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THE RESTING EXCHANGE OF RADIOACTIVE POTASSIUM IN CRAB NERVE

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This is the first of several papers describing some work with radioactive tracers on the ionic permeability of non-medullated invertebrate nerve fibres. The final objective was to determine the extent of the ionic movements during nervous activity, but preliminary experiments had to be done with resting nerve, in order to discover how reliable measurements could best be made of the amounts of ions passing through the nerve membrane under various conditions. It was essential to aim at determining the absolute magnitudes of the ionic fluxes as accurately as possible, since the results could not otherwise be correlated in a satisfactory way with observations made by other techniques. The purpose of most of the experiments discussed here was therefore to see what factors affected the resting exchange of ions, and how the rate of exchange could be measured most precisely.

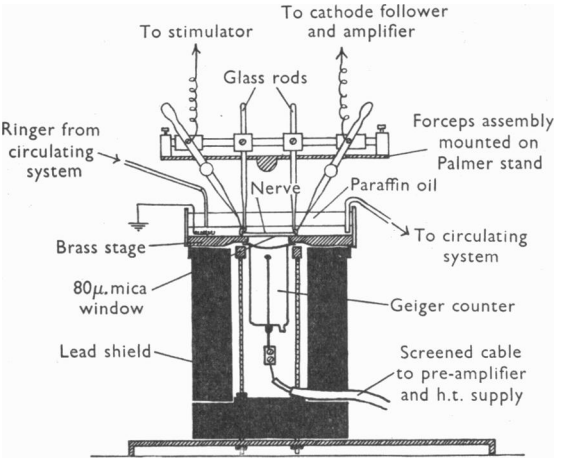
The work was begun with leg nerve from the Shore Crab, *Carcinus maenas*, partly because the animals were easy to obtain and keep alive in the laboratory, and partly because a certain amount of information was already available about the electrical characteristics of the 30μ. fibres which can be dissected from whole nerves (Hodgkin, 1947*a*). The method adopted was to observe the time course of exchange of radioactive ions by soaking nerves in radioactive solutions, mounting them in a thin-bottomed chamber over a Geiger counter, and then determining the rate at which radioactivity was lost when an inactive solution was circulated past them. The exchange rates under different conditions could be compared by taking several sets of counts for each nerve, a procedure which avoided much of the uncertainty due to variation between individual preparations. It was soon found that whole nerves were unsuitable for making absolute measurements of the ionic fluxes, chiefly because the sizes of the fibres in them covered rather a wide range. Experiments could not be done with single 30μ. fibres, owing to their small size, but by using bundles of about half a dozen, and K^{42} samples of high specific activity, it proved possible to make fairly reliable determinations of the exchange rates. Experiments with whole

nerves were continued in parallel, since there were some questions, such as the extent to which intracellular potassium was free to exchange with K^{42}, which could not conveniently be investigated with 30 μ. fibres.

At the time when this work was done it was difficult to obtain supplies of Na^{24} whose specific activity was high enough for experiments with 30 μ. fibres. This paper is hence concerned chiefly with the resting exchange of potassium, and only one experiment with Na^{24} is briefly mentioned. The effect of stimulation on the movements of potassium in crab nerve is considered in the following paper (Keynes, 1951*a*).

METHODS

Apparatus. Radioactivity measurements were made in two identical chambers, the apparatus being duplicated so that parallel experiments could be done on pairs of nerves. The chambers were built on old microscope stages. The side walls were glass and Perspex (see Text-fig. 1), and the



Text-fig. 1. Simplified diagram of chamber used for radioactivity measurements (not accurately to scale).

bottom was an 80 μ. sheet of mica stuck to the brass stage with vaseline. A brass ring soldered to the underside of the stage held the chamber on top of a 1 in. thick cylindrical lead shield in which a G.E.C. type GM2 copper-window Geiger tube was mounted upside-down. The nerves were held over the central hole in the stage in forceps mounted on a Palmer screw stand, and could be raised quickly out of the chamber and transferred to other solutions when necessary. Square pulses from a multivibrator circuit were applied to one pair of forceps, the shocks being synchronized with the a.c. mains so that they occurred at a frequency of 50/sec. or its submultiples. The other pair of forceps was connected via a cathode-follower to a d.c. amplifier and cathode-ray tube, so that the action potentials could be examined. The Ringer's solution in the chamber was earthed by a silver/silver chloride spiral, and was stirred by a circulating system driven by compressed air. This completely changed the fluid in the chamber in about 20 sec., and also served to keep it fully aerated. The total volume in circulation, 350 ml., was large enough to prevent the radioactivity of the solution from rising appreciably as ions from the nerve accumulated in it. The counting rates were measured by conventional scale-of-ten scalers, an automatic timing device being used to switch on one or both of them for predetermined time intervals.

Preparation of radioactive potassium. For the earliest experiments K^{42} samples were prepared by deuteron bombardment of $KHCO_3$ targets in the Cavendish Laboratory's cyclotron. The $KHCO_3$ was dissolved off the target in a small amount of distilled water, neutralized with standard HCl, and made up into *Carcinus* Ringer. For the later experiments with 30 μ. fibres it was necessary to increase the specific activity of the samples, and KBO_2 targets were therefore used, as suggested by Weil, Reid & Dunning (1947). These were much more stable than $KHCO_3$ during bombardment, and would stand the full deuteron beam without burning off the target. The targets were prepared by spreading a finely ground-up paste of KBO_2 in water on a copper sheet, drying overnight in a vacuum desiccator, and baking at 120° C. to remove last traces of water of crystallization. After bombardment, generally for 3 hr., the KBO_2 was dissolved in 2 ml. distilled water, and filtered to remove insoluble impurities from the target (chiefly CuO). The potassium was precipitated as $KClO_4$ by adding 0·5 ml. of 70 % perchloric acid, and cooled to 0° C. The precipitate was filtered off on a sintered glass filter, washed with ethyl alcohol saturated with $KClO_4$, dried, and reduced to KCl by heating in a weighed crucible with a trace of MnO_2. After reweighing, the KCl was dissolved in water, filtered into a 10 ml. volumetric flask, and made up to a standard solution. The total amount of KCl available was generally about 150 mg., which was enough to make 175 ml. of *Carcinus* Ringer. The average specific activity immediately after bombardment was about 10 μcuries/mg. K.

A few experiments were done with samples of $KHCO_3$ supplied by A.E.R.E., Harwell, which had been irradiated in a neutron pile. The potassium was precipitated as $KClO_4$ and then converted to KCl as described above.

Checks were made to see that there were no appreciable amounts of radioactive impurities, either short- or long-lived, by determining the half-life of each sample. This was always within 3 % of the correct value (12·4 hr.), and no evidence of any impurity was ever found. This test would not have shown up the presence of a small amount of Na^{24} in the samples, but all sodium would have been eliminated by the chemical procedure, since sodium is not precipitated as perchlorate.

Counting corrections. All counts were corrected for the decay of the K^{42}, using a table calculated from the theoretical decay constant. At counting rates greater than 1000 counts/min. a correction had to be applied for losses due to the dead time of the scaler, which was determined by a multiple pulse generator. All counts were also corrected for the unavoidable background due to stray radiation. This was generally about 12 counts/min., and frequent checks were made to see that it had not been increased by contamination of the apparatus with radioactivity. The figures given in the following sections have all been corrected for these three factors.

Composition of Ringer's solution. The inactive and radioactive solutions used in the experiments had the same composition, being based on the figures given by Pantin (1946), slightly modified for convenience in preparation by making the amounts of Mg^{++} and SO_4^{--} equivalent. They were made up from isotonic solutions, and their composition is shown in Table 1.

TABLE 1. Composition of *Carcinus* Ringer

	mM.		mM.
Na^+	495	Mg^{++}	18·3
K^+	11·3	Cl^-	532
Ca^{++}	12·6	SO_4^{--}	18·3

Experimental procedure. Nerves were dissected by the method described by Hodgkin (1938), except that the dissection was extended to the carpopodite as well as the meropodite, in order to obtain at least 3 cm. of nerve. For whole nerve experiments the nerve was ready for use as soon as the connective tissue sheath and any loose strands of fibres had been removed, though it was sometimes divided up into several bundles of fibres, only one of which was used.

To measure the rate of uptake of K^{42} the nerve was mounted on forceps and then immersed in a dish of K^{42} Ringer. At intervals of 20–30 min. it was quickly transferred to the chamber for measurement of its radioactivity. Loss of K^{42} during the counting period was minimized by making it as short as was consistent with reasonable accuracy—generally 2 or 3 min. When the internal

radioactivity had reached a suitable level the nerve was transferred permanently to the chamber, and a series of counts was taken while the K^{42} leaked out into inactive Ringer.

