"Methods of investigation of the Nervous System: the problem of the permeability of the frog sciatic nerve sheath".

A Thesis submitted in competition for the Ellis Prize in Physiology.

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INTRODUCTION

There is one aspect of the physiology of the peripheral nervous system which has been the subject of much controversy during the last few years. To what extent should the properties of the nerve sheath be taken into consideration in experiments on the function of electrolytes in relation to nerve activity? The matter is one of some importance as so much work has been carried out on whole nerves. Lorente de No, in particular, based most of his exhaustive analysis of nervous action (1947) upon the results of experiments done on whole frog sciatic nerves. If the nerve sheath should be proved to be an effective diffusion-barrier, and to determine partly the electrical characteristics of nerve, then, to quote Lorente de No (1950) "there would be in the literature on nerve physiology hardly a single important observation that could stand uncorrected."

Many of the studies in which the problem of the sheath arose were in relation to the action of sodium deficient solutions, and the part played by Na ions in nerve activity. They acquired a new importance in the light of the work of Hodgkin and his associates (reviewed fully by Hodgkin, 1951), one of the main postulates of which was that conduction cannot be maintained in the absence of extracellular Na ions.
Fig. I. Microscopic section of the peroneal branch of a frog sciatic nerve just below the bifurcation. Stained by the Haurob method X420. The epineurium is seen around the nerve. The perineurium and endoneurium stain darkly between the nerve fibres.
Previous evidence.

I) Histological evidence.

It has been known for a long time that peripheral nerves are partly composed of connective tissue. The first really extensive studies of this connective tissue (which have never been superseded) were done at about the same time by Ranvier (1878) and Key and Retzius, (1873).

The now standard description of the nerve connective tissue was suggested by Key & Retzius. It has three main components:

1) The endoneurium,

which covers and separates the individual nerve fibres.

2) The perineurium.

More or less continuous with the endoneurium, it forms a sheath around and between bundles of nerve fibres.

3) The epineurium,

a continuous external sheath which binds all the nerve bundles together.

Key and Retzius observed the lamellated structure of the perineurium; each lamella consists of a fibrillar network covered on both sides by a layer of nucleated endothelial cells. The endoneurial spaces were shown (by injection of fluid) to be continuous with the perineural spaces. The endoneurium was not a complete sheath, because fluid could be seen to come into direct relationship with
the neurilemma, but fluid practically never passed outward through the epineurium. The epineural lamellae apparently only differed from the perineural in being richer in connective and elastic fibres. However if a cannula was pushed into the epineurium and fluid injected, it did not travel up and down the perineural spaces, but formed a progressive, localized swelling, which eventually burst out on to the surface.

The lamellated sheath described so fully by Ranvier was the perineurium; the epineurium he called the "tissu conjonctif perifasciculaire". In his description of the perineurium he emphasised the honeycomb like nature of the network of anastomosing channels, lined by a continuous endothelial layer which forms a serous cavity. The endoneurium was essentially a continuation of the perineurium. The epineural lamellae were rather thicker than those of the perineurium. His injection experiments, on the whole, gave results like those of Key & Retzius, although passage of the fluid out through the epineurium seems to have occurred more easily. However, Ranvier gives no histological evidence to support his belief that there are lymph passages in the epineurium. Capillaries were usually found to be separated from nerve fibres only by a thin endoneural layer; even this was sometimes apparently absent.
II) Physiological evidence.

In the course of the great series of investigations on muscle and nerve physiology, Overton (1902) had observed that whereas a frog sartorius muscle becomes inexcitable in isotonic sugar solution within some 45 minutes, sciatic nerves showed no change even after 12 hours in an isotonic sugar solution. With muscle there was no change until the NaCl concentration of the medium became less than 0.15%. The excitability then decreased gradually, and became nil at about 0.07% NaCl, (i.e. c. 10% of normal). Other Na salts preserved the excitability; Li ions could replace Na.

Netter (1926) on the basis of measurements of the rate of change of the electrical resistance of frog nerve in isotonic sugar solutions, suggested that in studies of the effects of various solutions upon nerves, solutions should be applied for at least 2 - 3 hours. Lipoid soluble solutions seemed to accelerate the rate of exchange of "internal" electrolyte.

So far, the evidence given has only suggested that nerves might in fact behave differently if the epineurium were not present. Evidence as to the behaviour of a nerve under conditions in which the epineurium did not come into consideration has been given by several authors.

Rice & Davis (1928) noticed that chloral
hydrate acts much more rapidly upon bullfrog sciatic nerves deprived of their outer sheath, especially if the preparation was stripped clean.

