EXPERIMENTAL DIPHTHERITIC NEUROPATHY IN THE RAT

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THE rat is exceptionally insensitive to diphtheria toxin. This fact was discovered in the early days of experimentation with diphtheria toxin and has been confirmed several times since (Roux and Yersin, 1889, 1890 ; Goodman, 1907 ; Agarwal and Pryce, 1959 ; Kato and Pappenheimer, 1960). Figures quoted suggest that a lethal dose of toxin for a rat may be anything from 1000–4000 times that required to kill a guinea-pig.

In 1898 Roux and Borrel, however, found that the i.c. injection of relatively small quantities of toxin produced paralysis and death in rats. Agarwal and Pryce (1959) have confirmed this finding ; they stated that nerve cells only were damaged and that no demyelination occurred. Agarwal and Pryce also injected the sciatic nerve of one rat with 2 MLD diphtheria toxin and observed that paralysis ensued within a week, but no further examination of the nerve was reported.

In man, rabbits, guinea-pigs and chickens the lesion produced in peripheral nerves by diptheria toxin appears primarily to affect Schwann cells and to produce demyelination typically affecting some internodes and leaving others intact. Wallerian degeneration may also be found to a variable extent in some species.

The response of the peripheral nerve of the rat to diphtheria toxin seemed worthy of study in view of the resistance of the animal to parenterally injected toxin, whilst showing relative sensitivity to i.c. toxin.

Jacobs, Cavanagh and Mellick (1966) found that the method of intraneural injection gave easily reproducible results in the chicken and that this technique permitted a wider range of damage within the nerve than could be produced by parenteral injection without causing death of the animal. The method has been found to be equally reliable when applied to the rat.

This investigation has shown that intraneurally injected toxin damages the Schwann cells of the rat causing a typical segmental demyelinating lesion. Quantitative examination of this neuropathy revealed a selective toxicity to fibres of different sizes similar to that found by Cavanagh and Jacobs (1964) in the chicken ; it showed differences in response between young and mature animals and it demonstrated that the damaging process may take place over a considerable period of time. Furthermore, with suitable doses, diphtheria toxin can produce partial breakdown of myelinated segments allowing a closer examination of the response of a Schwann cell to damage of a part of itself.

MATERIALS AND METHODS

Animals.—WAG/C pure line strain rats of both sexes were used. Immature rats had been weaned for about a week and weighed between 65 and 100 g. Adults weighed from 220–320 g. The animals were housed in plastic cages and fed on Chardex.

Toxin and antitoxin.—Dr. Mollie Barr, of the Wellcome Research Laboratories, Beckenham, kindly supplied diphtheria toxin and antitoxin. The toxin contained 1 L^+ dose in 0·024 ml. By definition an L^+ dose is the least amount of toxin which when combined with one unit of antitoxin will kill a 250 g. guinea-pig in 5 days, (Ehrlich, 1906).

The use of partially neutralised toxin was considered to be superior to toxin alone in producing paralysis in experimental animals. Ransom (1900), however, in experiments using toxin with and without antitoxin found no evidence that the addition of antitoxin caused more paralysis.

Cavanagh and Jacobs (1964) found no difference in the development of paralysis in chickens given partially neutralised toxin or toxin alone. They ascertained that a given amount of toxin alone was equivalent to about 13 times the same amount of toxin when underneutralised by the addition of antitoxin according to the L^+ definition.

In this study toxin was used alone. The " L^+ " referred to has, therefore, about thirteen times the toxicity of a correctly defined L^+ dose.

Solutions of toxin were made up in sterile normal saline ; the dose to be injected was contained in a volume of 0·005 ml. Trypan blue (1 per cent) was added to give a final concentration of 1×10^{-6} g. in the injected volume. The trypan blue was used as an indicator of the site and spread of the toxin within the nerve.

Injection.—Rats were anaesthetised with ether and the hair was shaved from the lateral aspect of the thigh. The skin was cleansed with 70 per cent alcohol and an incision made into the skin of the thigh parallel to the femur and slightly posterior to it. A further incision was made in the gluteus maximus and the sciatic nerve was exposed.

Toxin was injected towards the trunk into the larger posterior branch of the sciatic nerve approximately midway between the sciatic notch and the lower end of the femur and usually a few mm. below the division of the sciatic nerve into 2 main branches.

The details of the injection procedure are similar to those described by Jacobs, Cavanagh and Mellick (1966). A volume of 0·005 ml. was injected into the nerve using an Agla micrometer syringe with a 27 gauge needle. The solution was seen to move rapidly up the nerve and in many cases the upper extent of the spread of dye was beyond the field of view. The dye was not seen to move more than a few mm. distal to the point of injection.

