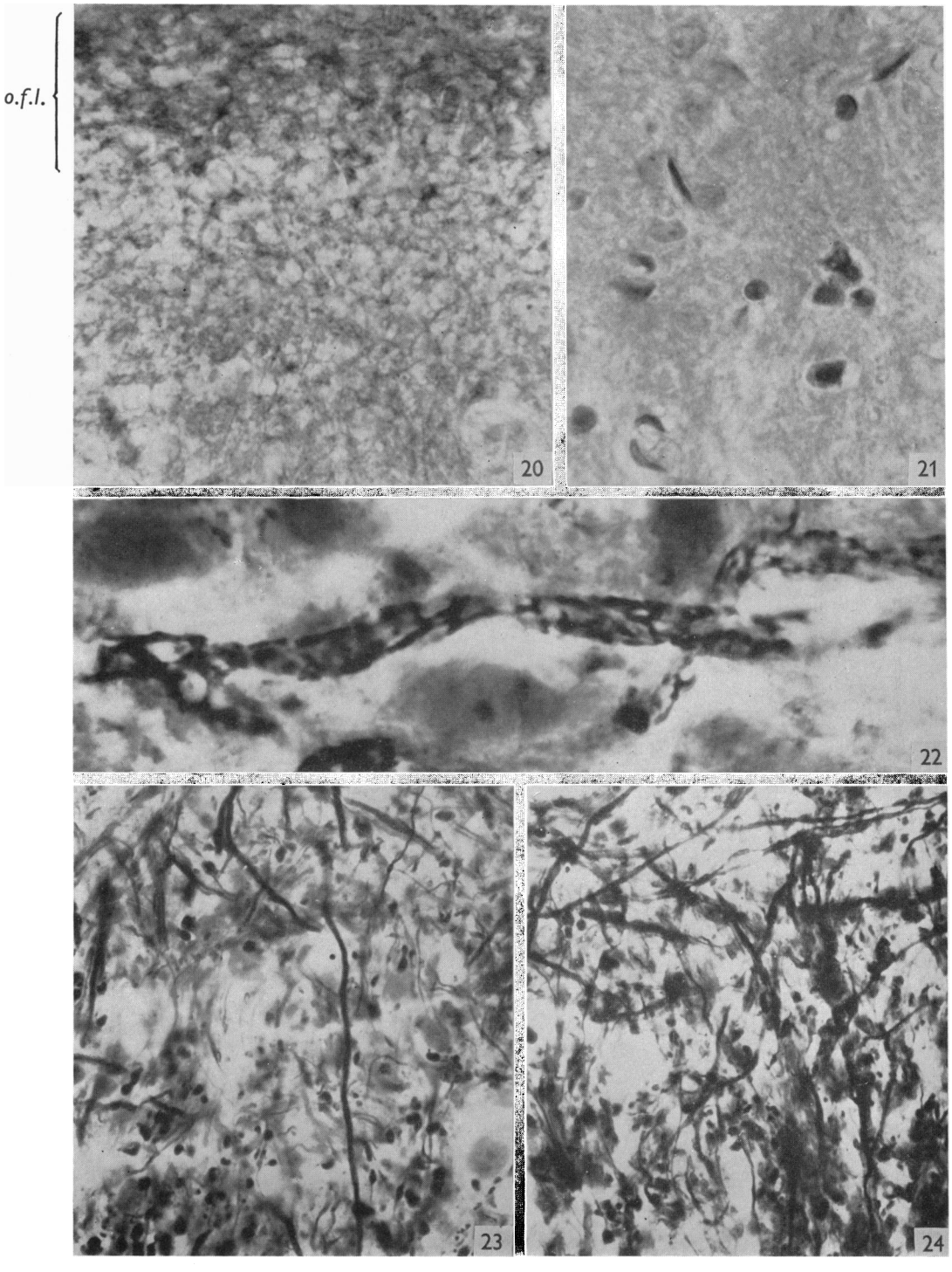








EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM



EVANS AND HAMLYN—SILVER DEGENERATION METHODS IN THE NERVOUS SYSTEM

Fig. 16. Cuneate tract, 5 days' degeneration. The degenerating axon in the centre of the field is seen to be surrounded by the reticulum. Nauta method preceded by mordanting and fat solvents. × 1080.

Fig. 17. Transverse section of medulla oblongata, 5 days after supra-nodose vagotomy, showing a degenerating vagal rootlet cut in longitudinal section. The staining of the normal fibres of the surrounding medulla is suppressed. Nauta method. × 552.

Fig. 18. Rabbit cerebral cortex, 5 days after a lesion of the contralateral hemisphere. No evidence of degeneration can be seen in the densely stained neuropil. Glees method. × 552.

Fig. 19. A section from the same block as fig. 18 showing some degenerating fibres. The staining of the normal neuropil is suppressed. Nauta method. × 552.

PLATE 4

Fig. 20. Avian optic tectum (control of fig. 7). No fibres are stained in the optic fibre layer (*o.f.l.*). Nauta method. × 690.

Fig. 21. Nucleus of the tractus solitarius, 170 days' degeneration. There is now no staining of degenerating material. Nauta method. × 690.

Fig. 22. A further view of the reticulum surrounding a longitudinally disposed normal axon in the nucleus cuneatus. Nauta method preceded by mordanting and fat extraction. × 1650.

Fig. 23. Cuneate nucleus, 5 days' degeneration. Numerous degeneration forms typical of the Glees method are seen. Glees method preceded by fat extraction. × 690.

Fig. 24. Section from the same block as fig. 23, stained by the Glees method without preliminary fat extraction. × 690.