Feng & Gerard (1930) first suggested that the frog nerve sheath may be a diffusion-barrier because they found that: i) Methylene blue did not penetrate into an intact nerve, but after slitting the sheath the axons were stained by the dye. ii) Isotonic glucose, CaCl₂ & KCl blocked conduction much more rapidly after slitting the sheath.

Bishop (1932) mentioned the more rapid action of concentrated KCl solutions on frog nerve roots than on nerve trunks and ascribed this difference to the latter's connective tissue sheath.

Kato (1936) showed that conduction in isolated bullfrog nerve fibres was blocked practically instantaneously by the application of isotonic grape sugar solutions. This blocking action was instantaneous whenever the NaCl concentration in the solution used was less than 1/10th of that in Ringer. Cocaine Ringer (concentration of cocaine as low as 0.005%) also produced instantaneous block.

Erlanger and Blair (1938) investigated the accurately localized action of isotonic sugar solutions upon bullfrog spinal nerve roots. When the spinal root was a very slender one, so that the activity of a single unit could be studied, it was possible to block conduction within 10 seconds if the Na free solution was applied to what were
postulated to be nodes of Ranvier. At intervening points of the root, the solution produced no block, even after 16 minutes. With a large sensory root bathed in the Na free solution, block was not quite complete after 6 minutes. The slower components of the compound action potential were noticed to disappear earlier than the faster. Erlanger & Blair also observed that if a root which had become inexcitable in an Na free solution was left in the air, conduction gradually returned spontaneously. The process was very much accelerated by Ringer containing the normal amount of Na, which produced recovery in about one second.

Feng & Li (1949) compared the rates at which different solutions produced inexcitability in normal and in desheathed toad nerves. A large variety of substances were tested on symmetrical nerves, and a ratio of the respective blocking times calculated. They found that with electrolytes, the "intact" blocking time was always considerably greater than the "sheathless" blocking time, thus giving a high ratio. Lipoid soluble substances such as acetone and ethyl alcohol, in contrast, seemed to produce inexcitability almost as rapidly in the intact nerve as in the sheathless one. e.g.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Intact nerve (mins)</th>
<th>Sheathless nerve (mins)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12 M KCl</td>
<td>13-15</td>
<td>3-1</td>
<td>15</td>
</tr>
<tr>
<td>2.5% Choline chloride</td>
<td>1200-1400</td>
<td>45</td>
<td>25-30</td>
</tr>
</tbody>
</table>
**T. Solution Intact nerve Sheathless nerve Ratio I/S**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Intact nerve</th>
<th>Sheathless nerve</th>
<th>Ratio I/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% Glucose</td>
<td>300-360 mins.</td>
<td>30 mins.</td>
<td>10-12</td>
</tr>
<tr>
<td>0.10M BaCl₂</td>
<td>500</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vol% in Ringer</td>
<td>5</td>
<td>2(\frac{1}{2})</td>
<td>2</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vol% in Ringer</td>
<td>17-20</td>
<td>7-8</td>
<td>2-3</td>
</tr>
</tbody>
</table>

Feng & Lim found that a sheathless nerve, unlike a normal nerve tended to increase in weight during the first few hours after being placed in Ringer. They also tested the permeability of the detached sheath to haemoglobin under pressure of a few centimetres of water, and found that the protein did leak out. Their conclusion, however, was that even if the nerve sheath did have some pores, they were sufficiently few to make the sheath a diffusion barrier.

Huxley & Stämpfli (1951) investigated the rate of action of electrolyte solutions on frog, isolated single, myelinated nerve fibres resting and action potentials. They observed that the effect of a change in the ionic composition of the medium developed in a time of the order of one second. Conduction was blocked by any solution containing less than 5-10 mm Na or Lithium.

Lundberg (1951) also tried desheathing the frog sciatic nerve, when he found that in an ionized
Na free solution (0.11 M diethanol dimethyl ammonium chloride) conduction was blocked in 3-4 minutes. If the sheath was replaced, complete block only occurred after 4-7 hours. In addition, he injected strong K solutions (0.11 M) into the aorta; these produced block in about 1 minute. Applying the solution directly to the nerve was only effective after 15-20 minutes.

Crescitelli (1951) produced similar evidence that anti-histamine compounds and other substances which have a depolarizing or blocking action on nerve act with very much greater speeds on desheathed than on intact or resheathed nerves.

Crescitelli (1952) has said that Na free solutions (Sucrose, Choline Chloride, Tetramethyl ammonium bromide) block conduction in desheathed frog sciatic nerve fibres in 2-15 minutes, and that conduction is restored by 0.011 M NaCl. (unpublished data quoted by Hodgkin, 1951) found that the penetration of Na\(^{2+}\) is accelerated by slitting or removing the nerve sheath.