No distension of the nerve was observed ; occasionally, especially in the small animals, there was slight leakage of toxin after the needle was withdrawn. After the injection the overlying muscle and skin were sutured, and plastic skin was sprayed over the wound.

Aseptic precautions were observed throughout the operations and no infections occurred.

Control injections of saline with trypan blue were made in 4 animals using the same volume as for the toxin. Two animals were given i.v. antitoxin 10 min. before intraneural injection of toxin. The amount of antitoxin given was calculated to produce a concentration of 10 times that required to neutralise a $1{\cdot}25 \times 10^{-2}$ L^+ toxin dose in the injected nerve. This calculation was based on the assumption that the antitoxin was equally distributed throughout the tissues after i.v. administrations.

Colchicine was given to all animals killed at 15, 22 and 31 days. A dose of 2 mg./kg. was injected s.c. 5 hr. before the animal was killed.

Functional disturbance.—Animals were examined twice weekly for signs of weakness or paralysis of the legs. No specific tests were made, the animals simply being observed while walking.

Disability appeared to commence with a loss of splay of the toes and foot drop ; at its most severe the feet became paralysed and were dragged behind as the animals moved about.

As a rough guide, the functional state was assessed on a 2 point scale corresponding to the 2 degrees of disability described above, (Tables I and II).

Preparation of tissues.—The rats were killed with an overdose of ether. The sciatic nerve from the sciatic notch to the level of the lower end of the femur was carefully removed and lightly stretched on a library card until adherent. In a few animals the posterior branch of the sciatic nerve was dissected down to the foot. The nerves were fixed in Helly's fluid for 24–48 hr.

After washing in water, about one cm. of the nerve was cut out above the injection site so as to avoid any local damage caused by the needle.

The epineurium and perineurium were removed from this portion of the nerve and the remaining bundle of fibres was slightly teased out to allow solutions to penetrate easily. This preliminary separation of fibres was done in such a way that the mass of fibres remained in one piece so facilitating its transfer from one solution to another.

The mass of fibres was treated with iodine and sodium thiosulphate to remove mercuric chloride deposit, and after washing thoroughly, was stained with OR[4]B in 60 per cent isopropyl alcohol for 45 min. at room temperature.

The fibres were rinsed for 5 sec. in 60 per cent isopropyl alcohol to remove excess dye, washed for 30 sec. in water and stained with Mayer's haemalum for about 7 min. After " blueing " in tap water the mass of fibres was transferred to 33 per cent glycerine.

Final teasing of the fibres was carried out in a drop of 33 per cent glycerine under a dissecting microscope with mounted sewing needles : the preparations were mounted under a circular coverslip and ringed with asphalt varnish.

Examination of tissues.—The teased nerve fibres were scanned systematically and measurements were taken of several parameters ; using a Leitz × 12·5 micrometer eyepiece with a ×57 objective.

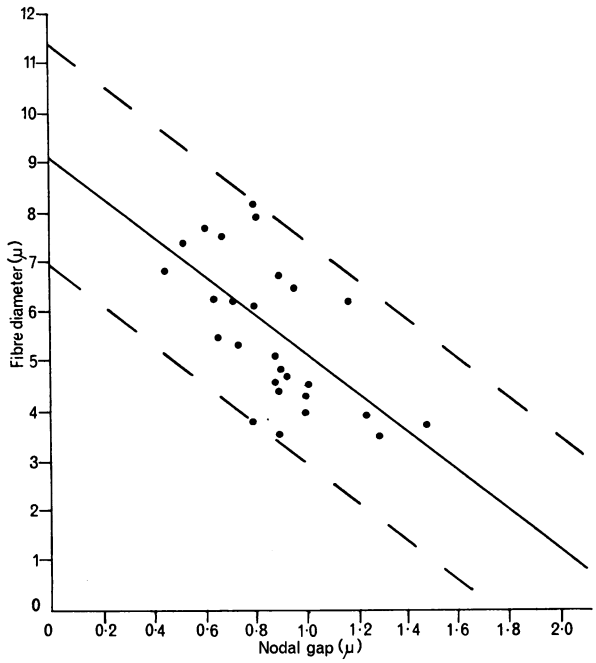


FIG. 1.—Graph of fibre diameter with calculated regression lines and two standard errors of estimate on either side.

Where damage was confined to the nodes of Ranvier measurements were taken of the external fibre diameter and also of the nodal gap.