When only the rate of loss of K^{42} was to be observed the nerves were put into K^{42} Ringer as soon as they had been dissected, and were left to soak for 1 or 2 hr. before being mounted on forceps and moved into the chamber. In most of the experiments on the resting exchange of K^{42} the nerves were stretched flat across the central hole in the chamber, and raised just above the mica bottom so that the Ringer could circulate freely round them. Excitability was tested at the beginning and end of each set of measurements by lifting the ends of the nerve just clear of the Ringer in order to examine the action potential. The paraffin oil layer shown in Text-fig. 1 was only necessary in the experiments on the effect of stimulation, where the ends of the nerve had to be insulated so that stimuli could be applied without moving them.

For experiments on 30 μ. fibres, three or four legs were taken from one crab, and the nerves were put in a large Petri dish containing K^{42} Ringer. The dissection of the 30 μ. fibres took several hours, so that by the time it was finished about four-fifths of the internal K had exchanged with K^{42}, and determination of the rate of K^{42} loss into inactive Ringer could be started immediately. The 30 μ. fibres were generally grouped in twos and threes, as can be seen from the photomicrographs in Pl. 1, and these bundles were kept intact. Loose pieces of connective tissue and smaller fibres were removed as far as possible, but no attempt was made to produce very cleanly dissected fibres, since it was of over-riding importance to have the fibres in good condition. Fortunately the 30 μ. fibres survived far better, even after they had been roughly treated in the dissection, than the smaller fibres of a whole nerve, probably because of their smaller surface/volume ratio. When about six fibres were ready, they were gathered together with a dissecting needle and mounted on the forceps. Their excitability was then tested with the ends lifted up into the air for a few seconds. By gradually increasing the stimulus strength and watching the individual action potentials appear on the cathode-ray tube, it was generally possible to tell how many of them were conducting impulses, though when there were more than about six it became increasingly difficult to count them. As long as at least five out of six fibres gave action potentials when tested at 1 impulse/sec. the experiment was continued; when fewer of the fibres were excitable they were rejected and a fresh batch was dissected, but this was not often necessary. After a pair of fine glass hooks had been put in position to hold down the central part of the fibres, the whole assembly was raised out of the Petri dish, swung round, and lowered into the measuring chamber. A layer of paraffin oil was poured on top of the Ringer, whose level was then lowered until the ends of the fibres were 2 or 3 mm. above the oil-water interface. The fluid-circulating device was switched on, and a series of counts was taken to determine the rate at which K^{42} was lost, first with the nerve resting in normal Ringer, then with altered conditions, and finally in normal Ringer again.

When these measurements were complete, the excitability of the fibres was tested again, and they were moved back into the Petri dish and taken off the forceps. The diameter of each fibre was measured in at least three places, using a micrometer eyepiece ($\times$ 20) and a high-power objective (16 mm.) in a Greenhough binocular microscope. In the first experiments the total diameter was measured, including the thin layer of connective tissue which surrounded each fibre. Later it was found that by careful observation the true diameter of the fibre, excluding the connective tissue, could be measured fairly accurately. Measurements on fixed sections of several nerves (see Pl. 1) showed that the average sheath thickness was 11·5 % of the overall diameter. This agrees with the estimate of Hodgkin & Huxley (1947) that it is 2–5 μ. thick for a 30 μ. fibre. The earlier measurements were corrected accordingly, so that the figures given in Table 5 represent the best estimate that could be made of the true fibre diameter. Individual fibres frequently varied in diameter by as much as a fifth at different points, so that the maximum error in the final result was probably about $\pm 15\%$.

Determination of total potassium exchange. The total exchangeable potassium in whole nerves was measured by comparing their radioactivity with standard K^{42} samples after they had been soaked for long periods in K^{42} Ringer. The nerves were mounted in a Perspex framework and put into a deep jar of K^{42} Ringer, which was kept thoroughly stirred by bubbling in compressed air. When soaking was complete, the nerves were cut through about 5 mm. from either end, in order to reduce

errors from the damaged ends, lightly blotted, and put on weighed nickel dishes. After reweighing they were dry-ashed in an oven for 5 hr. at a temperature rising slowly to 500° C. Their radioactivity was then compared with that of 0·05 ml. K^{42} Ringer samples, which were measured out with a micropipette on to nickel dishes and evaporated to dryness, using a conventional counting arrangement with a copper-window Geiger tube. The ash was finally washed off the dishes into platinum crucibles, and the total potassium present was determined by the dipicrylaminate method, as described below. Checks showed that incineration of the nerves on nickel rather than platinum dishes caused no appreciable loss of potassium. There may have been slight losses of potassium by volatilization during incineration, but this would have affected the radioactivity as well as the total potassium, and so would not have altered the figure obtained for the percentage of potassium exchanged.

Determination of potassium in nerve samples. Precipitation of potassium as the dipicrylaminate, which was first used for quantitative microanalysis by Kolthoff & Bendix (1939) and Harington (1941), has the advantages over the more commonly employed cobaltinitrite method that the precipitate has a constant composition, being a pure potassium salt, and is a strong orange-red in colour, so that it can be determined directly in a colorimeter. The procedure described by these authors was used, with some modifications, for almost all the analyses. The losses and errors at each stage were carefully checked with K^{42}, and a reliable routine was worked out. Only an outline of the method is given here; it will be discussed in greater detail elsewhere (Lewis, 1951).

The ash was dissolved in one drop of dilute HCl, excess acid was evaporated off in an oven at 110° C., and the residue was taken up in two drops of water. About 0·2 ml. of either sodium or lithium dipicrylaminate reagent (prepared by boiling 2·5 g. of dipicrylamine, previously recrystallized from glacial acetic acid, with 100 ml. of a 0·1 N solution of the alkali carbonate, and filtering the solution when cold) was then added. After standing for about an hour at room temperature the crucibles were put in an ice-bath and left in a refrigerator overnight. The supernatant solution was filtered off through a sintered-glass filter stick, and the precipitate was washed, first with two drops of ice-cold water, then with eight drops of a solution of potassium dipicrylaminate cooled to 0° C., and finally with another drop of water. The precipitate was next dissolved in the minimum quantity of acetone (about 2 ml.), and diluted to a standard volume, generally 50 ml., with 0·001 N-NaOH (to prevent hydrolysis). The depth of colour was determined in 1 cm. cells, using a single beam photoelectric colorimeter with an Ilford filter no. 621 (Bright Spectrum Violet). The method was standardized by precipitating known amounts of K from a mixture of K, Na, Cl, Ca, and phosphate in roughly the proportions found in crab nerve. The standard error was between $\pm 1{\cdot}5$ and $\pm 2{\cdot}5$ % for amounts down to 20 μg. of K, and slightly greater for amounts down to 10 μg. Standardization against solutions made up with weighed quantities of potassium dipicrylaminate gave results 2·5 % low, owing to inevitable losses in precipitation and washing.

THEORY

Before considering the experimental results, it will be helpful to derive equations for the time course of exchange of radioactive ions, particularly in the case of a cell which is not in a steady state. Suppose the cell has a volume V, surface area A, and that the intracellular and extracellular concentrations of the ion whose movements are being investigated are C_i and C_o respectively. The inward and outward ionic fluxes, which are not necessarily equal, are m_i and m_o. The internal concentration is changing at a rate given by

$$\frac{dC_i}{dt} = \frac{A}{V}(m_i - m_o). \qquad (1)$$

At time $t = 0$ the cell is immersed in a well-stirred radioactive solution containing the same total amount of ions, C_o, of which a proportion s_o are radioactive.

After time t, the resulting internal specific radioactivity is s_i. The total radioactivity of the cell, Y, is then given by

$$Y = s_i V C_i, \tag{2}$$

and this is changing at a rate

$$\frac{dY}{dt} = m_i s_o A - m_o s_i A, \tag{3}$$

or, from (2),

$$\frac{dY}{dt} = m_i s_o A - \frac{m_o}{C_i}\frac{A}{V} Y. \tag{4}$$

Conversely, if the cell is first allowed to accumulate some radioactive ions, and is then put in a well-stirred inactive medium for which $s_o = 0$, radioactivity is lost at a rate

$$\frac{dY}{dt} = -m_o s_i A, \tag{5}$$

or, from (2),

$$\frac{d}{dt}(\log_e Y) = -\frac{m_o}{C_i}\frac{A}{V}. \tag{6}$$

Equation (6) is of considerable practical importance, since the rate of loss of radioactivity in an inactive solution is a convenient quantity to determine experimentally. The value of $-\frac{d}{dt}(\log_e Y)$ will be termed the exchange constant, and denoted by k. It is a rate constant, or the reciprocal of a time constant, and its dimensions are therefore hours^{-1}.