Mullins (1950) has shown that removal of the connective tissue sheath increases the rate of P\(^{32}\) uptake to slightly more than twice the normal value.

The principal objections to the belief that the epineurium is a diffusion barrier have been raised by Lorente de Nó (1947, 1950, 1951, 1952, a,b.). They were based mainly on the following considerations.
1) Conduction in a bullfrog nerve placed in a sodium
   free solution is maintained for 8-12 hours.

2) Changes in the external concentration of K does
   not affect the resting potential immediately.
   But

3) The action of cocaine upon sodium deficient
   nerve is detectable within 5 seconds.

4) A nerve which has just become inexcitable may
   begin to conduct within one minute of being placed
   into a 0.015 M Na solution.

5) There is more connective tissue within the nerve
   than around it.

   He believes in consequence that neither Na
   nor K are directly concerned with the maintenance
   of the resting potential or the mechanism of nerve
   conduction. The changes produced by removal or
   even slitting of the sheath must be due to dis¬
   ruption of the normal arrangement of the fibres
   within the nerve and alteration of their properties. This
   opinion is supported by Feng & Liu's observation
   that the nerve increases in weight after de¬
   sheathing. Dissection of a single fibre would
   cause even greater damage; Lorente de No (1952, a)
   found that such single fibres showed considerable
   swelling.

   He maintains therefore that the epineurium
   cannot be an effective diffusion-barrier, and that
   "studies on the physiology of unaltered nerve fibres
can be carried out only when the epineurium has been left intact on the nerve". (1950)

It may be interesting at this point to review the principal methods and objects of study in different investigations on the action of various substances upon nerve activity and the function and structure of the connective tissue sheath.

1. Histological. Ranvier (1878), Key & Retzius (1873).

This includes staining methods, the fine dissection of the sheath into its component lamellae and the injection of fluids into the various compartments of the nerve.


It gives an independent measure of the ratio of travel of electrolytes out of the nerve.

4. Behaviour of the spinal roots. Bishop (1930); Erlanger & Blair (1938); Lorente de No (1950, 1951) The roots have no epineural sheath.

5. Behaviour of nerve with the sheath slit.
Feng & Gerard 1930.

6. Behaviour of nerve with the sheath removed.
Rice & Davis (1928); Feng & Liu (1949); Lundberg (1951); Crescittelli (1951, 1952); Lorente de No (1952b)

7. Behaviour of desheathed nerve, with sheath
replaced. Lundberg (1951); Crescitelli (1951).


9. Rate of movement of radioactive ions into and out of the nerve (Keynes, unpublished data, quoted by Hodgkin, 1951) Mullins (1950).

The histological method has been very informative and no doubt will provide still more evidence in the future, but it cannot describe the behaviour of the intact nerve under physiological conditions.

Method 2 in which the nerve is intact, may be considered as a control.

Method 3 is rather laborious, and gives results which have been, so far, only of indirect and limited value. The exact interpretation of changes in resistance in a complex conductor such as a nerve cannot be easily interpreted.

Method 4 does not involve any disturbance of the nervous structure, but Lorente de No claims that spinal root fibres have different properties from those of nerve trunks. (e.g. 1950, 1951).

Methods 5, 6, & 8. Lorente de No's objections here have been mentioned.

Method 7. This rather ingenious experiment might seem to provide an obvious answer to these objections. Lorente de No (1952, a,) however, claims that an important feature of the osmo-regulating functions of the epineurium, is the constant pressure it exerts upon nerve fibres. Desheathed and
resheathed nerve fibres would not then be really comparable. Such a method as No. 9 should be able to give a quantitative estimate of the movement of ions across the epineurium. As used by Keynes & Mullins, however, it fails to keep the nerve intact.

Since Lorente de Nó had stressed so strongly the importance of using an intact nerve, it was decided that perfusion of the nerve might provide unambiguous evidence of the permeability or impermeability of the epineurium. Work was begun with such a method before seeing the paper by Lundberg (1951) in which he mentions briefly the perfusion of a frog sciatic nerve with concentrated solutions of KCl.

**Frog perfusion methods.**

The first description of a method of perfusing frog hind limbs was given by Läwen, (1904). It was used to study the action of suprarenal extracts upon the peripheral circulation. A cannula was fixed into the descending aorta of bullfrogs; flow of perfusate was recorded at the exit from a cannula in the anterior abdominal vein. Trendelenburg (1910) used a similar bullfrog preparation to determine the adrenalin content of normal blood.