In more severely damaged fibres where whole internodes had broken down, it was usually possible to trace along such fibres to an undamaged internode. In this case, the external diameter of the normal internode was measured ; if the damaged segment had begun to remyelinate the external diameter of the new myelin was also measured. Frequently there was partial breakdown of a distal portion (paranode) of an internode ; this length was measured together with the diameter of the undamaged part of the segment. If remyelination had occurred in such a damaged segment, its external diameter was also noted.

In severely affected fibres, no normal internodes remained and therefore no measurements could be made.

The damage to fibres was graded in the following manner: (+) damage to nodal or paranodal regions not extending beyond 40 μ from the node; (++) partial breakdown of the internode, being greater than 40 μ from the node, but not affecting the whole internode; (+++) complete internodal breakdown.

In order to determine whether a nodal gap was widened beyond normal limits, measurements were made of the gap in normal animals. A graph was plotted of nodal gap against fibre diameter (Fig. 1). The gaps were found to be considerably smaller than those measured by Cavanagh and Jacobs (1964) using formal saline fixation and Sudan black B and carmalum staining. There are still, however, almost twice as large as those measured by Hess and Young (1952) after osmium tetroxide treatment.

The paranode is that region of the internode adjacent to the node of Ranvier, where the myelin sheath is folded to form grooves which contain abundant Schwann cell cytoplasm. These features have been demonstrated with light and electron microscopy by Williams and Landon (1963). The length of the paranodal region probably varies in fibres of different diameter, but since no measurements were available an approximate length of 40 μ was selected for this region.

At least 25, and usually more than 30 fibres were measured from each nerve. There was good agreement of the assessment of damage between different samples of fibres selected from the same piece of nerve; comparison of measurements made by two independent observers confirmed that the method was reliable.

RESULTS

Effect of dose

Histogram 1 (Fig. 2) shows the percentage of damaged fibres found in nerves of adult and immature animals at various times after injection with different doses of toxin.

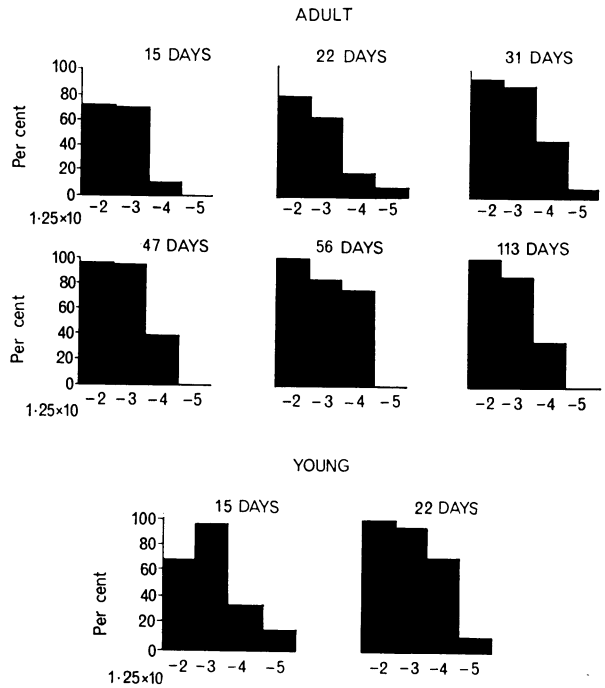


FIG. 2.—Histograms showing the percentage of damaged fibres in nerves of adult and immature animals at various times after injection with different doses of toxin.

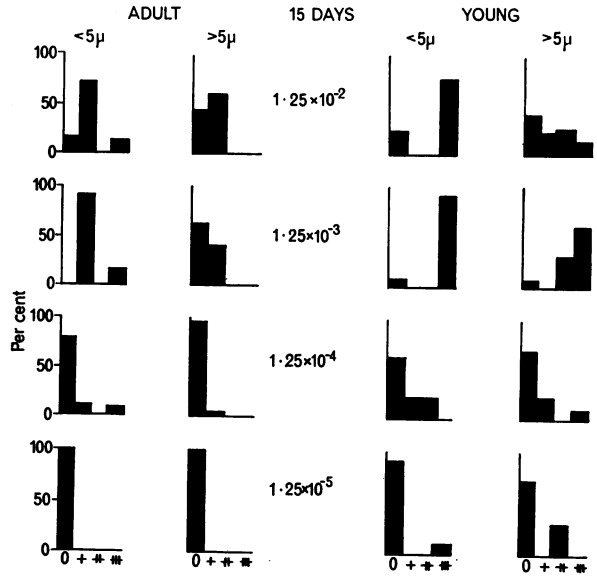
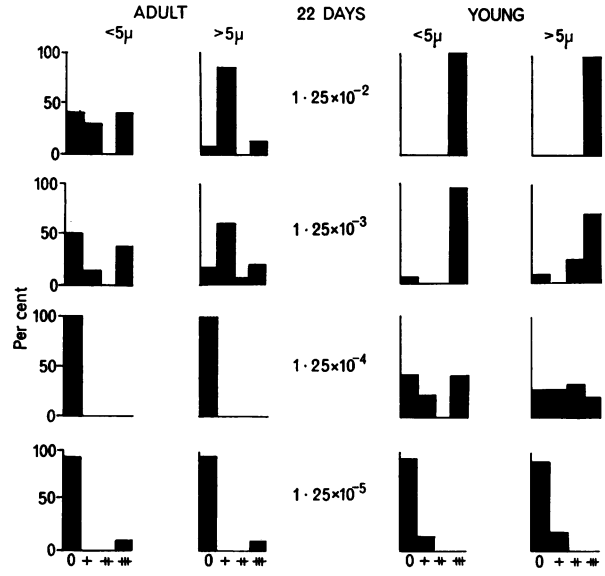