It is reasonable to assume, as Harris & Burn (1949) did in their treatment of the problem, that the outward flux is directly proportional to the intracellular concentration of the ion, so that

$$m_o = \kappa_2 C_i, \tag{7}$$

and hence

$$k = \frac{A}{V}\kappa_2 \tag{8}$$

(κ_2 thus has the same meaning as in Harris & Burn's paper, while k is the same as their k_2). It should be observed that even if m_o is actually a more complicated function of C_i and other variables such as the membrane potential, the expression

$$m_o = k\frac{V}{A} C_i \tag{9}$$

can be used to calculate the absolute size of the outward flux, as long as the value of C_i at the time of the measurements is known.

The total concentration of intracellular ions exchanged during a period of immersion T in a radioactive solution can be written as

$$C_i^* = C_i \frac{s_i}{s_o}. \tag{10}$$

If the net rate of entry of radioactive ions at time T, $(\mathrm{d}Y/\mathrm{d}t)_T$, is measured, and if the cell is then transferred to an inactive solution and the initial rate of loss of radioactivity, $(\mathrm{d}Y/\mathrm{d}t)_o$, is also measured, we have from (3), (5) and (10)

$$C_i = C_i^* \frac{m_o}{m_i}\left\{1-\frac{(\mathrm{d}Y/\mathrm{d}t)_T}{(\mathrm{d}Y/\mathrm{d}t)_o}\right\}. \tag{11}$$

This expression is independent of any assumptions about the relationships governing the sizes of the ionic fluxes, and is useful under certain circumstances for calculating the value of C_i in an axon which is not in a steady state (Keynes, 1951*b*).

In the steady state m_i and m_o are equal and constant, so that equations (4) and (6) can readily be solved, giving the usual simple exponential expressions (see Harris & Burn, 1949). A complete solution for the case of a cell not in a steady state would require a detailed knowledge of the relationship between ionic flux, ionic concentrations, membrane potential and membrane permeability, which is not at present available. Only the solution of one special case, which will be valuable in interpreting the results of some of the experiments, can be given here.

It was observed that the total potassium content of whole *Carcinus* nerves declined in a roughly exponential fashion (see Table 4) so that we can write

$$C_i = C_{i0}\exp\left[-\frac{A}{V}\kappa_3 t\right], \tag{12}$$

and hence

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = -\frac{A}{V}\kappa_3 C_i, \tag{13}$$

where $A\kappa_3/V$ is a rate constant for net potassium leakage similar to that for loss of K^{42} in an inactive solution, which is, from (8), $A\kappa_2/V$. Combining (1), (4), (12) and (13) it follows that for the entry of radioactive ions in K^{42} Ringer,

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = s_o A C_{i0}(\kappa_2-\kappa_3)\exp\left[-\frac{A}{V}\kappa_3 t\right]-\frac{A}{V}\kappa_2 Y. \tag{14}$$

Since $Y=0$ when $t=0$ the solution of this is

$$Y = V s_o C_{i0}\exp\left[-\frac{A}{V}\kappa_3 t\right]\left(1-\exp\left[-\frac{A}{V}(\kappa_2-\kappa_3)t\right]\right), \tag{15}$$

and from (2), (12) and (15) the change in intracellular specific radioactivity follows an exponential time course given by

$$s_i = s_o\left(1-\exp\left[-\frac{A}{V}(\kappa_2-\kappa_3)t\right]\right). \tag{16}$$

The total radioactivity of the cell thus depends on the product of two factors, one representing the total ionic content, and the other representing the specific activity of the cell contents, which both vary exponentially. Furthermore, the rate constant for the change in specific activity is simply the difference between

the rate constants for loss of K^{42} in an inactive medium and for net loss of potassium. This relatively straightforward solution is only obtained with the particular assumptions made above, and has no general validity, but it will be seen that it does describe the behaviour of the potassium in *Carcinus* nerves quite well.

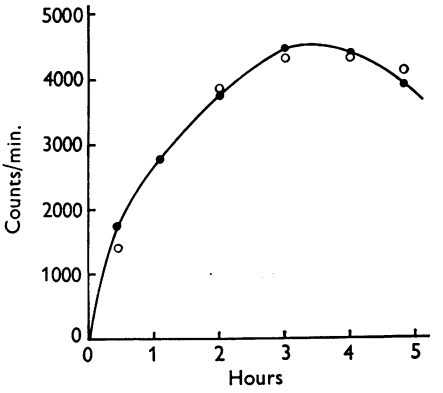
The steady state equations have been used in different guises by several authors, though their methods of deriving them and of expressing the results of their experiments have not always been the same. Krogh's (1946) permeability constant P, for example, has dimensions cm./hr., and using the notation above corresponds to the value of m/C_i. This method of expressing the results of permeability studies with radioactive tracers gives a false impression of the relative fluxes for different ions, in that cells containing much less sodium than potassium will have values of P much greater for sodium than for potassium even when the ionic fluxes are actually equal. Conway (1947) preferred to express the permeability constant as the flux per unit external concentration ($=m/C_o$), but this, too, gives misleading results when, for instance, chloride and potassium fluxes are compared. As Teorell (1949) has pointed out, it is dangerous to calculate such so-called permeability constants with reference only to the ionic concentrations, and without taking into account the electric field across the membrane. Moreover, different species of ions do not necessarily cross the membrane by the same type of pathway (Ussing, 1949), so that they may be influenced by different driving forces, in which case any comparison of their permeability constants has a rather restricted meaning. The results of the experiments considered here will be expressed in terms either of the ionic flux m, or of the exchange constant k, both experimentally determined quantities which do not imply any specific type of driving force.

RESULTS

The uptake of K^{42} *by resting whole nerves*

In the experiment illustrated in Text-fig. 2 a whole *Carcinus* nerve was soaked in K^{42} Ringer for nearly 5 hr., and its radioactivity was measured at intervals. The total amount of K^{42} in the nerve reached a peak after about 3 hr., and subsequently fell off again, despite the fact that it was still in K^{42} Ringer. Several other experiments gave similar results. This is clearly the type of behaviour which might be expected if there were a continuous net leakage of potassium from the nerve in addition to the resting exchange of ions, and it is interesting to see whether the results agree with the equations derived in the preceding section. Later experiments on the extent of potassium exchange in whole nerve showed that the total potassium content of nerves soaked in normal Ringer was reduced to half its initial value in about 5 hr. (see Table 4), so that the rate constant for net potassium leakage, $A\kappa_3/V$, was about 0·14 hr.$^{-1}$. Subsequent determination of the rate of loss of K^{42} in inactive Ringer for the

nerve of Text-fig. 2 gave an exchange constant, Ak_2/V, of 0·50 hr.$^{-1}$. Taking these two figures, and the total radioactivity at one point ($t=1{\cdot}1$ hr.) on the experimental curve, the theoretical uptake according to equation (15) was calculated, giving the points plotted as open circles. Considering the rough and ready nature of this procedure, the theoretical values fit reasonably well with the curve, and show that the time course of K^{42} uptake is at any rate



Text-fig. 2. Uptake of K^{42} by a whole *Carcinus* nerve. The nerve was in K^{42} Ringer throughout the experiment, except for the 3 min. periods in which the counts were taken. Temperature 18° C. The open circles are theoretical values made to fit the experimental curve at 1·1 hr., and calculated from $Y=10{,}000\ e^{-0{\cdot}14t}(1-e^{-0{\cdot}36t})$.

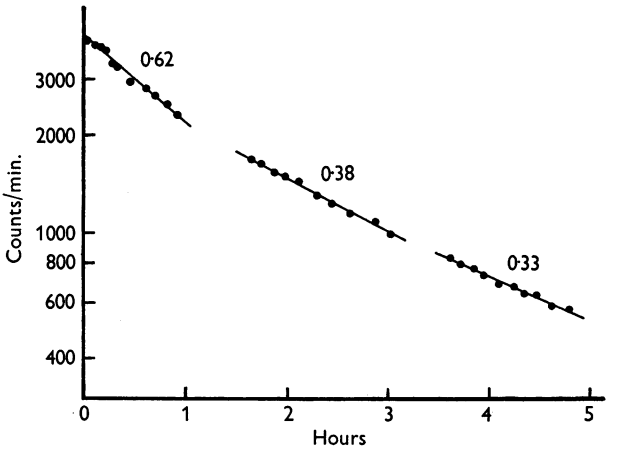
approximately that predicted by the theory. It was not worth while attempting to make any more detailed examination of uptake curves of this type, since too many variables were involved, and it would have been difficult to allow for the effects of variation in k with time and slowness of diffusion discussed in the next section. The experiments on extent of potassium exchange described later in this paper, in which the specific activity of the potassium in the nerves was measured as well as their total K^{42}, in any case provided better confirmation of the validity of the theory than could be obtained here.

The leakage of potassium was correlated with a steady decline in the size of the action potential, which was observed in every experiment on whole nerve. Cowan (1934) found a similar deterioration and loss of potassium in *Maia* nerve.