Frog perfusion methods have been used fairly frequently since. For instance, to investigate the mechanism of oedema formation: Drink (1927); Saslow (1938); Danielli (1940); Hyman & Chambers
(1943); Lundberg (1951) is apparently the first to have used such a method to study the behaviour of frog nerves.

**Frog sciatic nerve blood supply.**

Mies (1926) describes fully the vascular supply of the sciatic nerve in *Rana Temporaria* and *Esclulenta*. A large number of fine vessels travel to the nerve and its roots from the aorta, the common iliac and the sciatic arteries. When they reach the nerve, these vessels divide regularly into central and peripheral branches, which anastomose and form at the surface a continuous vascular network.

Blood vessels pass through the epineurium and into the nerve where they have a covering of perineurium. The capillaries bear a close relationship to nerve fibres from which they are usually separated by an endoneural layer; occasionally they come into direct relation with the neurilemma. Ranvier (1878); Key & Retzius, (1873).

According to Mies, vascular anastomoses in the frog are so rich that nothing less than ligation of the aorta at its origin can stop blood reaching the sciatic nerve.

**Present method.** (fig.2)

All the experiments were done on pithed frogs, *(R.Temporaria.)* during late winter and spring months.

The descending aorta was displayed by a dorsal exposure, involving mobilization of the vertebral
Fig. II. Diagram of the nerve bath and the moist chamber used in the experiments. The recording, earthing and stimulating electrodes, and the perfusion system can be seen.
column. The metal cannula was made from a No.1 hypodermic needle. It was inserted with the help of dissecting glasses (magnification c.2x.). This was the most difficult stage of the experiment, and one could never count upon its being successful; especially when dealing with small frogs. One of the common iliac arteries (usually the left one) was ligated, and the homolateral sciatic nerve dissected down to its terminal branches and excised. This nerve was mounted in a nerve bath with silver stimulating and recording electrodes, so that the roots were stimulated, and the action potentials recorded from one of the terminal branches. The bath had a middle compartment (capacity 7 c.c.) in which the middle 3 cms of the nerve lay, which could be filled with solutions, and emptied, very rapidly.

The other leg and the pelvis, with the attached cannula, were severed from the rest of the body and weighed. They were then placed in a chamber through which moist air circulated. It contained electrodes which were arranged so as to stimulate the nerve roots and record the action potentials from the peroneal nerve below the knee. The gastrocnemius, the peroneus, and the tibia anterior were ligated and resected to avoid interference by muscle potentials. Stimulation was always maximal, and at a rate of about 1 per second. The leg with the cannula was weighed again at the end of the experiment.

The short cannula (1.5 cms) was directly
connected to a 2-way tap, thus cutting down dead space to a minimum. The perfusates were run into the frog from bottles suspended at a height which varied between 30 and 50 cms. above the chamber. The height varied because of attempts to find the best balance between such conflicting factors as absence of oedema and high rate of flow. The situation is complicated by a time factor. The extent of oedema formation also depends upon the duration of each experiment. It is, of course, evident that the height of the perfusion bottle does not directly determine the pressure within the arterial system of the frog. The narrow cannula has a significant resistance and it is only by estimating the pressure drop across this resistance that a definite value can be given to the arterial perfusion pressure. This pressure drop should be directly proportional to the viscosity of the solution and to the flow rate

\[ P = \frac{8l \eta V}{\pi r^4} \]  

i.e. \( P \propto K \eta V \)

(from Poiseuille's equation, where

- \( P \) = pressure drop
- \( l \) = length of cannula
- \( \eta \) = viscosity of fluid
- \( r \) = radius of cannula
- \( V \) = volume of fluid/minute.

This proportion was confirmed by an experiment in which the lateral fluid pressures just before and
just after the cannula were measured, using a variable, artificial resistance to reproduce the resistance of the frog's circulating system. The height of the perfusion bottle, the rate of flow (via the resistance), and the viscosity were varied. It was found with Dextran that if the bottle was 50 cm above the cannula, the "arterial" pressure would rise from 20 to 35 cm. as the rate of flow decreased from 1.7 to 0.75 cc./min. When the height of the bottle was 35 cm. the same rise of pressure (20-35 cm.) was obtained by a decrease of flow from 0.8 to 0.0 cc/min. Figures for the frog's arterial blood pressure given in the literature usually lie between 10 and 40 mm of mercury, with an average between 20 and 30 mm. of mercury. (Burton-Opitz, 1920, Prosser et al. 1950). The perfusion pressure should therefore preferably be within the range 20-35 cm. of water. In practice, the perfusion flow was usually of the order of 1-1.5 cc./min., so that a height of about 50 cm. would be about optimum. It had to be kept in mind, however, that with lower rates of flow, the perfusion height should be reduced to some 30-40 cm.

