J. Physiol. (1954) 123, 338–356

SOME OBSERVATIONS ON PERFUSED FROG SCIATIC NERVES

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(*Received* 10 *August* 1953)

Many generations of physiologists have believed that the connective tissue sheath of the peripheral nerves is a diffusion barrier and possesses a high electrical resistance, and that its presence significantly modifies the apparent properties of the nerve fibres (e.g. Bunzen, 1807; Harless, 1846; Eckard, 1851; Key & Retzius, 1876; Overton, 1904; Bishop, Erlanger & Gasser, 1926; Rice & Davis, 1928; Erlanger & Blair, 1938; Rössel, 1943). This opinion has been challenged repeatedly by Lorente de Nó in recent years (1947, 1950, 1952), in spite of powerful additional evidence (Feng & Liu, 1949; Rashbass & Rushton, 1949; Lundberg, 1951; Crescitelli, 1951) based mainly upon experiments in which intact nerves were compared with nerves deprived of their sheaths by dissection. The validity of such experiments has been questioned by Lorente de Nó (1950, 1952) who claims that the presence of the sheath is of great importance for the normal function of the nerve fibres, and that a desheathed nerve is not a proper subject for the study of normal activity. He believes consequently that the delayed blocking action of Na-free and strong K solutions in intact frog nerves demonstrates the indirect connexion between Na and K and nerve conduction, and that the rapid onset of inexcitability shown by desheathed nerves in such solutions is a clear expression of the abnormal condition of the nerve fibres.

The purpose of the present experiments was to test the action of Na-free and other solutions introduced into nerves by their blood vessels, thus by-passing the sheath. Under these conditions, there is nothing to suggest that the nerve fibres are in a grossly abnormal state, and a comparison of the behaviour of such perfused nerves with that of control nerves should establish unambiguously whether the sheath is a barrier to diffusion.

Vascular perfusion methods have been used in frogs widely for a variety of purposes since the beginning of this century (Verworn, 1900) and the classical frog hind-limb 'Läwen-Trendelenburg' preparation (first described by Cushing,

1901) is well known. The latter has not been applied to the study of nerve physiology to any great extent. However, Lundberg (1951) noted the rapid action of strong solutions of K injected into the aorta of a frog, and Lorente de Nó (1952) described some experiments in the course of which whole frogs were perfused with Na-free solutions but total nerve block did not occur with great rapidity. A brief account of the method and of some of the results has been given previously (Krnjevic, 1952).

METHODS

All experiments were done with the common frog (*Rana temporaria*) during a period which included the four seasons; the preparations were on the whole more satisfactory and easier to handle during the colder months, but results did not differ significantly as far as the permeability of the sheath was concerned throughout the year. No discrimination was made between the sexes. The frog's brain and spinal cord were destroyed in every case.

Perfusion method. The metal cannula was made from a hypodermic syringe needle: two sizes were used, nos. 1 and 19, the larger one (no. 19) only occasionally when an exceptionally big specimen was available. The cannula was usually inserted into the descending aorta (in a few cases it was possible to use the common iliac artery) with the help of magnifying glasses (× 2). The dissection can be performed either from behind or from the front. The former is simpler, but the latter is safer when dealing with a small frog, in which the vessels are slender and the bifurcation of the descending aorta may be rather high.

One of the iliac arteries was ligated, and the homolateral sciatic nerve and its peroneal branch were removed to be used as a control. The opposite hind-limb and the pelvis, with the attached cannula, were severed from the trunk. The peroneal nerve was exposed and the gastrocnemius, peroneus and tibialis anterior muscles were ligated and resected to avoid interference by muscle potentials. After weighing, the part was mounted in a moist chamber with silver electrodes arranged so as to stimulate the cords of the lumbar plexus and record the action potential from the peroneal nerve, which was crushed between the recording electrodes.

The cannula (1·5 cm long) was directly connected to a 3-way tap controlling the choice of perfusate. The solutions were allowed to run into the hind-limb from bottles suspended at a height that varied between 30 and 50 cm above the chamber, according to the rate of flow, to keep the pressure within the aorta between 20 and 30 cm H_2O. Figures for the frog's arterial blood pressure in the literature are in the range 10–40 mm Hg (Burton-Opitz, 1902; Prosser, Bishop, Brown, Jahn & Wulff, 1950). The pressure in the aorta could be found from experimentally deduced curves relating flow rate to pressure drop for different viscosities. A continuous record of the perfusion flow was made by a conventional drop recorder situated under a hole in the floor of the chamber.

The preparations functioned well for many hours, but after some 6 hr the perfusion flow diminished appreciably, and this was associated with substantial oedema of the tissues and an increase in weight of the order of 30–50 %. It is significant that the perfused nerve did not show a corresponding increase in weight when compared with the control nerve.

Control nerve. The nerve was mounted in a nerve bath containing two sets of silver electrodes by means of which the nerve was stimulated proximally, and the action potential recorded distally from the peroneal branch. The bath had a central compartment of a capacity of about 7 ml. in which the middle 3 cm of the nerve lay. This stretch of nerve included the distal portion of the sciatic trunk and the proximal part of the peroneal nerve. The central compartment was connected with the perfusion system from which it could be rapidly filled with the appropriate solution. The solutions were changed frequently to ensure good mixing.

The temperature of the preparations and of the solutions was recorded at intervals; it was allowed to vary with room temperature, within a range of 18–23° C.

