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AN AUTORADIOGRAPHIC STUDY OF THE INCORPORATION OF NUCLEIC-ACID PRECURSORS BY NEURONES AND GLIA DURING NERVE REGENERATION

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Recent advances in quantitative microanalysis (Edström, 1953, 1960) have permitted measurement of change in ribonucleic acid (RNA) content of individual neurones after cutting their axons (Brattgård, Edström & Hydén, 1957). As measurement of a cell's content of RNA alone can give little indication of its dynamic intracellular turnover, the present investigation was undertaken with tritium (^3H) labelled precursors of RNA. Their incorporation into various subcellular compartments was detected autoradiographically. The investigation also includes a study of deoxyribonucleic acid (DNA) synthesis within perineuronal oligodendroglia.

METHODS

Animals. Most observations were made upon albino mice aged 3 months at the time of nerve division: they received standard food pellets and water supplemented with bread and milk *ad libitum*. The observations were repeated upon rats and rabbits of the same age.

Nerve division. Under general anaesthesia the left hypoglossal nerve was mobilized for about 5 mm where it crossed the interval between internal and external carotid arteries. In some animals the nerve was crushed with fine watchmaker's forceps for 10 sec; in others, the nerve was cut with sharp scissors, and the distal part avulsed from the tongue to impede regeneration. In a few animals the nerve was ligated with silk at this level. In all, care was taken to avoid traction upon the proximal portion of nerve. The animals were allowed to survive up to 80 days after the operation.

In other mice, the facial nerve was interrupted similarly where it passed superficially, inferior to the cartilaginous external auditory meatus and posterior to the common facial vein.

Isotope administration. Tritiated uridine (1·22 c/m-mole), adenosine (0·68 c/m-mole), and guanosine (89·3 mc/m-mole) were used as precursors of RNA. Tritiated thymidine (3·3 c/m-mole) allowed the synthesis of DNA in glial cells to be followed, and tritiated lysine (54·7 mc/m-mole) was used to indicate protein synthesis. All isotopes were obtained from the Radiochemical Centre, Amersham.

In early experiments the labelled compound was diluted with an electrolyte solution, such that the final composition represented that of cerebrospinal fluid (Mitchell, Loeschke, Massion & Severinghaus, 1963); in later experiments, water was used: no difference resulted from this change of solvent. The final activity of the solutions of [^3H]lysine, [^3H]adenosine, [^3H]guanosine and [^3H]uridine was 1 c/ml.: [^3H]thymidine was diluted to 0·25 c/ml. Twenty

microlitres of one of these solutions was injected into the lateral cerebral ventricle of each mouse (Haley & McCormick, 1957). Each rat received 50 μl. and each rabbit 100 μl. by cisternal injection under light ether anaesthesia.

Histological techniques. Between 2 hr and 48 hr after administering the isotope, the animal was anaesthetized with ether and exsanguinated: both hypoglossal nerves were removed and the animal was perfused with Carnoy's fixative. The medulla was excised, fixed for 1 hr, dehydrated and impregnated with wax: serial sections of 1 μ were obtained.

Each hypoglossal nerve was prepared by the method of Aitken, Sharman & Young (1947). A section about 4 mm distal to the site at which the nerve was crushed, just distal to the bifurcation of the hypoglossal nerve, was projected on to a sheet of bromide paper at a magnification of $\times$ 850. The internal diameters of all myelin rings were measured. Care was taken to avoid errors of magnification, and to use constant conditions of illumination: it was assumed that the diameter of the few crenated fibres had decreased by 10 % (Duncan, 1934).

Autoradiography. The transverse sections of medulla were rehydrated, agitated in 1 % perchloric acid at 4° C for 20 min to remove unincorporated nucleoside (Perry, Hell & Errera, 1961) and washed for 2 hr. Every fifth slide was digested with pancreatic ribonuclease, 50 μg/ml., in McIlvaine's buffer at pH 7·0 for 3 hr at 37° C (Edström, 1953) or with deoxyribonuclease, 50 μg/ml. in 0·02 M phosphate buffer, pH 7·0, with 0·003 M-$MgCl_2$. The slides were coated with Ilford G5 photographic emulsion, and exposed for between 3 days and 4 weeks, depending upon the nucleoside administered. They were developed in Kodak developer for 4 min at 18° C, fixed, washed, and stained either with methyl-green-pyronin, or with cresyl violet.

Only those neurones cut in the plane of their nucleoli were examined: when the same nucleolus was seen in adjacent serial sections, the section in which it was larger was chosen. No other selection of cells was permitted. At least twenty neurones seen consecutively were examined in each hypoglossal or facial nucleus. The area of each cell and of its nucleus was measured with a squared eyepiece graticule, the apparent length of the sides of each square being 1·8 μ: the diameter of the nucleolus was also measured with a graticule calibrated in apparent units of 0·36 μ. The silver grains over the nucleus, including the nucleolus, and over the cytoplasm were separately counted. The background count was determined close to each section, and all values presented were corrected for this.