A hole in the floor of the moist chamber allowed the perfusate to escape. An electronic drop recorder inscribed a continuous record of the rate of flow on a Kymograph (fig. 3).

The temperatures of the perfusates and of the interior of the moist chamber were recorded at intervals during experiments. They varied with room
temperature but the fluctuations were usually only of the order of 2-3°C. The average temperature during experiments was always between 18 and 22°C. These changes of temperature were of a long range nature: they could not affect the results of the individual perfusion experiments.

It should be mentioned that the preparations usually survived and functioned well for many hours (12-24 hours or more) even after having suffered the action of all kinds of nefarious solutions.

Perfusate

The solutions were usually made up in 6% deionized Dextran (Dextran Ltd.) In several experiments 0.5% and 1% gelatin (commercial) solutions were tried instead. These colloid solutions were employed to reproduce normal osmotic conditions as nearly as possible.

The normal standard solution contained the usual amounts of electrolyte. i.e. 

\[ \text{M} / \text{L} \]

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>111.0</td>
</tr>
<tr>
<td>K</td>
<td>2.5</td>
</tr>
<tr>
<td>Ca</td>
<td>1.0</td>
</tr>
<tr>
<td>Cl</td>
<td>111.0</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>2.5</td>
</tr>
<tr>
<td>Acid phosphate</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The buffering salts used were K salts.
The pH was checked by B.D.H. indicators. It was kept in the range 7.2 - 8.2 (The pH of frog plasma is given by White (1924), as 7.65).

The Na-free solutions retained all the other components of the standard. The Na substitutes tried were:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>8.0 g.%</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.0 g.%</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>1.5 g.% (0.11M)</td>
</tr>
</tbody>
</table>

Of these only the last one is ionized in solutions.

The Na contents of the deionized Dextran and the gelatin were estimated after ashing by the Uranyl Zinc photometric method. The Dextran contained less than 0.10mM/l. A simple mathematical interpretation of the diffusion of Na from a nerve in Na-free media suggested that an Na concentration of 0.35 mM would not increase the "blocking" time by more than about 10-15%.

Other test solutions were made up by displacing different amounts of Na.

From value of the molecular weight determined by its osmotic properties (given by Dextran Ltd. as in the region of 90,000), the colloid pressure of 6% Dextran was calculated to be about 15 cm. of water. In this respect, it is probably not very different from frog plasma. Values for the frog plasma colloid pressure given in the literature are:
Fig. III. Kymograph tracing made by the drop recorder.
Upper line: Above - 10 sec. time intervals.
Below - Drops, during an Na free gelatin perfusion.

Lower line: Above - 10 sec. time intervals.
Below - Drops, 2 min. after changing over to the standard perfusion.
5.5 - 6.0 cm water (Krogh 1922)
9.6 - 11.5 cm plasma (White 1924)
7.1 cm water (Churchill et al 1927)
11.5 cm water (Landis, 1927)

Using an Oswald viscometer, the viscosity of Dextran was found to be of the order of 4.5. This figure is rather higher than that in the only published data on frog blood viscosity, which give 2.4 (Burton-Opitz 1902). It would tend to slow down the flow, and may increase the pressure gradients within the circulation to some extent.

Gelatin was used mainly at the suggestion of Lorente de No (1952, b) who employed gelatin perfusates. It is rather troublesome because its viscosity increases rapidly on cooling, and it tends to set, with time, even at room temperature. When it was employed, results tended to be negative: blocking and recovery times were often considerably increased. The pH of the solutions could not be maintained at a steady value for long periods without rather more trouble than with Dextran. Finally, it was found on several occasions that in the presence of gelatin, Na free solutions (with glucose or with choline chloride caused a considerable decrease in perfusion flow which was reversible (fig. 3) but was not seen when Dextran was used. This slowing down may account, at least partly, for the relative ineffectiveness of gelatin perfusates. The explanation of this effect has not yet been determined. It may
indicate a change in the calibre of the frog vessels, an occlusion of the capillaries by gelatin or a change in the physical properties of the gelatin molecules, with a corresponding increase in viscosity.

The concentrations of gelatin used were 0.5% (Chambers and Zweifach, 1940) and 1% (Chambers and Zweifach, 1944). The solutions were sometimes autoclaved to increase their fluidity.

There was usually some degree of oedema formation after several hours of perfusion. The difficulty of preventing oedema in such experiments when synthetic substitutes are employed is well known; and different authors have given various explanations of this: lack of red blood cells (Krogh 1929), lack of serum, (Drinker, 1927) lack of red cells and oxygen, (Saslow, 1938) absence of platelets, (Danielli 1940). White (1924) had noticed that spring frogs show spontaneous oedema, and Danielli (1940) emphasised that oedema formation, under standard conditions is very much greater in spring frogs than in autumn frogs.