Solutions. The perfusates were mostly made up with Dextran, and on a few occasions with

gelatin; and in a further number of experiments, no colloid of any kind was used. The colloid solutions were employed to reproduce normal osmotic conditions as nearly as possible; this was especially important with Na-free and strong K solutions to prevent filtration of fluid from playing an important part in the exchanges between the capillaries and the tissues, and thus making an analysis of the rate of diffusion impossible.

The 'standard' solution contained the usual amounts of electrolyte, as follows:
Na^+, 110·0 mM; K^+2·65 mM; Ca^{2+}, 1·1 mM; Cl^-, 112·2 mM; HCO_3^-, 2·5 mM; $H_2PO_4^-$, 0·15 mM.

The buffers were salts of K; they gave a pH of 7·6–7·7. In all experiments, the acidity was controlled with B.D.H. indicators.

The Na-free solutions retained all the other standard components, with the addition of sucrose, glucose or choline chloride as Na substitutes, in isotonic concentrations.

Other test solutions were prepared by displacing equivalent amounts of Na, but the concentration of the latter was usually not allowed to fall below 10% of the normal, to avoid interference by the effects of Na lack. Ordinary commercial Dextran contains 0·9% NaCl. The Dextran employed was a deionized 6% solution made available by Dextran Ltd. The Na content of the deionized Dextran, estimated by the uranyl zinc acetate photometric method after ashing, was negligible (less than 0·01 mM).

The Dextran solution was diluted with half its own volume of water so as to approximate the viscosity (estimated to be 4·5 with an Ostwald viscometer) to that of frog blood (2·4 according to Burton-Opitz, 1902). The colloid osmotic pressure of the diluted Dextran was calculated (from its molecular weight) to be about 10 cm H_2O. This can be compared with the following measurements of the frog plasma colloid pressure: 5·5–6·0 cm H_2O (Krogh, 1922); 9·6–11·5 cm plasma (White, 1924); 7·1 cm H_2O (Churchill, Nakazawa & Drinker, 1927); 11·5 cm H_2O (Landis, 1927). Gelatin perfusates were used at the suggestion of Lorente de Nó (personal communication), but they were found less satisfactory than Dextran solutions because the viscosity increased rapidly at room temperature and the pH was more difficult to control over long periods. The concentrations of gelatin were 0·5% (Chambers & Zweifach, 1940) and 1·0% (Chambers & Zweifach, 1944). The commercial gelatin powder contained Na equivalent to 0·70 mM in the 1·0% solution. This can be ignored as it should not affect the results by more than 4%.

Recording and stimulating apparatus. This consisted of a conventional biological amplifier and display unit, and a stimulator triggered by the time base. Stimulation was always maximal, and at a rate of 1–5/sec. The A group of fibres was the only component of the compound action potential studied.

Desheathed nerves. The operation of desheathing was performed with the help of a binocular microscope, with fine watchmaker's forceps, and sharp needles. The sheath was freed at the main sciatic bifurcation and then rolled up or down the nerve as desired.

RESULTS

The results are based upon data obtained in sixty-eight perfusion experiments, forty-seven of which were successful. Failures were associated either with very low rates of inflow or with inadequate perfusion of the vessels of the sciatic nerve (confirmed subsequently by injecting the vascular system with a dye or with indian ink).

The contrast in the rates of onset of conduction block in perfused and non-perfused control nerves is seen most clearly in the accompanying figures.

The effect of Na*-free solutions* (Fig. 1)

Twenty-seven perfusions were done, with eleven failures.

Control nerve. The blocking times in Na-free solutions agreed with similar data in the literature. The range was between 3 and 8 hr, with a mean of $5\frac{1}{4}$ hr (S.E. $\pm\frac{1}{3}$).

The rate of recovery in a solution containing Na depended upon the time during which the nerve had been left in the Na-free solution. If the solution was changed as soon as conduction ceased, recovery began within 1 min.

Perfused nerve. It was always found that the muscles of the limb ceased to contract at least 2–3 min before the nerve stopped conducting.

The blocking time did not apparently vary with the Na substitute, but it was substantially and consistently longer with 1 % gelatin than with 0·5 %

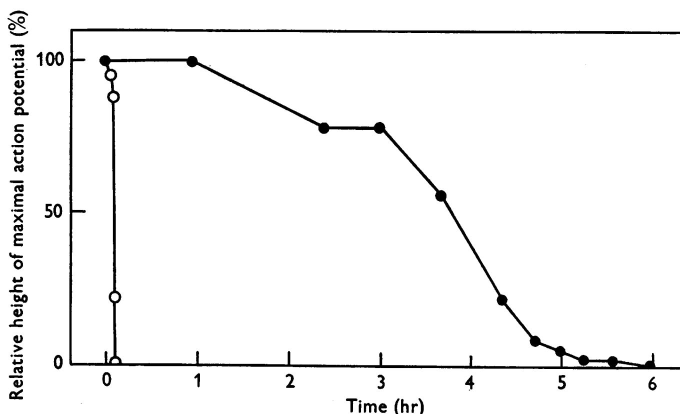

Fig. 1. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with a Na-free, isotonic choline chloride solution, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

gelatin or Dextran perfusates. Experiments with 1 % gelatin were therefore considered as failures. The mean of sixteen values of successful perfusions was 15·1 min (S.E. ± 1·5). As the efficiency of the vascular perfusion could only be estimated very approximately, it is probable that a somewhat inadequate perfusion in a number of cases caused the mean to be rather high. It should be noted that values of 10 min or less were seen in six experiments, and that blocking times of about 7 min were obtained with sucrose and choline chloride in Dextran perfusates, and with choline chloride in a ½ % gelatin perfusate. The time required for the first sign of recovery when perfusing with the standard solution did not vary very much, although it tended to be rather long with 1 % gelatin, as might be expected. The complete recovery time cannot be estimated exactly since there is no sharp change associated with full recovery.