ADULT
15 DAYS
YOUNG
<5μ
>5μ
<5μ
>5μ
Per cent
100
50
0
1·25×10-2
1·25×10-3
1·25×10-4
1·25×10-5
0 + + +

FIG. 3.


ADULT
22 DAYS
YOUNG
<5μ
>5μ
<5μ
>5μ
Per cent
100
50
0
1·25×10-2
1·25×10-3
1·25×10-4
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0 + + +

FIG. 4.

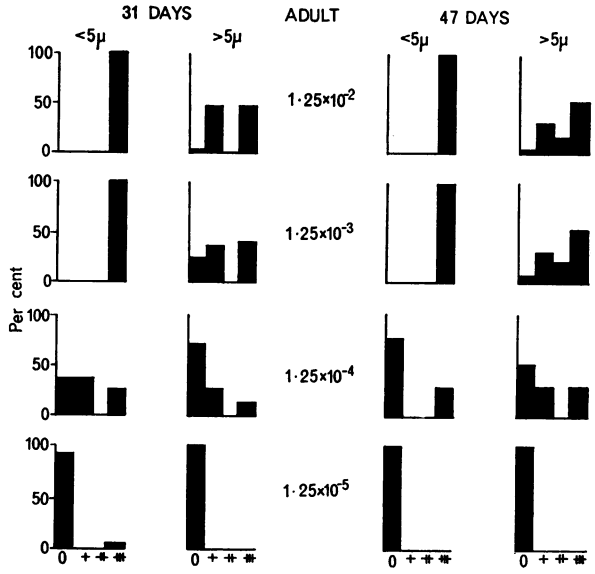


FIG. 5.

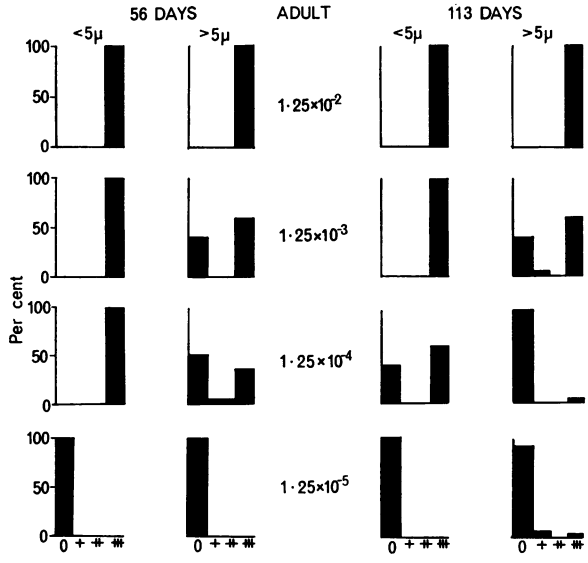


FIG. 6.

FIG. 3, 4, 5, 6.—Histograms showing the percentage of fibres with no damage (0) or with varying degrees of damage (+, ++, +++; see text for details) at various times after different doses of toxin in adult and young rats. Separate histograms are constructed for fibres of 5 μ diameter and under, and for fibres of over 5 μ diameter.

A similar pattern of graded response is seen in both young and adult animals. The range of concentrations of toxin employed was found eventually to produce 100 per cent damage at the highest dose and minimal damage at the lowest dose.

Effect of toxin upon nerve fibres of different sizes

Measured fibres from each injected nerve were divided into 2 groups : those of 5 μ external diameter and under, and those over 5 μ.

Histograms 2, 3, 4 and 5 (Fig. 3, 4, 5 and 6) were constructed for each of these fibre groups, to show the percentage of normal and damaged fibres. (The method of assessment of the degree of damage on a three point scale has been described above).