The loss of K^{42} *by resting whole nerve*

If the equations given in the theoretical section had been strictly obeyed, the exchange constant (k) for loss of K^{42} in inactive Ringer would not have altered with time. The experiment illustrated in Text-fig. 3, in which three consecutive determinations of k were made over a total period of 5 hr., showed that in fact k tended to decrease with time. This behaviour was apparently the general rule if the measurements were made under reasonably good

physiological conditions, and when k was determined in normal Ringer before and after making alterations of not too drastic a nature in the temperature or potassium concentration, the second value was usually the smaller. When k was measured under conditions which might have been expected to damage the fibres and increase their permeability, it often changed in the opposite direction. Thus in potassium-free Ringer it increased slightly with time, as is demonstrated by Text-fig. 4. In the experiments described in the following paper (Keynes, 1951*a*) it was observed that the value of k did not alter significantly after a period of stimulation in normal Ringer, but did increase appreciably after the nerve had been stimulated in potassium-free Ringer.

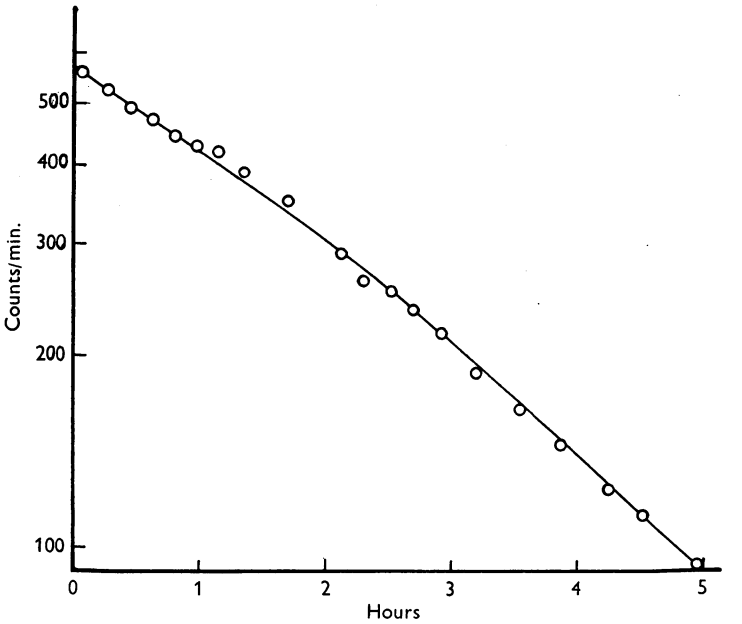


Text-fig. 3. The loss of K^{42} from a whole *Carcinus* nerve in inactive Ringer, plotted semi-logarithmically. After the first and second groups of counts the Ringer in the measuring chamber was changed, and the background count was checked. The figure against each set of counts is the exchange constant in hours^{-1}. Temperature 18° C.

A decrease in k with time does not necessarily mean that the value of m_o/C_i was reduced as C_i fell, although a failure of m_o/C_i to remain constant (which it was assumed to be in writing equation (7)) may explain some of the alterations observed. Non-uniformity in the exchange constants of the individual nerve fibres, arising either from the wide range of fibre sizes involved, or from variation in the membrane conductance for fibres of the same size (Hodgkin, 1947*a*), would be expected to produce an effect of this kind, since the sum of several exponentials plotted semi-logarithmically is a curve whose initial slope is greater than its final slope. Moreover, the membrane permeability may decrease with time, though the observation of Hodgkin & Rushton (1946) that the membrane conductance tended to increase in the course of their experiments rather suggests that the reverse is true. Any tendency of the fibres to swell as they lost their potassium might also make k fall. These factors must all have

been affecting k to some extent, but it was not worth while investigating their exact relative importance. Fortunately k seldom decreased to much less than half its initial value during an experiment, so that the effect did not prevent whole nerves from giving useful results in qualitative work, although it was one of the reasons why it was difficult to obtain reliable figures for the absolute size of the potassium flux in whole nerve.

Another possible source of error in working with whole nerve was that the apparent rate of exchange of ions may have been reduced below its true value by the slowness of diffusion between the closely packed fibres, as in frog muscle (Harris & Burn, 1949). When k for a very thin bundle of fibres was compared



Text-fig. 4. The loss of K^{42} from a whole *Carcinus* nerve in K-free Ringer, plotted semi-logarithmically. Temperature 16° C.

with k for the rest of the nerve from which the bundle was taken, the values were 0·64 and 0·47 hr.$^{-1}$ for the thin bundles, and 0·45 and 0·32 hr.$^{-1}$ for the thick ones. This would indicate a slightly slower exchange in the large bundles, though some of the difference may have arisen from the smaller average fibre diameter in the thin bundles, which contained none of the 30μ. fibres. It can also be seen on examining table 1 in the following paper (Keynes, 1951*a*) that thin bundles tended to give a higher exchange constant during stimulation than thick ones, the result obtained for the extra rate of loss of K^{42} being roughly doubled when the diameter of the nerve was reduced from 0·65 to 0·25 mm. (a t-test of the significance of the correlation gave $P = 0{\cdot}05$). Experiments with

Na^{24} (see below) confirmed that extracellular radioactivity was not lost very rapidly, but gave no figures on which calculations of the magnitude of the diffusion effect could be based. The question was further complicated by the fact that the nerves often split up into several fine strands when the connective tissue sheath was removed, as has happened in Pl. 1; the diameter of the separate strands may have been more important than their total number in deciding how much the exchange was slowed. Hence, although the evidence suggests that exchange was slightly hindered by the diffusion effect, no reliable correction can be made for it.

The average value of k from twenty-three experiments at temperatures between 14 and 22° C. was 0·49 hr.$^{-1}$. This figure was not much greater than the average value for 30μ. fibres, which was, from Table 5, 0·37 hr.$^{-1}$. The values of k for individual fibres must have varied over a much narrower range than their diameters, and the outward flux must therefore have been roughly proportional to the diameter (assuming that C_i was about the same in all fibres). Taking C_i as 270 mM. and V/A as 1·0μ. for the reasons discussed below, the average outward potassium flux in whole *Carcinus* nerves was estimated, using equation (9), to be $3{\cdot}7 \times 10^{-12}$ mol./cm.^{2}/sec. However, it will be clear that this figure can only be very approximate.

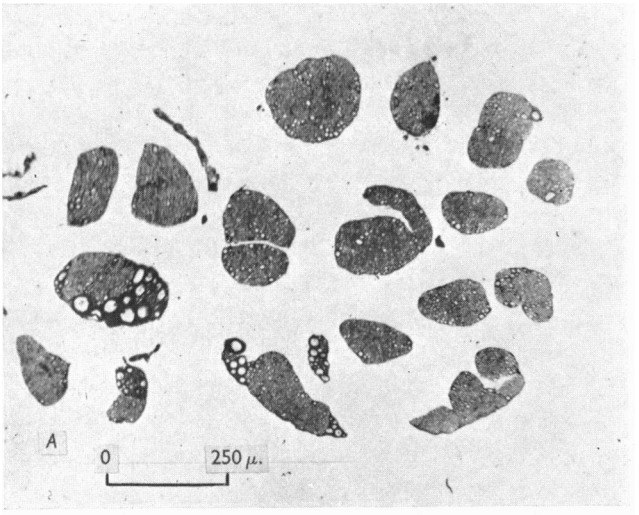
The fibre size distribution in Carcinus *nerve*

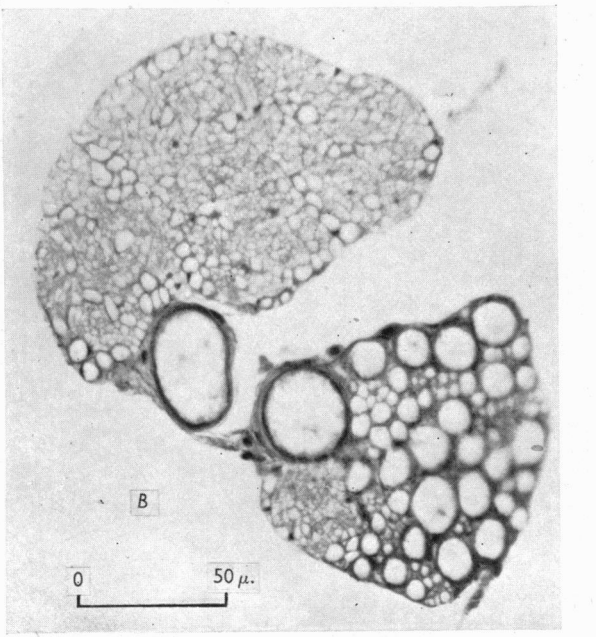
In a whole *Carcinus* nerve the diameters of the fibres range from about 1 to 30μ., as is clear in the photomicrographs shown in Pl. 1. A few measurements were made of the actual distribution of fibre sizes, in order to obtain some idea