Expression of results. The mean density of silver grains over the neurone cytoplasm was calculated by dividing the number of grains overlying the cytoplasm by its area. The density of grains over the nucleus, including the nucleolus, was similarly calculated. As each of these values depended upon the amount of isotope taken up by the cell, which was in part determined by factors other than intracellular synthesis of RNA (see Discussion), the ratio of cytoplasm grain density to nuclear grain density (cyt./nuc. ratio) was calculated.

Cell counts. The relative number of neurones surviving after nerve interruption was determined by comparing the number of neuronal nucleoli seen within the abnormal hypoglossal nucleus with the number observed on the normal side. The presence of multiple nucleoli did not invalidate this procedure as they were seen in only 0·2 % of cells, with equal frequency on the normal and abnormal side.

The number of perineuronal glial cells seen around each neurone was noted. After the administration of [^{3}H]thymidine, the number of labelled nuclei was expressed as a percentage of the total number of perineuronal glial cell nuclei.

RESULTS

Evaluation of methods. When the medulla of a mouse which had received no isotope was exposed the number of silver grains over the sections did not exceed the background count: it is therefore believed that none of the

grains was caused by chemical reduction of the photographic emulsion by the fixed tissue.

Adjacent sections of a given radioactive medulla were exposed for varying periods up to 4 weeks. The grain density was found to be proportional to the duration of the exposure, provided the density did not become so high that grains coalesced. There is therefore no evidence of fading of the latent photographic image within these limits.

The amount of [^{3}H]uridine given to each of a series of mice was varied within the range 5–50 μc. For mice killed at a given time after receiving the isotope (4, 6 or 8 hr) no significant variation was observed in the ratio 'grain density in cytoplasm to grain density in nucleus' (cyt./nuc. ratio), although the mean grain count per cell varied widely. This suggests that the cyt./nuc. ratio was not itself critically influenced by the amount of added nucleoside gaining access to the cell.

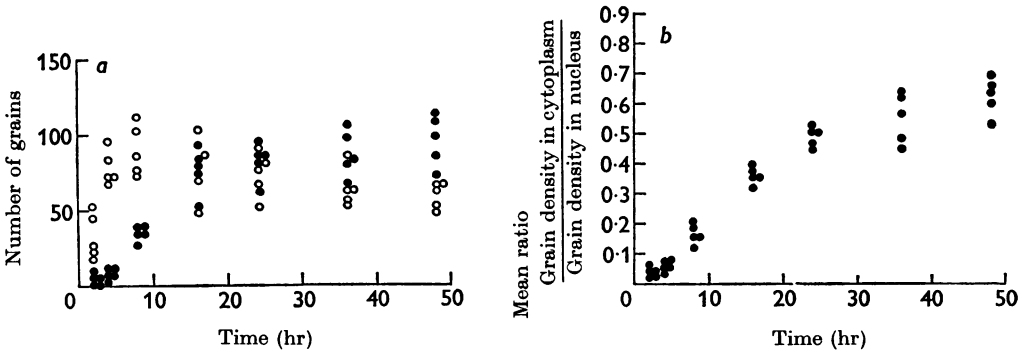
Axon regeneration. After a hypoglossal nerve had been crushed, no regenerating myelinated axons were seen distal to the lesion until the eighth day: until the twelfth day all axons were less than 2μ in diameter. The normal distribution of axon diameters was not restored until the fortieth day after nerve injury. After a hypoglossal nerve had been divided with avulsion of the distal part, no regeneration from the proximal stump could be seen.

When a hypoglossal nerve had been crushed, the number of neurones in the nucleus decreased between the fifth and the twentieth day to 60–90 % (mean 85 %) of the number on the normal side. Following complete division of the nerve with avulsion of the distal part the number of surviving neurones decreased over the same period to 22–30 % (mean 25 %) of the number on the normal side. Many of the surviving cells showed chromatolysis. Chromatolysis was also seen in about 5 % of neurones of the contralateral hypoglossal nucleus.

Within 2 days of crushing or dividing the axon, the area of the neurone seen on section began to increase, attaining a value 50 % greater than normal by the third day. On the eighteenth day, the area began to decrease, and was restored to its initial value by the fortieth day. The nucleoli of neurones began to increase in diameter on the third day after nerve injury, attained a maximum diameter 11 % greater than normal about the eighth day: they then became smaller, reaching their normal size about the eighteenth day.