To show that the loss of excitability was not caused by choline chloride, a nerve which had ceased conducting was perfused with a solution containing the usual amounts of choline chloride with the addition of 50 mM-NaCl. There was rapid recovery of conduction, and the compound action potential regained its original form, in spite of the hypertonicity of the perfusate.

If the Na-free perfusion was interrupted before, or soon after total block,

the action potential tended to recover spontaneously to some extent (cf. Lorente de Nó, 1952).

The effect of concentrated solutions of electrolytes

120 mM-KCl (Fig. 2)

Control nerve. The action potential began decreasing after about 8 min and disappeared altogether in about 25 min (mean of five cases: 22 min, S.D. $\pm 5{\cdot}5$). Recovery in the standard solution was slow; usually it began after some 20 min, but in one case the block was irreversible.

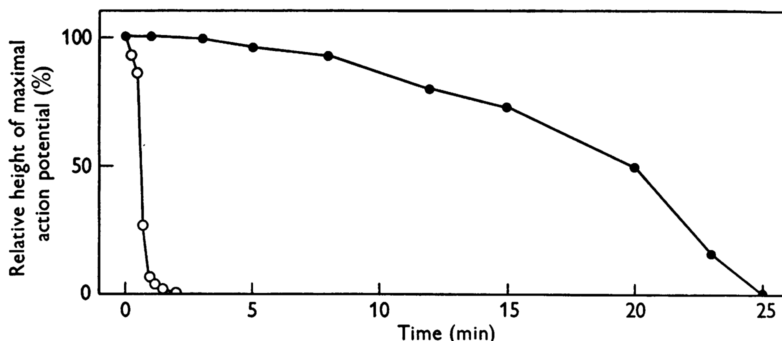

Fig. 2. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 120 mM-KCl, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

Perfused nerve. The first sign of a decrease in the action potential appeared within 30 sec and the block was always total within 1–1½ min. Conduction was first seen after some 5 min of standard perfusion, and it was normal after some 12 min.

60 mM-KCl

Control nerve. Complete block occurred after 1 hr, and recovery was first seen after 20 min, in two cases.

Perfused nerve. The block was complete within 1½ and 4 min respectively and recovery began after 4–5 min.

It was later found that the nerves of very emaciated autumn frogs are much more susceptible to the action of K. This will be described in the section dealing with desheathed nerves.

The results of experiments with RbCl, acid Ringer, $BaCl_2$, $HgCl_2$ and $CuCl_2$ are described adequately in Figs. 3–7. These were all single experiments, except in the case of $BaCl_2$ where the experiment was repeated. Conduction block was irreversible, except with RbCl and $BaCl_2$.

100 mM-NH_4Cl

Control nerve. Block was seen after 50–65 min.

Perfused nerve. Conduction did not stop until after some 40 min.

In neither perfused nor control nerves was recovery more than very slight and transient.

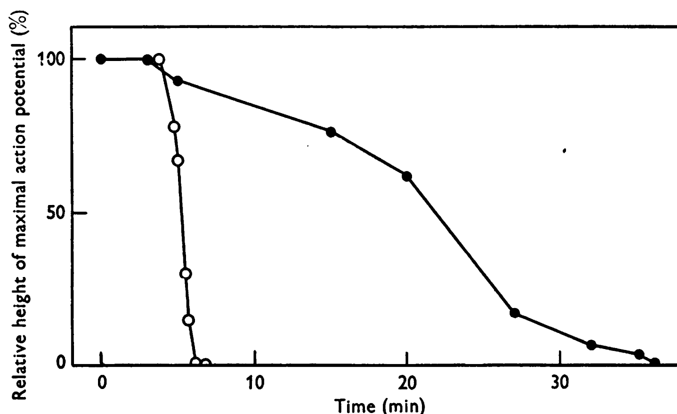

Fig. 3. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 100 mM-RbCl, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

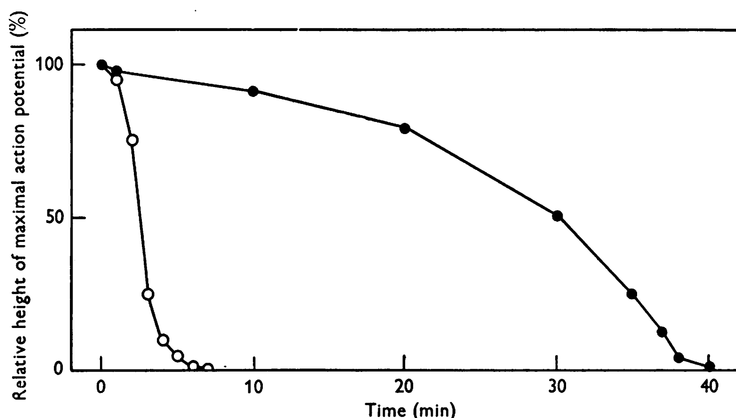

Fig. 4. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with acid Ringer (pH 3·0), and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

90 mM-$CaCl_2$

About 11 mM-NaCl was added to prevent interference by Na lack.

Control nerve. There was a very gradual loss of excitability which became complete after 5–6 hr. Recovery in the standard solution was negligible.

