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THE DISTRIBUTION OF Na AND K IN CAT NERVES

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Ever since the first full statement of the membrane theory (Bernstein, 1912) it has been widely believed that the mechanism of nervous conduction depends upon certain important differences between the concentrations of electrolytes inside and outside the nerve fibre. Bernstein had no evidence on this point and he relied for his argument on what evidence was available from analyses of muscle. We now know much about the composition of the intracellular fluid of invertebrate nerves (e.g. Lewis, 1952) and one analysis has been made of the electrolyte distribution in frog nerves (Fenn, Cobb, Hegnauer & Marsh, 1934). No study of the distribution of electrolytes in mammalian nerves, however, is apparently available.

Estimations of the total content of Na, K and Cl in various mammalian nerves have been made by several authors: Alcock & Lynch (1907): Cl; Tupikova & Gerard (1937): Na, K and Cl; Amberson, Nash, Mulder & Binns (1938): Cl; Fenn (1938): K; Manery & Hastings (1939): Na and Cl; Lissák & Kovács (1942): K; Davies, Davies, Francis & Whittam (1952): Na and K; McLennan & Harris (1954): Na and K. The information to be derived from these values is unfortunately greatly limited by complete uncertainty about the relative proportions of intra- and extracellular fluid and the relative proportions of total intra- and extracellular Na, K, or Cl. The present paper is an analysis of the distribution of Na, K and Cl in cat nerves, based upon: (*a*) a comparison of the total Na, K and Cl contents of intact nerve trunks and desheathed nerve bundles; (*b*) studies of the diffusion of Na from desheathed nerve bundles; (*c*) a direct estimate of the intracellular space obtained from photomicrographs.

METHODS

Animals. Most of the nerves analysed were from cats. A small number of nerves from rabbits, dogs and monkeys were also studied for comparison; the only estimations made, however, were of the total Na and K.

The majority of the nerves were removed from cats which were in good condition, with their circulation intact, in the course of experiments under general anaesthesia, or in some cases, after

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decerebration. The anaesthetics were ether, chloralose and pentobarbitone sodium (Nembutal). A smaller number of nerves were only removed after the death of the animal, but this was done as speedily as possible. Since the Na and K values obtained from these did not differ significantly from the others, they were all combined together. No record was kept of the sex of the cats.

Nerves. The intact nerves were portions of sciatic trunks and their two terminal branches (Pl. 1, fig. 1). The desheathed nerves were bundles of the medial or, more frequently, the lateral popliteal divisions carefully dissected out of the common trunk. The perineurium was freed by cutting around with fine scissors, and then rolled back like a sleeve with fine pointed forceps. By this method it is often possible to obtain 6 cm or more of nerve bundle free of its sheath. The success of this operation was subsequently checked whenever possible by a histological examination (Pl. 1, fig. 2). The surface of intact nerves was not cleaned unless there were present gross amounts of fat, as happened occasionally at the principal bifurcation. Intact nerves were sometimes rinsed quickly in mammalian saline if they were very obviously stained with blood. During desheathing, the nerve bundle was held away from the tissues, so that it could in no way become contaminated with blood and could not absorb fluid from anywhere.

All specimens were placed in platinum crucibles of known weights and weighed immediately. Intact nerves usually weighed 80–150 mg but occasionally as much as 300 mg, while desheathed nerves weighed 40–120 mg. The crucibles were left in an air oven at 100–110° C for at least 5 hr, and, after cooling in a desiccator, weighed again.

Na *and* K *estimations.* The dry nerves were ashed overnight in a muffle furnace at about 520° C. The ash was dissolved in hot nitric acid (0·75 N) and the solution made up to a known volume. Further dilutions were made so that the unknown solutions could be compared directly, by means of an Evans Electroselenium flame photometer, with a standard solution containing 5 mg Na/l. and 10 mg K/l. The Na values were obtained from the average of five readings, and the K values from the average of three readings, in each case.

Control runs, with known quantities of Na and K submitted to the same procedure, showed that the recovery of Na varied between 98·5 and 99·5%, and that of K between 96·5 and 98·0%. No systematic correction has been made to allow for the slightly imperfect recoveries. Some indication of the random error of the method was given by: (1) a series of eleven duplicate estimations of plasma Na (done by an exactly similar method) in which the S.D. of the twin readings was $\pm 1{\cdot}6\%$; (2) a comparison of the readings obtained with the same solution on two different days: this was done with four solutions and the S.D. was $\pm 1{\cdot}3\%$. It is difficult to avoid some personal bias in photometer estimations without taking very special precautions; allowing for this, it would seem justified to claim that the total S.D. of the method was unlikely to be more than $\pm 2\%$ for Na and $\pm 5\%$ for K.

Cl *estimations.* The weighed and dried nerves were left in 2–5 ml. distilled water for 24 hr or more, the solution being stirred at intervals. The Cl was then titrated electrometrically against standard $AgNO_3$ (B.D.H.) by a method similar to that described by Eggleton, Eggleton & Hamilton (1937). The accuracy was limited by the sharpness of the end-point rather than by the capacity of the burette to deliver small volumes. The error of the titre was therefore about $\pm 0{\cdot}01$ ml. which corresponded to an error in the estimation of the Cl concentration of ± 2–15%, according to the size of the nerve. In a series of eight titrations of known amounts of Cl, the average error was $\pm 3{\cdot}5\%$ and $\pm 10\%$ when estimating 100 and 5 μmole Cl respectively.

Diffusion of Na *from cat nerves.* The desheathed nerve bundle (about 6 cm long) was clamped in a special holder in which the two cut ends were grasped firmly over a distance of about 1–2 mm but the rest of the nerve remained quite free. The holder was originally made of Perspex but a stainless steel model was later found to be more satisfactory. The nerve and holder were immediately weighed, and then plunged in the diffusion bath.