Separate histograms were made for each dose of toxin employed and for each time interval in both adult and immature animals.

A comparison of small and large fibre groups reveals 3 points of difference.

Sensitivity to toxin.—Small fibres appear to be more susceptible to the effect of toxin than larger fibres. Thus, in many cases a higher proportion of small fibres is damaged ; this is illustrated by the histogram of the adults at 56 and 113 days with doses of $1{\cdot}25 \times 10^{-3}$ and $1{\cdot}25 \times 10^{-4}$ L^+ where 40–50 per cent of large fibres are unaffected, whereas no normal fibres remain in the below 5 μ group.

Extent of Schwann cell damage.—Cavanagh and Jacobs (1964) demonstrated in the chicken that when small fibres were damaged they underwent complete internodal breakdown in contrast to the larger fibres which often showed partial breakdown of an internode. In the rat, also, it was found that damage to small fibres always resulted in complete internodal breakdown. Although the majority of large fibres which were damaged eventually sustained complete internodal breakdown a small proportion was found in which myelin breakdown was restricted to a part of the internode.

Histograms show a small proportion of larger fibres with + or ++ degrees of damage at 113 days.

Damage to large fibres appeared to commence at the paranodal region and then gradually to spread along the internode towards the nucleus ; in the adult rat this process occurred over several weeks. In smaller fibres the whole internode appeared to fragment at one time.

Rate of myelin breakdown.—Small fibres reach a stage of complete internodal breakdown considerably more rapidly than do the larger fibres. This is exemplified by the adult rat injected with $1{\cdot}25 \times 10^{-2}$ L^+ toxin in which all the small fibres are damaged by 31 days, whilst the larger fibres were not found at this stage until the 56th day.

Comparisons of adult and young animals

Adult and immature rats do not appear to differ in their susceptibility to toxin. The striking difference in their response is the rapidity of myelin breakdown in the young animal compared with the adult in which the process is very slow.

In the adult, the percentage of damaged fibres is seen to grow higher with increasing time. In the larger fibre group from animals given $1{\cdot}25 \times 10^{-2}$ L^+ only about 50 per cent of fibres are damaged at 15 days and these show only nodal

or paranodal changes. By 22 days, more fibres are affected and in a few there is complete internodal breakdown. At 31–47 days a few normal fibres remain and in 50 per cent of fibres there is complete internodal breakdown. The full effects of the toxin are not manifest until 56 days when no fibre is free of damage.

With smaller doses evidence of continuing myelin breakdown is seen in a few fibres at 113 days (Fig. 7).

In the young animals, myelin has disappeared from many fibres in both large and small fibre groups by the 15th day ; indeed, new myelin is already apparent in a high proportion of fibres. By 22 days, no normal fibres remain in the animals that received $1{\cdot}25 \times 10^{-2}$ L^{+} and with lower doses few normal fibres are found.

Beyond 22 days, remyelination had progressed so rapidly that it was often difficult to distinguish remyelinating fibres from those that had not been damaged. Hence, the only means of identifying previously affected fibres would be to measure internodal lengths since it has been shown (Vizoso and Young, 1948) that these remain small when replacing damaged segments.

In view of the lack of visible evidence of further myelin breakdown in these fibres however, it seemed possible that by 31 days the damaging effects of toxin in the young animals was virtually complete. Furthermore, examination of nerves 31–47 days after injection with the lowest dose failed to show that myelin breakdown was continuing. Therefore, no attempt was made to quantitate damage to nerves in the young animals after 22 days.

Remyelination

The rapid appearance of new myelin in the immature animals has already been noted. By 47 days damaged fibres appear to have almost regained their original thickness.

The first signs of remyelination in the adult occur in a few fibres at 31 days. By 47 days approximately 50 per cent of damaged fibres were beginning to remyelinate and at 56 days new myelin was seen in all but those fibres in which recent damage had occurred.

Remyelination in the adult animals seems to be as low process and even by 113 days the damaged fibres had not nearly reached their original thickness (Fig. 8).

Functional disability

The rat is a difficult animal to examine for signs of functional disturbance ; it has short legs, and is unwilling to move about quickly. The method of observation while walking which was used to assess functional efficiency was relatively crude and hence small disturbances would probably have been missed.

Tables I and II show that observable symptoms were confined almost entirely to animals receiving the 2 highest doses of toxin ; these symptoms were not apparent until a high proportion of fibres were damaged.

The young animals became more severely affected than the adults, this was possibly because a greater length of nerve was damaged by the toxin. The times at which functional disability became obvious were different in young and adult animals and corresponded with periods in which a high proportion of fibres were damaged.