Perfused nerve. The action potential on both occasions decreased in height after 10 min for about 5 min and then remained more or less steady for the next 30–40 min. Perfusion with the standard solution brought about some recovery, which was not complete even after another 30 min.

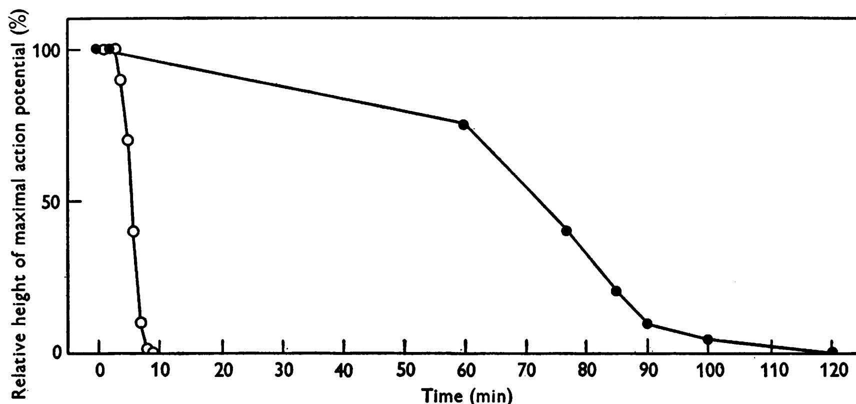

Fig. 5. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 100 mM-$BaCl_2$, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

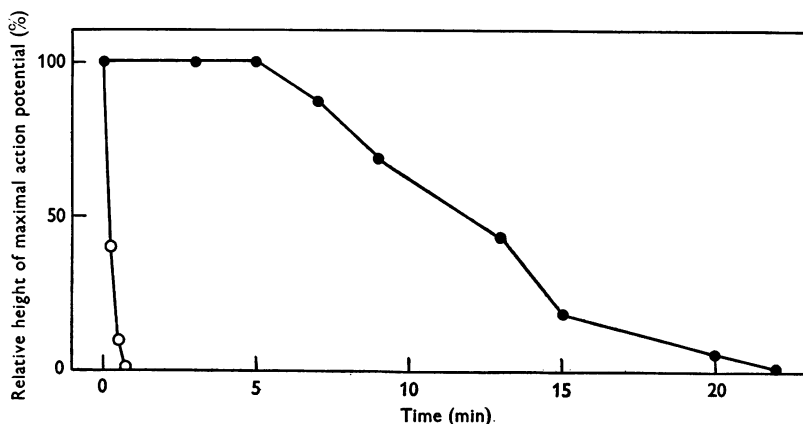

Fig. 6. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 100 mM $HgCl_2$, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—● control nerve.

In two other experiments the effect of 90 mM-$CaCl_2$ was studied in the absence of Na.

Control nerve. The gradual change led to complete block after 7 hr.

Perfused nerve. Conduction ceased after 9 and 3 min respectively. There was some recovery within 3 min of changing the perfusate but there was relatively little further change after 6 min.

The effect of lipoid-soluble substances

10% (v/v) *acetone* (Fig. 8)

Control nerve. The action potential obviously decreased after 1 min, and it always disappeared within 1½ min. Recovery only began after 40–50 min but was nearly full in 2 hr.

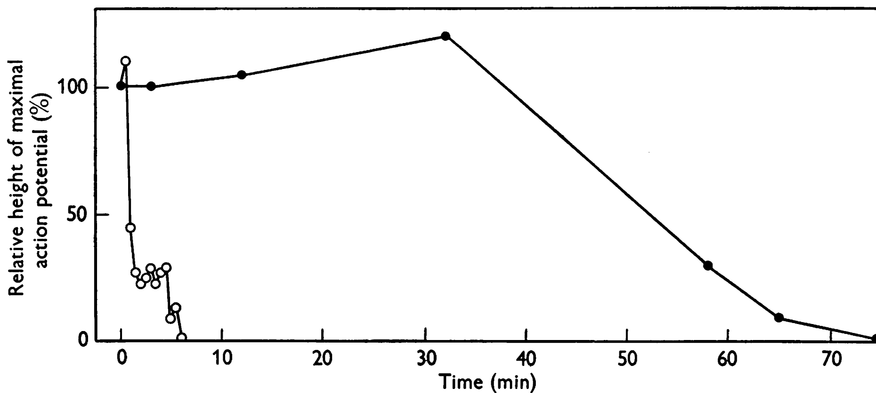

Fig. 7. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 100 mM-$CuCl_2$, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

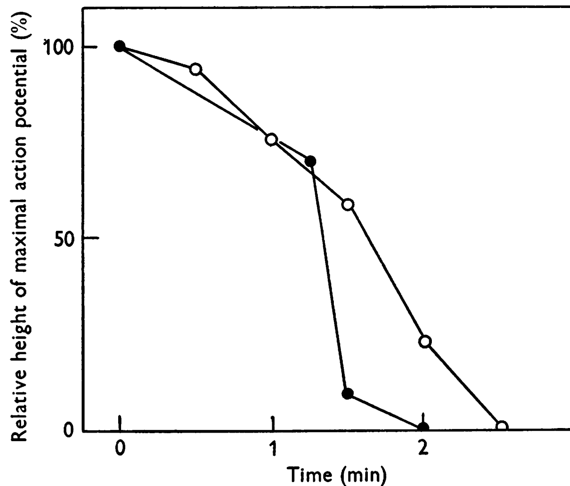

Fig. 8. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 10% (v/v) acetone, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

Perfused nerve. The conduction block was complete within 1½–2½ min. Recovery, however, was very much more rapid: it began after 2 min and was complete within 12 min. These results are based upon four experiments.