TABLE 2. Fibre size distribution in part of a *Carcinus* leg nerve. The nerve was fixed in 0·5% osmic acid in *Carcinus* Ringer, embedded in paraffin, and sectioned transversely. Sizes of all the fibres which could be picked out clearly were measured with a camera lucida arrangement. This bundle contained a higher proportion of fibres over 8μ. than the whole nerve

Fibre diameter (μ.)	No. of fibres	Fibre diameter (μ.)	No. of fibres
Less than 1·0	147	Less than 8	4
1·5	123	9	1
2·0	77	10	5
2·5	39	11	3
3·0	51	13	2
4	25	15	1
5	11	25	1
6	8	32	1
7	2		

of the ratio of total membrane area to axoplasm volume in a whole nerve. The particular bundle to which the figures in Table 2 refer was not quite representative, since the fibres over 8μ. in diameter contributed about half the total cross-sectional area, whereas other measurements showed that they occupied only about 12% of the volume of a whole nerve trunk. Examination of other bundles showed a similar distribution of the smaller fibres, though in one which was measured the most common diameter was 2·0 instead of 1·0μ. There may





Photomicrographs of transverse sections through *Carcinus* leg nerve. The nerves were fixed in 0·5% osmic acid in *Carcinus* Ringer, and stained with iron haematoxylin. *A*, low power, showing a whole nerve. *B*, high power, showing one bundle of fibres whose diameters range from 1 to 30 μ.

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have been a larger proportion of fibres below 1 μ. than is indicated in Table 2, since the very small fibres were difficult to distinguish when making the measurements. The largest fibres had much the same diameters as those isolated from nerves in living condition, so that there cannot have been any great change in size during fixation and embedding. The ratio of total volume to total surface for the bundle of Table 2 was 1·25 μ., and for another bundle containing fewer large fibres it was 0·75 μ. A fair estimate for V/A for the whole nerve trunk would be 1·0 μ. Since the extracellular space in *Carcinus* nerves was about 0·25 (see below) this means that there were about 7500 sq.cm. of membrane in 1 g. of nerve.

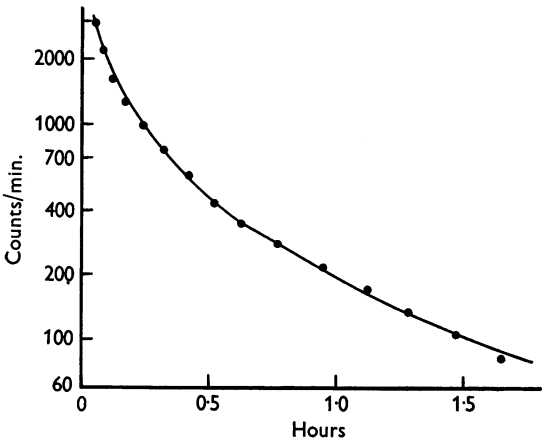
The potassium content of Carcinus *nerve*

As no analyses of *Carcinus* nerve are reported in the literature, the sodium and potassium contents of some freshly dissected nerve samples were determined by the method of activation analysis (Keynes & Lewis, 1950). For six nerves from different crabs the average potassium content was 257 ± 4 (S.E. of mean) m.mol./kg. wet weight, and the average sodium content was 152 ± 5 m.mol./kg. Twenty-four other analyses of the potassium content of *Carcinus* nerves by the dipicrylaminate method gave an average value of 262 ± 4 m.mol./kg. Cowan (1934) and Fenn, Cobb, Hegnauer & Marsh (1934) found somewhat lower values in other crustacean nerves, possibly because their material had been soaked in sea water for some time after being dissected and had lost some of its original potassium. The nerves analysed here were dissected very quickly, and the damaged ends were always cut off immediately before the nerves were blotted and weighed. A maximum value for the extracellular space can be calculated from the sodium figure, on the assumption that the sodium concentration outside the fibres was the same, 495 mM., as in Ringer. Even if there had been no intracellular sodium, the extracellular space could not have been less than 152/495, or 0·31. It is more probable, by analogy with analyses of squid axoplasm (Steinbach & Spiegelman, 1943) and of *Sepia* axons (Keynes & Lewis, 1950), that freshly dissected fibres contain about 40 m.mol./kg. of intracellular sodium, which would make the extracellular space 0·25. Fenn *et al.* (1934) obtained the same figure from analyses of potassium, sodium, and chlorine in crab and lobster nerves, and it should be fairly reliable. Taking the extracellular space as 0·25, the potassium content of the actual nerve fibres was 342 m.mol./kg. axoplasm, which is close to the results obtained for squid and *Sepia* axoplasm in the analyses quoted above—363 and 325 m.mol./kg. respectively. From considerations of osmotic balance none of the fibres can have contained much more potassium than this. As the smallest fibres occupied only a small fraction of the whole nerve, they could have contained less potassium than the large ones without greatly affecting the total potassium content of the nerve, but it seems unlikely that they contained less than 200 m.mol./kg.

The nerves used for these analyses were weighed and stored within a few minutes of dissection, and therefore contained more potassium than those on which the measurements of K^{42} loss were made, which had generally been dissected about 2 hr. earlier. A reasonable value for the potassium content 2 hr. after dissection would be 210 m.mol./kg., making C_i about 270 mM.

The exchange of Na^{24} *in whole nerve*

For qualitative experiments on potassium movements the effect of variation in k with time was not very troublesome, since the exchange constant could be determined by following the K^{42} loss for a relatively short period. In experiments with Na^{24}, however, it made the results extremely hard to interpret.

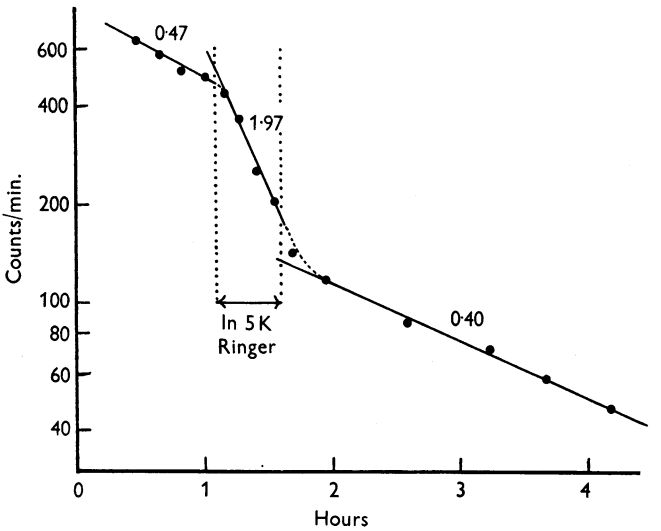


Text-fig. 5. The loss of Na^{24} from a whole *Carcinus* nerve in normal Ringer, plotted semi-logarithmically. Temperature 17° C.

Text-fig. 5 shows the loss of Na^{24} in inactive Ringer from a whole nerve which had previously been soaked for a short while in Na^{24} Ringer. Part of the relatively rapid initial loss of Na^{24} is certainly due to the diffusion of extracellular Na^{24} from the extracellular space, but the continued subsequent flattening of the curve makes it impossible to distinguish clearly between the exchange of intracellular and of extracellular sodium. It is instructive to compare this curve with the results obtained by Levi & Ussing (1948) and Harris & Burn (1949) for the loss of Na^{24} from frog sartorius muscles under similar conditions. In muscle, the fibre population is more homogeneous, so that the slow phase of loss of intracellular Na^{24} gives an almost straight line when plotted logarithmically, and can be easily differentiated from the initial loss of extracellular Na^{24}. Any tissue with less uniform fibres, such as crab nerve, is clearly unsuitable for quantitative experiments on sodium movements.

The effect of varying the external potassium concentration

In several experiments the resting value of k was determined first in normal Ringer, then in Ringer containing either no potassium or some multiple of the normal concentration, and finally in normal Ringer again. The value of k for each set of counts was worked out by dividing the counts into three roughly equal groups, averaging the figures for the first and last groups, and calculating k for an exponential decay. This process, although less rigorous than the method of least squares, was accurate enough for these experiments, and gave effectively the same answer, since the scatter of the individual counts was not



Text-fig. 6. The effect of potassium concentration on the loss of K^{42} from whole *Carcinus* nerve. Counts were taken first in normal Ringer, then in Ringer with 5 × normal [K], and finally in normal Ringer again. The figures against each group of counts are the values of the exchange constant in hours^{-1}. Temperature 17° C.

excessive. The ratio of k for altered conditions to its value in normal Ringer was then worked out, the two figures for normal Ringer being averaged so as to minimize systematic errors from variation in k with time or from irreversible effects produced by the abnormal solution. Even exposure to Ringer containing ten times the normal amount of potassium did not appear to damage the nerve, the average change in k after soaking in 10K Ringer being -10%, and the size of the action potential being effectively unaltered. In the solutions containing more than the normal amount of potassium, the extra potassium was additional to the usual constituents of the Ringer, and not substituted for them. Under these conditions there should be no shrinking or swelling of the fibres (Shanes, 1946).