The period of functional disability only continued for a few days in young animals but the adult animals were sometimes affected for a few weeks. Apparent loss of symptoms concurred with the appearance of new myelin although the fibres had by no means achieved their original dimensions.

TABLE I.—*Grades of Functional Disability at Various Times after Intraneural Diphtheria Toxin (Adult Rat)*

	Days	18	22	26	30	34	38	42	46	50	54	58	62
$1{\cdot}25 \times 10^{-2}$ L+	No.												
	2L	0	0	0	0	0	0	0	0	0	0	0	0
	10L	0	0	0	+	+	+	0*	..	..	..	..	..
	14L	0	0	0	0	+	+	+	+	+	+*	..	..
	16L	0	0	0	0	0	0	+	+	+	+	+	0
	18L	0	0	0	0*	..	..	..	..	..	..	..	..
	20L	0	+	+	+*	..	..	..	..	..	..	..	..
$1{\cdot}25 \times 10^{-3}$ L+	2R	0	0	0	0	0	0	0	0	0	0	0	0
	10R	0	0	0	0	0	0	0*	..	..	..	..	..
	14R	0	0	0	0	0	+	+	+	+	0*	..	..
	16R	0	0	0	0	0	0	0	0	0	0	0	0
	18R	0	0	0	0*	..	..	..	..	..	..	..	..
	20R	0	+	+	+*	..	..	..	..	..	..	..	..

(See text for description of grades of disability.)
* Animal killed.

TABLE II.—*Grades of Functional Disability at Various Times after Intraneural Diphtheria Toxin (Young Rat)*

	Days	0	2	4	6	8	10	12	14	16	18	20
$1{\cdot}25 \times 10^{-2}$ L	No.											
	7L	0	0	0	0	0	0	0	0	0	0	0
	8L	0	0	0	0	++	++	++	++‖	..	..	..
	35L	0	0	0	+	++	++	++	++	++	+	0
	36L	0	0	0	+	++	++	++	++	+	0	0
	38L	0	0	0	+	++	++	++	++	+	0	0
	49L	0	0	0	0	+	++	++	++‖	..	..	..
	51L	0	0	0	0	+	++	++	++	++	+	+
$1{\cdot}25 \times 10^{-3}$ L+	7R	0	0	0	0	0	0	0	0	0	0	0
	8R	0	0	0	0	+	++	++	+	+‖	..	..
	35R	0	0	0	0	+	+	+	+	+	0	0
	36R	0	0	0	+	++	++	++	+	+	0	0
	38R	0	0	0	+	++	++	+	+	+	0	0

EXPLANATION OF PLATE

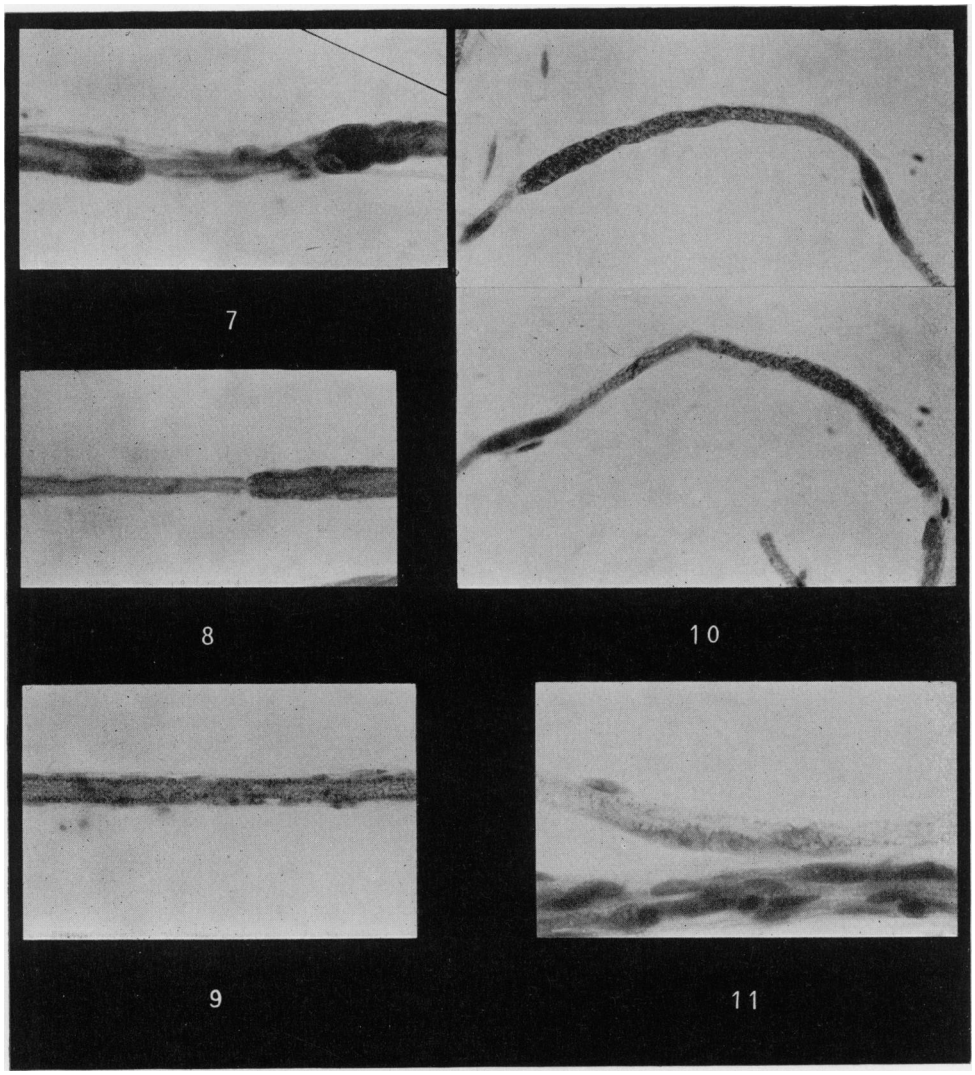
FIG. 7.—Partial internodal breakdown showing early remyelination and with further myelin disorganization at the node on the right. 113 days after injection. OR[4]B and haemalum. ×366.

