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THE RATE OF EXCHANGE OF ^{24}Na IN CAT NERVES

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The present investigation was begun in an attempt to verify a result of Manery & Bale (1941) that Na in mammalian (rabbit) nerve exchanges only very slowly with plasma Na *in vivo*. As the investigation proceeded it became clear that roughly quantitative information concerning the flux of Na across the nerve surface might be obtained. However it did not prove possible to make sufficiently accurate quantitative studies of the Na flux *in situ*, so a number of experiments on the rate of loss of ^{24}Na from desheathed cat nerves *in vitro* were carried out. From the results of the latter experiments estimations of the Na efflux were obtained.

METHODS

All nine cats used in the experiments were anaesthetized with intraperitoneal pentobarbitone sodium (Nembutal), small doses being subsequently given intravenously to maintain a light level of anaesthesia.

The solution injected was a mammalian saline (0·9% NaCl), made up from irradiated NaCl and obtained from the Atomic Energy Research Establishment, Harwell. At the time of injection, its activity varied between 0·40 and 0·50 mc/ml. The amount of solution injected was 5–7 ml., equivalent to a total of 2·15–3·50 mc of ^{24}Na.

Experimental procedure

^{24}Na *exchange* in situ. The solution was injected into a femoral or a jugular vein and washed in by a few ml. of inactive saline; the time required for this operation was about 1 min. At intervals after the injection, 4–5 cm long portions of the posterior tibial, the lateral popliteal, or, sometimes, the medial popliteal nerves were removed from one or the other hind limb; a sample of carotid arterial blood was obtained simultaneously. The nerves were rinsed very quickly in inactive saline to remove superficial contamination with blood, and, after lightly blotting with filter-paper, were immediately weighed in platinum crucibles of known weights. The crucibles were placed in an air-oven at about 100° C for at least 5–6 hr, and, after cooling in a desiccator, weighed again. They were then left overnight in a muffle furnace at a temperature not exceeding 550° C. In the morning, the ash was dissolved in 5 ml. nitric acid (0·75 N). A portion of this solution (about 3 ml.) was diluted about ×50, and the Na and K content then estimated by means of an Evans Electro-selenium flame photometer, with an accuracy rather better than 5% for Na and 10% for K. Details of the method and its accuracy are given in Krnjević (1955). The rest of the nitric acid solution (about 2 ml.) was used to determine the ^{24}Na content of the nerve; this was always done within 24 hr of the end of the experiment. The heparinized blood samples were centrifuged soon

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after removal, and 1·00 ml. samples of plasma pipetted into platinum crucibles; they were then submitted to a procedure exactly similar to that described above.

There were four nerve and blood samples in each experiment, and they were taken at intervals after the injection which were not the same throughout the series. The first was usually after about 5 min; the 2nd and 3rd were after intervals varying between 10 and 60 min, and the 4th was either after 60 or 75 min, or after some 5–5½ hr.

Loss of ^{24}Na *from desheathed nerves* in vitro. At least 4–5 hr after the injection of the active saline, one of the lateral popliteal nerves was dissected out and rapidly desheathed. About 6 cm of desheathed nerve was then fixed in a stainless steel clamp, which grasped firmly the two cut ends, and the nerve and clamp quickly weighed. Both were then plunged into a bath containing 10·0 ml. inactive Tyrode solution, stirred and oxygenated at 37° C. At intervals of about 5 min initially, later of 30 min, this bathing solution was replaced by 10·0 ml. fresh, inactive, Tyrode solution. The duration of this part of the experiment was 3 hr, and in this time twelve 10·0 ml. samples were collected. The radioactivity of these samples was always determined on the same day. A more complete description of the desheathing procedure, and the diffusion apparatus and method, is given by Krnjević (1955).

The composition of the Tyrode solution used was derived from one described by Strangeways (1924):

Na	149·2 m-mole	HCO_3	11·9 m-mole
K	2·7 m-mole	H_2PO_4	0·4 m-mole
Ca	1·8 m-mole	Glucose	100 mg
Mg	0·5 m-mole	Water to	1·0 l.
Cl	144·2 m-mole		

2% CO_2 in the oxygen bubbled through the solution, maintained the pH at about 7·4.

At the end of the experiment, the nerve was weighed again after blotting lightly, and then portions were kept for (1) Na and K determinations, and counting of activity; (2) confirmation of the effectiveness of the desheathing by a histological examination of paraffin sections, after fixing in Flemming's solution.

Determination of the volume/surface area ratio. This was carried out by measuring the internal diameters of all fibres in photomicrographs of paraffin sections of lateral popliteal nerves. The nerves had been fixed in Flemming's solution and dehydrated in dioxan. The magnification at which the photographs were taken was about ×700. According to Sanders (1947) the correction for the shrinkage of fibres caused by dehydration and paraffin embedding, after fixation in Flemming's solution, is not greater than 7%. This correction has been ignored in the present calculations.