The bath was a 7 cm long portion of 1·5 cm diameter Pyrex glass tubing closed at the lower end with a rubber stopper, and with an outer jacket containing a heating element immersed in liquid paraffin. The heating element was the cathode load of the cathode follower output of a simple d.c. amplifier across the input of which was a bridge with a thermistor in one of its arms. The

thermistor was placed in the solution inside the bath and the bridge adjusted to maintain a constant temperature of $37 \pm 1°$ C. A needle pushed through the rubber stopper supplied a stream of a suitable gas mixture for oxygenation, pH control and stirring.

The solution which had been heated to about 37° C was delivered into the bath in samples of 10·0 ml. with an accuracy rather better than $\pm 0{\cdot}1$ ml. The whole operation, of emptying the bath by letting the solution run out, and then refilling it, took about 10 sec. It was found that between 0·38 and 0·40 ml. of solution remained in the bath after emptying, by estimating the Na concentration of samples of distilled water after samples of saline. A correction was accordingly made to all results. Using small drops of a dye solution, mixing was shown to be complete, as far as the eye could judge, in less than 5 sec.

The solution into which diffusion took place contained 1% sucrose and K buffers to maintain the pH between 7·3 and 7·5. In a small number of experiments in which the outward diffusion of K was also studied, no buffers were used.

In every experiment, some 12–14 samples of solution were obtained, first at intervals of 2–5 min, later of 30 min. Diffusion was allowed to go on for at least 2 hr. The Na (and sometimes K) concentration of the samples was estimated with the flame photometer, each value being calculated from the average of three readings. Controls were made to determine (*a*) the interference effect of sucrose on the Na readings ($+2\%$), (*b*) the degree of contamination with Na of the sucrose solution, which was never quite negligible. Suitable corrections were applied to all readings.

At the end of each experiment, the nerve was placed in a fixing solution so that the effectiveness of the desheathing could be confirmed later by a histological examination.

In each case, an exactly similar desheathed nerve, from the opposite limb of the same cat, was analysed for Na and K, as described above, as a control for calculating the initial Na and K content of the nerves used in the diffusion study.

Inulin space. Some 30 ml. of 20% inulin were injected intravenously in cats anaesthetized with Nembutal, both renal pedicles having been tied. After about 4 hr the sciatic nerves were removed, together with a sample of blood which was heparinized. Each nerve was ground to a paste and the inulin extracted with hot water and vigorous stirring. Proteins in the plasma sample and in the nerve extract were precipitated with $ZnSO_4$ and NaOH, and the filtrates (diluted if necessary) incubated with HCl at 80° C for 15 min in the presence of $0{\cdot}05\%$ $FeCl_2$ and alcoholic resorcinol ($0{\cdot}2\%$). After cooling, the inulin concentration was estimated with the help of a Hilger photometer (filter 603) and a calibration curve obtained from samples containing known amounts of inulin (2, 4 and 6 mg%) submitted to this procedure. Control experiments with two nerves containing no inulin, showed that cat nerves normally give a photometer reading equivalent to only about 0·1 mg % inulin.

Direct estimation of extracellular space. This depended on a direct comparison of intra- and extracellular areas in photomicrographs of intact cat nerves. The latter were fixed in Flemming's fluid, and paraffin sections were later photographed, magnified about 700 × (Pl. 1, fig. 3). Details of the histological procedure are given later; it is similar to that used by Sanders (1947) in a study of rabbit nerves, in which, after fully considering and checking all possible errors, he came to the conclusion that the only serious systematic error introduced by the method was a general shrinkage of all elements, which can be allowed for by multiplying all data by 1·07. It is true that Sanders was concerned particularly with the dimensions of the nerve fibres, but there is no reason to believe that the apparent arrangement of the fibres would be greatly altered in good preparations, which are cut cleanly and with no evident disruption of the tissue. The hydrostatic pressure of the circulation may possibly tend to increase slightly the dispersion of the nerve fibres *in situ*, but this cannot be a very large effect since the perineurium limits outward expansion.

The photomicrographs included only parts of a bundle; they were selected to give variable proportions of large and small fibres. The intra-axonal areas were cut out with scissors and the relative proportions of intra- and extracellular areas found by weighing. Each photomicrograph contained about 150 nerve fibres; to exclude personal bias, several were also cut by an independent observer. The corresponding twin figures had a S.D. of $\pm 4\%$. Prolonged drying of the paper after

cutting (as was done by Ellison (1910) in a similar study of horse nerves) was found to make practically no difference to the final ratio, and was therefore unnecessary.

Histological techniques. All nerves were mounted on small glass frames and fixed in Flemming's fluid, glacial acetic acid being added before sealing the tube with paraffin wax. The duration of fixing was from 12 to 36 hr; it depended upon the thickness of the nerve, and also upon whether the latter had been desheathed or not. After washing, progressive dehydration in alcohol, or, more often, in dioxan, preceded embedding in paraffin blocks, from which 8–12μ sections were cut. A connective tissue stain, like that of Mallory, which differentiated the perineurium clearly, was commonly used.

RESULTS

Na *and* K *content of intact nerves*

The average Na concentration in fifty-one cat nerves was 96·2 m-mole/kg fresh nerve (S.E. $\pm$ 1·2), and the average K concentration in fifty-three cat nerves 46·2 m-mole/kg fresh nerve (S.E. $\pm$ 0·8). The average water content of these nerves was 68·9% (S.E. $\pm$ 0·4).

Na *and* K *content of desheathed nerves*

The average Na concentration in sixteen cat nerves was 99·3 m-mole/kg fresh nerve (S.E. $\pm$ 3·0) and the average K concentration in fifteen cat nerves 76·7 m-mole/kg. fresh nerve (S.E. $\pm$ 2·6). The average water content of the desheathed nerves was 63·6% (S.E. $\pm$ 1·0).