Isotope administration. The tritium label was first detected within neurones 15–30 min after the injection of RNA precursor. The mean grain count per neurone then increased to reach a maximum value in 12–24 hr. Initially the grains lay predominantly over the nucleus, but after about 4 hr the cytoplasmic grain count increased: after 16–24 hr most grains lay

over the cytoplasm (Text-fig. 1*a*). The cyt./nuc. ratio increased between 2 hr and 24 hr after isotope administration (Text-fig. 1*b*). Incubating the slides in ribonuclease completely removed the labelled compound: incubating in buffer alone did not significantly decrease the grain count.

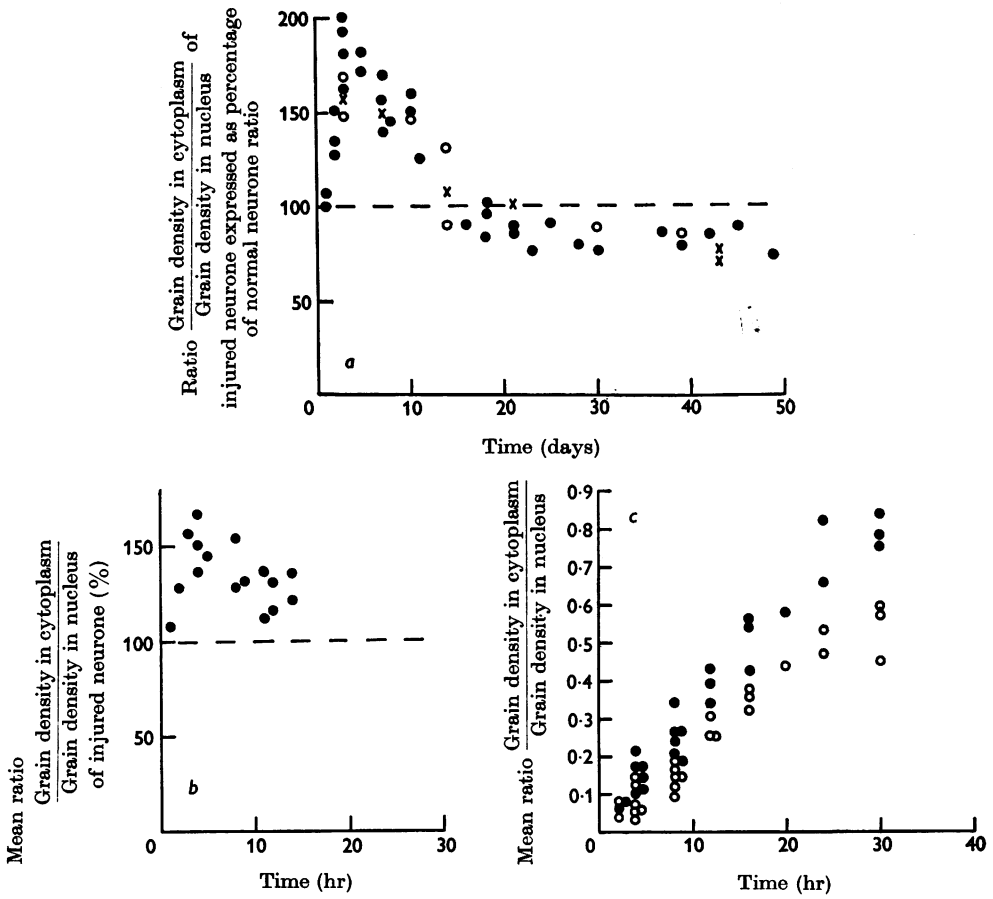


Text-fig. 1. *a*. The mean number of silver grains over the nucleus, including the nucleolus, or over the cytoplasm is expressed graphically against the duration of survival after intraventricular injection of [^{3}H]-uridine. Each point represents values obtained from one mouse. Twenty neurones of each hypoglossal nucleus were counted. ●, grains in cytoplasm; ○, grains in nucleus.

b. The ratio 'density of grains in cytoplasm/density of grains in nucleus' is expressed graphically against the duration of survival after intraventricular injection of 20 μc[^{3}H]uridine.

Between 2 and 20 days after the hypoglossal nerve had been crushed the uptake of labelled nucleoside and of labelled lysine per neurone was greater on the injured side. The cyt./nuc. ratio, measured 8 hr after injecting tritiated nucleoside, increased within 48 hr of crushing or dividing the nerve, and reached a maximum value about the third day (Text-fig. 2*a*, *b*; Pl. 1). This increase resulted principally from an increased density of grains over the cytoplasm and, to a lesser extent, from reduction of nuclear density. Text-fig. 2*c* shows values obtained for the cyt./nuc. ratio on the third day after nerve division at varying times after the administration of [^{3}H]uridine, and compares these values with the normal. This curve, relating the ratio to the time following isotope administration, is not simply shifted to the left after nerve division, but its gradient is also increased.