Counting procedures. Using a 0·100 ml. graduated pipette, about 0·070 ml. of the ashed nerve solution was pipetted on to a standard counting planchette. The drop of solution was evaporated to dryness and a measure of the amount of radioactivity obtained by counting, in a standard geometrical arrangement, with a GM 4 end-window counter and standard equipment (A.E.R.E. Type 1033 A power unit, E. K. Cole Type N 526 scaler and A.E.R.E. Type 1014 A probe unit). The measure of radioactivity, in counts/min, was corrected for lost counts (due to the finite 'dead time' of the GM 4 and probe unit), background counts and decay of the ^{24}Na. No correction was necessary for source thickness.

The radioactivity of the plasma samples was determined in a similar way.

The radioactivity of the 10 ml. liquid samples collected during the *in vitro* efflux experiments was determined by counting in a M 6 liquid counter. This method gave a perfectly standard geometrical arrangement and the factor to convert counts/min. ml. in this geometry to counts/min. ml. in the GM 4 geometry was determined experimentally. The same counting equipment was used with both the M6 and the GM 4 counters, and the usual corrections for dead time, background and decay were always made.

In all cases, at least 1000 counts were recorded so that the standard deviation was never more than 3%.

RESULTS

Rate of change of specific activity of nerve Na in situ

The specific activity (S) of a nerve sample was calculated from its total Na content and its activity, and the Na content and activity of the corresponding plasma sample. The specific activity of the latter was taken as 100%.

TABLE 1. The mean values of the specific activity of cat nerves at intervals after the intravenous injection of ^{24}Na saline. The corresponding activity of plasma is 100%.

Time (min)	Mean activity (%)	S.E. of mean	No. of nerves
5	45·2	9·0	6
10	55·2	9·2	4
21	71·0	6·0	3
33	70·8	7·0	5
68	88·5	10·4	8
330	101·1	15·0	4

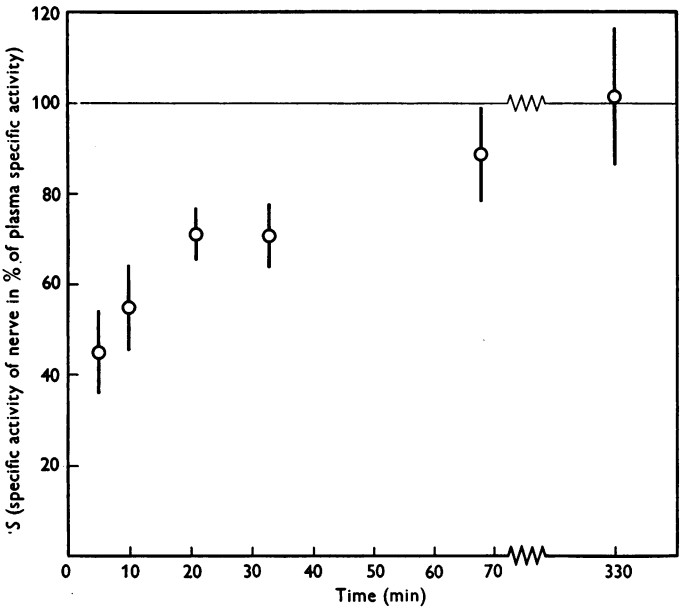

Fig. 1. Values of S, the mean specific activity, of cat nerves removed at various intervals after the intravenous injection of ^{24}Na saline, plotted as a function of time after removal. The specific activity of plasma is taken as 100%. The vertical lines show $\pm$S.E. of the mean.

All the data obtained in this way were grouped according to the time of removal after injection; it was possible to arrange thirty results from nine cats in six groups, each containing at least three results. The averages of these groups, together with the S.E.'s, are given in Table 1. The values of S, plotted as a function of time, are shown in Fig. 1.

Rate of loss of ^{24}Na *from desheathed nerves* in vitro

The method employed, which was similar to that of Levi & Ussing (1948), gave values for the loss of ^{24}Na during various time intervals. It was necessary to convert these figures into rates of loss of ^{24}Na at various times.

The radioactivity in counts/min of each 10 ml. sample was a measure of the amount of ^{24}Na which had left the nerve in a given interval of time, say from t_1 to t_2 sec. The number of counts/min divided by $t_2 - t_1$ gives an approximate measure (P) of the rate of loss of ^{24}Na at time $(t_1 + t_2)/2$ sec. To find more accurate values of the rates, the following procedure was adopted. P was plotted on semi-logarithmic graph paper as a function of time $(t_1 + t_2)/2$. Except for the first point, these approximate rates fitted two straight lines, so that the function was of the form $A\,e^{-K_1 t} + B\,e^{-K_2 t}$. Using the values of K so determined, a more accurate value (Q) of the rate was found by multiplying P by the factor

$$\frac{K(t_2 - t_1)/2}{\sinh K(t_2 - t_1)/2}$$

derived as follows:

the observed number of counts appearing in the interval t_1 to $t_2 = N\,e^{-Kt_1} - N\,e^{-Kt_2}$ assuming that the ^{24}Na is lost according to a simple exponential law. Therefore

$$P \text{ (approximate rate)} = \frac{N\,e^{-Kt_1} - N\,e^{-Kt_2}}{t_2 - t_1}$$

$$= \frac{N\,e^{-K(t-\Delta t/2)} - N\,e^{-K(t+\Delta t/2)}}{\Delta t}, \quad \text{where} \quad \begin{cases} t_2 - t_1 = \Delta t \\ (t_1 + t_2)/2 = t \end{cases}$$

$$Q \text{ (true rate)} = KN\,e^{-Kt}.$$

Therefore

$$Q/P = \frac{KN\,e^{-Kt}\Delta t}{N\,e^{-Kt}[e^{K\Delta t/2} - e^{-K\Delta t/2}]}$$

$$= \frac{K\Delta t/2}{\sinh K\Delta t/2}.$$

Straight lines drawn to fit the amended points (Q) did not differ sufficiently from the original lines to justify repetition of the whole procedure.

