The Effect of Temperature on the Content and Turnover of Sodium and Potassium in Rabbit Nerve

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The maintenance of a high potassium and a low sodium concentration is a feature of many cells. As tracer studies have shown that this is not the result of an impermeability to these ions, it follows that a continuous expenditure of energy is required to potentiate the flux of one or other, if not both, species of ions. It is now well known that interference with metabolism by such means as temperature reduction (Aebi, 1953), poisons (Hodgkin & Keynes, 1953), or in oxidative systems by anoxia (Whittam & Davies, 1953), brings about changes in the rates of turnover of the alkali ions, and therefore in the amounts of them present in the tissue.

This study is concerned with the effect of temperature on the Na and K of rabbit nerve, the behaviour of which proves to be qualitatively similar to that of other mammalian tissues.

EXPERIMENTAL

Materials

Pieces of the cervical vagus nerve of the rabbit, weighing about 5 mg. (about 2 cm. in length), were used. The radioactive alkali salts were obtained as bicarbonates from the Atomic Energy Research Establishment, Harwell. These were treated with sufficient HCl to convert the K salt into chloride and the Na salt into a mixture of 25% bicarbonate and 75% chloride.

Methods

The pieces of nerve were provided with cotton loops at their ends so that they could be fitted when desired between two pegs in a flat plastic base which could then be slid beneath an end-window Geiger tube (General Electric type GM-4). In order to follow the uptake of Na or K from solutions containing a proportion of the respective radioactive ion, the tissue was immersed in about 50 ml. of one of the following media: for 0°, HCO₃ 100, Cl 41.6; for 18°, HCO₃ 40, Cl 101.6; for 37°, HCO₃ 30, Cl 111.6; plus in each case Na 142, K 3.6, Ca 1, Mg 0.5, SO₄ 0.5, phosphate 3, glucose 10, all in m-mol./litre. The solutions were stirred by the introduction of O₂ with 5% CO₂. The pH of each mixture was near 7.4. Media containing radioactive ions had an activity at the start of an experiment of approximately 5 μ C/ml.

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At intervals during their exposure to the radioactive media the nerves were removed and washed for exactly 2 min. in about 10 ml. of a non-radioactive solution of similar composition. Following this wash they were fitted on the plastic bases and the radioactivity measured for two periods each of 1 min. The counts, except at the very beginning of the experiment, were some thousands per minute. Following the assay of radioactivity the specimens were returned to the labelled soaking solutions.

In order to follow the loss of radioactivity from specimens previously treated in a radioactive solution it was only necessary to use a large volume (relative to the volume of tissue, i.e. 30 ml.) of non-radioactive solution and measure the residual activity in the tissue at intervals; no wash was given.

At the end of either uptake or loss experiments the specimen was washed for exactly 2 min. in about 10 ml. of fresh isotonic (0.32M) sucrose solution and then was dissolved in 0.1 ml. of nitric acid which had been distilled in silica vessels. A sample of the diluted solution of the nerve was taken for assay so that a direct comparison could be made of its activity with that of a dilution of the radioactive treatment solution. Then by proportion it was possible to estimate the quantity of radioactive Na or K derived from the original solution which was present in the nerve at the time it was dissolved.

Radioactivity measurements were corrected for blank, decay, and resolution time. Analyses for Na and K were carried out on the digestion residues from the nitric acid solutions by dissolving them in water and using a flame photometer. The flame photometer was of our own construction, but was similar in principle to that described by Domingo & Klyne (1949). Its limit of sensitivity was about 0.03 μ equiv./ml. of either Na or K.

RESULTS

Sodium and potassium contents of nerve

Table 1 shows the results of analyses of nerves taken from a given rabbit, and analysed either directly or after storage. After storage at 0° both the total base content and the fraction of this made up by K are reduced as compared with the quantities found either directly or after storage at 18° or 37°, although even at the higher temperatures the K content of the tissue is reduced by storage below that found in freshly excised tissue. There does not appear to be any difference between the two higher temperatures in these respects. Keeping the nerve at 0° brings about more loss of K than gain of Na;

Table 1. *The effect of temperature on the Na and K contents of rabbit nerve*

Na and K values in $\mu\text{equiv./g.}$ fresh tissue. All nerves rinsed 2 min. in isotonic sucrose. The horizontal lines show analyses for samples from individual rabbits.