The counts taken in one of the experiments are plotted in Text-fig. 6. In this experiment the values of k in normal Ringer were 0·47 and 0·40 hr.$^{-1}$, and the value in Ringer containing five times the normal amount of potassium was 1·97 hr.$^{-1}$. The change in k on altering the potassium concentration was not immediate, but occurred with a delay of 5 or 10 min. This was observed in all the experiments with five and ten times normal potassium, but not in those with twice normal potassium, when the change in k was not large enough for the effect to have been easily detectable. The first count taken after changing the potassium concentration was therefore ignored in working out the value of k. Shanes & Hopkins (1948) found a very similar time lag in the change of resting potential of crab nerve when the external potassium concentration was altered. The lag may be due partly to a diffusion effect, or to an actual change in the permeability of the membrane to K^+ ions, but it seems more likely that it is due chiefly to the time taken to establish a new Donnan equilibrium for K^+ and Cl^- across the membrane. On increasing the external potassium, there is probably some immediate drop in membrane potential due to the change in potassium concentration ratio, followed by a further delayed drop as potassium chloride diffuses into the fibres and the chloride concentration ratio falls, and these changes in membrane potential are accompanied by parallel changes in the potassium flux.

TABLE 3. The effect of potassium concentration on the rate of loss of K^{42} from whole *Carcinus* nerve. Each figure for the exchange constant in normal Ringer is the mean of two values, determined before and after the exchange constant with altered potassium concentration

Ratio of [K] to normal	Exchange constant for [K] normal (hr.$^{-1}$)	Exchange constant for [K] altered (hr.$^{-1}$)	Exchange constant ratio
0	0·30	0·18	0·60
0	0·29	0·21	0·73
0	0·45	0·36	0·80
0	0·46	0·41	0·90
2	0·43	0·73	1·71
2	0·48	0·86	1·79
2	0·89	1·40	1·57
2	0·33	0·56	1·71
4	0·36	1·32	3·69
5	0·40	2·37	5·87
5	0·39	2·00	5·17
5	0·44	1·97	4·50
10	0·41	2·68	6·52
10	0·35	3·11	8·88
10	0·53	3·11	5·82

Table 3 shows the results of all the experiments on the effect of varying the potassium concentration. Near the normal potassium concentration, the outward flux did not alter much when the concentration was changed, but at higher concentrations it followed the changes more closely. This behaviour is somewhat reminiscent of the relationship between resting potential and potassium concentration reported by Shanes & Hopkins (1948), and between

membrane conductance and potassium concentration (Hodgkin, 1947*b*), probably because the flux is related to both these quantities.

The principal object in making these measurements was to verify that the exchange constant in high potassium Ringer was increased roughly in proportion to the concentration change. This was the case, so that in the interpretation of the results discussed in the following section, the exchange constant in 2K Ringer may be taken as 1·7 times its normal value, and in 5K Ringer as 5·1 times normal.

The extent of potassium exchange in whole nerve

In the type of experiment considered in this paper, the absolute value of the outward potassium flux could only be calculated from the exchange constant when the true internal potassium concentration was known. The figure taken for C_i was based on analyses of *Carcinus* nerve, but it should strictly refer only to potassium free to exchange with K^{42}, which is not necessarily identical with the total potassium content of the nerve. The reason for suspecting that the potassium might not all be freely exchangeable was that there have been several reports of an incomplete exchange of K^{42} in nerve and muscle (Hevesy & Hahn, 1941; Rothenberg, 1950). The validity of some of the earlier work has been questioned on technical grounds (Dean, Noonan, Haege & Fenn, 1941), and there are considerable difficulties in accepting the idea of 'bound' potassium (Krogh, 1946), but it nevertheless seemed necessary to determine the extent of potassium exchange in *Carcinus* nerve by a reliable method.

TABLE 4. The extent of potassium exchange in *Carcinus* nerve. Each figure is an average for three or four nerves. The first five results were obtained with nerves from one group of crabs, and the remaining four with nerves from another group. The percentage exchange was worked out by averaging the values for the individual nerves, and the standard error of the mean is shown

[K] in Ringer (mM.)	Time in K^{42} (hr.)	K exchanged (m.mol./kg.)	K content (m.mol./kg.)	Exchange of K (%)	Rate constant for entry of K^{42} ($hr.^{-1}$)
11·3	0	—	297	—	—
11·3	3·7	105	171	63±5	0·28
11·3	6·3	95	124	78±8	0·24
11·3	9·3	86	94	91±2	0·26
11·3	15·3	73	76	95±2	0·20
11·3	0	—	260	—	—
22·6	5·6	181	192	94±1	0·50
22·6	9·4	157	171	93±3	0·29
56·5	6·4	290	298	97±2	0·53

The experimental procedure, which involved parallel determinations of K^{42} and of total potassium in nerves soaked in radioactive Ringer for long periods, has been described in the section on 'methods'. Each figure in the results given in Table 4 is an average for three or four nerves taken from four different crabs. All the experiments in which there was a normal potassium concentration in the Ringer were done with one set of crabs. The experiments with raised potassium

concentrations were done with another set. This method of averaging had the object of reducing errors due to variation between individual crabs, since it had been found that there was much less variation between nerves from one crab than between nerves from different crabs.

The results in normal Ringer show that there was a steady decrease in the K^{42} content of the nerves after they had been soaked for about 4 hr., as had been found in the experiments on K^{42} uptake already described. At the same time there was an even greater decrease in the total potassium content of the nerves, so that the specific activity of the internal potassium continued to rise. After 15 hr. the percentage of potassium exchanged, which was given by $100s_i/s_o$, had reached about 95%. The change in specific activity followed a roughly exponential time course with a rate constant of about 0·26 hr.$^{-1}$, as is shown by the figures in the last column. The last value for the rate constant was less reliable than the others, since analytical and counting errors became more important when exchange was nearly complete.

Another less complete experiment, conducted on the same lines but at a lower temperature (18° instead of 25° C.), gave very similar results, the rate constant for the change in specific activity being estimated as 0·20 hr.$^{-1}$. It was shown in the theoretical section that the sum of the rate constants for specific activity and for net potassium leakage should be equal to the exchange constant for loss of K^{42} in inactive Ringer. These experiments gave average values of 0·14 hr.$^{-1}$ for the net leakage rate constant, and 0·23 hr.$^{-1}$ for the specific activity rate constant. This corresponds to a calculated value for k of 0·37 hr.$^{-1}$, which is not far from the average value quoted earlier, 0·49 hr.$^{-1}$.

In Ringer containing twice and five times the normal amount of potassium, the exchange of K^{42} was faster. In such solutions the net potassium leakage is somewhat slower than in normal Ringer, as the figures in Table 4 show, so that the specific activity rate constant should increase by rather more than the factors found in the experiments described in the last section. This means that although the rate constant for the nerves soaked 5·6 hr. in $2K^{42}$ Ringer was consistent with the value in normal Ringer, the other two sets of nerves gave results less than half as great as those expected. It is obvious from the sizes of the standard errors that this may have been due solely to experimental error, though the possibility cannot be excluded that the final 3% of the potassium was really (or apparently) slower to exchange than the rest.

It was difficult, using this technique, to obtain trustworthy evidence about the behaviour of the last fraction of potassium to exchange, because of the inevitable errors in counting and in chemical analysis. One experiment was done by a method designed to reduce such errors to an absolute minimum. After being soaked in K^{42} Ringer as before, the nerves were incinerated, and their potassium was precipitated as the dipicrylaminate. A small portion of the precipitate was put on a nickel dish, and its radioactivity was measured.

It was then washed off the dish, and the amount of dipicrylaminate present was determined colorimetrically. The specific activity of the potassium in the nerve could thus be compared with that of the original K^{42} sample, which was similarly treated. This procedure gave no figure for the total potassium content, but enabled the proportion of potassium exchanged to be determined fairly accurately. Three nerves soaked for 5·2 hr. in 5 K^{42} Ringer were found by this method to have exchanged $97{\cdot}5 \pm 0{\cdot}6$ % of their potassium.

The fact that the exchange never quite reached 100 % was probably caused by dilution of the K^{42} with inactive potassium, from the nerves themselves or present as an impurity in the sodium chloride used in making up the Ringer, either of which might well have reduced the specific activity by 1 %. Although it remains possible that there is a trace of tightly bound potassium, forming not more than 3 % of the whole, in the nerve, there can be no doubt that it is justifiable for the present purpose to assume that all the potassium in *Carcinus* nerve can exchange readily with K^{42}.