In this way, except for the first point, the rate of loss curves, an example of which is shown in Fig. 2, were expressed in the form $A\,e^{-K_1 t} + B\,e^{-K_2 t}$. The first point lay above this curve, but this is to be expected since the 'fast' part of the curve is to be interpreted as a diffusion process and hence must be expressed as a solution of the diffusion equation for a cylinder; this is not a single exponential except for large t.

The values of K deduced from the slopes of curves based upon five experiments are shown in Table 2. The mean value of the slow K (K_s) was $(4{\cdot}35 \pm 0{\cdot}19) \times 10^{-4}$ sec^{-1}, and of the fast K (K_F) $(32{\cdot}2 \pm 2{\cdot}1) \times 10^{-4}$ sec^{-1}. (The quoted errors are standard errors of the means.)

Fraction of ^{24}Na *in the slow component.* The equations of the five curves were integrated and the fraction of ^{24}Na in the slow component calculated from the constants of the new equations, suitable corrections being made to allow for the first point of the fast curve. The mean value was 23·9% of the total ^{24}Na, with a S.E. of 4·9.

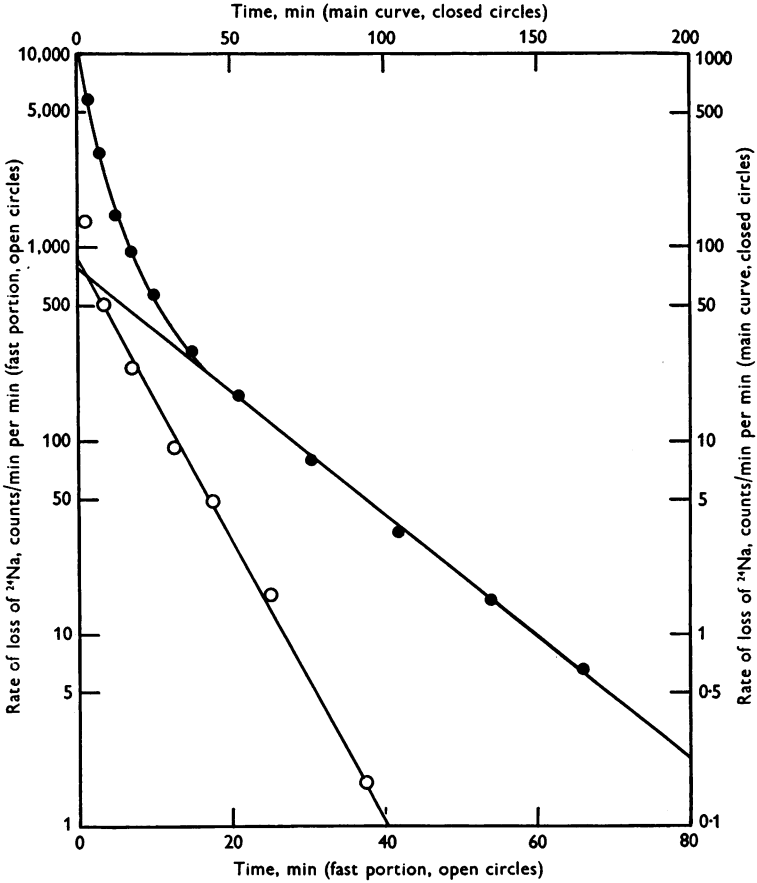

Fig. 2. ●—●, Curve of rate of loss of ^{24}Na from a desheathed cat nerve at various times after being placed in a Tyrode solution. ○—○, Curve of fast component of the main curve, obtained by subtraction of straight line produced back to time zero. The two curves have different scales both in the abscissa and the ordinate.

TABLE 2. Values of K_s and K_F, the rate constants of the slow and fast components governing the loss of ^{24}Na from desheathed cat nerves *in vitro*. K_F was deduced from the straight portion of each diffusion curve.

$10^4 K_s$ (sec^{-1})	$10^4 K_F$ (sec^{-1})
4·25	32·6
3·93	28·7
4·92	28·1
4·75	31·5
3·91	39·9

Rate of loss of ^{24}Na from an 'intact' nerve

In one case the desheathing procedure was incomplete, so that the perineurium apparently remained intact, only superficial, epineural tissue being removed. The corresponding ^{24}Na rate of loss curve is shown in Fig. 3. The values of K_s and K_F derived from this curve were $0{\cdot}995 \times 10^{-4}$ sec^{-1} and $48{\cdot}2 \times 10^{-4}$ sec^{-1} respectively.