The most striking feature of these results is the negligibly small difference between the Na concentrations in intact and desheathed nerves, particularly when compared with the difference between the respective K concentrations. Comparison of the Na and K concentrations in a few nerves from other mammals revealed the same feature. For instance, in a monkey, a dog and a rabbit, the Na concentrations of intact and desheathed nerves were as follows: 121·0 and 115·8; 85·4 and 95·1; 75·4 and 76·4 m-mole/kg fresh weight, whereas the K concentrations of intact and desheathed nerves were: 44·7 and 57·9; 26·0 and 44·5; 53·6 and 80·7 m-mole/kg fresh weight.

Cl *content of intact and desheathed cat nerves*

These estimations were made on a smaller number of nerves by a somewhat less exact method; the results are correspondingly less accurate. They are principally of interest in giving an indication as to the probable composition of the interstitial fluid in the nerve.

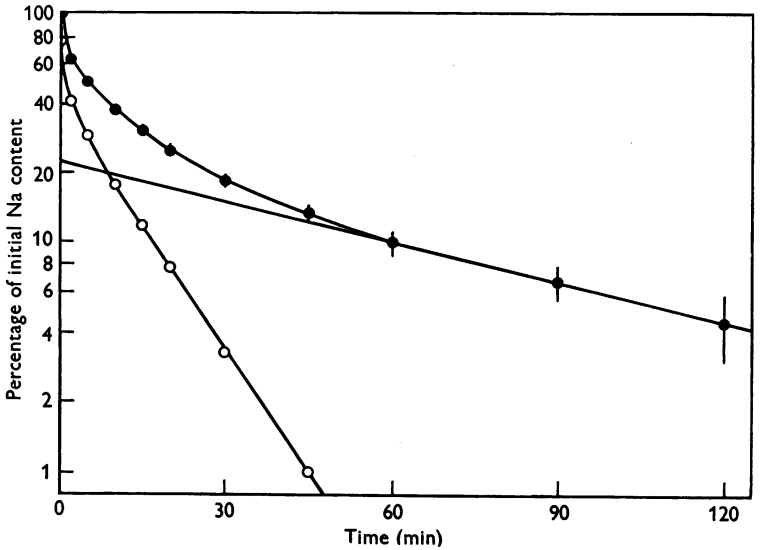
The average Cl concentration in seven intact nerves was 62·8 m-mole/kg fresh nerve (S.E. $\pm$ 3·7), and in six desheathed nerves 62·1 m-mole/kg fresh nerve (S.E. $\pm$ 2·6). The average water contents were 63·8% (S.E. $\pm$ 0·7) and 66·7% (S.E. $\pm$ 0·9) respectively.

Diffusion of Na *and* K *from desheathed cat nerves into a sucrose solution*

The rate of diffusion was obtained by plotting the percentage of the initial Na or K content still remaining in the nerve against time. The initial content

was calculated from the weight of the nerve and the concentration of Na or K found in the control nerve removed from the opposite limb.

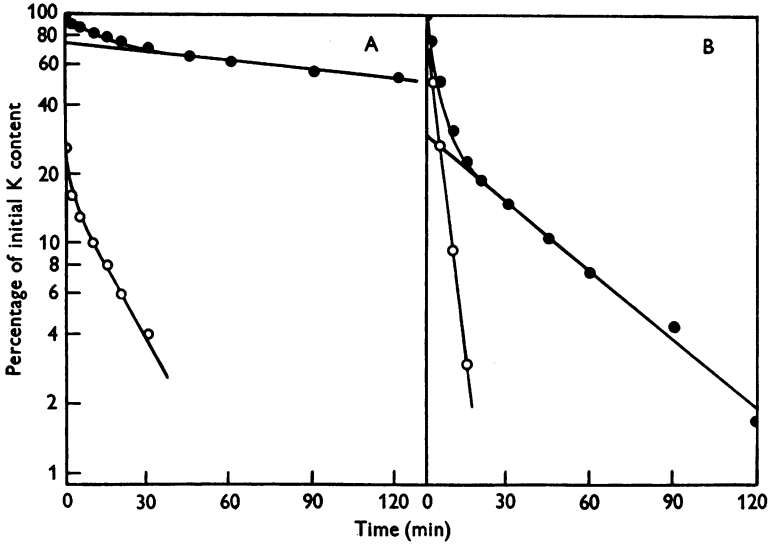
Nearly all the Na curves became excellent straight lines after about 1 hr when plotted on semi-logarithmic paper, and the curves were easily divided into a fast and a slow component by subtraction. The slow component gave by extrapolation to zero time a Na percentage, which is assumed to be related to the intracellular content whose mean value, obtained from seven nerves, was 24·0% (S.E. ± 2·0) of the total. From Text-fig. 1, which shows the curve obtained from the average of seven curves, it can be seen that the loss of intracellular



Text-fig. 1. ●—●, curve showing diffusion of Na from desheathed cat nerves in isotonic sucrose. The Na content of the nerves is expressed as a percentage of the initial content, which was itself deduced from the Na content of bilateral, control nerves. Each point is the mean of seven experiments, and the S.E. is indicated. ○—○, curve of fast, initial component obtained from first curve by subtraction of straight line.

component had a half-period of about 50 min, whereas the fast component, which is complex initially, as might be expected of a diffusion process, later decayed with a half-period of about 8 min.

The escape of K from two nerves gave curves which were much flatter and which could not be so easily analysed into two components (Text-fig. 2). They showed, however, that about 10% of the total K left the nerve within 10 min, the remainder coming out with a half-period of approximately 4 hr. If the nerve was exposed to chloroform fumes at 37° C for about 20 min beforehand, 75% of the K diffused out with a half-period of 3·5 min, and the remaining 25% with a half-period of only 35 min.