The increase in weight after a 6-8 hours experiment was usually only of the order of 30-40%. The sciatic nerve itself, however, never seemed oedematous. This significant observation was confirmed by Lorente de Nó (1952, b). The recording and stimulating apparatus.

The electronic recording and stimulating apparatus was built for these experiments. It
consists of a two channel, R.C. coupled, differential input preamplifier and main amplifier, whose output is displayed on a double-beam oscilloscope, where it can be photographed. The stimulator provides two independently variable condenser-discharge type stimuli, triggered by the time-base. A crystal controlled series of dividing circuits supplies milli-second and other intervals for time-marking.
Fig. IV. Action potentials and stimulus artefacts from a non-perfused frog sciatic nerve at intervals after being placed in an Na free solution and in the standard solution.

The first potential is a normal control in the standard solution, at the beginning of the experiment.

Read each record from right to left.

The amplification was varied.
RESULTS

A. With Na free solutions.

1. Non-perfused nerve (fig. 4).

The first change in the action potential commonly began to be obvious after about 2 - 3 hours. Complete block, however, did not take place until a total time of some 6 hours had elapsed. Shorter blocking times of 3 and 4½ hours were recorded also.

The components of the compound potential gradually dispersed, and the later components disappeared before the earlier. The conduction intervals of the components could be followed and seen to become progressively longer.

Recovery in the standard solution was fairly rapid. The first sign of conduction could be seen within 2 - 5 minutes. The whole process was reversed, with the faster components reappearing first.

2. Perfused nerve (fig. 5).

With a Dextran perfusate.

<table>
<thead>
<tr>
<th>Na substitute</th>
<th>Blocking time (minutes)</th>
<th>First recovery time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>21</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Glucose</td>
<td>15</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Glucose</td>
<td>14</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>14</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>10½</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6½</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>6 - 7</td>
<td>5 - 10</td>
</tr>
</tbody>
</table>
Fig. V. Action potentials and stimulus artefacts from a frog sciatic nerve at intervals after beginning perfusion, with an Na free solution, and with the standard solution.

The first potential is a normal control, perfused with the standard solution, at the beginning of the experiment.

Read each record from left to right.

There was some 50 cycle mains interference.
With a gelatin perfusate.

<table>
<thead>
<tr>
<th>Gelatin Concentration</th>
<th>Na Substitute</th>
<th>Blocking time (minutes)</th>
<th>First recovery time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>Choline Chloride</td>
<td>32</td>
<td>60</td>
</tr>
<tr>
<td>1%</td>
<td>&quot;</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>1%</td>
<td>&quot;</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>0.5%</td>
<td>&quot;</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>0.5%</td>
<td>&quot;</td>
<td>7½</td>
<td>10</td>
</tr>
</tbody>
</table>

It can be seen that the blocking times obtained with 1% gelatin perfusates are on the whole rather long, and the recovery rates correspondingly slow.

However, it should be noted that a blocking time of the order of 7 minutes was recorded both with Dextran and with gelatin perfusates, in the former case with sucrose and with choline chloride as Na substitutes.

It was shown in one experiment that the conduction block cannot be due to choline chloride by demonstrating that perfusion with the full concentration of choline chloride plus half the normal concentration of Na produced rapid recovery in an inexcitable nerve.

As with the non-perfused nerve, the blocking process affects the later components of the action potential before the others. The first effect of the Na free solution may be seen within 2 minutes (fig. 5) as a broadening of the action potential. The potential usually returned to its full height
Fig. VI. Action potentials and stimulus artefacts from a "non-perfused" frog sciatic nerve at intervals after being placed in 0.12 M KCl, and in the standard solution.
First potential is a normal control in the standard solution at the beginning of the experiment.
Read each record from right to left.
There was no recovery in this case.
Fig. VII. Action potentials and stimulus artefacts from a frog sciatic nerve at intervals after beginning perfusion with 0.12 M KCl and with the standard solution.

First potential is a normal control, perfused with the standard solution, at the beginning of the experiment.

Read each record from left to right.
after about 1 minute of the standard perfusion, but it took several more minutes to regain its original conduction rate.

B. With 0.12 M KCl.

1. Non-perfused nerve (fig.6).

The action potential started decreasing after about 8 minutes, and disappeared in about 25 minutes. The blocking action seemed to affect the compound potential as a whole. With 2 nerves, there was some recovery after about 20 minutes, but in one case, there was none even after 14 hours in the standard solution.

