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ELECTRON MICROSCOPIC AND ELECTROPHYSIOLOGICAL STUDIES ON THE CAROTID BODY FOLLOWING INTRACRANIAL SECTION OF THE GLOSSOPHARYNGEAL NERVE

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SUMMARY

1. The innervation of carotid body Type I cells has been investigated in seventeen cats. At a sterile operation the glossopharyngeal and vagus nerve roots were cut intracranially on one side.

2. From 1½ to 378 days after the operation the carotid bodies were fixed *in situ* and prepared for electron microscopy. Nerve endings on Type I cells were found to degenerate with a prolonged time course. In each cat there was a decrease in the number of nerve endings on the operated side as compared with the non-operated side.

3. Before the carotid bodies were fixed, recordings were made from chemoreceptor, and baroreceptor, afferent fibres in the sinus nerve on the operated side. The chemoreceptors responded in the usual way to changes in arterial O_2, CO_2 and pH; the injection of cyanide evoked a brisk response.

4. It is concluded that the nerve endings on Type I cells are efferent rather than afferent and the cell bodies of their axons are probably in the brain stem.

INTRODUCTION

Carotid body receptors detect changes in the O_2, CO_2 and hydrogen ion concentrations in arterial blood, and the afferent fibres from these receptors are in the carotid sinus nerve. It is currently held that the Type I, or glomus, cells of the carotid body are the receptors and that nerve endings on these cells are terminals of the afferent fibres. This hypothesis is supported by the work of De Castro (1926, 1928) who demonstrated that the nerve endings related to these cells degenerated after the sinus nerve was cut. Further than this, he reported that the nerve endings had not degenerated when the carotid body was examined up to 12 days after the

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glossopharyngeal and vagal nerve roots were cut intracranially, a procedure which would eliminate efferent fibres in the nerve.

In recent years, with the advent of the electron microscope, the structure of the carotid body has been examined in much greater detail than was possible for De Castro. The recent studies include Biscoe & Stehbens (1965, 1966, 1967), De Kock & Dunn (1966), Hess (1968) and Al-Lami & Murray (1968). It has been shown that the nerve endings on the Type I cells have features which are ordinarily associated with efferent nerve endings (Gray & Guillery, 1966), namely micro-vesicles 500×10^{-10} m diameter, an aggregation of mitochondria, glycogen, etc. However, it is not possible to assert on the basis of the structure alone that the nerve endings are afferent or efferent. The possibility that these nerve endings may be efferent was suggested by Biscoe & Stehbens (1966). It is apparent that in this case there should be an efferent pathway in the sinus nerve; a centrifugal pathway has been demonstrated in the sinus nerve (Biscoe & Sampson, 1967, 1968). Also Neil & O'Regan (1969) have shown that stimulation of the sinus nerve can depress chemoreceptor activity which is in keeping with the view that sinus nerve efferents pass to the carotid body. Sampson & Biscoe (1970) confirmed this finding and further showed that the pathway in the sinus nerve had tonic effects on the receptor response to O_2.

Thus, whereas the 1928 experiments of De Castro seemed to have been conclusive regarding the origin of the nerve endings on the glomus cells, there is a dissonance between his results and the more recent studies. Biscoe & Stehbens (1967) and Hess (1968) were able to confirm that the nerve endings on the Type I cells degenerate after the sinus nerve is cut, but the intracranial nerve section experiment, which is crucial to the argument concerning the origin of the endings, has been repeated only by De Castro & Rubio (1968) who claim to have confirmed the earlier studies of De Castro (1928).

In the present paper we report the results of experiments in which the glossopharyngeal and some of the roots of the vagus nerve were cut intracranially and the carotid bodies later examined under the electron microscope.

Some of the results have been communicated to the Physiological Society (Biscoe, Lall & Sampson, 1969).

METHODS

Intracranial section of glossopharyngeal and vagus nerve roots. Thirty cats were anaesthetized with sodium pentobarbitone (Diabutal, Diamond Laboratories), 30 mg/kg body wt. given intravenously. Under sterile conditions the right lambdoidal ridge was exposed by separating the muscles overlying it and scraping their

periostial insertion away from the bone. The posterior fossa was entered through a craniotomy made at the junction of the parietal bone, the occipital bone and the mastoid portion of the petrous temporal bone. The craniotomy was extended caudally across the lambdoidal ridge at a horizontal level about that of the zygomatic arch and dorsal to the occipital condyle which was usually exposed during removal of the muscle. There was often a venous sinus lying in the dura mater beneath the lambdoidal ridge; the sinus was clipped or tied when the dura mater was cut. The cerebellum was displaced medially by gentle pressure, and the roots of the glossopharyngeal, vagus and spinal accessory nerves were identified. Overlying the roots there was invariably an artery running caudorostrally within the arachnoid; this artery was gently pushed medially in order to display the region of the jugular foramen. The intracranial configuration of the cranial nerves was usually one of those described by Cottle (1964), with the first type predominating. However, in one cat the glossopharyngeal nerve emerged from a separate foramen (cf. Cottle, 1964, Fig. 1, type 3), but there was an additional component that emerged with the roots of the vagus, a configuration not described by Cottle. After the various roots were identified, the glossopharyngeal nerve was avulsed from the brain stem and cut above the jugular foramen resulting in the removal of 1–2 mm of nerve. In some cats, the differentiation between the glossopharyngeal and vagus nerve roots was obscure, and the upper roots of the vagus nerve were also removed. A piece of polyethylene sheet (3 mm × 5 mm) was placed between the jugular foramen and the brain stem in order to prevent re-innervation. The skull was closed with bone wax. A small amount of penicillin and streptomycin powder was dusted into the wound and the muscles and skin were sutured in layers.

The animals that recovered had some slight cerebellar damage as indicated by ataxia for 3–4 days. The over-all mortality rate, which was directly attributable to the operation, was thirteen out of thirty cats. The cause of death was damage to the artery overlying the nerve roots or excessive haemorrhage from some other cause.

Fixation of the carotid body. The seventeen surviving cats were again anaesthetized with intraperitoneal sodium pentobarbitone, 30 mg/kg, at 1½, 3, 3, 6, 7, 20, 25, 27, 34, 35, 36, 44, 57, 105, 128, 312 and 378 days after the operation. The trachea was cannulated and the carotid sinus regions exposed by retraction of the larynx and pharynx in the mid line. Sinus nerve action potentials were recorded on the operated side from cats killed 3, 7, 20, 25, 34, 35, 44, 105, 128, 312 and 378 days after the operation (see below) and from all of these, except for the cats killed after 25, 34, 128 and 312 days on the non-operated side. The elapsed time between giving the anaesthetic and commencing fixation of the carotid body was not more than 2½ hr in the experiments where electrophysiological records were made and ¾ hr when recordings were omitted. The common carotid arteries were exposed and cannulated and perfusion with warm (37–39° C) fixative was commenced immediately, whereupon the external carotid arteries were tied off. Perfusion was continued for 2–4 min until 10–20 ml. fixative was injected. The perfusion pressure was sufficient to just prevent reflux of blood up the carotid body vein or veins when viewed through an operating microscope. The fixative was of the following composition: glutaraldehyde, 4%; paraformaldehyde, 2%; sodium cacodylate, 0·067 M at pH 7·4; potassium chloride, 0·0073 M; calcium chloride, 0·0018 M; sucrose, 1%. After removal, the carotid body was stored overnight in fresh fixative. The next morning extraneous tissue was removed and the carotid bodies were diced into four to five blocks. The tissue blocks were post-fixed for 1 hr with 1% osmium tetroxide in veronal acetate buffer at pH 7·4 (Palade, 1952).

The fixed tissues were stained *en bloc* with uranyl acetate by the method of Kellenberger, Ryter & Sechaud (1958), modified according to Farquhar & Palade

(1965). The blocks were dehydrated in acetone and embedded in Epon (Shell Chemicals) according to Luft (1961). Thin sections $400-800 \times 10^{-10}$ m in thickness were mounted on copper grids coated with a parlodion film stabilized with carbon. They were stained sequentially with 5% uranyl acetate at 60° C for 60 min and in lead citrate (Venable & Coggeshall, 1965) for 15 min. The latter step was carried out in a humid oxygen atmosphere to avoid formation of lead carbonate crystals on the sections. Sections from two to three out of the four to five blocks were viewed and photographed on a Siemens Elmiskop I microscope.