10 % (*v*/*v*) *ethanol*

Control nerve. Inexcitability was rapid in onset; it was complete in 5 min. Recovery was greatly delayed and was incomplete.

Perfused nerve. The block was complete after 8 and 2 min, but recovery was nearly full in 25 and 5 min respectively. The control refers only to the first of these two nerves.

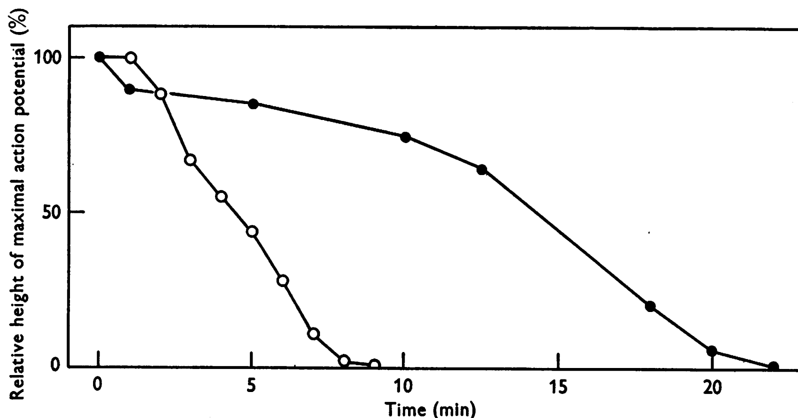

Fig. 9. Comparison of rate of loss of conduction in a frog sciatic nerve perfused with 1/5000 veratrine HCl, and in a control nerve placed in the same solution. ○—○, perfused nerve; ●—●, control nerve.

The action of 50 % ethanol was also tried in one experiment; the control nerve maintained conduction for 2½ min, but the injected nerve stopped conducting after 1 min.

10 mM-*cocaine* HCl

Control nerve. The blocking times of six nerves varied between 3 and 11 min, and even symmetrical nerves showed a difference of as much as 100 %; recovery was slow and only partial.

Perfused nerve. The blocking times of four nerves tended to be longer than in the control experiments; the range covered was 12–25 min. Recovery was imperfect. In one experiment, the non-perfused and perfused blocking times were 11 and 12 min respectively.

1/5000 *veratrine* HCl (Fig. 9)

Control nerve. The action of the alkaloid was quite rapid: conduction ceased after 22 min. There was no recovery.

Perfused nerve. The action potential disappeared after about 8 min. There was only partial recovery with the standard solution. This was a single observation.

Desheathed nerves

The effect of Na-free and strong KCl solutions upon several desheathed sciatic-peroneal nerves was studied for comparison with intact control and perfused nerves. The results of these experiments agreed with those of similar experiments described in the literature (e.g. Crescitelli, 1952). Five desheathed nerves placed in Na-free sucrose, or choline chloride solutions ceased conducting within 2–8 min (mean: 4 min; S.D. ± 3). Considerably longer blocking times were always associated with failure to remove the perineurium, confirmed by a histological examination.

When a phalangeal preparation, with only one or two active units, was desheathed distally to the level of the ankle and placed in a Na-free solution, there was conduction block within 15–45 sec. There was always some recovery of conduction within 5 sec when desheathed nerves were replaced in solutions containing Na (110 mM).

120 mM-KCl produced block in about 25 sec, and 60 mM-KCl in about 50–55 sec, in desheathed sciatic nerves. Recovery began within 15–50 sec in normal Ringer.

It was found later that, when sciatic nerves from very emaciated autumn frogs were used for experiments, the blocking times obtained with 60 mM-KCl were considerably shorter, being about one-fifth of the values already given. With intact nerves, conduction ceased in 11 min (mean of five nerves; S.D. $\pm 3{\cdot}2$) while symmetrical desheathed nerves all blocked within 10–15 sec. Similar reductions in blocking times of both perfused and control nerves were seen with 120 mM-KCl. Although the absolute values of diffusion coefficients calculated from data obtained with these nerves would be substantially different, the relative rates of diffusion in control, perfused and desheathed nerves remain remarkably constant.

DISCUSSION

The experimental results have shown clearly that many solutions which interfere with the ability of the frog sciatic nerve to conduct impulses do so much more rapidly when they are introduced into the vascular system of the nerve than when they are simply allowed to act upon the whole intact nerve. These are: Na-free solutions, isotonic solutions of KCl, RbCl, acid Ringer (pH 3), $BaCl_2$, $HgCl_2$, and $CuCl_2$. It is well known that experiments in which intact and desheathed frog nerves are compared give very similar results (Feng & Liu, 1949).

A perfused nerve can be considered as a system in which the sheath is bypassed and therefore equivalent to a desheathed nerve. However, in one case, there is diffusion between the whole nerve and the surrounding fluid, whereas in the other case, diffusion takes place between each capillary and a small part

of the nerve. It is therefore unjustified to compare the results of desheathing and perfusion experiments directly; the only comparison which can be made is between the respective diffusion coefficients, calculated from results obtained in experiments with intact, desheathed and perfused nerves.