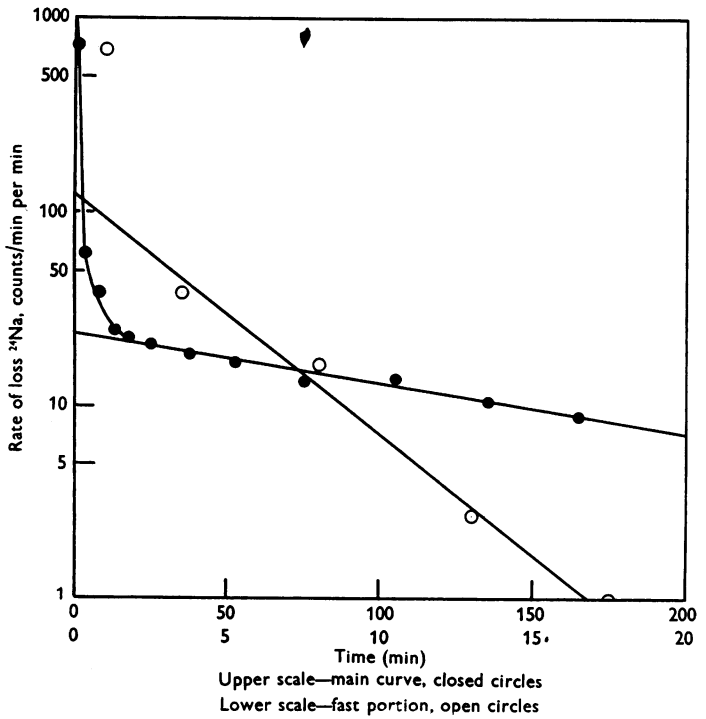

Fig. 3. ●—●, Curve of rate of loss of ^{24}Na from an 'intact' cat nerve at various times after being placed in a Tyrode solution. ○—○, Curve of fast component of the main curve, obtained by subtraction of straight line produced back to the time zero. The two curves have different time scales.

Change in weight of desheathed nerves in Tyrode

Of the five desheathed nerves studied, four were weighed before and after the *in vitro* experiment. All showed an increase in weight which varied between 15 and 49% of the initial weight (mean value: 35%). The average water content of the nerves after the experiment was 74·8% and the Na and K concentrations were 112·6 and 32·4 m-mole/kg nerve respectively.

^{24}Na *space in cats*

The proportion of the total body weight which may be considered as ^{24}Na space was calculated from the volume and activity of the ^{24}Na saline injected, the activity of plasma samples, and the body weight. It was assumed that the Na was singly distributed in body water. The mean value of the ^{24}Na space in eight cats was 36·5% (ml./100 g). (The standard error of this mean value was 3·6.) In two kittens included in the above, the values were 43·6 and 58·1%.

Volume/surface area ratio of cat nerve fibres

Three groups of measurements of the diameters of axons in different photomicrographs of lateral popliteal nerves were made by two observers. Every visible fibre was measured, i.e. about 150 in each photomicrograph. When the sections of the axons were not reasonably circular, two measurements were made at right angles to each other, and the mean value taken. The overall volume/surface area ratio for each group was calculated from the following equation:

$$V/A = \sum_i n_i d_i^2 / 4 \sum_i n_i d_i,$$

where d_i is the diameter and n_i the respective frequency. The three values of the ratio obtained agreed very well with each other: $1{\cdot}97 \times 10^{-4}$, $2{\cdot}15 \times 10^{-4}$ and $2{\cdot}20 \times 10^{-4}$ cm. For subsequent flux calculations a value of V/A equal to $2{\cdot}1\mu$ was taken, but this choice needs some qualification. The nerve fibres contain an inhomogeneous population of cells, for the axon diameters varied between 1 and 16μ. It might be better, therefore, to use in the flux calculations a larger value of V/A than $2{\cdot}1\mu$, which was obtained by summing over all axons. For example, if only axons with diameters greater than 5μ are considered then V/A is about $2{\cdot}8\mu$; these axons would comprise about 80% of the total volume and hence, presumably, 80% of the intracellular Na. However, throughout this paper the volume/surface area ratio will be taken as $2{\cdot}1\mu$, but the correct value to use could be as high as 3μ.

DISCUSSION

For convenience the ^{24}Na was obtained from A.E.R.E. in the form of saline made up from irradiated NaCl. Irradiation of the chloride produces a small amount of ^{32}P which might interfere with the interpretation of the experimental results. However, by measuring the decay of a large number of various samples, it was verified that the amounts of long-lived ^{32}P were small enough to be neglected.

^{24}Na *exchange in nerves* in situ

The exchange was more rapid than that described by Manery & Bale (1941) in which the specific activity of a single nerve was only about 50% after

68 min. It can be seen from Fig. 1 that the specific activity of the nerves in the present experiment reached 50% in less than 20 min. It was hoped the *in vivo* experiments would produce more definite evidence concerning the existence of a blood-nerve barrier as suggested by Davson (1951). However, the low accuracy of the experimental points (see Fig. 1 and Table 1) precludes any clear-cut decision on this, though the fact that the experimental points are below a curve constructed from the *in vitro* data on desheathed nerves does support the blood-nerve barrier hypothesis to some extent.