In thirteen of the seventeen cats, the carotid bodies from both the operated and non-operated sides were removed, and in the remaining four, killed after 6, 27, 36 and 312 days, the carotid body on the operated side only was removed.

Recording of sinus nerve action potentials. Electrical activity was recorded from the sinus nerve on the operated and the non-operated side prior to fixation of the carotid body in the cats listed above. The sinus nerves were dissected under either liquid paraffin or 0·9% NaCl solution at 36–38° C. Action potentials were recorded from the whole nerve and from fine strands of it. Conventional techniques were used as described in recent papers (Biscoe & Purves, 1967; Biscoe & Sampson, 1968). In brief, the electrical signal from the nerve was displayed on an oscilloscope and photographed on moving film. A rate-meter was used to monitor the discharge frequency of the nerve impulses. One femoral artery was cannulated and blood pressure was recorded through a Statham P 23 Gb strain gauge. End-tidal CO_2 was monitored continuously with a Beckman LB 1 physiological gas analyser. The outputs of the transducers were recorded on a Grass Polygraph. Arterial O_2 tension and pH were measured with a Beckman medical gas analyser and associated electrodes. In some experiments, the animal was given gallamine triethiodide (Flaxedil), 5 mg/kg, and artificially ventilated so that the arterial O_2 tension could be varied and the end-tidal CO_2 kept constant.

The body temperature of the cats was maintained in the range 36–38° C with the aid of a heating pad and infra-red lamp.

Quantitative estimation of degeneration of nerve endings. The number of nerve endings within the basement membrane around a Type I–Type II cell complex was counted in sections of the carotid body taken from the operated and the non-operated sides. Electron micrographs of the carotid body were taken at magnifications of 3000–4000 and were enlarged three times on photographic paper. The cells were selected as they came into view whilst scanning the grids on the microscope. A Type I cell was included only when the plane of section passed through its nucleus and no attempt was made to select these cells other than by this criterion. The section was thus assumed to be through the main body of the cell and fragments of cells were excluded from the counts. Nerve endings were identified by their position closely applied to a Type I cell and were those structures which contained microvesicles 500×10^{-10} m diameter, small mitochondria and sometimes the other cytoplasmic constituents described by, for example, Biscoe & Stehbens (1966). The number of nerve endings on the perimeter of a Type I cell was counted. Nerve endings lying outside the basement membrane of the Type I–Type II cell cluster were not counted. All nerve endings within the basement membrane were counted whether or not they showed degenerative changes.

RESULTS

Degenerative changes will be seen in the carotid body after the glossopharyngeal nerve is cut intracranially if any one of the following situations obtain: (1) if there were nerve fibres or endings in the carotid body con-

nected with an efferent pathway in the sinus nerve (e.g. the centrifugal fibres described by Biscoe & Sampson, 1967, 1968); (2) if afferent cell bodies in the glossopharyngeal ganglia were injured during the operation; (3) if the cell bodies for chemoreceptor afferent fibres were not in the ganglia but were in the brain stem. It follows in the first case, in which the degenerative changes would occur in an efferent pathway, that the chemoreceptor afferents should continue to function, and it should be possible to record their electrical discharges. In the second and third cases, the chemoreceptor afferent fibres would have ceased to function and it should not be possible to record action potentials from them. In addition baroreceptor fibres may also be affected in the second case as well as in the parallel third case and their activity would be unrecordable.

The morphological results are presented and are followed by the results of nerve recording experiments.

Morphological changes

The nomenclature for cells in the carotid body is that used by Biscoe & Stehbens (1965, 1966, 1967): the Type I cell is equivalent to the glomus, epithelioid, chemoreceptor or enclosed cell; the Type II cell is equivalent to the supporting, sustentacular or enclosing cell.

Quantitative data were obtained by making counts of the number of nerve endings of the micro-vesicle containing type to be found on Type I cells at various times after the operation. These results are given first and are followed by a description of the character of the ultrastructural changes which may be seen.

Effects of intracranial section of IX and X nerve roots on the number of nerve endings on Type I cells

At the present time it has not been shown how many nerve endings are normally present on a Type I cell. There may be only one coursing over the surface of the cell (see, for example, Biscoe & Stehbens, 1966, Fig. 2). More than one may appear to be present if the single ending were to be cut across in several places. The problem can be resolved only by serial section studies and we have not investigated it. Accordingly all structures which have the appearance of a nerve ending were counted.

The mean and standard deviation of the number of nerve endings per Type I cell are given in Table 1 for the carotid bodies taken from both the operated and the non-operated sides of eleven cats at various times after the operation. These counts show that there were always fewer nerve endings on the operated than the non-operated side. The mean counts were compared using Student's t test (Student, 1908) and the value for t and the probability of this value occurring by chance (P) are also given in Table 1.

After 44 days the mean number of nerve endings per Type I cell on the operated side was always significantly less than the mean number on the control side. It was also significantly less in the cat killed 20 days after operation.

TABLE 1. Comparison of the number of surviving nerve endings in the denervated and control carotid bodies in the same cat

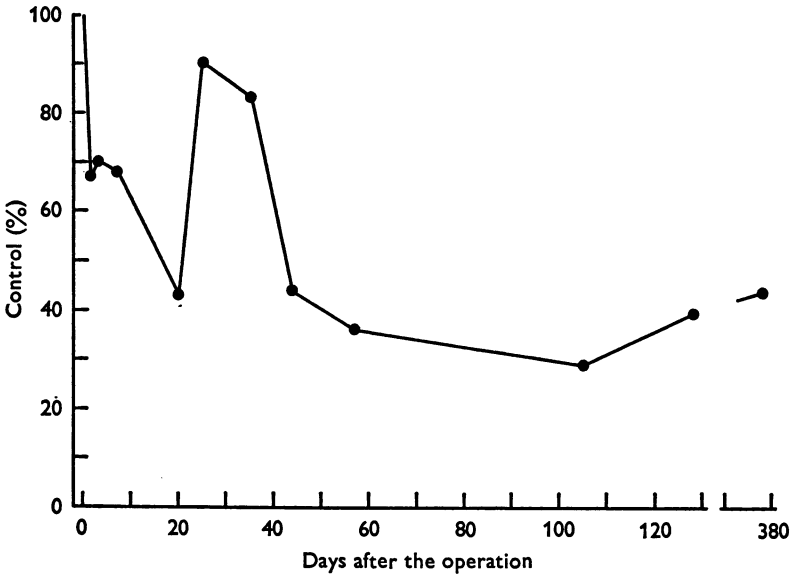
	Control carotid body		Denervated carotid body		
Number of days after denervation	Cells counted	Nerve endings/cell (mean ± S.D.)	Cells counted	Nerve endings/cell (mean ± S.D.)	*P*
1·5	22	2·00 ± 1·47	41	1·34 ± 1·13	< 0·1
3	27	2·44 ± 1·42	10	1·70 ± 1·00	< 0·2
7	22	1·27 ± 0·86	22	0·86 ± 0·86	< 0·2
20	14	2·14 ± 1·45	38	0·92 ± 1·10	< 0·01
25	31	1·51 ± 1·18	30	1·36 ± 1·16	< 0·7
35	26	1·65 ± 1·10	16	1·37 ± 0·99	< 0·5
44	9	1·55 ± 0·95	16	0·68 ± 0·76	< 0·05
57	24	2·00 ± 1·32	54	0·72 ± 1·04	< 0·001
105	27	1·92 ± 0·97	37	0·56 ± 1·02	< 0·001
128	26	1·88 ± 1·62	66	0·74 ± 0·85	< 0·001
378	36	1·38 ± 0·97	49	0·61 ± 0·72	< 0·001

It should be noted that if chance selection were operating the prediction would be made that in some cases the count on the operated side should exceed that on the non-operated side and that the results would be evenly distributed between the two groups. In the best case there would be five to six cats with more nerve endings per Type I cell on the operated side than on the non-operated side. The value for χ^2 with the observed distribution by this test is $> 9{\cdot}2$ and the probability of this result occurring by chance is $0{\cdot}005 > P > 0{\cdot}001$.

These results are represented in graphical form in Text-fig. 1 where the number of nerve endings on the operated side is expressed as a percentage of the number present on the non-operated side of the same cat. The progressive decrease of nerve endings on the operated side is apparent; no claim is made for the validity of the seemingly triphasic nature of the time course of the disappearance of nerve endings. This variation may be accounted for by variation in rate of degeneration in different cats.