It is reasonable to consider the whole problem as one of diffusion. Diffusion measurements are sometimes distorted by the movement of a solution as a continuous phase resulting from differences in hydrostatic and osmotic pressures. This can only take place through a membrane if sizable pores are present which allow both solvent and solute particles to pass. The ability of the frog perineurium to prevent the outward passage of solutions of methylene blue even at high pressure (Krnjevic, 1954) suggests that such pores, if at all present, must be rather fine. Further, the rigidity of the sheath tends to keep the volume of the nerve constant so that significant inward movement of water can only take place after desheathing (Lorente de Nó, 1952). Considerable transport by filtration through the capillary pores during perfusion is unlikely, especially as there is no accumulation of fluid in the nerve. Pappenheimer, Renkin & Borrero (1951) have shown that even under conditions of excessive filtration in the perfused hind-limb of the cat, the exchange of NaCl by diffusion is about 260 times greater than by filtration.

Of the various results described earlier only two groups, those dealing with Na-free and with KCl solutions, lend themselves to mathematical analysis. It is only in these two cases that there is good evidence for correlating conduction block with a definite local concentration of the diffusing substances. An attempt at evaluating a coefficient of diffusion in the nerve will therefore only be made for Na and for K.

Equations which can be used for the study of the kinetics of diffusion in intact and desheathed nerves were described by Hill (1928) and by Carslaw & Jaeger (1947). For the case of the perfused nerve, the conditions are somewhat more complex. If, however, the nerve is considered as an aggregation of smaller cylinders, each having a capillary at its centre, then it is possible to deal with the problem by the type of equation described by Muskat (1937) and by Roughton (1952) for a system of two concentric cylinders. The approximation is that made by Krogh (1922) when calculating the rate of diffusion of O_2 in muscle; with a nerve, which has a smaller concentration of capillaries, the approximation is, no doubt, a little less exact but the capillaries are sufficiently uniformly distributed to give an answer of the correct order of magnitude. It will be noticed that this treatment requires that no diffusion takes place through the outer boundary of each system of concentric cylinders. This can be assumed to be true at the axis of the nerve.

Since the various fibre types are distributed evenly throughout the nerve trunk, it is assumed that the onset of complete inexcitability is associated with the attainment of threshold concentration at the axis of non-perfused nerves.

In the perfused nerve, the corresponding time is the attainment of threshold concentration at the outer boundary of each set of concentric cylinders.

The apparent diffusion coefficients of K *and* Na *in the frog nerve*

Intact control nerve in 120 mM-KCl *and* 60 mM-KCl *solutions.* The corresponding equation was given by Hill (1928, p. 71, no. 47). The assumption made here, that there is no conduction when the interstitial K concentration reaches the value of 20 mM, is based upon the results of experiments with whole nerves (Lorente de Nó, 1947) and with single fibres (Huxley & Stämpfli, 1951). Taking an average value for the radius of the peroneal nerve (0·015 cm) and blocking times of 25 min for 120 mM-KCl, and 60 min for 60 mM-KCl, the respective diffusion coefficients are 10×10^{-7} and $5{\cdot}8 \times 10^{-7}$ cm^2/min.

Intact control nerve in a Na*-free solution.* In this case, Na diffuses from the nerve into the surrounding solution, in which its concentration is maintained at 0. It is assumed that complete block of conduction occurs when the concentration at the axis of the nerve becomes 1/10 of the original (Overton, 1902; Lorente de Nó, 1947; Huxley & Stämpfli, 1951). With a typical blocking time of 300 min the diffusion coefficient is $3{\cdot}8 \times 10^{-7}$ cm^2/min.

Desheathed nerve in 120 mM-KCl *and* 60 mM-KCl. Similar assumptions are made, and with a blocking time of 25 sec for 120 mM-KCl, and 50 sec for 60 mM-KCl, the coefficients are $3{\cdot}7 \times 10^{-5}$ and $3{\cdot}8 \times 10^{-5}$ cm^2/min respectively.

Desheathed nerve in a Na*-free solution.* A blocking time of 4 min gives a diffusion coefficient of $2{\cdot}8 \times 10^{-5}$ cm^2/min.

Nerve perfused with 120 mM-KCl *and* 60 mM-KCl *solutions.* Roughton's equation (1952, 3.10, p. 207) was used with data obtained from a large number of counts of vessels in frog sciatic nerves. The average of the highest concentration of vessels in different nerves (68/mm^2) gave a value of 70 μ for the radius of the larger cylinder. The radius of the inner cylinder is that of a capillary, i.e. 5 μ.

Blocking times of 1 min for 120 mM-KCl and 1·5 min for 60 mM-KCl are equivalent to coefficients of $1{\cdot}4 \times 10^{-5}$ and $1{\cdot}6 \times 10^{-5}$ cm^2/min respectively.

Nerve perfused with a Na *free solution.* With the same equation, and the average of the shortest blocking times, 7 min, the diffusion coefficient is $1{\cdot}6 \times 10^{-5}$ cm^2/min. Taking the general average, 15 min, the value is $7{\cdot}5 \times 10^{-6}$ cm^2/min.

It follows from these results that, whereas the rates of diffusion in desheathed nerves are only 2–3 times greater than those in perfused nerves, the latter are between 13 and 42 times greater than those in intact control nerves. These are facts which are very remarkable in several respects.

(*a*) The structure of the perfused nerve has not been altered in any way, so that the rapid rate of diffusion seen in such a nerve can only be ascribed to the absence of the sheath as an effective barrier. Since the sheath is only

2–6 μ thick, it is evident that it must in fact be very impermeable indeed to be responsible for such low apparent rates of diffusion for the nerve as a whole.