The increase of cyt./nuc. ratio following nerve division or crushing never preceded cell swelling. The ratio decreased from its maximum value while the cell size remained large, and about the time the nucleolus attained its maximum diameter.

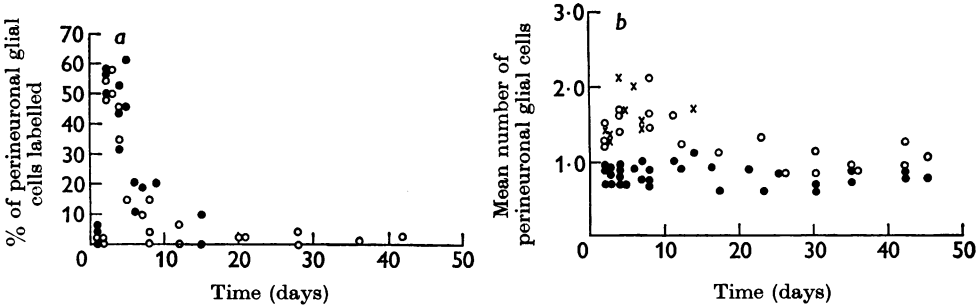


Text-fig. 2. *a*. The mean ratio 'density of grains in cytoplasm/density of grains in nucleus' of injured hypoglossal neurones is expressed as a percentage of the mean ratio of normal hypoglossal neurones. This percentage is expressed graphically against the duration of survival after crushing the nerve. All values were obtained from mice 8 hr after injections of tritiated nucleoside. ●, [^{3}H]uridine; ○, [^{3}H]adenosine; ×, [^{3}H]guanosine.

b. As in Text-fig. 2*a*, except that the nerve was divided with avulsion of the distal part, and not crushed.

c. The mean ratio 'density of grains in cytoplasm/density of grains in nucleus' is expressed graphically against the duration of survival after intraventricular injections of [^{3}H]uridine (as in Text-fig. 1*b*). All values were obtained from mice injected with [^{3}H]uridine 72 hr after crushing a hypoglossal nerve. ○, values for normal (right) hypoglossal neurones; ●, values for crushed (left) hypoglossal neurones. Note that the ratios found in right hypoglossal neurones do not differ from those of normal mice (Text-fig. 1*b*) and that any given ratio is achieved by left (injured) hypoglossal neurones in about half or two-thirds of the time taken by right hypoglossal neurones.

Neuroglia. In the normal mouse, less than 1 % of perineuronal glial cells incorporated [^{3}H]thymidine. Forty-eight hours after crushing or dividing the nerve, 50 % of these cells within the nucleus of the injured hypoglossal nerve had incorporated [^{3}H]thymidine given 16 hr before death (Pl. 2*a*): by the eighth day after nerve injury the uptake of [^{3}H]thymidine was again low (Text-fig. 3*a*). In the contralateral hypoglossal nucleus 5 % of the neurones were surrounded by glia labelled in a similar proportion: these contralateral neurones lay on the medial side of the nucleus and most showed chromatolysis or cell swelling. This label was completely



Text-fig. 3*a*. The number of perineuronal glial cell nuclei labelled 16 hr after intraventricular injections of 5 μc [^{3}H]thymidine is expressed as a percentage of the total number of perineuronal glial cells seen. This percentage is expressed graphically against the duration of survival following nerve injury. ○, nerve crushed; ●, nerve divided, with avulsion of distal part.

b. The mean number of perineuronal glial cells seen in association with each neurone in each 1 μ section is expressed graphically against the duration of survival following nerve injury. ●, normal (right) hypoglossal nucleus; ○, left hypoglossal nucleus after nerve crushed; ×, left hypoglossal nucleus after nerve divided with avulsion of distal part.

removed by deoxyribonuclease digestion, but not by ribonuclease or by incubation in buffer alone. When a mouse was killed as early as 4 hr after injecting [^{3}H]thymidine, 48 hr after nerve division, fewer glial cells were labelled but all were perineuronal. If a mouse survived 8, 16 or 24 days after [^{3}H]thymidine was given, the labelled cells were still found only in this situation. It is therefore probable that the perineuronal glial cells themselves divide and do not then migrate elsewhere. [^{3}H]thymidine was never incorporated by perineuronal glia before the mean area of the neurones had increased. Apparently random variation in size of normal neurones prevented direct comparison of the increase in size of each neurone with the number of labelled glial cells around it.

The mean number of glial cells seen in association with each neurone increased between the second and fifth days after nerve injury, a period

corresponding with the increased incorporation of [^{3}H]thymidine (Text-fig. 3*b*).