Text-fig. 2. A: ●—●, curve of escape of K from desheathed cat nerves in isotonic sucrose. The K content of the nerves is expressed as a percentage of the initial content, calculated from that of bilateral control nerves. ○—○, curve of initial, fast component obtained from first nerve by subtracting straight line. B: both curves as above, but nerves had previously been exposed to hot chloroform fumes for 20–25 min. In both A and B, each curve is the mean of two experiments.

Extracellular space estimations

Inulin. The mean value of the extracellular water percentage in seven nerves was only 11·0 (S.E. ± 1·0); when allowance was made for the water content of the nerves, the extracellular water expressed as a fraction of the total nerve water was still only 16·5%.

The total extracellular volume of the body, calculated from the plasma–inulin concentration, the amount of inulin injected, and the body weight, was found to vary between 16·2 and 17·8% in four adult cats; it was 23·8% in a young kitten.

Photomicrographs. The results are based upon the relative weight of intracellular areas in nine photomicrographs, from two nerves, fixed and sectioned independently. Each photomicrograph covered an area of nerve bundle which included at least 150 axons (Pl. 1, fig. 3). The mean value of the intracellular area was 43·4% (S.E. ± 1·3). The intrafascicular extracellular space, which includes the myelin sheaths, was accordingly 56·6%.

DISCUSSION

Na *and* K *concentrations in intact nerves*

For comparison with the data presented here, Na and K concentrations found in various mammalian nerves by other observers are given in Table 1.

All the nerves analysed by McLennan & Harris (1954) were apparently first rinsed in isotonic sucrose for 2 min, and this caused the loss of one-third of the total Na, enough to account for the discrepancy.

TABLE 1. Na and K concentrations found in intact mammalian nerves by other observers

Observers	Na concentration (m-mole/kg fresh weight)	K concentration (m-mole/kg fresh weight)	Species
Fenn (1938)	—	48·5	Cat
Lissák & Kovács (1942)	—	37·9–103	Cat
Lissák & Kovács (1942)	—	42·3–106	Rabbit
McLennan & Harris (1954)	59 (S.D. ± 14)	38·5 (S.D. ± 10)	Rabbit
Manery & Hastings (1939)	77·7	—	Rabbit
Tupikova & Gerard (1937)	137–156	33–38	Dog
Davies *et al.* (1952)	93 (S.E. ± 4·8)	40 (S.E. ± 2·9)	Ox

Na *and* K *total in intact nerves*

Several authors have commented previously on the high content in mammalian nervous tissue of Na and K, which is particularly striking when it is considered that nervous tissue contains only some 60–70% water (e.g. Davies *et al.* 1952; Harris & McLennan, 1953). In the present case, the Na plus K total amounted to 142 m-mole/kg in fresh cat nerves, which is equivalent to about 210 m-mole/kg nerve water. The mean values of the Na and K concentrations in fifteen samples of cat plasma were 148·1 m-mole/l. (S.E. ± 1·9) and 4·4 m-mole/l. (S.E. ± 0·19). Allowing for the water content (water makes up 92·7% of cat blood serum according to Dukes, 1935) the Na and K concentrations were equivalent to 161 and 4·8 m-mole/kg water, in good agreement with previous estimations (Robertson & Dunihue (1954) found Na and K concentrations of 159 and 4·8 m-mole/kg serum water). Clearly, the Na plus K concentration in the intact nerve water exceeded that in plasma by about 25%.

The Na + K concentration was even higher in the monkey nerve (165 m-mole/kg); in the rabbit nerve, it was also rather high (130 m-mole/kg), but this was not the case in the single intact dog nerve analysed. It may be said that a general, but possibly not a universal, feature of intact mammalian nerves is that their Na + K content is to a varying extent higher than one would expect from the Na + K concentration of plasma.

Na *and* K *total in desheathed nerves*

The so-called intact nerve usually consists principally of several bundles, containing axons, endoneurium, interstitial extracellular fluid, and some blood vessels, each bundle being limited externally by a substantial, organized sheath, the perineurium, and the several bundles held together by a variable amount of comparatively loose epineural tissue (Pl. 1, fig. 1). Since both perineurium and epineurium may be regarded as indifferent connective tissue, it was felt

that an analysis of the nerve bundle after desheathing, i.e. after removal of the external sheaths, should give a more revealing picture of the internal composition (Pl. 1, fig. 2).

Before analysing the results obtained, one source of error in the experiments, which was probably not negligible, must be considered. The greater size, better protection and easier dissection of the intact nerve made very small the likelihood that a substantial fraction of the total weight was lost by drying, before the nerve was weighed. This is confirmed by comparing the mean value of the water concentration obtained here ($68{\cdot}9 \pm 0{\cdot}4\%$) with other data for cat nerves in the literature (Alcock & Lynch, 1907: $67{\cdot}3 \pm 1{\cdot}0\%$; Fenn, 1938: $66{\cdot}2 \pm 0{\cdot}07\%$). Desheathed nerves are, of course, appreciably smaller, have no external envelope, and require a longer time for their preparation. Hence drying is more likely to be important and to have an appreciable effect on the total apparent weight. In fact, the mean water concentration of the first series of desheathed nerves was $63{\cdot}6 \pm 1{\cdot}0\%$. This might have been a genuine difference, but in a later series of six desheathed nerves (used for Cl determinations) in which very special precautions were taken to avoid drying by working as quickly as possible near the damped balance, the mean water content was $66{\cdot}7 \pm 0{\cdot}9\%$ (S.E.). Observations of the rate of drying on the balance showed that the latter figure was certainly within 1–2% of the true value.