(*b*) The values of the coefficients of diffusion of NaCl and KCl from 0·1 M solutions into pure water at 18° C, given in the International Critical Tables are $7{\cdot}8\times10^{-4}$ and $9{\cdot}1\times10^{-4}$ cm^2/min respectively. The substantial difference between these figures and those calculated for desheathed and perfused nerves is perhaps greater than might be expected. However, the presence of the large number of closely packed fibres which are effectively impermeable to Na and K cannot be neglected. Their principal effect is perhaps to lengthen the total diffusion path by a factor which cannot be determined very exactly, but which might be of the order of $\frac{1}{2}\pi$ if the fibres could be simply considered as closely packed cylinders. This is probably too great a simplification, and does not take into account any complications introduced by the presence of endoneural sheaths or septa. The electrolyte and protein content of the interstitial fluid (although not known exactly) must raise its specific viscosity, and thus decrease the rate of diffusion, by a factor of perhaps as much as 2. It may well be that the spaces between the tightly packed fibres are sufficiently small in relation to the mean free paths of the ions to interfere with their diffusion.

(*c*) The small difference between rates of diffusion in desheathed and perfused nerves is rather surprising, especially as the assumptions made in the calculations all tended to exaggerate any difference. The desheathed nerve suffers some loosening of its structure which probably increases exchange by mixing to a variable degree, and so gives a rather higher diffusion coefficient. In the case of the perfused nerve no allowance was made for the dead space of the perfusing system, and it was assumed that the capillary concentration was always at the maximum level, ignoring any arteriovenous gradient. This, of course, would give rather low diffusion coefficients. It has been claimed (Chambers & Zweifach, 1947; Pappenheimer *et al.* 1951) that only a small fraction of the capillary wall is available for the diffusion of water and electrolytes (about 0·2% of the surface area according to Pappenheimer and his co-workers). If this were true, then one would expect to find a considerable difference between the calculated rates of diffusion in desheathed and in perfused nerves, since the capillary was assumed to be freely permeable.

At least part of the explanation for the discrepancy can be traced to the assumption made by Pappenheimer *et al.* (1951) that the capillary and the surrounding tissues can be considered as a system of two well-stirred compartments separated only by the capillary wall. This assumption seems quite unjustifiable, except on the grounds of convenience, since it ignores the geometry, the dimensions and the nature of the block of tissue which is effectively supplied by each capillary. The rather low figure given by the authors for the total capillary surface area in the perfused hind-limb of the cat (70 cm^2/g muscle) suggests that the ultimate diffusion pathway (Δx) in their

experiments was of the order of 40μ (initially, of course, Δx is a function of time). Using their own equation, this would give a value nearer 8% for the fraction of the capillary wall available for diffusion. The contrast between the rates at which lipoid-soluble and lipoid-insoluble substances leave the circulation (Renkin, 1952, 1953) does not support the thesis of Pappenheimer and his colleagues as directly as they suggest. The lipoid-soluble substances diffuse out much more rapidly not only because the whole capillary wall is available to them, but also because the surrounding muscle fibres are permeable, as Renkin himself showed.

It is not claimed that the entire frog capillary wall is freely permeable to electrolytes, but rather that the results of the present experiments do not seem consistent with a very high degree of impermeability.

The permeability of the nerve sheath to electrolytes and to lipoid-soluble substances

The interpretation of the mode of action of various substances upon intact and desheathed nerves given by Feng & Liu (1949) can be used to explain several features of the perfusion experiments. When there is a great difference between the blocking times in perfused and in control nerves, this indicates that the substance in question has a rapid action upon nerve fibres, but is usually prevented from reaching them by the impermeable sheath. To this group belong the Na-free, KCl, RbCl, acid Ringer, $BaCl_2$, $CuCl_2$ and $HgCl_2$ solutions. If the difference between the blocking times is not very striking, there are two possibilities. The substance may have a rapid action and penetrate the sheath readily (like acetone, alcohol and possibly the alkaloids, cocaine and veratrine) or it may have a very slow action (like $CaCl_2$ and NH_4Cl). Clearly none of the electrolyte solutions gave any evidence of being able to penetrate the sheath at all easily. The rapid blocking action of isotonic $CaCl_2$ described by Feng & Liu (1949) may have been due to a failure to preserve an adequate concentration of Na.

Cocaine did not cause a rapid block in perfused nerves. This suggests that desheathing may sensitize the nerve fibres to its action. At a physiological pH, cocaine is in the form of the amine (Woods, Cochin, Fornefeld, McMahon & Seevers, 1951) which has a high oil-water partition coefficient and to which the sheath should be permeable. Renkin (1952, 1953) has demonstrated very clearly the direct relationship which exists between the ability of many substances to penetrate cellular barriers and their oil-water partition coefficient, independently of their molecular weight. The high values of the coefficients of diffusion of O_2 (Gerard, 1927) and CO_2 (Fenn, 1928) in intact frog nerves agree fully with this.

It has been shown that the lack of Na and an excess of K prevent conduction as rapidly in intact perfused nerves as in desheathed nerves. This supports the

ionic theory of nerve conduction presented by Hodgkin (1951). The early failure to contract shown by muscles perfused with Na-free solutions demonstrates the importance of Na for muscular activity, and also the greater abundance of the capillary blood supply in muscles.

The high values of the diffusion coefficients of Na in nerves calculated by Lorente de Nó (1952) are based upon very arbitrary estimates of the interstitial Na concentrations. Most of the phenomena described in his papers can be accounted for by the dramatic response of nerve fibres to very small changes in the Na concentration near the threshold for conduction. For instance, the spontaneous recovery seen when a Na deficient nerve is exposed to the air is probably caused by drying, and perhaps also by the escape of Na from connective tissue cells.