Hypoglossal nerve of rat. After crushing the nerve in the rat the cell bodies began to swell at 48 hr, as in the mouse. The incorporation of [^{3}H]thymidine by glial-cell nuclei reached a peak between the third and fifth days, when 40–60 % of cells were labelled: after 12 days, the incorporation of [^{3}H]thymidine was again at its normal low value. The neuronal cyt./nuc. ratio found 8 hr after injecting [^{3}H]uridine increased by 40–90 % between the third and seventh day after nerve division, and then decreased. By the twenty-fifth day the ratio was not significantly different to that found on the normal side.

Hypoglossal nerve division in rabbit. After crushing the hypoglossal nerve the neurone cell bodies began to swell after 24 hr, and attained a maximum value on the third or fourth day. This was accompanied by an increase in the cyt./nuc. ratio most marked between the third and fifth days: it then decreased as in the mouse. The uptake of [^{3}H]thymidine by glial cells occurred between the second and fifth days. Endothelial cells of capillaries traversing the nucleus of the injured hypoglossal nerve incorporated [^{3}H]thymidine at this time (Pl. 2*b*): this was not found on the normal side, and was not apparent in rats or mice.

Facial nerve division in mouse. Changes occurring in the facial nucleus of the mouse after crushing the nerve were not significantly different from those observed after crushing the hypoglossal nerve.

Nerve ligation. After ligation of the hypoglossal or facial nerve of the mouse, the changes occurring in the first 5 days precisely resembled those following crushing the nerve.

DISCUSSION

Histological artifact. Following the preparative procedure used, linear shrinkage of nerve cells is about 20–25 %. Such a change in the mean diameter of a neurone would reduce its area seen on section by 40 % and its volume by 55 % (Brattgård *et al.* 1957). This reduction in cell size may be accompanied by a small increase in nuclear volume. As the density of silver grains is based upon areas measured in fixed tissues, it is probable that the cytoplasmic density has been over-estimated, and possible that the nuclear density is really greater than stated. The cyt./nuc. ratio within a given fixed cell is therefore probably about twice that existing before fixation. If it is valid to assume that normal and injured nerve cells behave similarly in this respect (Friede, 1963), then this factor will be the same in each. As the control neurones were in the same section as the injured the preparative procedures must have been identical.

Although no loss of acid-insoluble macromolecular RNA occurs from nerve cells prepared in this way (Edström, 1957; Stenram, 1958; Jarlstedt, 1962), it is possible that the intracellular distribution of labelled molecules is altered by fixation and dehydration. Labelled molecules would have to pass between nucleus and cytoplasm after death in order to affect the cyt./nuc. ratio measured. As the labelled RNA seen in the nucleus 2–4 hr after isotope administration does not enter the cytoplasm during fixation, and as the cytoplasmic labelled RNA seen 16 hr or more after isotope administration remains confined to the cytoplasm it is probable that errors from this source are negligible.

The limitations and possible errors arising from the techniques used to measure axon diameters are fully discussed by Aitken *et al.* (1947).

Isotope uptake. When isolated cells are cultured, it may be assumed that isotope added to the medium becomes rapidly and uniformly diluted, and has equal access to all cells (Perry *et al.* 1961; Kimball & Perdue, 1962; Feinendegen & Bond, 1963). This cannot be assumed for cells of the central nervous system following intraventricular or intracisternal injection; some isotope certainly leaks back along the needle track, and some may be absorbed by vessels of the brain and meninges. Autoradiographs of the liver can be obtained after administration by these routes. It is unlikely that the concentration of isotope in the fourth ventricle is the same as that administered, for the injection displaces some cerebrospinal fluid from the site of injection, and the rate at which mixing occurs with such displaced fluid is not known.

The concentration of tracer substance in the immediate vicinity of hypoglossal neurones is even less certain. It is probable that a concentration gradient exists across each hypoglossal nucleus, for the neurones lying nearest the ependyma usually show the highest activity. Any assumption that the tracer has equal access to the cell bodies of both normal and abnormal hypoglossal neurones is probably invalid, for it is not known how cell swelling, reactive division of oligodendrocytes and perhaps altered glial motility would influence the penetration of tracer substances from the inner or outer surfaces of the brain. Furthermore, regional vascular changes following altered metabolic activity of nerve cells could result in an increased removal of tracer substance administered intraventricularly, just as it may increase the local concentration following intravascular administration (Altman, 1963). The observation that DNA synthesis is occurring more in endothelial cells of capillaries passing through the nucleus of injured neurones suggests that there may be local hyperaemia.