A separate estimate of the number of nerve endings was made in the cat where degeneration was allowed to proceed for 105 days. Here the total number of nerve endings was counted in electron micrographs from the two carotid bodies. The area of Type I cell to be seen on the pictures was then measured by cutting out with scissors all Type I cell outlines, excluding supporting cell and other tissue. These pieces of paper were weighed and

the areas they represented calculated from the weight of a known area. The total areas inspected were 4900×10^{-6} m^2 for the operated side and 6500×10^{-6} m^2 for the non-operated side at a magnification of $9000\times$. The results showed 19·6 nerve endings per 10^{-6} m^2 on the normal, non-operated side and 3·1 nerve endings per 10^{-6} m^2 on the operated side.



Text-fig. 1. Graph to show the time course of the disappearance of nerve endings on the Type I cells after the glossopharyngeal nerve roots were cut in eleven cats. The ordinate is the number of nerve endings per cell on the operated side expressed as a percentage of the number on the non-operated side; the abscissa is time in days after the operation.

Qualitative observations on the ultrastructure

Type I and Type II cells, blood vessels, perivascular nerve plexuses and connective tissue elements in the carotid bodies of the operated side in all cats were normal and indistinguishable from those of the non-operated side. All the cell inclusions which have been previously described, by, for example, Biscoe & Stehbens (1966), were seen.

For purposes of comparison, a nerve ending on a Type I cell of the carotid body from the non-operated side of one of the cats used in this study is presented in Pl. 1*A*. The nerve ending contains micro-vesicles, small mitochondria and glycogen granules and has a number of electron dense junctional regions along the synaptic cleft.

The changes which occur on the operated side. When examining the sections it was our practice to work in pairs, only one of us knowing whether the tissue under view was from the operated or the non-operated side. In all

cases, having seen from two to four sections, the viewer ignorant of the origin of a given grid was able to state correctly its source.

Pathological changes were seen in association with the nerve endings on Type I cells, or in positions appropriate to nerve endings. The changes were not uniform, and a few normal nerve endings were present even after 128 days (cf. Biscoe & Stehbens, 1967). At any one time, and especially 20 days after the operation, an array of changes was seen becoming less obvious between 50 and 60 days post-operatively. The changes were: aggregation and increased density of mitochondria with loss of internal structure (Pls. 1*B*, 2*A*, *B*) and sometimes loss of internal structure without an increase in electron density (Pl. 2*C*); clumping or swelling of synaptic vesicles(Pl.2*A*) which were sometimes enclosed in a vacuole (Pl. 1*B*); vacuolation and fragmentation of nerve (Pls. 1*B*, 3*A*); extracellular formation of vacuoles between Type I and Type II cells and between Type II cells (Pls. 1*B* 2*A*, 3*A*).

The most convincing evidence that these changes *are* associated with the nerve endings can be seen where such changes are contiguous with a nerve ending (Pl. 1*B*). In this case the nerve ending contains micro-vesicles and small mitochondria and has an electron dense junctional region with the Type I cell. Within the ending is a small aggregation of electron dense mitochondria, M, and a vacuole, V, having a double layered membrane containing a number of vesicles 500×10^{-10} m diameter. Away from the Type I cell, the ending merges into an area of vacuolation and fragmentation of tissue with no interposed membrane. Some of the fragments are parts of a nerve fibre, NF. There are electron dense figures in this area, and the intercellular space between the Type II cell and the nerve ending is much above the normal 200×10^{-10} m at one point. The whole area is partially enclosed by the Type II cell and the basement membrane, BM. A similar appearance may be seen in Pl. 2*A*.

Table 1 and Text-fig. 1 show that after 128 days some 39% of endings persist. In fact it should be emphasized that after two months it is unusual to find normal nerve endings (all structures recognizable as nerve endings were counted). The nerve endings contain irregularly shaped membranous sacs with indistinct outlines which may represent remnants of vesicles. Vesicles when present were irregular in shape and size and often larger than normal synaptic vesicles. There is also an increase in cytoplasmic density. The indistinct character of such a structure is illustrated in Pl. 2*D*. Clumping of micro-vesicles was also a feature.

All the pathological changes so far discussed were associated with non-myelinated fibres in Type I–Type II cell groups. Similar changes to these were seen in some of the Schwann cells containing non-myelinated fibres (Pl. 3*B*). In this micrograph there is also a small unaffected Schwann cell

adjacent to the Schwann cell containing fragmented nerve fibres having a pathological appearance. Such changes in non-myelinated fibres are thus not seen uniformly throughout the structure. We have not seen similar changes in myelinated fibres in the parenchyma of the carotid body.

When electrophysiological recordings were made, the sinus nerve on the non-operated side was always dissected first. Accordingly, if there had been any morphological changes caused by local trauma, prolonged exposure to liquid paraffin or saline, these should have been most obvious on the non-operated side since they would have had the longest time to develop. Nevertheless, the changes are on the operated side. In some experiments no nerve recording was undertaken and in these animals the changes were as marked as elsewhere in the series (Pls. 1*B*, 2*A* and 3).

Changes in the sinus nerve. From 3 days after the operation granulocytes were seen in the nerve and between endothelial cells of blood vessels in the nerve on the operated side. Myelinated fibres could very occasionally be seen partially engulfed by macrophages. To be certain which groups of fibres are involved would require fibre diameter histogram studies of the nerve made many months after the intracranial operation at such a time as the changes were complete. We have not done this.

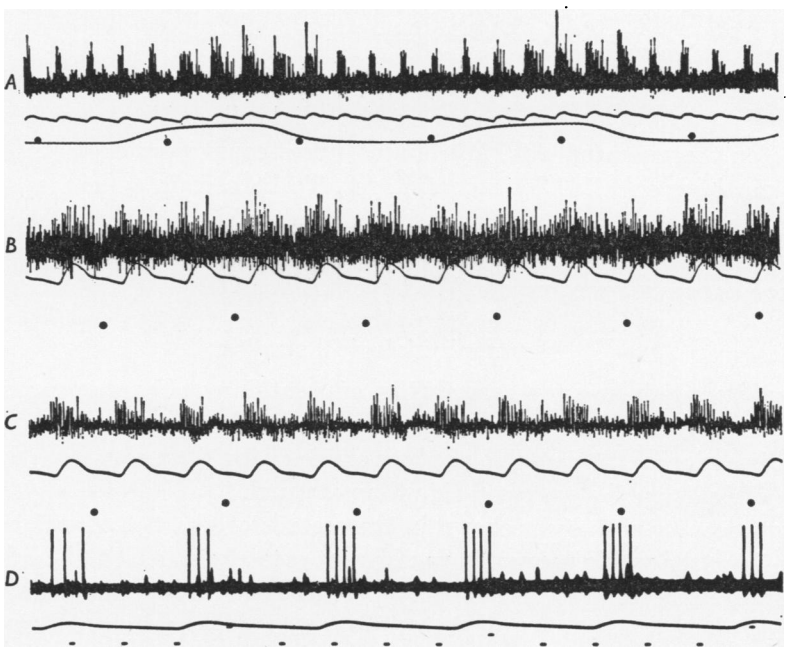
Electrophysiological studies

Nerve potentials were recorded from afferent fibres of baroreceptors and chemoreceptors in the sinus nerves on the operated and non-operated sides. The dissection of the nerve to produce single or few fibre responses was no more difficult on the operated side than is usual.

Baroreceptor activity is shown in the records of Fig. 2*A*, *B* which were made from the whole nerve of two different cats after the sheath had been removed from the nerve. Bursts of potentials in time with the arterial pressure pulse were obvious, and there were many, generally smaller potentials in diastole. The discharge of baroreceptor afferents increased during expiration (Text-fig. 2*A*) in the usual way. The potentials seen in the diastolic period diminished in frequency when arterial O_2 tension was raised. This cannot be regarded as conclusive evidence for the presence of chemoreceptor afferent fibres in the nerve, however, because the arterial pressure also often falls under these conditions. In Text-fig. 2, for example, the mean arterial pressure fell from 133 mm Hg in *B* to 116 mm Hg in *C* when the P_{a,O_2} was raised from 132 to 410 mm Hg. Recordings were made from single baroreceptor afferent fibres with the usual facility (Text-figs. 2*D* and 4*G*).