The resting potassium flux in 30 μ. fibres

In attempting to determine the absolute value of the potassium flux in whole nerves, the chief sources of error were the wide range of fibre sizes, the rapid deterioration in condition of the fibres, and the possibility that exchange

TABLE 5. The resting exchange of K^{42} in 30 μ. *Carcinus* fibres. Figures bracketed together are independent measurements on different bundles of fibres from the same crab. The potassium content of the fibres was taken as 255 mM. The average half-time corresponds to the average of the exchange constants, and is not the average of the individual half-times. Temperature 14–19° C. The standard errors of the exchange constants were calculated from the observed scatter of the counts

Exp. no.	No. of fibres	No. of action potentials	Mean diam. (μ.)	Half-time of K^{42} loss (hr.)	Exchange constant ($hr.^{-1}$)	Calculated outward potassium flux ($mol./cm.^2/sec.$)
53	6	6	35·9	2·21	$0{\cdot}31 \pm 0{\cdot}02$	$19{\cdot}7 \times 10^{-12}$
56	6	5	33·1	2·36	$0{\cdot}29 \pm 0{\cdot}03$	17·0
57A	6	6	35·1	1·69	$0{\cdot}41 \pm 0{\cdot}06$	25·5
57B	6	5	34·5	1·69	$0{\cdot}41 \pm 0{\cdot}03$	25·0
60A	5	5	36·1	2·17	$0{\cdot}33 \pm 0{\cdot}01$	21·1
60B	5	4	36·5	2·33	$0{\cdot}30 \pm 0{\cdot}02$	19·4
65A	9	8	34·6	1·96	$0{\cdot}35 \pm 0{\cdot}02$	21·4
65B	7	7	32·9	2·51	$0{\cdot}28 \pm 0{\cdot}03$	16·3
68B	4	4	38·2	1·41	$0{\cdot}49 \pm 0{\cdot}07$	33·1
68C	5	4	31·6	1·36	$0{\cdot}51 \pm 0{\cdot}05$	28·5
78B	5	5	27·1	1·73	$0{\cdot}40 \pm 0{\cdot}03$	19·2
Average			34·1	1·85	0·37	$22{\cdot}4 \times 10^{-12}$

was slowed by diffusion effects. These drawbacks could largely be overcome by working with isolated 30 μ. fibres, whose diameter and action potential could be individually examined, and which were virtually in direct contact with the surrounding medium. Table 5 shows the results of eleven determinations of the

resting exchange constant for K^{42}. The later experiments (from Exp. 65A onwards) were primarily concerned with the loss of K^{42} during activity, and the values of k given in the table are those worked out from the first periods of resting leakage, before the fibres had been stimulated. The exchange constants were all calculated by the method of least squares, on the assumption that there was a linear relationship between t and $\log_e Y$ over the period of the measurements. It was also assumed that the weight of each value of $\log_e Y$ was the same, which would only have been perfectly true if the total number of disintegrations recorded in each counting period had been constant. But the length of the counting periods was generally somewhat increased as the counting rate fell, so that the assumption of constant weight cannot have led to any great errors. The standard errors of the values of k could be derived in two ways—either from the scatter of the observed results, or from the fact that the standard error of each determination of the counting rate was simply the square root of the total number of disintegrations recorded, divided by the counting time. The figures given in the table were calculated from the observed scatter, but the other method gave the same answers to within about 0·01 hr.$^{-1}$ in every case. This confirms that the principal source of uncertainty in the determination of k was the statistical variation in the counting rates due to the random nature of the radioactive disintegration process. The standard errors ranged from ± 3 to $\pm 15\%$ of k, with an average value of $\pm 9\%$. The exchange constants of two sets of fibres from one crab were always nearly equal, but this may have been accidental, and too few figures were available to make it worth applying a statistical test. It is also clear from the sizes of the standard errors that there was a real variation in the values of k for the different bundles of fibres.

The potassium content of 30μ. fibres was investigated by a method similar to that used to determine the extent of potassium exchange in whole nerve. Fibres from two crabs were soaked in K^{42} Ringer for periods, including the time taken for dissection, of between 9 and 12 hr., with an average of 10·6 hr. They were then mounted on forceps singly or in pairs in order to test their excitability; all gave good action potentials. After their lengths and diameters had been measured they were rinsed for 1 min. in inactive Ringer and put on standard mica slips so that their radioactivity could be compared with that of the Ringer. The average amount of potassium which had been exchanged in nine samples (a total of 16 axons) was 206 ± 14 m.mol./l. axoplasm, with extreme values of 154 and 266 m.mol./l. These fibres were evidently more nearly in a steady state than the smaller fibres of whole nerve, the rate constant for net leakage being of the order of 0·05 hr.$^{-1}$. The exchange constant for loss of K^{42} was, from Table 5, 0·37 hr.$^{-1}$, so that the rate constant for K^{42} entry was about 0·32 hr.$^{-1}$. In 10·6 hr. 97% of the internal potassium should therefore have been exchanged, making the estimated value of C_i 212 m.mol./l. Another similar but less reliable experiment was done, in which some of the fibres were

soaked for shorter periods in K^{42} Ringer, so that larger corrections had to be made for the incompleteness of exchange. The result was, nevertheless, almost the same, C_i being estimated as 221 m.mol./l.

The fibres were too small for any chemical analyses to be made of their potassium content, so that no direct comparisons could be made between the K^{42} exchanged and the total potassium in the axoplasm. From the analyses of freshly dissected whole nerve the initial potassium content of the axoplasm was estimated as 342 mM., so that in 10·6 hr. the 30 μ. fibres must have lost about 130 mM. of potassium, which corresponds to a net leakage of 12 mM./hr. The measurements of k listed in Table 5 were made on average 7 hr. after beginning the dissections, so that in order to calculate the absolute size of the outward potassium flux C_i was taken as 255 mM. This leads to an average result of 22×10^{-12} mol./cm.^{2}/sec. at a temperature of 17° C. A net leakage of 12 mM./hr. in a 34 μ. fibre would correspond to a net outward potassium current of 3×10^{-12} mol./cm.^{2}/sec., so that the inward potassium flux was about 19×10^{-12} mol./cm.^{2}/sec. The net outward potassium current was thus only about one-seventh as great as the flux of ions moving in either direction.

The temperature coefficient of potassium exchange

The temperature coefficient of the potassium flux in whole nerves was determined by measuring k first at room temperature, then 10° C. lower, and finally at room temperature again. In two of the experiments the order of temperature changes was reversed. The first and last values of k were averaged in order to reduce as far as possible any systematic errors due to variation of k with time, and the Q_{10} was then calculated. The average result from six experiments was $2{\cdot}18 \pm 0{\cdot}13$.

Five determinations of the Q_{10} were made for 30 μ. fibres. Only two measurements of k were made in each experiment—one at room temperature, and another about 10° C. lower. The exchange constant should vary less with time than for whole nerves; any decrease during the experiment would make the result too high. The average result was $1{\cdot}70 \pm 0{\cdot}21$.

It would be unwise to attach too much significance to these results, since it was impossible to determine the Q_{10}'s very accurately, chiefly because the standard error of k at the lower temperature was always rather large. Application of a t-test shows that the coefficients for 30 μ. fibres and for whole nerve do not in fact differ significantly ($P = 0{\cdot}08$).

Radiation effects

Some of the experiments with 30 μ. fibres required K^{42} samples of high specific activity, and since the fibres were soaked in K^{42} Ringer for several hours they received appreciable doses of radiation. On several occasions the radioactivity of the Ringer was about 4 mcuries/l. Mullins (1939) found that the rate of

penetration of Na^{24} into *Nitella* cells was reduced if the radioactivity of the medium exceeded 1 mcurie/l. It was therefore necessary to make sure that the irradiation undergone by the *Carcinus* nerves did not affect them adversely.

Pairs of nerves soaked in dishes of K^{42} Ringer with different specific activities showed no appreciable difference afterwards in their excitability or rate of loss of K^{42}, but only large effects could have been detected. A better test was provided by irradiating nerves with 218 kV. X-rays for an hour while they were soaked in K^{42} Ringer, and then comparing their action potentials and exchange constants with those of controls. Even a dose of 2000 r. had no apparent effect. It can be calculated from the formula given by Evans (1944) that 4 mcuries/l. of K^{42} β-radiation corresponds to a dose rate of about 10 r./hr., so that the intensity of radiation in all the experiments with K^{42} was safely below the level at which the nerves might have been damaged. This is confirmed by the finding of Rothenberg (1950) that the penetration of Na^{24} into squid axons was only slightly increased by 50,000 r. of X-rays, although 125,000 r. produced a fairly large effect.