It was essential to consider this source of error, as it would tend to exaggerate the Na and K concentrations in the desheathed nerves, and, even more, the concentrations calculated for the desheathed nerve water. In the following analysis, a correction has been made in all cases for the probable effect of drying by assuming that the water contents of the intact and desheathed nerves were equal.

In all nerves examined, the effect of desheathing was to increase very appreciably the K concentration, as one would expect. It is more surprising that there was no comparable decrease of the Na concentration. If the nerve fibres contain much K but little Na, removal of the indifferent connective tissue, which is presumably rich in Na, might be expected to keep the Na and K total more or less constant: in fact, in every case it was increased to an even higher value, so that even in the dog nerve the concentration in the water exceeded substantially that in plasma water. The corrected value of the Na plus K concentration in cat nerves was now 243 m-mole/kg water. Only two explanations were possible; either the intracellular concentration of Na was very high, very nearly as high as in plasma, or the interstitial extracellular fluid had a much higher concentration of Na than plasma.

Intracellular Na

To decide which of these two explanations was more likely to be correct, it was necessary to know how much of the total nerve Na was intracellular. Two

methods were employed to obtain this information, both depending upon an analysis of the diffusion of Na from desheathed nerves.

In the first method, the diffusion of Na into isotonic sucrose gave the results described earlier. The fast component in Text-fig. 1 agrees reasonably well with the theoretical curve of diffusion from a cylinder given by Hill (1928) in his fig. 5, p. 70. The corresponding diffusion coefficient (D) can be calculated by taking the value of Dt/α^2 as $\frac{1}{8}$ when 10% of the initial Na remains, α being the radius of the cylinder and t the time. The diameters of the nerve bundles used in the experiments varied between 0·50 and 1·30 mm, so that D must have been between $2{\cdot}0 \times 10^{-7}$ and $1{\cdot}4 \times 10^{-6}$ cm²/sec. This range of values extends between $\frac{1}{65}$ and $\frac{1}{8}$ of the coefficient of diffusion of NaCl from a 0·1 M solution into water, at 18° C. It overlaps at the lower end the values of D for Na found by Krnjević (1954*a*) in desheathed and perfused frog nerves, by Shanes (1954) in desheathed toad nerves, and by Harris & McLennan (1953) in mammalian sympathetic ganglia; at the upper end it is about half the value of D for extracellular Na estimated by Keynes (1954) in frog muscle. Diffusion within a nerve bundle is slowed down not only by an increase in the path length due to tight packing of the fibres, but also probably by endoneural partitions, which are well developed in cat nerves (Pl. 1, fig. 1). Where diffusion takes place into isotonic sucrose, the requirements of electrical and osmotic equilibrium may also be a hindrance, especially if interstitial Na is associated to any extent with slowly diffusing anions.

From these considerations, it seems likely that the slow component in the diffusion curve (Text-fig. 1) does in fact correspond to the intracellular Na. It is interesting that this component should fit an exponential so well, since this suggests that the Na efflux from the nerve fibres was a simple function of the intracellular Na concentration. This would seem to argue against an exchange diffusion mechanism such as that postulated by Levi & Ussing (1948).

To derive a value for the intracellular Na the mean Na percentage ($24{\cdot}0 \pm 2{\cdot}0$) obtained by extrapolation of the slow component must be corrected for the slowness of diffusion of Na from the extracellular spaces. The application of the correction (see Dainty & Krnjević, 1955) leads to a value for the percentage of the nerve Na which is intracellular of 18 ± 3. This agrees with the value ($18{\cdot}1 \pm 3{\cdot}3$%) found by the second method, which involved similar experiments with desheathed nerves and ^{24}Na (Dainty & Krnjević, 1955). The intracellular Na may then be taken as 18% of the total: this suggests that the excess Na is to be found in the interstitial fluid, but an exact evaluation requires a knowledge of the relative amounts of intra- and extracellular water. The slow rate of loss of nerve K confirms the belief that most of it is intracellular.

Extracellular water

Inulin space. The values of the total extracellular volume in the cat (16–24 %) seem to agree fairly well with estimations of the inulin space in man (15–16 %, Gaudino, Schwartz & Levitt, 1948) and in the dog (17·5–21·8 %, Gaudino & Levitt, 1949).

The small inulin space in cat nerves, equivalent to only 16·5 % of the total water, presumably reflects the failure of inulin to penetrate into the tissue. The above-cited authors emphasized the fact that the large inulin molecule diffuses very slowly. In an illuminating study of the behaviour of inulin, Nichols, Nichols, Weil & Wallace (1953) showed that inulin penetrates remarkably slowly into water associated with connective tissue. It is not surprising, therefore, that the inulin space should be so small in nerves which contain a relatively high proportion of connective tissue.

Photomicrographs. All the methods of estimating the extracellular space of a tissue by allowing equilibration of some normal or abnormal constituent of plasma are based upon the assumption that the concentrations in the extracellular fluid are the same as those in plasma. In the case of cat nerves, however, there was good reason for believing that the interstitial fluid might not be similar to plasma in composition. It was therefore decided that a more direct estimation, from the respective intra- and extracellular areas in photomicrographs of sections of cat nerves, would probably be more reliable.

The value of $43{\cdot}4 \pm 1{\cdot}3$ % for the intracellular space of a desheathed nerve can only be converted into an intracellular water percentage if we know the proportion of water within that space. Fortunately, there is available an estimate of the water content of the axoplasm of myelinated frog nerves: 91–92 %. This is based upon the X-ray absorbing properties of the tissue (Engström & Lüthy, 1950). If we then assume that 91 % of the axoplasm in cat nerves is water, and that the total water content of the desheathed nerve is 69 % by weight (see pp. 479 and 480), the intracellular water is found to make up 57 % of the total water.