Discharge from chemoreceptor afferent fibres could be recorded with no more than the usual difficulty, though this is of course a subjective assessment. Chemoreceptor afferents showed responses to changes in O_2

tension at constant arterial CO_2 tension, and some examples from four cats are shown in Text-figs. 3 and 4 where a decrease in the frequency of action potentials as the P_{a,O_2} was raised is shown for single fibres from different cats. The decrease in frequency with an increase in O_2 tension is shown for a multifibre strand from another cat in Text-fig. 3*G* to *J*. In addition, the change in rate of discharge with an increase in inspired P_{O_2} occurred as rapidly as in other non-operated cats, usually within 10 sec. In the example illustrated in Text-fig. 3*K*, the rate began to decrease within 7 sec of the start of the inhalation of 100% O_2.

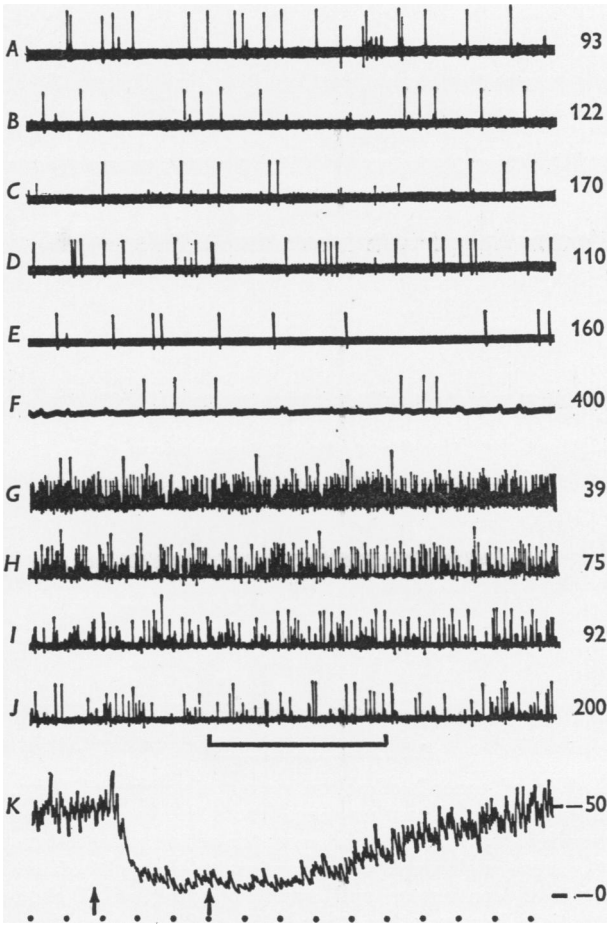


Text-fig. 2. Filmed records of baroreceptor action potentials recorded from the sinus nerve of three cats whose glossopharyngeal nerve roots on the side of these recordings had been cut intracranially. In each case the upper record is of the nerve potentials and below it is the arterial pressure wave, the lowest trace in *A* indicates the phase of respiration with expiration upwards. *A*, operation 34 days previously, mean arterial pressure 119 mm Hg. *B* and *C*, operation 35 days previously, mean arterial pressure in *B* 133 mm Hg, in *C* 116 mm Hg; P_{a,O_2} in *B* 132 mm Hg, in *C* 410 mm Hg. *D*, operation 105 days previously, mean arterial pressure 95 mm Hg. Time scale 500 msec in *A* to *C*, 100 msec in *D*.

The O_2 response curves plotted for single chemoreceptor afferent fibres from the sinus nerve on the operated side (Text-fig. 5) were found to be similar to those described for chemoreceptor afferent fibres studied pre-

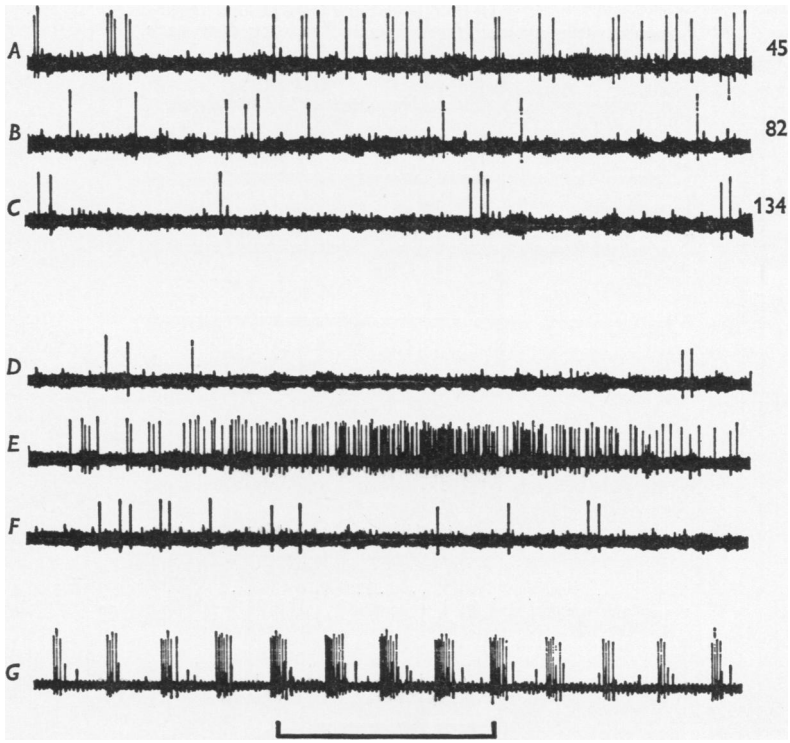
viously in normal cats (Biscoe, Sampson & Purves, 1967; Biscoe, Bradley & Purves, 1970; Biscoe, Purves & Sampson, 1970).

The discharge of chemoreceptor afferents on the operated side also increased when the P_{I,CO_2} was raised with associated changes in arterial pH.



Text-fig. 3. Filmed records of chemoreceptor action potentials (*A* to *J*) and rate-meter record of chemoreceptor discharge (*K*) recorded from three cats whose glossopharyngeal nerve roots on the side of these recordings had been cut intracranially. The numbers to the right in *A* to *J* show the P_{a,O_2} in mm Hg. *A* to *C*, operation 44 days previously; *D* to *F*, operation 105 days previously; *G* to *J*, operation 34 days previously. Time scale: 1 sec for *A*, *B*, *D*, *E*, *G* to *J*, 2 sec for *C*, *F*. In *K* the inspired gas was changed from air to O_2 at the first arrow and back to air at the second arrow; the scale to the right indicates 0–50 impulses/sec; recording made from a second strand in the cat operated on 44 days previously (*A* to *C*); time scale is 10 sec.

The response of chemoreceptor fibres arising from the denervated carotid body to intravenous injection of sodium cyanide (20–25 μg/kg) was also used as a routine test; in all cases sodium cyanide evoked a marked, but short lasting increase in chemoreceptor afferent fibre discharge (Text-fig. 4*D* to *F*).

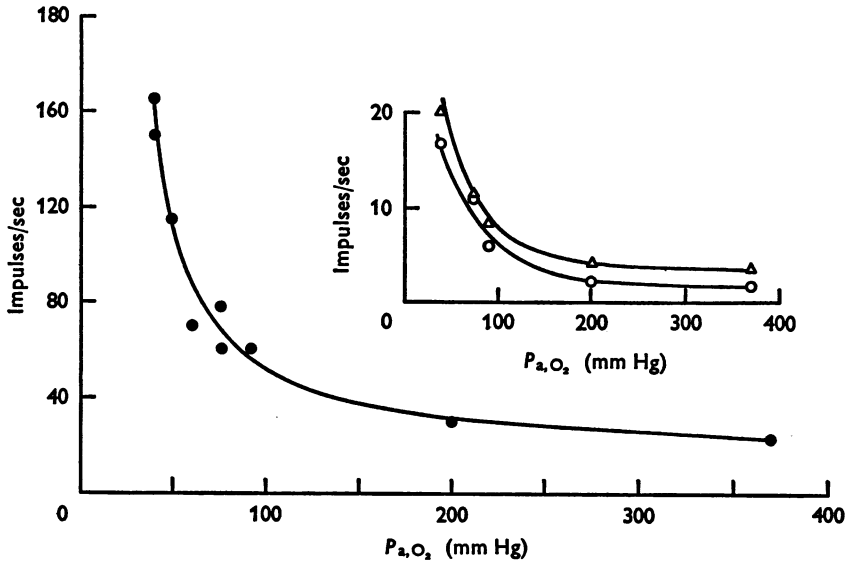


Text-fig. 4. Filmed records of action potentials recorded from the sinus nerve of a cat whose glossopharyngeal roots had been cut intracranially 378 days previously. *A* to *C*, chemoreceptor action potentials recorded at different O_2 tensions shown by the numbers to the right of the Figure in mm Hg. *D* to *F* continuous and overlapping record of chemoreceptor action potentials from another strand; 50 μg NaCN was injected intravenously at the beginning of record *D*. *G* is a record of baroreceptors and shows the bursts with each pressure pulse wave and the variation with respiration. Time scale is 2 sec for *A* to *F* and 1 sec for *G*.