Fresh		0°			18°			37°		
Na	K	Na	K	Hours stored	Na	K	Hours stored	Na	K	Hours stored
—	—	64	15	4	61.5	27.5	5	—	—	—
—	—	78	10	7	51	37	7	—	—	—
—	—	—	—	—	73	29	5	65	24	5
—	—	—	—	—	62	25	7	47	25	6
52.5	23.5	—	—	—	51.5	26.5	6	49	16	6
82.5	46.5	61	10	7	65	31	6	—	—	—
—	—	62	7	4	48	24	4	—	—	—
48	40	56	18	23	56.5	26.5	23	—	—	—
70	35	70.5	15.5	5	—	—	—	73.5	31.5	5
46	35	67	11	21	65	24	22	73	31	7
52.5	50.5	78	10	4	70	29	4	58	36	4
Av. 59	38.5	67	12	—	60	28	—	61	27	—
S.D. 14	10	8	4	—	8.5	4	—	11.5	7	—

Fig. 1. The time course of the uptake of Na into a rabbit nerve incubated at 18°, from a saline medium containing a proportion of ^{24}Na . The nerve was rinsed for 2 min. in a non-radioactive solution before counting in order to remove adhering drops of radioactive medium.

thus at 18° the mean K content is 28 $\mu\text{equiv./g.}$, and the mean Na content 60 $\mu\text{equiv./g.}$ Cooling causes the K to fall to 12, but the Na only rises to 67. These changes are not associated with any large or consistent weight alteration.

The effect of the wash in sucrose solution, besides removing adhering drops of saline solution, is to remove one-third of the total Na of the tissue, and about 0.5 $\mu\text{equiv./g.}$ of K. The loss of intracellular material, judged from the K analyses, is thus negligible, since this amount of K would be expected to come from the extracellular phase.

Table 2. *Movement of sodium in nerve*

Influx was measured directly from a plot similar to that of Fig. 1, by drawing a tangent to the curve at the 2 hr. point. From the flux so obtained the first-order rate constant k_1 was calculated from the relation: $\text{flux} = k_1 [\text{Na}_0]$. The rate constant k_2 governing efflux was obtained directly from a logarithmic plot similar to that of Fig. 2.

Influx			
0°		18°	
Observed flux ($\mu\text{equiv./g./hr.}$)	Calc. k_1 (hr.^{-1})	Observed flux ($\mu\text{equiv./g./hr.}$)	Calc. k_1 (hr.^{-1})
6.3	0.045	8.5	0.060
6.4	0.045	4.7	0.034
5.9	0.042	4.6	0.034
Av. 6.2	0.044	5.9	0.042
Efflux			
Observed k_2 (hr.^{-1})		Observed k_2 (hr.^{-1})	
0.26		0.53	
0.41		0.92	
0.46		0.72	
0.29		0.77	
0.65		0.91	
0.15		0.44	
Av. 0.37		0.72	

Movement of sodium

When the nerve is immersed in saline medium containing ^{24}Na ions it takes up the radioactive material; the quantities found after successive times of soaking (all measured after a 2 min. wash) fall on a curve similar to that of Fig. 1. The quantity increases rapidly at first, and then more slowly. After 2 hr. the faster turnover processes seem to have become substantially complete: the rate of the residual slow process has been estimated by

drawing tangents to the curves at the 2 hr. point, and the results are set out in Table 2. The results are expressed both as the rate of Na entry (turnover) per g. of tissue and as the first-order rate constant obtained by dividing these figures by the external Na concentration ($142 \mu\text{equiv./ml.}$). In all the present experiments it has been assumed that it is permissible to apply first-order kinetics to the results. Strictly the rate of gain per unit volume of cell fluid, or better, per unit area of cell surface, should be computed, but in the absence of data to provide the requisite conversion factors it has been necessary to express the figures per g. of tissue. The rate constant referring to unit volume of cell water will be higher than the figure in Table 2 by the inverse of the proportion of cell water in the tissue.

The values of the rate of Na uptake do not vary consistently with temperature, and the means of three paired experiments are nearly equal at the two temperatures (0° and 18°).

When a nerve is kept 7 hr. or longer in ^{24}Na -labelled saline, the content of ^{24}Na corresponds to complete equilibration of the Na of the tissue with that in the solution. Thus there is no suggestion of there being inexchangeable Na.

The time course of the loss of activity to a non-radioactive solution from a nerve previously treated for 4 hr. with a solution containing ^{24}Na is illustrated in Fig. 2. The logarithmic plot can be analysed into three parts in the case of an 18° experiment. The respective rate constants applying to these are: >10 , 1.88 and 0.86 hr.^{-1} . It must be stressed that such complex behaviour admits of a number of

interpretations, which are considered in the discussion. The fraction with the intermediate time constant cannot be distinguished at 37° , perhaps because its rate is less temperature dependent than is the rate of the slow process. The results given in Table 2 (second part) are for the rate constant applying to the movement of the slow fraction, and these are found from the slope of the logarithmic plots. By extrapolation of the lines relating to exit of the slow fraction back to $t=0$ it is possible to estimate the quantity of Na so moving: this came to $7.5 \mu\text{equiv./g.}$ (mean of 4). This figure is close to the one obtained by balancing inflow against outflow at 18° . We have: slowly moving Na inflow $= 5.9 \mu\text{equiv./g./hr.}$, outward rate constant 0.72 hr.^{-1} , therefore slowly exchangeable Na $= 5.9 / 0.72 = 8.2 \mu\text{equiv./g.}$