The value of the specific activity (S) at 330 min was 101% (± 15). Thus it can only be claimed from this result that at least 70% of the nerve Na is free to exchange.

It is not possible to calculate a value for the Na efflux from the results illustrated in Fig. 1 because of the large standard errors.

^{24}Na *exchange in desheathed nerves* in vitro

Initial exchange. The first part of the curve of loss of ^{24}Na (Fig. 2) is considered to represent the diffusion of ^{24}Na from the extracellular spaces. The desheathed nerve can be considered as a cylinder, radius a cm, with an initial, uniform, extracellular ^{24}Na concentration C'_o. After a time t sec the average concentration will be

$$\bar{C}' = 4C'_o \sum_{n=1}^{\infty} \frac{1}{\beta_n^2} e^{-\beta_n^2 D' t/a^2}, \tag{1}$$

where β_n is the nth zero of

$$J_0(\beta) = 0 \quad (\beta_1 = 2{\cdot}405), \tag{2}$$

and D' is the effective diffusion coefficient of ^{24}Na in the extracellular space (Hill, 1928, p. 71; Keynes, 1954).

After a short time (here about 2 min) only the first term of the series is important and equation (1) reduces to

$$\bar{C}' = \frac{4C'_o}{5{\cdot}783} e^{-5{\cdot}783 D' t/a^2}. \tag{3}$$

The fast parts of the curves (see, for example, Fig. 2) thus give the quantity $5{\cdot}783D'/a^2$ and a value of D', the effective diffusion coefficient of ^{24}Na in the extracellular space can be calculated. The radii, a, which ranged from 420 to 560μ, were calculated from the weights of the nerves. The mean value of D' was $(1{\cdot}5 \pm 0{\cdot}3) \times 10^{-6}$ cm^2/sec (range 1·0 to $2{\cdot}2 \times 10^{-6}$ cm^2/sec). This is to be compared with the self-diffusion coefficient of Na^+ in 0·1 M-NaCl of about 15×10^{-6} cm^2/sec. The difference between these two values probably arises from the inexactness of the model chosen, which takes no account of the obstacles to diffusion present in a cylindrical bundle of nerve fibres.

From this value of the effective coefficient of ^{24}Na in the extracellular space of the nerve, it is possible to estimate how far diffusion affects the apparent

sodium efflux from the nerve fibres. A theory of this estimation has been given by Keynes (1954, pp. 376–379). By trial and error using Keynes's equations (9), (16) and (18) a mean value for the ratio of apparent efflux to true efflux equal to 0·96 was found.

Slow exchange. The loss of ^{24}Na from nerve fibres into an inactive medium is given by

$$Y = Y_0 e^{-Kt} \quad \text{(Keynes, 1951, p. 14, eqn. (7))}, \tag{4}$$

where K, the rate constant, is given by

$$K = MA/C_i V \quad \text{(Keynes, 1951, p. 14, eqn. (8))}, \tag{5}$$

where M = efflux of Na in mole/cm^2.sec, V/A = volume/surface area ratio in cm, C_i = intracellular Na concentration in mole/c.c.

The mean value of K ($=K_s$) from measurements on five desheathed nerves was $4{\cdot}35 \times 10^{-4}$ sec^{-1}. V/A is to be taken as $2{\cdot}1 \times 10^{-4}$ cm and $C_i = 41 \times 10^{-6}$ mole/c.c. (Krnjević, 1955). From these figures $M = 3{\cdot}75 \times 10^{-12}$ mole/cm^2.sec. (This calculation assumes that the whole surface of the nerve fibre is available for Na exchange.) This figure should be increased by about 4% to take account of the fact that exchange takes place into the extracellular fluid from which ^{24}Na has to diffuse; the corrected value of the efflux, M, is $3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec. Combining the standard errors of K_s, V/A and C_i gives a standard error for M of about $0{\cdot}6 \times 10^{-12}$. It should be remembered that there may be a systematic error of as much as 40% in the chosen value of V/A because of insufficient weighting of the larger fibres. The effect of the wide distribution of fibre size did not, however, reveal itself in any clear deviation from a simple exponential rate of loss of ^{24}Na.

McLennan & Harris (1954) have carried out a somewhat similar experiment using pieces of the cervical vagus nerve of the rabbit. At 0° C they found a value of $K_s = 1{\cdot}0 \times 10^{-4}$ sec^{-1} and at 18° C a value of $K_s = 2{\cdot}0 \times 10^{-4}$ sec^{-1}. These values agree rather well with our value of $K_s = 4{\cdot}35 \times 10^{-4}$ sec^{-1}, which was obtained at a temperature of 37° C. However, the nerves used by McLennan & Harris were not desheathed, so their figures should perhaps be compared with our figure of $K_s = 0{\cdot}99 \times 10^{-4}$ (at 37° C), which was obtained from a single experiment with an intact nerve (see below and Fig. 3). No exact comparison can, however, be made between the two experiments, which used different nerves and different methods of loading the nerves with ^{24}Na.