DISCUSSION

The results of the series of experiments described here show that the number of nerve endings on the Type I cells is reduced after the glossopharyngeal and some of the vagus nerve roots are cut intracranially. Furthermore, the morphological changes which occur in these nerve

endings are similar to those which are recognized as an indication that degeneration is occurring in nerve tissue (Gray & Hamlyn, 1962; Colonnier & Gray, 1962; Colonnier, 1964; Hunt & Nelson, 1965; Ralston, 1965; Walberg, 1964, 1965; Alksne, Blackstad, Walberg & White, 1966; Gray & Guillery, 1966; Laatsch & Cowan, 1967; Hamori, Lang & Simon, 1968; Glees & Hasan, 1968). Thus we conclude that following intracranial section of the glossopharyngeal and vagus nerves the micro-vesicle containing nerve endings on the Type I cells degenerate.



Text-fig. 5. Graph to show the rate of discharge of chemoreceptor action potentials in impulses per sec against the arterial O_2 tension (mm Hg). The glossopharyngeal nerve of the cat had been cut intracranially 34 days previously. ●, discharge rate in the whole strand read off the ratemeter. ○, △, discharge rate in two single fibres measured by counting the number of action potentials on the filmed records.

This conclusion from the results runs counter to that originally proposed by De Castro (1928) who claimed that the innervation of the carotid body was intact within 12 days of the intracranial operation. Our evidence would suggest that he may not have waited long enough to see degeneration. However, De Castro & Rubio (1968) waited up to 30 days and they claim that there was no nerve degeneration. It is difficult to judge the state of the nerve endings in their work because the quality of the fixation shown in their published pictures was not good enough to allow a judgement. The cell cytoplasm lacks detail and the electron dense cored vesicles are uniformly depleted of their contents; often the internal mitochondrial structure is absent. Under these circum-

stances, the validity of their conclusion that there is no degeneration of nerve endings on Type I cells is questionable.

Time course and appearance of degeneration. Possibly the major difference between our findings and those of authors who have studied degeneration elsewhere in the nervous system is the prolonged time course of the changes in the carotid body, though this particular aspect has not usually been investigated in the C.N.S. The time course is much slower than that claimed by Hess (1968). He cut the sinus nerve and states that at '7 days no nerve fibres or synapses are seen in or around the glomera' (Hess, 1968, p. 53). This result conflicts with the evidence of Biscoe & Stehbens (1967) who found occasional nerve endings on Type I cells in the carotid bodies from cats whose sinus nerves had been cut up to 97 days previously. We see a similar prolonged time course for degeneration but this is not unique in the nervous system. Thus Spoendlin & Gacek (1963) found that non-myelinated nerve terminations in the organ of Corti may persist 3 months after the olivocochlear bundle was cut, and Walberg (1965) has found that nerve endings may still be recognized in the inferior olive up to 112 days after cutting afferent fibres. It should also be noted that Szentagothai (1965) allows 2–3 months to elapse in his work on the central nervous system before degeneration is regarded as complete. Walberg (1965) suggests that the long time that may be required for changes in the inferior olive to occur may be related to the fact that the predominant glial cells are fibrous astrocytes (Walberg, 1963*a*). In this tissue Walberg (1963*b*) has noted an absence of glial changes and of phagocytic activity. It may be that in the carotid body there is no cell capable of phagocytosing the nerve endings rapidly and this may account for the prolonged time course of degeneration.

The characters of the pathological changes we have seen have all been described at other sites in the nervous system. However, there are differences between the carotid body and the central nervous system in the incidence of changes. Very striking alterations in the mitochondria are often a feature of the published micrographs of the authors listed above, but aggregations such as they describe were uncommon in this study while fragmentation of nerve accompanied by the appearance of large vacuoles in intercellular space is perhaps more common. Furthermore, we have never seen accumulations of neurofilaments as described by Gray & Hamlyn (1962), Colonnier & Guillery (1964), Guillery (1965), Smith, O'Leary, Harris & Gay (1964), Smith & Rasmussen (1965), Dowling & Cowan (1966), though this is not a uniform finding and is not seen in mammalian cerebral cortex (Colonnier, 1964), inferior olive (Walberg, 1964, 1965) and the hippocampus (Blackstad, 1965).

There are a number of possible interpretations of the conclusion that

the Type I cell nerve endings degenerate. The degeneration may be said to occur because the glossopharyngeal ganglia are in some way damaged during the operation and hence degeneration is to be expected. In this case, chemoreceptor and baroreceptor activities should be absent. However, we have demonstrated that both chemoreceptor and baroreceptor activity persist in the sinus nerve even at a time when nerve endings on the Type I cells are degenerating. Furthermore, the glossopharyngeal ganglia are completely enclosed in bone at the base of the skull and hence remarkably well protected from trauma. During the operation great care was always taken to ensure that no blood vessels were damaged intracranially; in fact if they were damaged the cats always died. It is not certain where the blood supply to the ganglia arises and we have not seen an intra-dural vessel passing into the foramen from which the glossopharyngeal and vagus nerves emerge.

A second explanation is that the cell bodies of the afferent fibres are in the brain stem and that the nerve endings in the carotid body will necessarily degenerate when the nerve roots are cut. In such a case, which must be considered unlikely, the same argument concerning the presence of chemoreceptor activity applies; namely, if the sensory nerve endings are degenerating, how is it that chemoreceptor activity may still be recorded?

The third possible explanation of the results is that the nerve endings on the Type I cells are part of an efferent pathway to the carotid body whose cell bodies are located in the brain stem; hence when the intracranial operation is performed, the nerve endings are severed from their cell bodies. In this situation degeneration could occur without affecting chemoreceptor or baroreceptor activity. The explanation has the merit that it fits the known facts about the structure of the nerve endings on the Type I cells (see, for example, Biscoe & Stehbens, 1965, 1966, 1967; De Kock & Dunn, 1966, 1968; Hess, 1968; Al-Lami & Murray, 1968), since this structure is similar to that of efferent nerve endings elsewhere in the nervous system (see the review of Gray & Guillery, 1966).

Transneuronal degeneration may also be said to account for the degenerative changes in the carotid body. In this case a cell body in the petrosal ganglion would be synaptically related to axons in the cut nerve roots. The petrosal cell bodies concerned could then be efferent neurones and the nerve endings on the Type I cells efferent. Gray & Guillery (1966) have discussed the literature on this problem and concluded that degenerative responses would appear to be primarily located in dendrites. There is no evidence that changes would be expected to occur at an axonal terminal some 30 mm distant from the cell body. In any event the most likely situation appears to be one where efferent cell bodies are in the brain stem

while the transneuronal argument, if valid, does not materially affect the conclusions of this paper.

According to our data all nerve endings have not degenerated on the Type I cells even 378 days after cutting the glossopharyngeal nerve roots. However, it should be remembered that in order to try and avoid subjective errors we counted all structures that in any way resembled nerve endings. Nonetheless, some nerve endings appeared normal. It is possible that these endings do not degenerate because they arise from efferent fibres that join the sinus nerve extracranially. Biscoe & Sampson (1968) reported that post-ganglionic fibres from the superior cervical ganglion join the glossopharyngeal nerve extracranially and course down the sinus nerve towards the carotid body. However, a sympathetic origin of the nerve endings that did not degenerate is unlikely, because previous studies have shown that removal of the superior cervical ganglion does not affect nerve endings on Type I cells (Biscoe & Stehbens, 1967). It is possible that efferent fibres from other nerves (e.g. vagus) join the glossopharyngeal nerve extracranially and terminate in the carotid body; this remains to be investigated. We conclude that although some nerve endings on Type I cells survive up to 378 days after section of the IXth nerve intracranially, all are efferent.