The functional significance of the connective tissue sheath of the peripheral nerve

The present work has shown that the frog nerve sheath acts as a barrier to the diffusion of electrolytes under experimental conditions which closely approximate the normal. It remains to be considered why there is such a diffusion barrier around the peripheral nerves.

Histological observations (Krnjevic, 1954) suggest that the diffusion barrier is to be found in the continuous endothelial cell layers of the perineurium, described so clearly by Key & Retzius (1876) and Ranvier (1878). The perineurium surrounds bundles of nerve fibres and is the most striking element in the nerve connective tissue sheath, particularly where only one bundle is present. Its regular and compact structure contrasts with the loose, irregular structure of the epineurium, which covers the nerve as a whole. In the frog the epineurium consists of only poorly defined tissue lying outside the perineurium. It may be added that desheathing, when successful, always removes the perineurium. Causey & Palmer (1953) have claimed that desheathing only removes the epineurium, but plate 1 in their paper shows a frog nerve which has been cleaned but not desheathed, and which would not behave as a desheathed preparation, while their desheathed rabbit nerves have clearly lost both epineurium and perineurium.

The question whether it is the epineurium or the perineurium which is removed is not of purely academic interest. A continuous sheet of cells as in the perineurium might well be a diffusion barrier, and in having such a structure, the perineurium resembles another well-known diffusion barrier, the arachnoid. This similarity is rather significant as there is good evidence that both membranes are developed from the same ectodermal embryonic tissue, the neural crest. Harvey & Burr (1926), Harvey, Burr & van Campenhout (1933) and Lear & Edwards (1933) have shown that the leptomeninx (pia-arachnoid) is essentially of ectodermal origin, being formed from migratory cells of the

neural crest, whereas the dura is largely mesodermal. Masson (1942), on the other hand, has demonstrated the importance of such migratory neural crest cells in the development of the peripheral nerves in human embryos; all the connective tissue elements of the peripheral nerves, except the mesodermal epineurium, are apparently derived from these cells which have been named the primitive Schwannoglia.

It would seem, therefore, that the ectodermal tissues of the entire nervous system, central and peripheral, are separated from the surrounding mesodermal elements by a continuous system of ectodermal sheaths. In this light, the significance of the peripheral nerve sheath becomes more evident. The arachnoid, as a component of the blood-brain and blood-cerebrospinal-fluid barrier, plays an important part in the maintenance of the internal environment of the central nervous system. It may be supposed that the perineurium preserves the constancy of the internal environment of the peripheral nervous system, by shielding it from the products of excessive activity, or of pathological processes in surrounding tissues. A striking example of this was described by Hoyle (1953), who has shown that such a system of sheaths, acting as a diffusion barrier, enables the avascular nervous tissues of the insect to function normally in spite of enormous variations in the K concentration of the blood.

There is no doubt that removal of the sheath does affect the behaviour of the nerve fibres; the myelin sheaths swell, there are gradual changes in the fast electrotonus, the fibres deteriorate relatively quickly (Lorente de Nó, 1952) and apparently become very sensitive to cocaine. It is not clear whether these are only osmotic effects to be ascribed entirely to the absence of the elastic sheath, or whether they also demonstrate the loss of some essential factor, normally present in the interstitial fluid of the nerve. The similarity in the behaviour of spinal roots and desheathed nerves is of some interest in this respect.

It can be concluded that the function of the peripheral nerve sheath as a diffusion barrier is probably to preserve the specific character of the internal environment of the nervous system, and that the elastic pressure it exerts upon the contents of the nerve may be of some importance for the regulation of their osmotic balance.

SUMMARY

1. A perfusion method suitable for the study of the properties of the frog sciatic nerve is described.

2. Perfusion with a Na-free solution can block conduction reversibly within 6–7 min; perfusion with isotonic KCl blocks conduction reversibly in about 1 min.

3. Perfusion with isotonic RbCl, acid Ringer (pH 3·0), $BaCl_2$, $CuCl_2$ or $HgCl_2$ also blocks conduction rapidly, but perfusion with isotonic $CaCl_2$ or NH_4Cl has only a slow effect on conduction.

4. Most electrolyte solutions act much more rapidly in perfused nerves than in control nerves simply exposed to the same solutions. Solutions of lipoid-soluble substances act nearly equally rapidly in the two cases.

5. An analysis of the diffusion coefficients of Na and K in control, desheathed and perfused nerves shows that, whereas the rate of diffusion in control nerves is about $\frac{1}{13}$ to $\frac{1}{40}$ of that in perfused nerves, there is remarkably little difference between the rates of diffusion in perfused and desheathed nerves.

6. These results confirm the belief that the peripheral nerve connective tissue sheath is a barrier to the diffusion of electrolytes. They do not agree with the hypothesis that only a very small portion of the capillary wall is available for the diffusion of electrolytes.

7. It is suggested that the perineurium is a diffusion barrier as part of an extensive system of ectodermal sheaths which cover the entire nervous system, central and peripheral, and which probably serve to maintain the specific character and the constancy of its internal environment.

I am indebted to Prof. D. Whitteridge and Dr P. Eggleton for much advice and guidance, and to Mr W. T. S. Austin for his help in the construction and maintenance of the recording apparatus. Part of the work was performed during the tenure of a Goodsir Memorial Fellowship.

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