DISCUSSION

There is general agreement that nerve membranes are relatively permeable to K^+ ions, and that the resting membrane potential is at any rate partly due to the difference in potassium concentration between the inside and outside of the fibres (Ussing, 1949). It follows that there must be a dynamic rather than a static equilibrium across the membrane, and the existence of a continuous flux of K^+ ions, passing in and out through the membrane, which these experiments reveal, is therefore not unexpected. The evidence presented here is consistent with the view that potassium is able to traverse the membrane passively, with no driving forces other than concentration and potential differences.

As far as whole nerve is concerned, it is not possible to support this assertion by any quantitative arguments, but the observations on 30μ. fibres enable a useful comparison to be made between potassium flux and membrane resistance. The electrical conductance of the surface membrane depends on the ease with which ions can pass through it, and is therefore directly related to the ionic flux as measured with radioactive tracers. The current which must be drawn through the membrane when the conductance is measured is carried by several different types of ion, whose contributions to the total conductance depend on their separate permeability coefficients and concentrations. A complete correlation between conductance and flux cannot be made until the fluxes of all the ionic species have been determined, and until it has been decided whether the whole of the flux in each case is due to the passage of free ions. A sodium flux due to the operation of an exchange diffusion mechanism of the type proposed by Ussing (1949) would not, for example, contribute to the conductance, and a sodium pump mechanism or sodium entering by the

type of carrier system discussed by Hodgkin, Huxley & Katz (1949) might even contribute a negative conductance. In the meantime, Hodgkin & Katz (1949) suggested from their data on squid axons that about half the membrane conductance is due to K^+ ions, and it is worth seeing whether this is also true for *Carcinus* axons, which may reasonably be expected to behave in a similar way since, as has been shown here, the ionic concentration differences across the membrane are roughly the same.

It can be shown that for a fibre in ionic equilibrium with its surroundings

$$m_K = \frac{RT}{F^2} G_K,$$

where m_K is the resting potassium flux determined with K^{42}, and G_K is the component of the membrane conductance due to current carried by K^+ ions. The derivation of this expression, which is due to Hodgkin & Huxley (1951), will be discussed more fully in a later paper (Keynes, 1951*b*). It has a general validity in that it can be derived without making any assumptions as to the distribution of electric field across the membrane, but it applies only to the movements of ions along electro-chemical gradients, and is not valid, except in certain circumstances, for the passage of substances in combination with a carrier. No excised tissue is quite in a state of ionic equilibrium, but for isolated *Carcinus* fibres it has been seen that the net outward potassium current was only one-seventh of the total resting potassium flux, so that in this case the assumption that there is an ionic steady state will not lead to any serious discrepancies.

At 17° C. the value of RT/F is 25 mV., and RT/F^2 is therefore $2{\cdot}6 \times 10^{-7}$. The resting outward potassium flux in 30μ. *Carcinus* fibres was 22×10^{-12} mol./cm.^{2}/sec., so that

$$G_K = \frac{22 \times 10^{-12}}{2{\cdot}6 \times 10^{-7}} = 8{\cdot}5 \times 10^{-5} \text{ mho/cm.}^2.$$

The membrane resistance of 30μ. fibres was estimated by Hodgkin (1947*a*) as 7500 ohm.cm.^{2}, corresponding to a total conductance of $13{\cdot}3 \times 10^{-5}$ mho/cm.^{2}. Katz (1948) found a rather higher conductance for the same fibres, his average value being $30{\cdot}6 \times 10^{-5}$ mho/cm.^{2}. The calculated figure for the potassium conductance therefore lies between 0·28 and 0·64 of the total conductance. Although lack of information about the fluxes of other ions makes it impossible to extend this comparison any further at present, it is at least satisfactory to find that the potassium flux is of the order expected from electrical measurements. The similarity of the effects of external potassium concentration on membrane conductance (Hodgkin, 1947*b*) and on potassium flux also supports the idea that K^+ ions make a large contribution to the conductance.

The finding of greatest practical interest was that there were no large

quantities of tightly bound potassium in crab nerve. This already seemed unlikely from considerations of osmotic balance (Boyle & Conway, 1941) and of the sizes of resting and action potentials (Hodgkin & Katz, 1949), which suggest that all the intracellular sodium and potassium in nerve and muscle must be freely ionized. Moreover, the intracellular potassium would probably exchange fairly rapidly with intracellular K^{42} ions even if it were combined with an organic compound. Thus in bacteria, which appear to retain their potassium in chemical combination rather than by a selectively permeable membrane, the internal potassium exchanges completely with K^{42} in a few minutes when the cells are metabolizing normally (Roberts, Roberts & Cowie, 1949; Eddy & Hinshelwood, 1950). Potassium combined in this way would not behave as 'bound' potassium in the sense intended by those who have found an incomplete exchange of K^{42} in tissues. In any case, the experiments of Rothenberg (1950) on squid axons, which are the only evidence of an incomplete exchange of potassium in invertebrate nerves, are not really conclusive. It is obvious from the theory of exchange discussed earlier that in a cell for which the outward potassium flux exceeds the inward flux, the total radioactivity taken up in a medium containing K^{42} ceases to increase when the ratio of internal to external specific activity becomes equal to the ratio of inward to outward flux, since the cell is then gaining and losing K^{42} at equal rates. In whole *Carcinus* nerves, for example, it is shown by Text-fig. 2 and Table 4 that the total K^{42} content reached a peak when exchange was only about half completed, so that the outward potassium flux must have been about twice as great as the inward flux. In squid axons the resting membrane potential is much lower than the theoretical potassium diffusion potential, so that there is a large net leakage of potassium, and the outward flux is probably at least four or five times as great as the inward flux (Hodgkin & Katz, 1949; Keynes, 1951*b*). Rothenberg's report that uptake of K^{42} ceased when only 25 mM. of the internal potassium had been exchanged is therefore not proof that the rest of the intracellular potassium was unable to exchange. Furthermore, he found that when the external potassium concentration was doubled, the axons exchanged over 50 mM. of potassium in 80 min. The curve drawn through the experimental points was made to flatten off at this level, but it is evident from the results that the total K^{42} in the axoplasm was actually still rising. The axons can only have contained about 200 mM. of potassium, since they readily exchanged 180 mM. of sodium, and the sum of sodium and potassium contents of squid axoplasm is less than 400 mM. (Steinbach & Spiegelman, 1943; Keynes & Lewis, 1950). Rothenberg's results are thus not inconsistent with the evidence presented here that all the intracellular potassium is free to exchange with K^{42}.

The only recent evidence for the existence of bound potassium in muscle is that Wesson, Cohn & Brues (1949) found two phases of exchange of K^{42} in

chick embryo muscle cultures. It is perhaps worth suggesting that this may have been due to the presence of cells whose individual time constants covered quite a wide range, rather than to the existence of two intracellular potassium fractions. In experiments involving large numbers of cells there is likely to be some variation in the exchange time constants of individuals, even when they are uniform in size, and it is therefore dangerous to attribute deviations from a perfectly exponential exchange to such factors as the existence of two forms of the substance being investigated, until it has been made certain that the cell population is really homogeneous.

SUMMARY

1. The passage of K^+ ions through nerve membranes was studied under various conditions by measuring the rate of change of K^{42} content in nerves mounted over a Geiger counter in inactive Ringer's solution.

2. In whole *Carcinus* leg nerves accurate interpretation of the results was impossible, owing to the wide range of fibre sizes, and the rapid net leakage of potassium from excised nerves. The rate constant for loss of K^{42} in inactive Ringer was 0·49 hr.$^{-1}$, corresponding to a half-time of 1·4 hr.

3. Analyses showed that whole nerves contained 260 m.mol. K/kg. wet weight and 152 m.mol. Na/kg. The extracellular space was estimated as 0·25, and the initial potassium content of the axoplasm as 342 m.mol./kg.

4. More exact results were obtained by working with bundles of about six isolated 30μ. axons. These had a rate constant for K^{42} loss of 0·37 hr.$^{-1}$. Fibres soaked for 10·6 hr. in K^{42} Ringer exchanged 206 m.mol. K/l. axoplasm. The potassium fluxes were estimated as 22×10^{-12} mol./cm.^{2}/sec. outwards and 19×10^{-12} mol./cm.^{2}/sec. inwards.

5. The rate of loss of K^{42} from whole nerves was roughly proportional to the external potassium concentration, except that in K-free Ringer it was only reduced to about three-quarters of its value in normal Ringer.

6. Parallel determinations of K^{42} exchanged and of total K content in whole nerves soaked for long periods in K^{42} Ringer showed that at least 97% of the intracellular potassium could exchange readily with K^{42}.

7. The Q_{10} for loss of K^{42} in inactive Ringer was $2{\cdot}18 \pm 0{\cdot}13$ (six experiments) for whole nerves, and $1{\cdot}70 \pm 0{\cdot}21$ (five experiments) for 30μ. fibres.

8. Control experiments showed that the nerves were not appreciably affected by the doses of radiation which they received in the course of the experiments.

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