2. Perfused nerve (fig.7).

The first sign of a decrease in the action potential appeared within 30 seconds, and block was complete within 1 - 1\(\frac{1}{2}\) minutes. Conduction was first restored after some 5 minutes of standard perfusion, and it was normal after some 12 minutes.

C. With 0.10 M BaCl\(_2\)

The test solution contained one fifth of the normal concentration of Na; an equivalent solution (without BaCl\(_2\)) was formed by experiment to preserve conduction.

1. Non-perfused nerve. (fig.8).

There was definite evidence of block after 1 hour in the BaCl\(_2\) solution. Conduction was completely lost by the end of 2 hours. First signs
Fig. VIII. Action potentials and stimulus artefacts from a "non-perfused" frog sciatic nerve at intervals after being placed in 0.10 M BaCl₂, and in the standard solution.

The first potential is a normal control in the standard solution, at the beginning of the experiment. Read each record from right to left.
Fig IX. Action potentials and stimulus artefacts from a frog sciatic nerve at intervals after beginning perfusion with 0.10 M BaCl₂, and with standard solution.

The first potential is a normal control, perfused with the standard solution, at the beginning of the experiment.

Read each record from left to right.
of recovery in the standard solution were seen after 6 minutes; it was quite substantial after 15 minutes. During recovery, many spontaneous impulses were seen at high amplifications, and it was observed that one stimulus caused a considerable increase in this spontaneous activity, lasting for some 10-15 seconds.

2. Perfused nerve. (fig.9).

The late components of the action potential seemed to be affected before the main group, which only began decreasing after 3 minutes. However, there was no tendency towards dispersion of the various components as in Na free media. Block was complete after 8 minutes. Recovery with the standard perfusion began within one minute, and it was nearly complete after 5 minutes.

D. 10 vol.% acetone (in standard solution).

1. Non-perfused nerve (fig.10).

The action potential had obviously decreased after 1 minute, and it disappeared after 2 minutes. Recovery only began after 40 minutes in the standard solution, and it was not complete until after 1 ½ hours.

2. Perfused nerve (fig.11).

As with the above, block was practically complete in 2 minutes. Recovery, on the other hand, was very much more rapid: it began after 2 minutes, and was complete after 12 minutes.

Preliminary experiments have given similar results with 10 vol.% ethyl alcohol, i.e. with
Fig. X. Action potentials and stimulus artefacts from a "non-perfused" frog sciatic nerve at intervals after being placed, in 10 vol.% acetone, and in the standard solution.

The first potential is a normal control in the standard solution, at the beginning of the experiment. Read each record from right to left.
Fig. XI. Action potentials and stimulus artefacts from a frog sciatic nerve at intervals after beginning perfusion with 10 vo.% acetone, and with standard solution.
The first potential is a normal control, perfused with the standard solution, at the beginning of the experiment.
Read each record from right to left.
little difference between perfused and non-perfused blocking times, but a considerable difference in the recovery rates.

There might seem to be some discrepancy as far as the NaCl ratio is concerned. It should be noted, however, that only one experiment was done with NaCl, and that Feng and Liu only gave one set of figures for NaCl.

As far as liquid soluble substances are concerned, it is clear that the rate of onset of inactivity is very much the same with perfused and non-perfused nerves. This also agrees closely with Feng & Liu (1949) who found an 1/3 ratio of 1-3 with similar organic solvents.

One is inevitably led to the conclusion that the mechanism in the structure which prevents the rapid action of electrolyte solutions upon intact spinal nerves. The present results were obtained without interference to the structure of the nerve, and with perfusions which were approximately normal as far as the osmotic pressure, electrolyte content, pH and temperature were concerned. The perfusion pressure
DISCUSSION

The ratio of the respective blocking times of perfused and non-perfused nerves exposed to the action of Na free, strong KCl and BaCl₂ solutions correspond on the whole to those of Feng & Liu (1949).

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Na free</th>
<th>0.12 MKCl</th>
<th>0.10M Ba Cl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.E/R</td>
<td>C. 30</td>
<td>25</td>
<td>15</td>
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</table>

I/S     10-30  15  80  (Feng & Liu 1949.)

There might seem to be some discrepancy as far as the Ba Cl₂ ratio is concerned. It should be noted, however, that only one experiment was done with Ba Cl₂, and that Feng and Liu only gave one set of figures for Ba Cl₂.

As far as lipoid soluble substances are concerned, it is clear that the rate of onset of inexcitability is very much the same with perfused and non perfused nerve. This also agrees closely with Feng & Liu (1949) who found an I/S ratio of 1.3 with similar organic solvents.