FIG. 8.—Part of an undamaged internode on the right; a remyelinating internode on the left. OR[4]B and haemalum. ×220.

FIG. 9.—Part of a large diameter fibre about to break down. Numerous small nuclei are seen in the Schwann cell cytoplasm. OR[4]B and haemalum. ×265.

FIG. 10.—Whole internode shown in two parts. Nodal gaps are widened at both ends. The Schwann cell nucleus, seen in both photographs, has divided and the daughter nuclei appear to be separating. OR[4]B and haemalum. ×233.

FIG. 11.—Schwann cell nucleus of large diameter fibre which showed paranodal damage. The nucleus has divided. Two and possibly three daughter nuclei can be seen. OR[4]B and haemalum. ×385.



Jacobs.

Effects of antitoxin

No damaged fibres were found in sciatic nerves from two rats which had received ten times the neutralising dose of antitoxin ten minutes before intraneural injection of $1{\cdot}25 \times 10^{-2}$ L^+ toxin.

DISCUSSION

Several studies on the mechanisms of action of diphtheria toxin have confirmed that it causes inhibition of protein synthesis.

In 1962, Kato showed that diphtheria toxin produced inhibition of incorporation of ^{14}C labelled amino-acid into microsomal protein of normal guinea-pigs liver cells and Ehrlich ascites tumour cells. He suggested that toxin had a specific inhibitory effect on the transfer of amino-acids from soluble RNA to microsomal protein.

Recently Collier and Pappenheimer (1964) have also demonstrated inhibition of uptake of amino-acids into protein, using cell free extracts of HeLa cells and rabbit reticulocytes ; they emphasize that this inhibitory effect is only produced in the presence of a co-factor, nicotinamide adenine dinucleotide (NAD).

Pappenheimer (1947) deduced from the role of iron in toxin production that diphtheria toxin might be the protein moiety of cytochrome B of the diphtheria bacillus and suggested that its toxic property might be due to an abnormal synthesis of cytochrome B or a closely related enzyme. Van Heyningen (1950) criticised this view on biochemical grounds and Pope, Stevens, Caspary and Fenton (1951) considered that the purified toxin which they had isolated by electrophoresis was not a protein of cytochrome B because of its strong enzymic activity.

Strauss and Hendee (1959), studying the effects of diphtheria toxin on the metabolism of HeLa cells obtained no evidence that the resulting inhibition of protein synthesis was associated with an interference with energy metabolism. Although Kato and Pappenheimer (1960) showed a decreased ^{32}P uptake itdo ATP by MK cells treated with toxin, inferring that oxidative phosphorylation was affected, Collier and Pappenheimer were unable to confirm these results.

Strauss (1960) in a study of the effects of toxin on nucleic acid metabolism concluded that toxin did not act by uncoupling oxidation from phosphorylation.

In summary, therefore, it appears that diphtheria toxin causes inhibition of protein synthesis possibly due to some effect upon the mobilisation of amino-acids. for the synthesis of polypeptides ; it seems unlikely that oxidative phosphorylation is involved.