Intracellular and extracellular concentrations of Na *and* K

It is now a matter of simple arithmetic to calculate the Na and K concentrations in the intracellular and interstitial water. For instance:

$$Na_i \times 0{\cdot}57 + Na_o \times 0{\cdot}43 = 136 \text{ m-mole/kg of desheathed nerve water*.} \quad (1)$$

We know that $Na_i \times 0{\cdot}57 =$ the total internal $Na = 0{\cdot}18 \times 136$, hence we can easily find the values of Na_i and Na_o (the intracellular and interstitial concentrations of Na).

The interstitial Na concentration comes out to be 258 m-mole/kg and is

* From the total Na and water contents given on p. 476, with a correction for drying.

greater than that of plasma (160 m-mole/kg) by a factor of about 1·6. When calculating K_i, the concentration in the intracellular water, one can either assume that K_o, the interstitial concentration, is equal to K_p, the plasma concentration (4·8 m-mole/kg), or that K_o is also greater than K_p by the factor of 1·6 already cited ($K_o = 7·7$ m-mole/kg). The respective values of K_i differ only slightly: they are 183 and 181 m-mole/kg intracellular water.

The fractions of extra- and intracellular water in intact nerves can now be calculated. It is assumed in the following that the intact nerve water consists of three independent components, the intracellular water ($1-S$), the interstitial, intrafascicular, extracellular water (S_i) and the extrafascicular, extracellular water (S_o); the last two together make up the extracellular water (S). It is also assumed that the composition of the last component (S_o) is similar to that of plasma. If the difference between the Na and K concentrations in intact and desheathed nerves are wholly consistent with each other, the following simultaneous equations should yield figures for S_o and S_i which have a general validity, at least within the scope of the present experiments.

$$K_i \times (1-S) + K_o \times S_i + K_p \times S_o = K_N, \tag{2}$$

$$Na_i \times (1-S) + Na_o \times S_i + Na_p \times S_o = Na_N, \tag{3}$$

(where K_N and Na_N are the total concentrations in intact nerve water and $S_o = S - S_i$). Furthermore, if Z is the extracellular fraction of the *desheathed* nerve water, it can easily be shown that the following, independently derived, equation should also be true,

$$S_o = \frac{Z - S_i}{Z}, \tag{4}$$

It is perhaps asking rather much that the various concentrations should correspond precisely and, in fact, if the values of K_N and Na_N obtained from the results are used (67 and 140 m-mole/kg), equations 2, 3 and 4 yield values of S_o which are not exactly similar. However, only a slight adjustment abolishes this discrepancy. Thus, if the figure for the concentration of Na in desheathed nerves is decreased from 136 to 130 m-mole/kg (a decrease of only 4·5%) and the corresponding values of Na_i and Na_o employed, fully consistent values of S, S_i and S_o are obtained. These are:

$$S = 0·65, \quad S_i = 0·26, \quad S_o = 0·39.$$

Na_i and Na_o are now 41 and 245 m-mole/kg respectively, so that the ratio of Na_o/Na_p becomes 1·53. That only such a small correction is necessary to give perfect correspondence between intact and desheathed nerve data seems in itself a justification of the method. It should be made clear that it is only the relative values of S_i and S_o which are sensitive to small variations of concentrations. S is a much more stable element, comparatively independent of minor adjustments. Likewise, the relative values of Na_i, Na_o and K_i are not

disturbed fundamentally even by appreciable changes in the original estimate for the extracellular water in the nerve bundle. The concentrations given here are almost certainly of the correct order of magnitude, and they probably do not differ from true average values by more than $\pm 10\%$.

Significance of results

The high concentration of Na and K in the nerves studied is of great interest when considered in conjunction with a recent controversy about the permeability of the nerve sheath. A great majority of authors have now agreed that nerves are surrounded by a relatively impermeable external sheath, and it has been suggested (Krnjević, 1954*a*) that the function of such a sheath may be to preserve the specific character of the internal environment of the nervous tissue. Evidence has been presented that the frog nerve is surrounded by a continuous layer of epithelium at the inner margin of the perineurium (Krnjević, 1954*b*) such as might well be a diffusion barrier. It has long been known that the mammalian perineurium is rich in cellular elements, which usually form distinct lamellae. There is some doubt, however, whether passages are present between such lamellae in all the layers; the evidence for their presence in all layers depends upon the forcing of a fluid injected into the bundle out through the perineurium. This is a dubious procedure, and it cannot extinguish the fact that an external barrier to diffusion has been demonstrated around mammalian nerves (Causey & Palmer, 1953). Close examination of the sheath shows that the cellular elements are concentrated at the innermost boundary of the perineurium where they appear to form a continuous system (Pl. 1, fig. 4), and it seems reasonable to suppose that this is the site of the diffusion barrier, as in frog nerves.

An external cellular membrane is then probably in some way connected with the specific composition of the interstitial fluid. How does this specific composition arise? There are at least two possible explanations:

(1) Endoneural connective tissue probably consists largely of collagen, which is a protein with an isoelectric point at a low pH. At the normal pH of the body, its free anion groups would be available for combination with Na^+, which would be immobilized in a Donnan system. According to Tristram (1953), collagen has 77·2 free anion equivalents/10^5 g protein; from this, it can be shown that if all these groups are available, the amount of collagen necessary to produce the effect observed would only be about 3% of the nerve weight. A Donnan excess of internal osmotic pressure might be neutralized by the inextensibility of the perineurium; the consequent rise in internal hydrostatic pressure, however, could presumably only be tolerated up to a limit set by the requirements of the blood supply.

(2) Active secretion of water by the nerve would maintain a general increase

in the concentration of all the solutes in the nerve. There is at present no direct evidence of such a mechanism.