The conclusion that the nerve endings on the Type I cells are efferent raises a number of important questions: what is the function of the Type I cells and which structures are the chemoreceptors? At present it is only possible to speculate upon the answers to these problems though it seems unlikely that the Type I cells can be receptors and more likely that they are part of an efferent system whose function is to control receptor performance. This view is supported by the observation that there is a centrifugal pathway in the sinus nerve (Biscoe & Sampson, 1968) and that stimulation of the peripheral end of the sinus nerve depresses chemoreceptor activity (Neil & O'Regan, 1969; Sampson & Biscoe, 1970). Interruption of this pathway also increases the responses of chemoreceptors to hypoxia (Sampson & Biscoe, 1970). Thus spontaneous activity in the pathway does have tonic effects. Catecholamines or 5-hydroxytryptamine are very likely to play a role in this efferent modulation of the chemoreceptors since these substances are present in the Type I cells (see, for example, Chiocchio, Biscardi & Tramezzani, 1967) though the precise mode of action is uncertain.

The question of which structure is the chemoreceptor remains to be determined and is discussed elsewhere (Biscoe, 1970).

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REFERENCES

ALKSNE, J. F., BLACKSTAD, T. W., WALBERG, F. & WHITE, L. E. JR. (1966). Electron microscopy of axon degeneration: a valuable tool in neuroanatomy. *Ergbn. Anat. EntwGesch.* **39**, 1–32.

AL-LAMI, F. & MURRAY, R. G. (1968). Fine structure of the carotid body of normal and anoxic cats. *Anat. Rec.* **160**, 697–718.

BISCOE, T. J. (1970). Carotid body. Structure and function. *Physiol. Rev.* (In the Press.)

BISCOE, T. J., BRADLEY, G. W. & PURVES, M. J. (1970). The relation between carotid body chemoreceptor discharge, carotid sinus pressure and carotid body venous flow. *J. Physiol.* **208**, 99–120.

BISCOE, T. J., LALL, A. & SAMPSON, S. R. (1969). On the nerve endings associated with the carotid body glomus cells of the cat. *J. Physiol.* **200**, 131–132*P*.

BISCOE, T. J. & PURVES, M. J. (1967). Observations on the rhythmic variations in the cat carotid body chemoreceptor activity which has the same period as respiration. *J. Physiol.* **190**, 389–412.

BISCOE, T. J., PURVES, M. J. & SAMPSON, S. R. (1970). The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded *in vivo* with intact circulation. *J. Physiol.* **208**, 121–131.

BISCOE, T. J. & SAMPSON, S. R. (1967). Spontaneous activity recorded from the central cut end of the sinus nerve of the cat. *Nature, Lond.* **216**, 294–295.

BISCOE, T. J. & SAMPSON, S. R. (1968). Non-rhythmical spontaneous activity recorded from the central cut end of the sinus nerve. *J. Physiol.* **196**, 327–338.

BISCOE, T. J., SAMPSON, S. R. & PURVES, M. J. (1967). Stimulus response curves of single carotid body chemoreceptor afferent fibres. *Nature, Lond.* **215**, 654–655.

BISCOE, T. J. & STEHBENS, W. E. (1965). Electron microscopic observations on the carotid body. *Nature, Lond.* **208**, 708–709.

BISCOE, T. J. & STEHBENS, W. E. (1966). Ultrastructure of the carotid body. *J. cell Biol.* **30**, 563–578.

BISCOE, T. J. & STEHBENS, W. E. (1967). Ultrastructure of the denervated carotid body. *Q. Jl exp. Physiol.* **52**, 31–36.

BLACKSTAD, T. W. (1965). Mapping of experimental axon degeneration by electron microscopy of Golgi preparations. *Z. Zellforsch. mikrosk. Anat.* **67**, 819–834.

CHIOCCHIO, S. R., BISCARDI, A. M. & TRAMEZZANI, J. H. (1967). 5-Hydroxytryptamine in the carotid body of the cat. *Science, N.Y.* **158**, 790–791.

COLONNIER, M. (1964). Experimental degeneration in the cerebral cortex. *J. Anat.* **98**, 47–53.

COLONNIER, M. & GRAY, E. G. (1962). Degeneration in the cerebral cortex in electron microscopy. *Fifth Internat. Congr. for Electron Microscopy*, vol. 2, P.U.-3, ed. BREESE, S. S., Jr. New York: Academic Press.

COLONNIER, M. & GUILLERY, R. W. (1964). Synaptic organization in the lateral geniculate nucleus of the monkey. *Z. Zellforsch. mikrosk. Anat.* **62**, 333–355.

COTTLE, M. K. (1964). Degeneration studies of primary afferents of IXth and Xth cranial nerves in the cat. *J. comp. Neurol.* **122**, 329–345.

DE CASTRO, F. (1926). Sur la structure et l'innervation de la glande intercarotidienne (glomus caroticum) de l'homme et des mammifères, et sur un nouveau système d'innervation autonome du nerf glossopharyngien. Etudes anatomiques et expérimentales. *Trab. Lab. Invest. biol. Univ. Madr.* **24**, 365–432.

DE CASTRO, F. (1928). Sur la structure et l'innervation du sinus carotidien de l'homme et des mammifères. Nouveaux faits sur l'innervation et la function du glomus caroticum. Etudes anatomiques et physiologiques. *Trab. Lab. Invest. biol. Univ. Madr.* **25**, 331–380.

DE CASTRO, F. & RUBIO, M. (1968). The anatomy and innervation of the blood vessels of the carotid body and the role of chemoreceptive reactions in the autoregulation of the blood flow. In *the Proceedings of the Wates Foundation Symposium on Arterial Chemoreceptors*, ed. TORRANCE, R. W., pp. 267–277. Oxford and Edinburgh: Blackwell Scientific Publications.

DE KOCK, L. L. & DUNN, A. E. G. (1966). An electron microscope study of the carotid body. *Acta anat.* **64**, 163–178.

DE KOCK, L. L. & DUNN, A. E. G. (1968). Electron-microscopic investigation of the nerve endings in carotid body. In *The Proceedings of the Wates Foundation Symposium on Arterial Chemoreceptors*, ed. TORRANCE, R. W., pp. 179–187. Oxford and Edinburgh: Blackwell Scientific Publications.

DOWLING, J. E. & COWAN, W. M. (1966). An electron microscope study of normal and degenerating centrifugal fibre terminals in the pigeon retina. *Z. Zellforsch. mikrosk. Anat.* **71**, 14–28.

FARQUHAR, M. & PALADE, G. E. (1965). Cell junctions in amphibian skin. *J. cell Biol.* **26**, 263–291.

GLEES, P. & HASAN, M. (1968). The signs of synaptic degeneration—a critical appraisal. *Acta anat.* **69**, 153–167.

GRAY, E. G. & GUILLERY, R. W. (1966). Synaptic morphology in the normal and degenerating nervous system. *Int. Rev. Cytol.* **19**, 111–182.

GRAY, E. G. & HAMLYN, L. H. (1962). Electron microscopy of experimental degeneration in the avian optic tectum. *J. Anat.* **96**, 309–316.

GUILLERY, R. W. (1965). Some electron microscopical observations of degenerative changes in central nervous synapses. In *Progress in Brain Research*, ed. BARGMANN, W. & SCHADE, J. P., vol. 14, pp. 57–76. Amsterdam: Elsevier.

HAMORI, J., LANG, E. & SIMON, L. (1968). Experimental degeneration of the preganglionic fibers in the superior cervical ganglion of the cat. An electron microscope study. *Z. Zellforsch. mikrosk. Anat.* **90**, 37–52.

HESS, A. (1968). Electron microscopic observations of normal and experimental cat carotid bodies. In *The Proceedings of the Wates Foundation Symposium on Arterial Chemoreceptors*, ed. TORRANCE, R. W., pp. 51–56. Oxford and Edinburgh: Blackwell Scientific Publications.

HUNT, C. C. & NELSON, P. G. (1965). Structure and functional changes in the frog sympathetic ganglion following cutting of the presynaptic fibres. *J. Physiol.* **177**, 1–20.

KELLENBERGER, E., RYTER, A. & SECHAUD, J. (1958). Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. *J. biophys. biochem. Cytol.* **4**, 671–678.

LAATSCH, R. H. & COWAN, W. M. (1967). Electron microscopic studies of the dentate gyrus of the rat. II. Degeneration of commissural afferents. *J. comp. Neurol.* **130**, 241–262.