Even if all these assumptions were valid, change in isotope incorporation, expressed simply as the activity of each cell, cannot be equated with altered sythesis of RNA, or of protein, unless it is demonstrated that the relevant intracellular 'pool' of unlabelled precursor has remained constant

under these circumstances (Robertson, 1957). It is at least possible that the nucleoside 'pool' has changed as a result of altered respiratory coenzyme synthesis following nerve division (Geiger & Yamasaki, 1957; Friede, 1959). Simple measurement of the activity of precursors incorporated into RNA or protein (Brattgård, Hydén & Sjöstrand, 1958; Fischer, Lodin & Kaloušek, 1958) is therefore of little value.

Of greater significance is the fate within the cell of ribonucleic acid into which a labelled precursor has been incorporated. The neurone resembles nearly all other cell species studied, in that the precursor is incorporated first within the nucleus, then into the cytoplasm (Goldstein & Plaut, 1955; Woods & Taylor, 1959; Fitzgerald & Vinijchaikul, 1959; Zalokar, 1959; Goldstein, Micou & Crocker, 1960; Jacob & Monod, 1961; Gros, Hiatt, Gilbert, Kurland, Risebrough & Watson, 1961). Although all cytoplasmic RNA originates within the nucleus, not all nuclear RNA passes to the cytoplasm (Harris, 1963).

An increase in the cyt./nuc. ratio may result either from an increased rate of transfer of RNA from nucleus into the cytoplasm, or from an increased period of survival of such RNA within the cytoplasm. An increase in the area of the cytoplasm, such as occurs after axon division will by itself decrease the ratio.

Consequences of nerve division. Following division of the left hypoglossal nerve no change occurred in the cyt./nuc. ratio of the right hypoglossal nucleus; this permitted each right hypoglossal nucleus to act as a control for the left. The changes observed in the cyt./nuc. ratio of the left hypoglossal nucleus following nerve division may be interpreted in conjunction with measurements of the RNA content of injured neurones (Brattgård *et al.* 1957). These authors observed an increase in the RNA content beginning about twelve days after nerve division, after the initial period of axon outgrowth and accompanying early maturation. The neurone's content of RNA attained a value twice normal between the twenty-seventh and forty-eighth day, and then decreased to normal. As most of the RNA present in cells is ribosomal, and as the base content of the total RNA extracted from normal neurones and from neurones 42 days after nerve division resembles that of isolated ribosomes, it is probable that the changes observed by Brattgård *et al.* (1957) reflect alteration in the ribosomal RNA of injured neurones. If the change in cyt./nuc. ratio indicates synthesis of this RNA, then the greatest increase in the ratio should coincide with the greatest rate of increase of RNA content found on the fourteenth day. In contrast, the cyt./nuc. ratio increased to a maximum value before the period of axon outgrowth, and then decreased to normal by the twelfth day.

If the increased cyt./nuc. ratio found between the second and the eleventh day after nerve division represents increased synthesis of RNA and its transfer to the cytoplasm, it must be accompanied by an increased destruc-

tion of RNA, as the neurone's RNA content does not change. Studies using actinomycin D to inhibit DNA-primed RNA synthesis, and using the incorporation of [^{3}H]lysine into protein to follow the 'decay' of labile RNA suggest that the survival time of such RNA is markedly decreased during this phase (unpublished observations). Brattgård *et al.* (1957) observed an increase in the protein content of injured neurones which preceded the increased RNA content. They equated this with an increased protein synthesis, an assumption not necessarily confirmed by finding an increased incorporation of labelled amino acid (Brattgård *et al.* 1958), and advanced the working hypothesis that the constant amount of RNA formed more proteins during the phase of axon outgrowth by 'changing its state of particulate aggregation from an active to a more active form'. The results reported here indicate that during this phase there is an increased formation and destruction of RNA accompanying this increased protein synthesis.

The return of the cyt./nuc. ratio to its normal value before the fourteenth day, when the RNA content of the neurone is increasing most rapidly, probably results from the increased area of the cytoplasm, due to cell swelling: this corresponds to the finding of Brattgård *et al.* (1957), that the concentration of RNA in injured neurones is less than normal at this stage, despite an increasing RNA content.

DNA synthesis. [^{3}H]thymidine is incorporated by cells synthesizing DNA. Glial cells begin to synthesize DNA about 48 hr after dividing or crushing the hypoglossal or facial nerve. This synthesis is confined to glial cells surrounding damaged neurones, and never precedes cell swelling. Glial cell division accompanies the DNA synthesis and occurs equally around neurones after crushing, cutting or ligating the nerve. Uptake of [^{3}H]thymidine by glial cells is most marked about 4–6 days after nerve injury, and by the tenth day has decreased to its normal very low incidence. If the perineuronal oligodendroglia divide only to act as macrophages to remove dead neurones, DNA synthesis would be expected to be more marked after nerve division when 75% of the neurones die, than after crushing the nerve when 85% of nerve cells survive. Glial division occurs around all chromatolytic neurones in the damaged nucleus regardless of their individual fates. Furthermore, the period of cell division precedes the principal period of neurone death after nerve division by several days.