The efflux of Na from the nerve fibres is $3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec if it is assumed that the whole surface of the axons is uniformly available for the exchange of Na. If only a region near the nodes of Ranvier allows free exchange, then the Na efflux is considerably greater, but in this case it is not immediately obvious that the rate constant (K_s) governing the slow part of the curve of the loss of ^{24}Na can be used to determine the Na efflux, for diffusion along the internodes might be the rate-determining process. In order to investigate

which is the rate-determining step, it is sufficient to calculate the change in concentration of ^{24}Na in a cylinder, length $2l$ cm and cross-section 1 cm^2 whose surface is impermeable except at the two ends, at which permeation takes place at a rate proportional to the concentration gradient into an outside medium of zero ^{24}Na concentration.

The equation to be solved is thus

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}, \tag{6}$$

with the initial condition:

$$c = C_0 \quad \text{at} \quad t = 0, \; |x| \leqslant l$$

and the boundary conditions:

$$\frac{\partial c}{\partial x} = \pm hc \quad \text{at} \quad x = \mp l, \tag{7}$$

where $h = M/DC_i$, and M = Na efflux across the ends of the cylinder, D = diffusion coefficient of ^{24}Na in axoplasm, C_i = concentration of Na in axoplasm in moles/c.c.

The solution to this problem is (Carslaw & Jaeger, 1947, p. 100)

$$\frac{c}{C_0} = \sum_{n=1}^{\infty} \frac{2h \cos \alpha_n x}{[(h^2 + \alpha_n^2)l + h] \cos \alpha_n l} \exp[-D\alpha_n^2 t], \tag{8}$$

where α_n is a root of $\alpha \tan \alpha l = h$. The rate of loss of ^{24}Na at time t

$$= 2D\left(\frac{\partial c}{\partial x}\right)_{x=-l}$$

$$= 2DC_0 \sum_{n=1}^{\infty} \frac{2h^2 l}{h^2 l^2 + \beta_n^2 + hl} \exp[-D\beta_n^2 t/l^2], \tag{9}$$

where

$$\beta_n = \alpha_n l \text{ is a root of } \beta \tan \beta = hl. \tag{10}$$

The roots of equation (10) have been tabulated for various values of hl (Carslaw & Jaeger, 1947, App. iv, table I).

If $hl < 10^{-2}$ then equation (10) reduces to $\beta_1^2 \simeq hl$, $\beta_n \simeq (n-1)\pi$; it is therefore justifiable to ignore all terms in equation (9) other than the first, so that equation (9) reduces to

$$\text{Rate of loss of } ^{24}Na = 2DC_0 h \, e^{-Dht/l}$$

$$= 2M \frac{C_0}{C_i} e^{-Mt/lC_i}.$$

This is the equation to be expected when the rate-determining step is the efflux of Na across the membrane at the end of the cylinder. Thus the use of K_s to find the efflux from a node of Ranvier is certainly valid if $hl < 10^{-2}$. If $l = 0{\cdot}075$ cm, $D = 10^{-5}$ cm^2/sec and $C_i = 41 \times 10^{-6}$ mole/c.c., then this condition corresponds to $M < 0{\cdot}55 \times 10^{-10}$ mole/cm^2.sec and $M/lC_i < 2 \times 10^{-5}$ sec^{-1}.

The experimental value of M/lC_i (K_s) is about 20 times greater than this limit, corresponding to a value of M of the order of 10^{-9} mole/cm^2.sec and $hl = 10^{-1}$. Table 3, calculated from Carslaw & Jaeger, App. iv, table I, shows how β_1^2/hl depends on hl in the range (10^{-2} to 1) appropriate to the present problem.

TABLE 3. Values of β_1^2/hl as a function of hl

hl	0·01	0·02	0·04	0·06	0·08	0·10	0·20	0·40	0·60	0·80	1·00
β_1^2/hl	0·998	0·994	0·987	0·980	0·974	0·968	0·937	0·880	0·829	0·782	0·740

It is clear that when $hl \sim 10^{-1}$, i.e. $M \sim 10^{-9}$ mole/cm^2.sec and $M/lC_i \sim 4 \times 10^{-4}$ sec^{-1}, β_1^2 is only 3–4% less than hl, *or* the value of the efflux calculated from K_s will be only 3–4% too low because of the effects of internal diffusion. Even if $M/lC_i \sim 40 \times 10^{-4}$ sec^{-1}, i.e. $hl \sim 1{\cdot}0$, then the flux would be only about 25% too low.

Thus, at least as far as the present discussion goes, the effects of internal diffusion along the internodes can be neglected. (It can also be shown that for values of $hl < 1$ all terms higher than the first in equation (9) are unimportant.)