Cl *concentration in nerves.* It seemed likely that an estimation of the Cl concentration in either intact or desheathed nerves would give some indication as to which of the two explanations offered above is nearer the truth.

The Cl concentration found in intact nerves, 62·8 m-mole/kg fresh nerve (S.E. ± 3·7), was similar to that found in mammalian nerves by most previous authors (Table 2).

If the value of Na_o is kept higher than that of Na_p by the presence of indiffusible anions, then we may expect to find $Cl_p/Cl_o = Na_o/Na_p$; taking Cl_p as 127 m-mole/kg, Cl_o is about 83 m-mole/kg. When this value, together with values given earlier for the intra- and extracellular water, and the total concentration of Cl in intact and desheathed nerve water (99 and 93 m-mole/kg) are used to calculate Cl_i, the value of the latter is at least 100 m-mole/kg.

TABLE 2. Cl concentrations found in intact mammalian nerves by other observers

Observers	Cl concentration (m-mole/kg fresh weight)	Species
Alcock & Lynch (1907)	60·5	Cat
Amberson *et al.* (1938)	62	Cat
Manery & Hastings (1939)	50·2	Rabbit
Tupikova & Gerard (1937)	63	Dog

TABLE 3. Probable distribution of Na, K and Cl inside a cat nerve bundle.

	Intracellular (m-mole/kg water)	Extracellular (m-mole/kg water)
Na	41	245
K	181–183	7–5
Cl	0–17·5	190–210

If we test the assumption that $Cl_o = Cl_p = 127$ m-mole/kg (Robertson & Dunihue, 1954), we still find a rather high value of Cl_i (50–60 m-mole/kg). On the other hand, if the second explanation is put to the test by taking $Cl_o/Cl_p = Na_o/Na_p$ (which makes $Cl_o = 194$ m-mole/kg), we obtain 0–17·5 m-mole/kg for Cl_i in intact and desheathed nerves, which is the order of value to be expected if K and Cl are distributed in the intracellular water according to a Donnan equilibrium, such as has been claimed to exist in frog muscle (Boyle & Conway, 1941). It is generally agreed that most cells are deficient in Cl, and is therefore probable that Cl_o is, in fact, of the order of 190 m-mole/kg.

Weight changes in isolated nerves

It is known that frog nerves gain weight rapidly in 'isotonic' Ringer after desheathing. Shanes (1953) found that the increase in weight was 40% of the original weight after 3–4 hr. In the experiments of Dainty & Krnjević (1955), desheathed cat nerves in a standard Locke solution showed an average increase

in weight of 35% after 3 hr. It has also been shown by Alcock & Lynch (1907) that cat nerves which have been partly desheathed tend to increase in weight in 'isotonic' NaCl, but maintain a constant weight in 200 mM-NaCl. It does not follow that this last concentration is exactly that which would have been isosmotic with the normal contents of the nerve because the nerves studied by Alcock & Lynch were allowed to soak in saline for 15 min before the initial weighing. Nevertheless, it indicates the order of magnitude to be expected.

These facts are consistent with the suggestion made here that the nerve bundle contains a hypertonic solution, but they would also seem to indicate that at least some of the excess Na is bound to protein because: (1) if all the Na were free to diffuse rapidly away, there should be little swelling, and (2) nerves containing 240 mM-Na might well be isosmotic with 200 mM-NaCl, since the protein anion would produce only a small osmotic effect.

All the swelling in desheathed frog nerves apparently takes place in the interstitial spaces (Shanes, 1953). This suggests that as the interstitial Na concentration decreases, ions leave the axoplasm thus lowering the internal ionic concentration. The K concentration of desheathed nerves soaked for 3 hr by Dainty & Krnjević (1955) was only 43 m-mole/kg water, which corresponds to $K_i = 105$ m-mole/kg water, instead of the initial value of 181 m-mole/kg. As Na_i had apparently increased to about 80 m-mole/kg, this was equivalent to the loss of about 40 m-mole/kg internal cation and must have been accompanied by an equivalent amount of anions.

CONCLUSION

Cat nerves are bathed in an extracellular fluid which is substantially more concentrated than plasma. The high content in Na and K, which amount together to about 250 m-mole/kg, is probably associated with the connective tissue protein of the endoneurium, but the presence of a simple Donnan system is made unlikely by the comparatively high Cl content. The difference in ionic content between plasma and the nerve water (90 or more m-mole/kg) would in any case result in an internal hydrostatic pressure incompatible with the supply of blood. One cannot avoid the conclusion that some active mechanism, situated perhaps in the sheath, may be essential for the maintenance of this system by pumping water out fast enough to prevent a large increase in pressure.

SUMMARY

1. The mean concentrations of Na in fifty-one intact and sixteen desheathed cat nerves were 96·2 and 99·3 m-mole/kg fresh nerve respectively.

2. The mean concentrations of K in fifty-three intact and fifteen desheathed cat nerves were 46·2 and 76·7 m-mole/kg fresh nerve.

3. From the outward rate of Na diffusion from desheathed nerve it was concluded that 18% of the total is intracellular.

4. Even after 4 hr, inulin is present in the same concentration as in plasma in only 16·5% of the nerve water.

5. The mean relative intracellular area in photomicrographs of bundles of cat nerves was 43·4%.

6. On the basis of the data in 1, 2, 3 and 5, the following estimates have been made of the distribution of Na and K inside the nerve bundle: extracellular Na – 245 m-mole/kg water; extracellular K – 5–7 m-mole/kg water; intracellular Na – 41 m-mole/kg water; intracellular K – 183–181 m-mole/kg water. The true values are probably within ± 10% of these.

7. The fractions of extra- and intracellular water in desheathed nerves were apparently 43 and 57% respectively, and in intact nerves, 65 and 35% respectively. The extracellular water in intact nerves may be divided into intra- and extrafascicular fractions which were about 26 and 39%.