The lesion produced in the rat by diphtheria toxin in peripheral nerve appears to be one specifically affecting Schwann cells. Signs of Wallerian degeneration were limited to a few fibres found within the injection site and distal to the damaged region. These were no more numerous in the toxin injected animals than in those given saline with trypan blue. They were probably caused by trauma from the needle.

Electron microscopy of the Schwann cell has shown that cytoplasmic inclusions associated with the synthesis of protein are present in the form of ribosomes in the opened out loops of myelin at the nodes of Ranvier (Landon, personal communication), and as endoplasmic reticulum in the perinuclear region.

The protein formed in these sites may be destined either to enter into the structure of myelin or to replace enzymes concerned with the metabolism of the cell. If protein synthesis is indeed the metabolic activity which is disturbed in diphtheritic intoxication of Schwann cells its effect might be produced at either or both of these sites where protein is required.

The Schwann cells of an immature animal must synthesize considerable quantities of protein for incorporation into new myelin. In the adult, when myelination has ceased, protein will only be required for replacement within the sheath. The young animals might, therefore, be expected to respond more promptly to any cessation of protein synthesis, and the results reported have confirmed this suggestion.

The prolonged process of myelin breakdown which occurs in the adult is evidence for a slow turnover of protein within the sheath.

In this paper it is shown that with the largest dose of toxin employed there is complete breakdown of myelin along whole internodes ; with lower doses partial breakdown of myelin may occur. In both instances the Schwann cells survive, indeed in the latter case there is evidence to suggest that the nucleus may divide to produce the new Schwann cell nucleus which will remyelinate the damaged segment. This suggests that diphtheria toxin is more likely to be affecting the protein associated with the myelin sheath than the enzymes that are controlling essential metabolic processes within the cell.

Fibres of small diameter were found to be more severely affected than those of larger diameter. Two alternative hypotheses may account for this selectivity. Firstly, the fibre groups may differ quantitatively. For example, Weller (1965) has suggested that differences in distribution of Schwann cell cytoplasm may be the factor determining the localisation of myelin breakdown.

Alternatively, the effect may be due to differences in surface area/volume ratio between the two groups of fibres. If molecules of toxin became equally distributed and fixed to the surface of the Schwann cell, a small fibre, because of its greater surface area/volume ratio would be affected by a larger number of molecules of toxin than would a fibre of greater size.

Reference has been made to the occasional finding of damage to a part of an internode. It should be recalled that the myelin sheath of each internode is formed by a single Schwann cell. When damage to this cell results in breakdown of a part of the internode, a new Schwann cell nucleus arrives at the damaged area ; it stations itself at the mid-point of this damaged site and forms a new myelin sheath ; thus, an intercalculated internode is formed.

The origin of the Schwann cell nucleus which forms the intercalculated internode is of some interest. Lubinska (1961*a*, *b*) has studied the formation of intercalated internodes in the proximal internodes of a crushed nerve. She surmised that the new Schwann cell nucleus was derived from the original Schwann cell nucleus, although she apparently had been unable to observe the origin of the new nucleus. It is, of course, conceivable that a cell could penetrate the basement membrane of the Schwann cell which still covers the demyelinated part of the internode. Many nuclei are found within the Schwann cell of an internode which is in the process of breaking down and some of these almost certainly originate extratubally (Fig. 9).

With the fixation and staining method employed in this study the nuclei are observed more easily than with the osmium tetroxide method used by Lubinska.

Where partial breakdown has occurred it is often possible to see a double Schwann cell nucleus in its normal position at the mid-point of the internode (Fig. 10). The nuclei may seem to be overlapping or in the process of separating (Fig. 11).

Colchicine was used in this study in the hope of arresting these Schwann cells in mitosis; however, no convincing demonstration of mitosis was obtained. Since further work is in progress on mitosis in Schwann cells it is not proposed to discuss the significance of these observations in detail. However, it may be noted that these observations imply that fully differentiated cells, apparently successfully maintaining the greater part of their substance, are capable of mitotic division.

SUMMARY

Intraneural injection of diphtheria toxin damages Schwann cells specifically. The cells are about 1000 times more resistant to toxin than chicken Schwann cells.

Quantitative studies of this neuropathy show it to be dose dependent and easily reproducible.

Small diameter fibres are more severely damaged and respond more rapidly than larger diameter fibres.

Adult and young animals differ in their temporal response to toxin. The damaging process may continue for several months in adult rats.

The use of toxin to produce damage to part of a Schwann cell is described; some observations on the reactions of these Schwann cells are recorded briefly.

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