LUFT, J. H. (1961). Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* **9**, 409–414.

NEIL, E. & O'REGAN, R. G. (1969). Effects of sinus and aortic nerve efferents on arterial chemoreceptor function. *J. Physiol.* **200**, 69*P*.

PALADE, G. E. (1952). A study of fixation for electron microscopy. *J. exp. Med.* **95**, 285–298.

RALSTON, H. J. (1965). The organization of the substantia gelatinosa Rolandi in the cat spinal cord. *Z. Zellforsch. mikrosk. Anat.* **67**, 1–23.

SAMPSON, S. R. & BISCOE, T. J. (1970). Efferent control of the carotid body chemoreceptor. *Experientia.* (In the Press.)

SMITH, J. M., O'LEARY, J. L., HARRIS, A. B. & GAY, A. J. (1964). Ultrastructural features of the lateral geniculate nucleus of the cat. *J. comp. Neurol.* **123**, 357–378.

SMITH, C. A. & RASMUSSEN, G. L. (1965). Degeneration in the efferent nerve endings in the cochlea after axonal section. *J. cell Biol.* **26**, 63–77.

SPOENDLIN, H. H. & GACEK, R. R. (1963). Electron microscopic study of the efferent and afferent innervation of the organ of Corti in the cat. *Ann. Otol. Rhinol. Lar.* **72**, 660–686.

STUDENT (1908). The probable error of a mean. *Biometrika* **6**, 1–25.

SZENTAGOTHAI, J. (1965). The use of degeneration methods in the investigation of short neuronal connections. In *Degeneration Pattern in the Nervous System*, ed. SINGER, M. & SCHADE, J. P., pp. 1–32. Amsterdam: Elsevier.

VENABLE, J. H. & COGGESHALL, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J. cell Biol.* **25**, 407–408.

WALBERG, F. (1963*a*). An electron microscopic study of the inferior olive in the cat. *J. comp. Neurol.* **120**, 1–17.

WALBERG, F. (1963*b*). Role of normal dendrites in removal of degenerating terminal boutons. *Expl Neurol.* **8**, 112–124.

WALBERG, F. (1964). The early changes in degenerating boutons and the problem of argyrophilia. Light and electron microscopic observations up to 10 days after lesion. *J. comp. Neurol.* **122**, 113–137.

WALBERG, F. (1965). An electron microscopic study of terminal degeneration in the inferior olive of the cat. *J. comp. Neurol.* **125**, 205–222.

EXPLANATION OF PLATES

PLATE 1

A, section of carotid body from the non-operated side. The Type I cell, I, is surrounded by a Type II cell, II. The usual cytoplasmic constituents may be seen including the Golgi apparatus, G, the endoplasmic reticulum, mitochondria, electron dense cored vesicles, and a cilium, C. There is a large nerve ending, NE, which contains small mitochondria, micro-vesicles approximately 500×10^{-10} m diameter and glycogen. The nerve ending has several electron dense junctional regions with the Type 1 cell. The complex folding of the Type II cell membranes may be seen adjacent to the nerve ending. In the space between the two adjacent Type I–Type II cell complexes collagen is present. Small nerve fibres may be seen at several sites. × 12,000. The horizontal line is 1 μ in each plate.

B, electron micrograph from the carotid body of the operated side, intracranial operation performed 27 days previously. There is a nerve ending, NE, adjacent to a Type I cell and partially enclosed by Type II cell processes; fine filaments may be seen in the Type II cell cytoplasm. The nerve ending contains a small aggregation of electron dense mitochondria, M, and a vacuole, V, containing an aggregation of micro-vesicles.The ending is contiguous with a large vacuolated area which contains electron dense figures. To the left a nerve fibre, NF, is adjacent to this vacuolated area. BM is a part of the basement membrane which surrounds the Type I–Type II cell complex. × 16,800.

PLATE 2

A, intracranial operation performed 6 days previously. The nerve ending, NE, shows electron opaque mitochondria and clumping of the synaptic vesicles and has an adjacent vacuolated area containing tissue debris. × 15,000.

B, intracranial operation performed 44 days previously. The nerve ending, NE, shows degenerating, electron dense mitochondria. × 18,000.

C, intracranial operation performed 105 days previously. The nerve ending shows swollen mitochondria with loss of the internal structure. × 21,600.

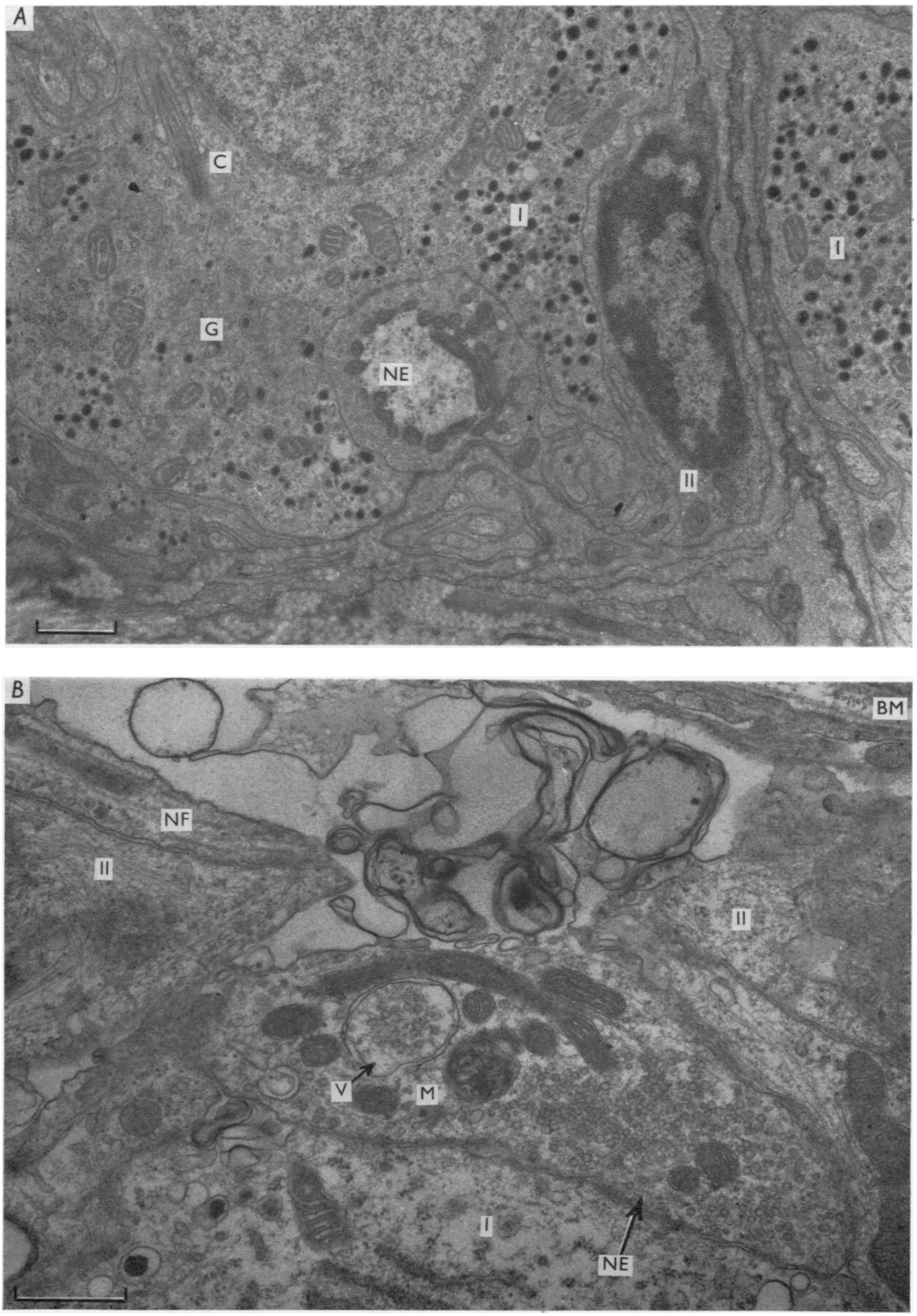
D, intracranial operation performed 128 days previously. The presumed nerve ending shows loss of detailed cytoplasmic structure and appears to contain small irregularly shaped vesicles and membrane fragments. The nerve ending has no mitochondria or glycogen particles and contains two dense bodies near the upper end. Near the lower right edge are small myelinated nerve fibres enclosed by processes of Type II cells. These fibres appear normal. × 16,000.