At 0° the analyses show that the tissue gains Na, and as the mass does not change appreciably, it is unlikely that it is due to changes in the extracellular fluid proportion. Experimentally it is found that the outward rate constant at 0° is only 0.37 hr.^{-1} , while the inflow is about the same as at 18° . Thus the slowly moving Na content will come up to a level of $15.2 \mu\text{equiv./g.}$ ($= 8.2 \times 0.72 / 0.37$) to regain the steady state. Since this level is $7 \mu\text{equiv./g.}$ above the 18° value, it would be expected that cooled specimens after some hours storage would contain this additional amount of Na, which is in agreement with the results in Table 1.

Movement of potassium

Measurements of K uptake and loss were made by methods similar to those used in the Na experiments. Table 3 (first part) summarizes the results obtained for the K inflow into unit mass of nerve at 0° and 18° , and it will be seen that temperature has a large effect. The rate constants were found as before by division of the K inflow by the external K concentration ($3.6 \mu\text{equiv./ml.}$), again assuming the applicability of first-order kinetics to this system. Again the rates refer to a time 2 hr. after the beginning of the experiment. The uptake of K is more linear with respect to time than is Na uptake, so that the selection of the 2 hr. point does not imply that other more or less mobile fractions are ignored.

When the rate constants governing the outflow of labelled K are measured the unusual result is found that at 0° the rate constant is about twice that holding at 18° (Fig. 3 and Table 3, second part). At 18° , if one equates the known rate of uptake to the rate of loss, according to the steady state which the analyses in Table 1 indicate pertains at this temperature, then the content of exchangeable K per g. of tissue can be calculated, for the rate constant governing the efflux is known. That is, inflow ($2.8 \mu\text{equiv./g./hr.}$) = efflux ($0.31 \text{ hr.}^{-1} \times \text{content of}$

Fig. 2. The time course of the loss of Na from nerves at 18° (○—○) and 37° (●—●). The nerves had been previously incubated in radioactive medium until a steady level of tissue radioactivity corresponding to complete exchange of the tissue Na with the ^{24}Na of the medium had been attained.

exchangeable K). This content comes to $9.1 \mu\text{equiv./g.}$, and is much less than the K content indicated by analysis ($28 \mu\text{equiv./g.}$). Since the K found in the analyses includes about $1 \mu\text{equiv./g.}$ of extracellular K, taking the space as 50% by volume, the proportion of exchangeable K in the cellular fraction is only $9.1/27 = 34\%$. The corresponding ratio for total K is 36%. This result, which resembles those found both for mammalian sympathetic ganglia

(Harris & McLennan, 1953) and for frog muscle (Harris, 1953), was confirmed by another method. Nerves were kept at 18° in the radioactive solution for 24 hr., and it was then confirmed that no further increase in labelled K content was in progress; at this point the ratio of labelled K derived from the solution to total K in the tissue was 36%. That an important net loss of K was not in progress was shown by the fact that the K content found analytically in these stored nerves was as high as in specimens stored for a much shorter time (Table 1).

At 0° K uptake drops to $0.6 \mu\text{equiv./g./hr.}$, and from this and the observed rate constant governing K efflux it is found that the content of exchangeable K at 0° in the steady state will be $0.9 \mu\text{equiv./g.}$, which is small compared with the K content of nerve equilibrated at this temperature ($12 \mu\text{equiv./g.}$). This small amount of K moving with a high-rate constant would be expected to be present also at 18° , which does in fact seem to be the case. If a nerve is soaked long enough at 0° it would be expected from these figures that only the $0.9 \mu\text{equiv./g.}$ plus the K in the extracellular space (about $1 \mu\text{equiv./g.}$) would become labelled, making the degree of exchange 16%. In each of three runs lasting 24 hr. the degree of exchange reached was found to be 12%, in fair agreement with expectation.