8. The mean Cl concentrations in seven intact and six desheathed nerves were 62·8 and 62·1 m-mole/kg fresh nerve. If it is assumed that $Cl_o/Cl_p = Na_o/Na_p$, so that Cl_o is 194 m-mole/kg water, the value of Cl_t approximates that required by a K and Cl Donnan equilibrium across the nerve membranes (0–17·5 m-mole/kg water).

9. It was concluded that endoneural protein is probably at least partly responsible for the hypertonic nature of the nerve fluids, but that some additional active mechanism in the nerve may well be necessary to account for all the features described.

I am grateful to members of this Department who gave me ready access to cat nerves, often at very inconvenient hours. I am especially grateful to Miss J. W. Bowyer for her help in the dreary task of cutting holes out of photomicrographs.

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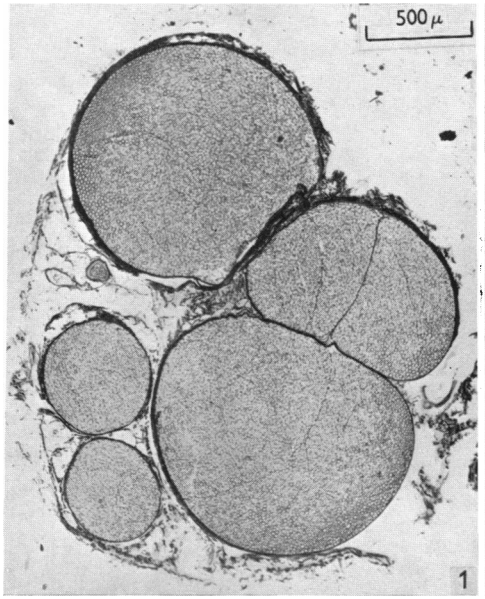
EXPLANATION OF PLATE

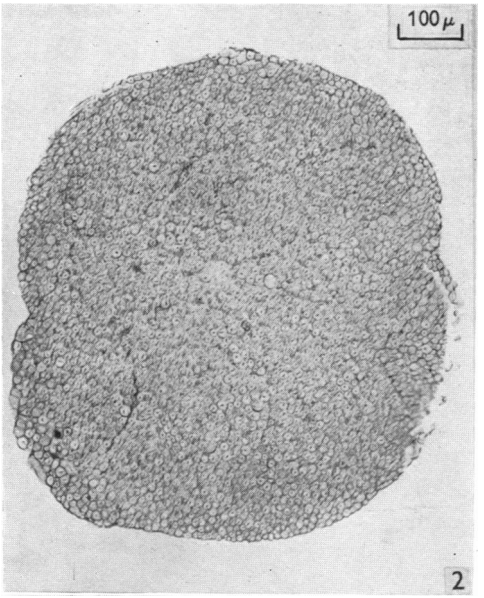
Fig. 1. Low-power view of section of sciatic nerve trunk from a cat, showing division of the nerve into separate bundles, the perineurium around bundles, and the comparatively loose epineurium, mainly between bundles. Some endoneural septa are also visible. Nerve fixed in Flemming's solution, and paraffin sections stained with ponceau fuchsin and aniline blue.

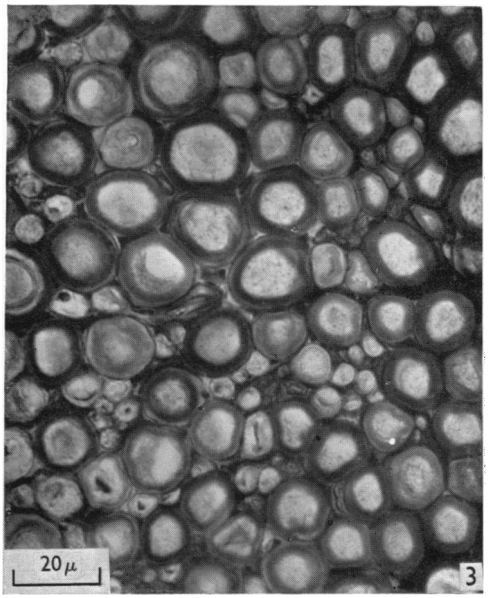
Fig. 2. Section of desheathed lateral popliteal nerve of cat, prepared as above.

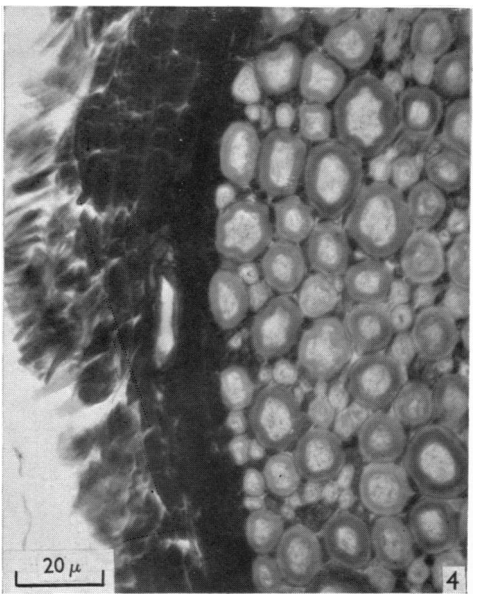
Fig. 3. High-power view of nerve fibres in a bundle from a section of an intact cat sciatic nerve, prepared as above. One of the photomicrographs used in the determination of the nerve intracellular area.

Fig. 4. High-power view of the edge of an intact cat sciatic nerve bundle, prepared as above. The innermost layer of the perineurium is very compact, and stains darker. This is probably the site of the diffusion barrier. Endoneural fibrils (the Fibrillenscheide of Key and Retzius) may be seen as dark dots between nerve fibres.









To face p. 488