I and II are Type I and II cells.

PLATE 3

A, intracranial operation performed 27 days previously. Between the Type I and Type II cells there is an area containing fragmented nerve fibres, NF, some of which appear swollen. They are related to a vacuolated area containing some small electron dense figures. × 14,400.

B, intracranial operation performed 27 days previously. Schwann cell, *S*, which contains fragmented degenerating nerve fibres, vacuoles, V, and a myelin figure, M. To the right is another smaller Schwann cell containing nerve fibres, NF, which have a normal appearance. × 14,400.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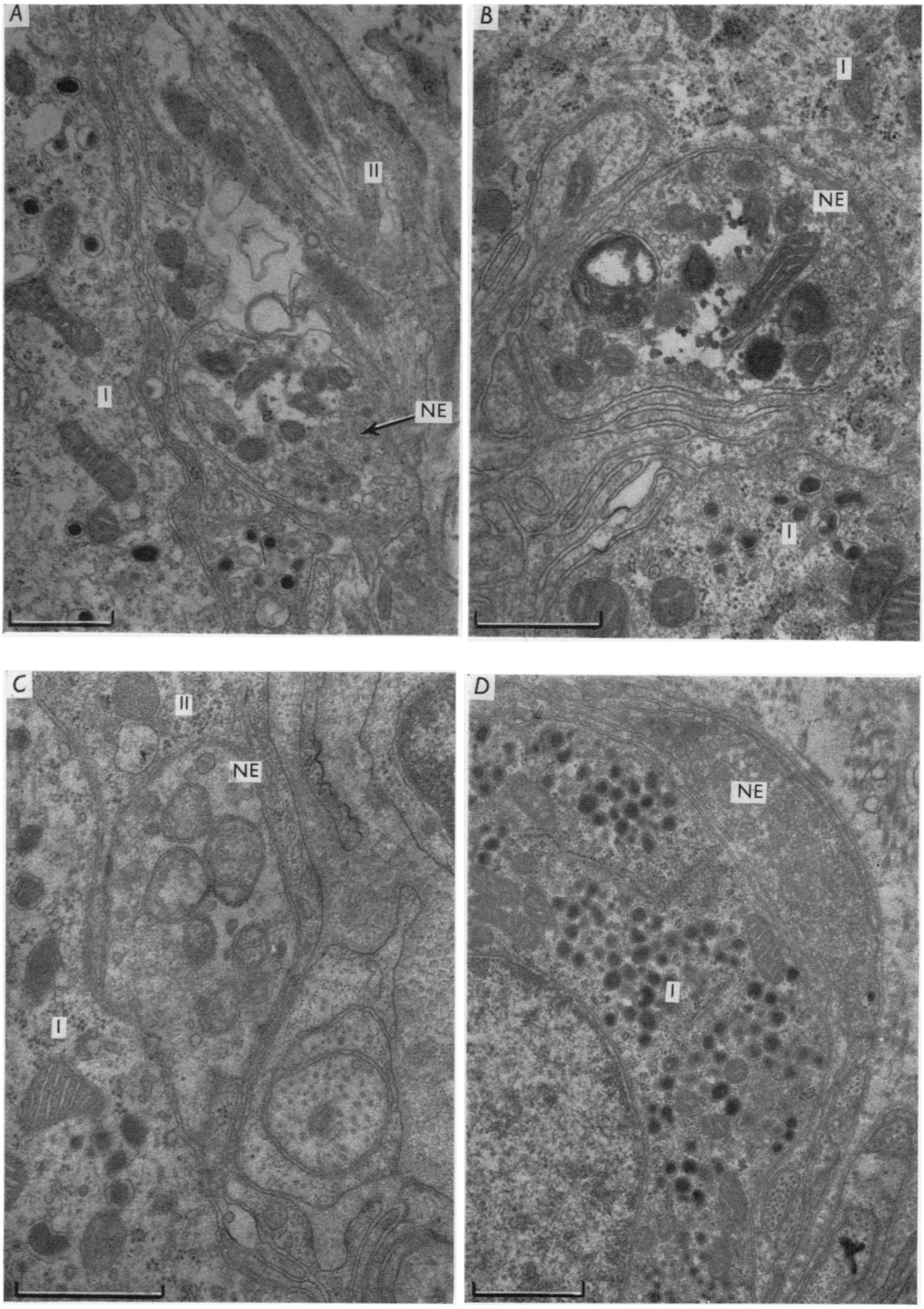
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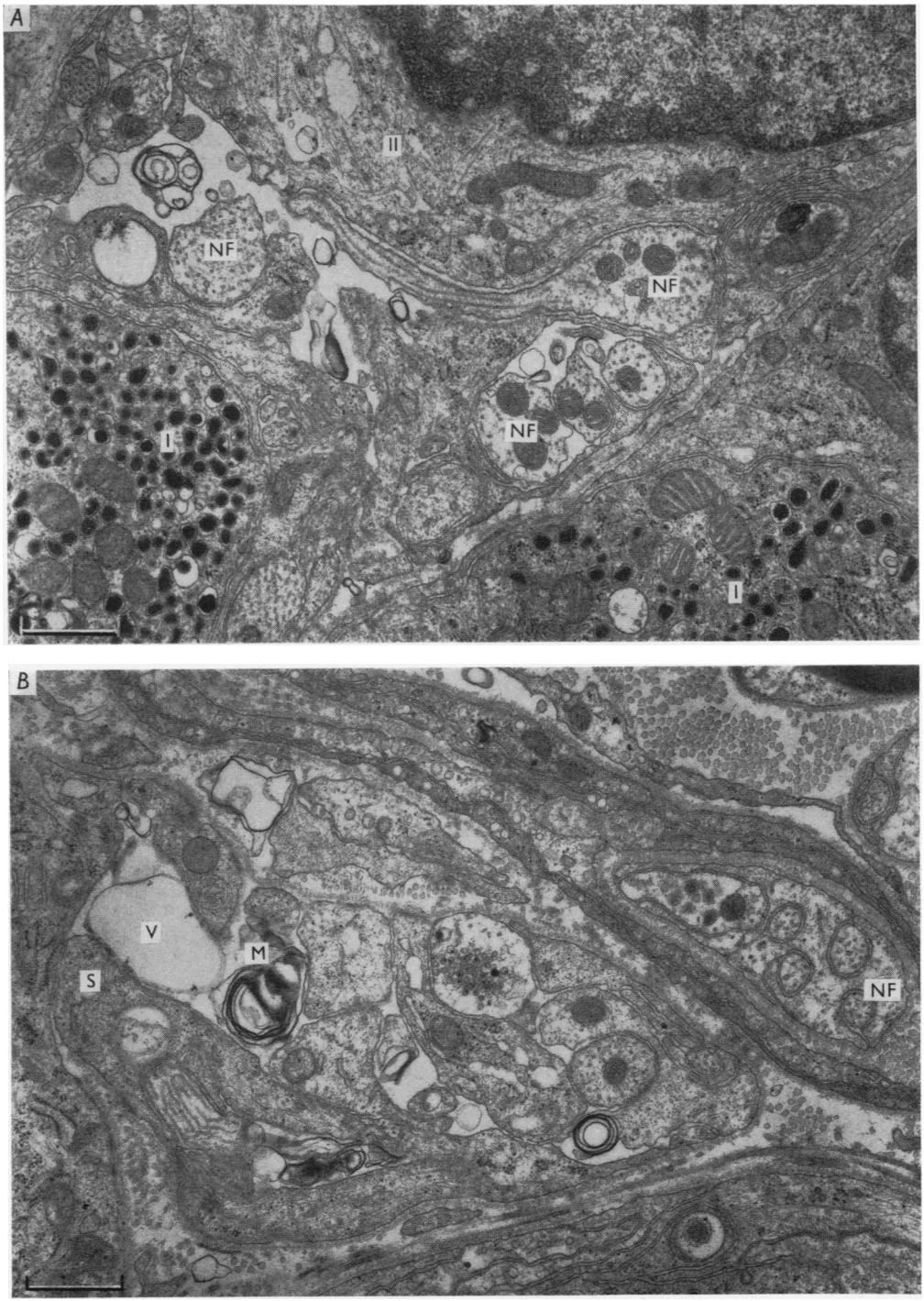

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