There is evidence that neurones and perineuronal oligodendroglia may act in respiration symbiosis when the metabolic demands of nerve cells are increased by stimulation (Hydén & Pigón, 1960; Lasansky, 1961). While the activity of oxidative enzymes is known to increase in astrocytes responding to injury (Friede, 1962), similar data upon oligodendroglia are lacking. It is therefore suggested that the early division of perineuronal glial cells found after injury is primarily a response either to increased

metabolic activity of the neurone, or to a decreased efficacy of the neuronal respiratory enzymes as a result of cytoplasmic swelling.

SUMMARY

1. One hypoglossal or facial nerve was cut, crushed or ligated in rodents, which were permitted to survive for varying periods. [^{3}H]uridine, [^{3}H]guanosine, [^{3}H]adenosine, [^{3}H]thymidine or [^{3}H]lysine was given by injection into the cerebral ventricles or cisterns. The uptake of tritium by neurones and glia was measured autoradiographically, and its distribution between intracellular compartments determined.

2. In both normal and injured neurones, RNA precursors were incorporated first within the nucleus, and later within the cytoplasm. This change of distribution represented an apparent transfer of RNA from nucleus to cytoplasm.

3. After nerve division the uptake of RNA precursors and of [^{3}H]lysine increased within 48 hr. The rate of apparent transfer of RNA from nucleus to cytoplasm also increased markedly. This change always accompanied cytoplasmic swelling of the neurone.

4. During the period of axon outgrowth preceding maturation of the regenerating nerve the rate of apparent transfer of RNA from nucleus to cytoplasm decreased to a value less than normal, and remained low for the remaining period of maturation.

5. These results are discussed in relation to measurements of the RNA and protein contents of regenerating neurones.

6. Between 2 days and 5 days after crushing, dividing or ligating the nerve the number of perineuronal glial cells increased and they synthesized DNA. This change always accompanied cytoplasmic swelling of the neurones.

7. In the rabbit, endothelial cells of capillaries of the nucleus of the injured nerve synthesized DNA.

8. These results are discussed in relation to the probable changes in metabolic activity of the injured nerve cells.

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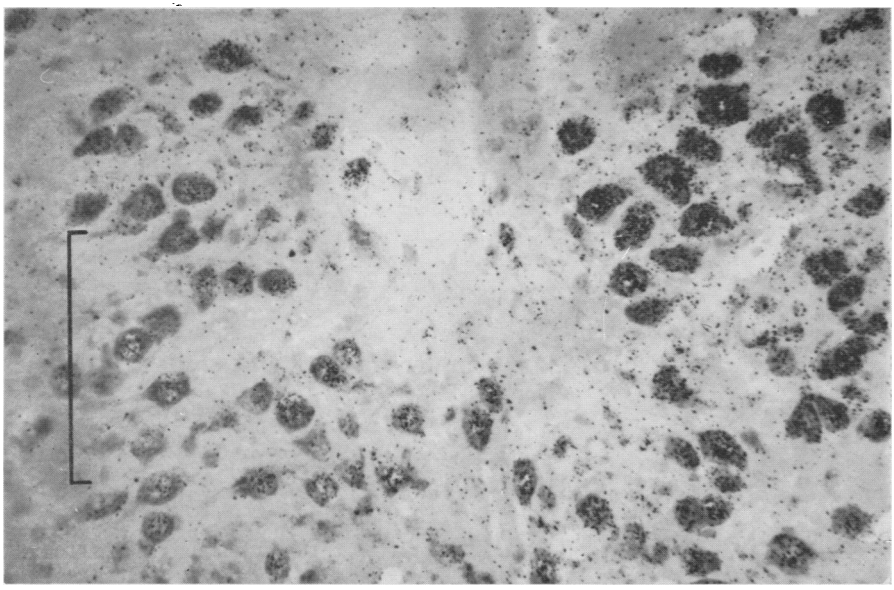