An estimate of the magnitude of the Na efflux, assuming that it takes place only in the region of the nodes of Ranvier, can now be made. At the nodes the myelin-free gap is of the order of $0{\cdot}5\,\mu$ (Hess & Young, 1952; Stämpfli, 1952). The appropriate average diameter of the axons is $8{\cdot}4\,\mu$ as determined from photomicrographs (see above). The approximate relationship between internodal distance (L, mm) and fibre diameter (d, μ) is $L = 0{\cdot}1d$ (Stämpfli, 1952). Thus the average internodal distance is 1·5 mm if it is assumed that an $8{\cdot}4\,\mu$ axon has an external diameter of $15{\cdot}5\,\mu$ (Hess & Young, 1952, table 2, p. 309). If, therefore, Na exchange is confined to the myelin-free gap the Na efflux must be $1500/0{\cdot}5 = 3000$ times greater than $3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec, the value calculated on the assumption that the whole surface of the axon is permeable to Na. On the other hand, a length of the order of $10\,\mu$ in the region of the nodes may be permeable, for the membrane covering this length has different staining properties from the rest of the axon membrane (Hess & Young, 1952). If this $10\,\mu$ length is the permeable region then the efflux is $1500/10 = 150$ times greater than $3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec. Both these values of the efflux should perhaps be multiplied by 2, for there is some evidence that the axon is half as wide near a node. Thus the approximate efflux values are:

$3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec if the whole axon surface is available for Na exchange,

$(1{\cdot}2\text{–}2{\cdot}4) \times 10^{-8}$ mole/cm^2.sec if only the myelin-free gap is available,

$(0{\cdot}6\text{–}1{\cdot}2) \times 10^{-9}$ mole/cm^2.sec if a $10\,\mu$ length in the region of a node is available.

Significance of the results in relation to the saltatory theory of nerve conduction

The value of M calculated on the assumption that the whole nerve fibre takes part in the exchange of Na is of the same order as those values found by previous authors in other excitable tissues; but if it is assumed that exchange of Na can take place only at the nodes, M is of the order of 1000×10^{-12} mole/cm^2.sec. If the latter is true the value of the resting membrane Na conductance (near a node of Ranvier) is very much greater in the mammalian myelinated nerve than in those described previously (e.g. Hodgkin, 1951).

It would be rather surprising if the mammalian myelinated nerve membrane were to be so different, and it might well be rather difficult to explain the mechanism of such concentrated, intense activity. This might then seem to

argue against the saltatory conduction theory. However, another explanation is possible.

According to Hodgkin (1951, p. 364, eqn. (19)), the Na conductance of the nerve membrane in a steady state is given by

$$G_{Na} = \frac{F^2 M_{Na}}{RT}.$$

Substitution of the appropriate values (including $M = 3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec) yields a figure for G_{Na} of about $1{\cdot}4 \times 10^{-5}$ mho/cm^2. In the same article (table 7, p. 394) Hodgkin quotes a figure of $0{\cdot}16 \times 10^6$ ohm.cm^2 for the resistance of the myelin sheath of a frog nerve fibre recalculated from Huxley and Stämpfli. This is equivalent to a conductance of $6{\cdot}25 \times 10^{-6}$ mho/cm^2. If this value may be used here for comparison, it becomes clear that the myelin sheath conductance is probably of the same order of magnitude as G_{Na}, and hence also not very different from the total resting membrane conductance. This suggests that the series resistance provided by the myelin sheath only becomes an effective restriction during activity by passively preventing a flow of current adequate for local depolarization.

One great advantage of this explanation is its simplicity; there is no need to postulate that the mammalian myelinated axon differs in any fundamental respect from the unmyelinated axon, and no special properties are required of the membrane at the nodes.

Intracellular ^{24}Na

The fraction of ^{24}Na in the slow component of the rate of loss curves was calculated to be $(23{\cdot}9 \pm 4{\cdot}9)\%$. It has been shown that the apparent flux of Na from the cells is about 96% of the true flux. This difference, which is due to the slowness of the diffusion of ^{24}Na from the extracellular fluid to the bathing fluid, implies that the mean specific activity of extracellular Na is about 4% of that of intracellular Na. Thus, when t is large, the extracellular ^{24}Na content is about $4\gamma\%$ of the intracellular ^{24}Na content, where γ is the ratio of extracellular to intracellular Na. Since γ is of the order of 4, about 13% of the activity in the slow component, when t is large, is extracellular. Also, during the first few minutes, the extracellular activity is much greater than this because some of the ^{24}Na initially in the extracellular fluid is still present; this high extracellular specific activity at small values of time decreases the rate of loss of intracellular ^{24}Na to values below those obtained by a linear extrapolation of the slow component to zero time.

For these two reasons it is incorrect to say that a linear extrapolation of the slow component to zero time gives a value for the fraction of intracellular ^{24}Na. An exact analysis, to be published elsewhere, shows that the amount by which such a linear extrapolation must be corrected to give the intracellular ^{24}Na

depends, essentially, on the ratio K_s/K_F, and that the correction can be quite large. In the present case the average correction is about 25%, the actual figure for the intracellular ^{24}Na being (18·1 ± 3·3)% of the total ^{24}Na (3·3 is the S.E. of the mean of five observations).