One is inevitably led to the conclusion that the epineurium is the structure which prevents the rapid action of electrolyte solutions upon intact sciatic nerves. The present results were obtained without interference to the structure of the nerve, and with perfusates which were approximately normal as far as the osmotic pressure, electrolyte content, pH and temperature were concerned. The perfusion pressure
was also very near the normal arterial pressure as far as this is known. The blocking times recorded agree fairly closely with those of previous observers.

Na free: Erlanger & Blair (1938); Crescitelli (1951, 1952); Lundberg (1951).

0.12 MKCl: Lundberg (1951).

The very much more rapid rates of blocking by Na free solutions seen with single nerve fibre preparations one could not expect to reproduce under these conditions. It was found by other observers (including Overton, 1902; Huxley & Stämpfli, 1951, and Crescitelli, 1952) that conduction block only occurs when the Na concentration in the external medium falls to at least 10% of normal. In a perfused whole nerve, this cannot happen instantaneously: the return of excitability on the other hand, should be very rapid, as was indeed the case. The converse is seen with 0.12 MKCl perfusions where block is very rapid, but recovery is prolonged.

The close agreement with the results of Erlanger & Blair (1938) working on spinal roots suggests that there is no significant difference between the properties of the root fibres and those of the trunk, in spite of the claim made by Lorente de Nó that the area of greater sensitivity to lack of sodium is not related to the anatomical distribution of the epineurium (1951).

The high rate of penetration of organic solvents into intact nerves is very significant. It is
precisely what one would expect if the nerve had as a sheath a continuous, cellular envelope. That such an endothelial envelope probably exists within the epineurium is made clear in the histological work of Ranvier (1878), and Key & Retzius (1873). Further, they give evidence that the perineurium and endoneurium although similar in basic structure, have open passages within them. It may be pointed out here that in microscopic sections of the frog sciatic nerve and its branches (fig.1), which were examined in these experiments, there was little tendency for the fibres to be grouped in large bundles, and the perineurium was correspondingly little differentiated from the endoneurium.

There would seem to be little ground left for Lorente de No's belief that the epineurium is ineffective as a diffusion barrier. In a recent personal communication (1952, b), Lorente de No mentioned the results he obtained in similar perfusion experiments with Na free solutions: blocking times were of the order of 20 minutes, and therefore he believed that the mechanism of conduction block in perfused nerves is different from that in desheathed nerves, in which block takes place in 5 minutes. He did not mention what concentration of gelatin was used in his perfusates, a point which has been shown to be of some importance. Further, the blocking times for desheathed preparations in Na free media.
given by Crescitelli (1952) are 2-15 minutes. These values overlap considerably those obtained in the present series of experiments, the lowest of which was only 6½ minutes. The process of desheathing may disrupt the normal arrangement of the nerve fibres somewhat, and render them more accessible. In any case, the small difference between 5 and 6½ minutes does not suggest that the mechanisms of conduction block in the two preparations are fundamentally different.

The slowing down of conduction seen with Na free solutions is probably explained by the decreased influx of Na ions during activity and the consequent slower rate of depolarization of adjacent nodes of Ranvier. It is less easy to explain the greater sensitivity of slower fibres to lack of sodium. Erlanger and Blair (1938) showed that Na free solutions act at nodes of Ranvier, and are ineffective at other points along the nerve fibre. They suggested that in a large group of fibres, the slower, with their greater number of nodes would tend to be affected first because the probability that some of their nodes were reached would be correspondingly greater. The explanation, however, may be a different one. Smaller nerve fibres have a relatively greater surface area. The ionic fluxes per unit area should be smaller in proportion since the internal ionic concentrations are probably
the same as in larger fibres. If this assumption is correct, one might expect these smaller fibres to be more sensitive to changes in the external ionic concentrations. Even though their nodes are closer together, the margin of safety may well be less.

The spontaneous activity noticed in nerves under the action of BaCl₂ has been observed before by Lorente de No and Feng (1946).
SUMMARY

1. Previous evidence on the question of the permeability of the frog sciatic nerve sheath is reviewed.
2. Methods used by previous investigators are also reviewed.
3. A perfusion method for the study of the behaviour of the intact frog sciatic nerve, with Dextran and gelatin perfusates and a non-perfused symmetrical control is described.
4. Na free perfusates block conduction in as little as 6½ minutes.
5. Na free, 0.12 M KCl and 0.10 M BaCl₂ solutions produce block very much more rapidly in perfused than in non-perfused nerves.
6. 10 vol.% acetone and 10 vol.% ethyl alcohol block perfused and non-perfused nerves with about equal rapidity.
7. It is suggested that the results obtained are good evidence that the frog sciatic nerve sheath is an effective barrier to the diffusion of electrolytes.
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