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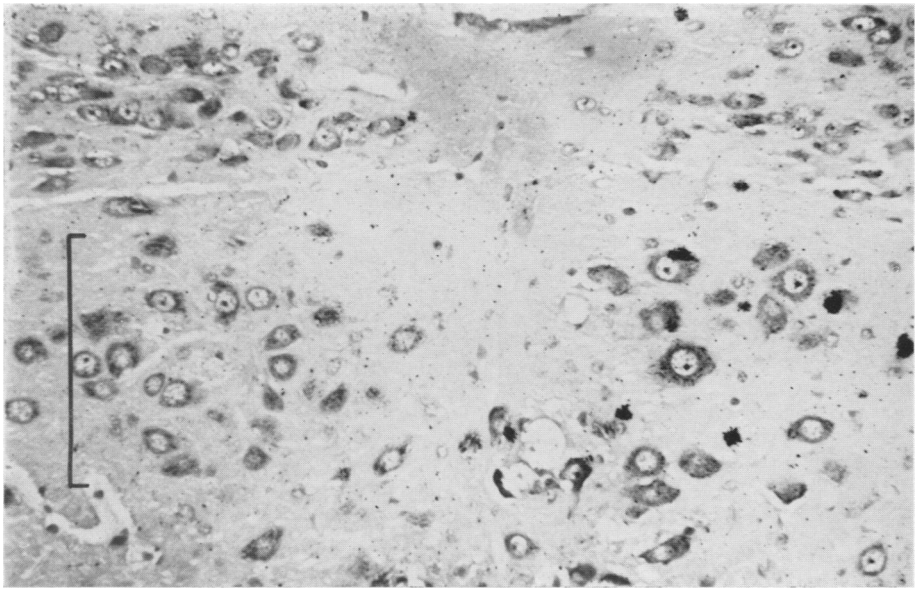
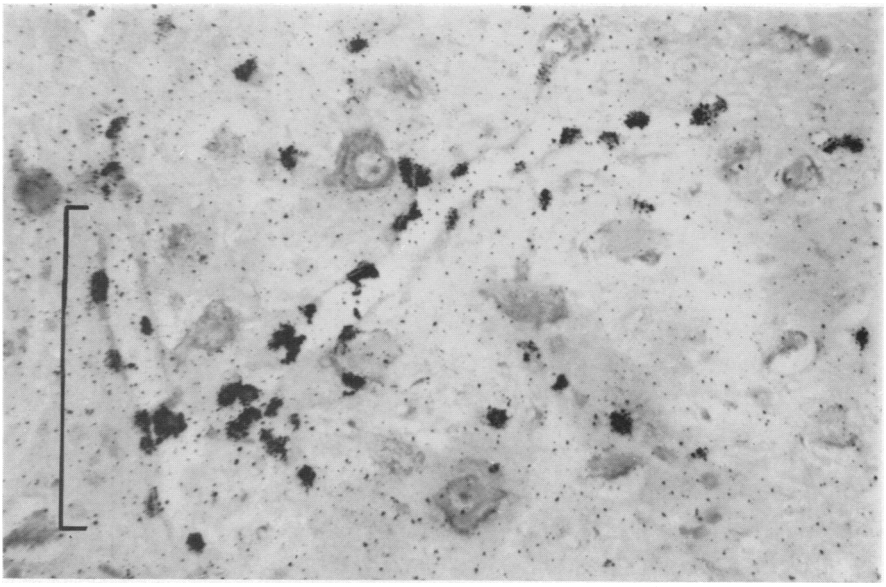


(*a*)



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EXPLANATION OF PLATES

Pl. 1. Autoradiograph of transverse section of mouse medulla, 0·5 mm anterior to obex. The left hypoglossal nerve had been cut 3 days previously, and [^{3}H]uridine had been injected 8 hr before death. The median plane lies centrally and vertically. The nucleus of the injured left hypoglossal nerve lies on the right of the illustration. Note that the injured neurones are larger, have taken up more [^{3}H]uridine and that the cyt./nuc. ratio appears greater on the injured side. The mean ratio of 100 normal neurones of this mouse was 0·17, and of 100 injured neurones 0·30 ($P < 0{\cdot}001$). Scale 100 μ. Stained methyl green-pyronin.

Pl. 2*a*. Autoradiograph of transverse section of mouse medulla, 0·5 mm anterior to obex. The left hypoglossal nerve had been cut 3 days previously and [^{3}H]thymidine had been injected 16 hr before death. The median plane lies centrally and vertically. The nucleus of the injured left hypoglossal nerve lies on the right of the illustration. Note the dense clusters of silver grains at the periphery of neurones: exposure of this section was prolonged to accentuate their distribution. High-power examination of normally exposed sections shows the activity to overlie oligodendroglial nuclei. Some clusters of grains apparently remote from neurones lie against a neurone seen in an adjacent section. Note the scattered grains over neurones and neuropil: these are removed by ribonuclease, and probably result from conversion of some [^{3}H]thymidine into [^{3}H]uridine. Scale 100 μ. Stained methyl green-pyronin.

Pl. 2*b*. Autoradiograph of transverse section of rabbit medulla, 1 mm posterior to obex, confined to injured left hypoglossal nucleus. The nerve had been cut 3 days previously and [^{3}H]thymidine had been injected 16 hr before death. Note the capillary traversing the injured nucleus, and the uptake of [^{3}H]thymidine by capillary endothelium. Deliberately over-exposed. Scale 100 μ. Stained methyl green-pyronin.