Change in weight of desheathed nerves in Tyrode

The increase in weight of 35% compares well with the value of 40% found in studies of desheathed bullfrog nerves by Shanes (1953). It is known that all the swelling in desheathed nerves takes place in the interstitial tissue (Lorente de Nó, 1952; Shanes, 1953); therefore the extracellular water content of the desheathed nerve must increase from 43% of the total water (Krnjević, 1955) to about 62% after 3 hr in Tyrode solution. From these figures and the values of the K concentration (32·4 m-mole/kg nerve) and water content (74·8%) of the nerve it can easily be shown that the intracellular K concentration must have been reduced from a normal level of about 180 (Krnjević, 1955) to about 110 m-mole/kg water.

Since there is nothing in the structure of the fibre that could prevent appreciable swelling, it is reasonable to suppose that the axoplasm remains in osmotic equilibrium with the surrounding fluid. Krnjević (1955) mentions evidence that the intracellular fluid is isosmotic with 200 mM-NaCl, despite the fact that the total intracellular concentration (Na + K) is about 223 m-mole/kg water. If this relationship still held for the swollen desheathed nerves then the intracellular (Na + K) concentration would fall to 173 m-mole/kg when the extracellular fluid was Tyrode solution (155 mM). On this assumption the intracellular Na concentration must have increased from the normal value of 41 to about 63 m-mole/kg water.

However this, rather flimsy, argument seems to underestimate the increase in intracellular Na concentration; for the measured Na concentration in the swollen desheathed nerves was 150 m-mole/kg water. The obvious conclusion to derive from this figure is that the intra- and extracellular Na concentrations were both equal to about 150 m-mole/kg water. Thus is seems likely that, during the 3 hr exposure to Tyrode solution, there has been a net increase of Na of between 20 and 110 m-mole/kg water in the nerve axons. This increase would correspond to an average net influx of between $0{\cdot}4 \times 10^{-12}$ and $2{\cdot}2 \times 10^{-12}$ mole/cm^2.sec; however it is not known how the net influx varied during the three hours.

^{24}Na *exchange in intact nerve* in vitro

The slow rate of loss of ^{24}Na from a nerve which had not been adequately desheathed (Fig. 3) agrees with present ideas about the permeability of the nerve sheath (particularly the perineurium). The small fast initial component of the curve presumably represents the diffusion of ^{24}Na adhering to, or actually present within, the superficial layers of the perineurium. The difference between

the rates of escape of ^{24}Na from this and from the other nerves may be compared with that found between intact and desheathed toad nerves in a similar study by Shanes (1954).

^{24}Na *space in cats*

The rather high mean value for the ^{24}Na space (36·5%) is almost certainly caused by the inclusion in the series of two young kittens; the ^{24}Na spaces in these latter were 43·6 and 58·1%. It is well known that young individuals have a much higher apparent extracellular space than adults of the same species (Manery, 1951). If the values obtained from the six adult cats only are used, the mean is 31·7%; this is a little higher than the values found by other observers in rabbits, dogs and man, quoted by Manery (1951), which is perhaps not surprising since the calculations in the present paper are based upon the activity of the last plasma sample, usually removed after 5–6 hr.

SUMMARY

1. The exchange of Na between cat nerve fibres and the surrounding fluids has been studied using ^{24}Na.

2. The accuracy of the *in situ* experiments was low, but it could be concluded that 50% of the nerve Na was exchanged in less than 20 min and that at least 70% was exchanged in 5–6 hr.

3. The loss of ^{24}Na from desheathed nerves to Tyrode solution *in vitro* was attributed to two processes: a rapid diffusion from the extracellular fluid and a slower loss from the nerve fibres. From the 'fast' diffusion curves a mean value for the effective diffusion coefficient of ^{24}Na in the extracellular spaces equal to $(1{\cdot}5 \pm 0{\cdot}3) \times 10^{-6}$ cm^2/sec was calculated. From the 'slow' part of the curves a value for the rate constant (K_s) governing the loss of ^{24}Na from the nerve fibres was deduced; this was $K_s = (4{\cdot}35 \pm 0{\cdot}19) \times 10^{-4}$ sec^{-1}. It was calculated from the parameters of these curves that $(18{\cdot}1 \pm 3{\cdot}3)$% of the ^{24}Na in the nerve was intracellular. (The quoted errors are standard errors of the means of five observations.)

4. From measurements on photomicrographs of lateral popliteal nerve sections the average value of the ratio of the total volume of the nerve fibre axons to the total surface area was found to be 2·1 μ.

5. A value of $3{\cdot}9 \times 10^{-12}$ mole/cm^2.sec for the efflux (M) of Na from the desheathed nerves to Tyrode solution was calculated from the values of K_s and V/A given in paragraphs 3 and 4, taking the intracellular Na concentration to be 41×10^{-6} mole/c.c. This value of M was obtained on the assumption that the whole surface of the nerve fibre is available for the exchange of Na. If only a region near the nodes of Ranvier is available, then $M = (500\text{–}25{,}000) \times 10^{-12}$ mole/cm^2.sec.

6. The significance of these values of M for the saltatory theory of nerve conduction is discussed.

7. After the desheathed nerves had soaked for 3 hr in Tyrode solution their weight increased by 35%, the intracellular K concentration decreased from 180 to 110 m-mole/kg water and the intracellular Na concentration increased.

8. The 'Na space' of the cats studied was equal to 36·5% (ml./100 g). (S.E. of mean of eight observations = 3·6.)

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