The respective changes in total and exchangeable internal K brought about by cooling to 0° can now be evaluated. Total K falls from 28 to $12 \mu\text{equiv./g.}$, exchangeable K from 9.1 to $0.9 \mu\text{equiv./g.}$, so that cooling in fact causes a release of $7.8 \mu\text{equiv./g.}$ of the K which is inexchangeable at 18° , together with a loss of $8.2 \mu\text{equiv./g.}$ of the exchangeable fraction. The rate constant determining the loss of the latter fraction was evaluated in a separate experiment in the following way. A nerve was soaked at 18° to constant radioactive K content, corresponding to 36% turnover. It was then cooled to 0° in a medium of the same specific activity. In this way any loss of activity from the tissue represents a net loss of K from the exchangeable fraction. The quantities $*K(t=0) - *K$ (where $*K$ is the tissue radioactivity) were plotted on a logarithmic scale against time on a linear scale, and from the slope the rate constant for this process was found to be 0.19 hr.^{-1} . This rate constant must be carefully distinguished from the one applying to the turnover of the small quantity of exchangeable K which remains eventually; and, it is worth repeating, represents a lower ratio of labelled K to total K than had been attained before changing over to the solution at 0° . In an experiment in which a nerve was first equilibrated with labelled K at 18° and then put in a non-active solution at 0° one would see a composite process made up of a rapid turnover of the $0.9 \mu\text{equiv./g.}$ and slower net loss of $8.2 \mu\text{equiv./g.}$ of the labelled K. The rate constant governing the

Table 3. *Movement of potassium in nerve*

Influx was determined by drawing a tangent to the uptake curve at the 2 hr. point, and from this the rate constant k_1 from the relation: $\text{flux} = k_1 [K_0]$. The rate constant governing efflux was determined directly from logarithmic plots of the type shown in Fig. 3.

Influx			
0°		18°	
Observed flux ($\mu\text{equiv./g./hr.}$)	Calc. k_1 (hr.^{-1})	Observed flux ($\mu\text{equiv./g./hr.}$)	Calc. k_1 (hr.^{-1})
0.3	0.08	2.0	0.56
1.0	0.28	2.2	0.62
0.4	0.12	2.9	0.81
0.7	0.20	4.2	1.17
0.5	0.14	2.7	0.75
Av. 0.6	0.17	2.8	0.78
Efflux			
Observed k_2 (hr.^{-1})		Observed k_2 (hr.^{-1})	
0.72		0.34	
0.61		0.28	
Av. 0.67		0.31

Fig. 3. The time course of the loss of K from nerves at 0° (●—●) and 18° (○—○). The nerves had been previously incubated in a radioactive medium in order to introduce a proportion of ^{42}K into the cells.

net loss of the inexchangeable part ($7.8 \mu\text{equiv./g.}$) has to be determined from analyses made after various times of soaking at 0° . It is not experimentally distinguishable from the constant applying to the net loss of the exchangeable K, so that both these fractions are lost with a rate constant of 0.19 hr.^{-1}

That the failure of much of the nerve K to exchange with labelled K in the solution is not due to a contaminant in the radioactive K preparation is shown by the following facts. The decay period of the radioactivity was correct, and the use of a strong solution of the K (as isotonic K phosphate, pH 7.4) did bring about complete turnover within 1 hr. at both 0 and 18° , even when this treatment was applied to the nerve after it had been 24 hr. in a saline medium. Once the K had been mobilized by the application of phosphate it did not appear to become bound again when the nerve was replaced in a saline solution.

DISCUSSION

The most outstanding result found in this investigation is the fact that reduction of the temperature from 18 to 0° greatly diminishes the rates of movement of both K (4.5-fold) and Na (2-fold) ions against the respective concentration gradients, and in addition it brings about the liberation of much K which at 18° does not turnover with the K in the external solution. Owing to our ignorance of the internal-external potential difference in these myelinated nerves, it is not possible to decide how much of the exchangeable K may be held in the electrical asymmetry, but it seems likely that a large proportion is so held if one may draw conclusions from non-myelinated nerve or muscle. Two fundamental points governing the ionic distribution in the rabbit nerve therefore are: (a) there is an extrusion of Na against gradients of electrical potential and concentration, and (b) much of the K is inaccessible to K ions derived from the external solution. Despite the binding or imprisonment of some of the K, the actual content of base in the nerve is low, so that even when 50 % is allowed for the extracellular space (Manery & Hastings, 1939) and 15 % by weight is deducted for the protein and lipids present (estimated by analogy with other nervous tissue) the total base, i.e. Na + K, concentration in the cell water only comes to $100 \mu\text{equiv./g.}$ This is very different from the sympathetic ganglia recently studied, in which the total base concentration could reach as much as $300 \mu\text{equiv./g.}$ cell water.

The measured rate of movement of the ions might conceivably be determined by the presence of the epineurium. Recently, Shanes & Berman (1953) have found that the rates of movement of certain

substances from the intact frog sciatic nerve are the same as from the lumen of the epineurium. However, the epineurium, if acting purely as a resistive sheath without electrical or chemical asymmetry, would not be capable of retaining a solution with sufficiently high K and low Na to account for the results obtained here. The pronounced effect of temperature on the K influx and Na efflux also is indicative of a chemical process being involved in the movement of these ions. One is led therefore to two alternative proposals: either (a) the epineurium is not a purely passive membrane, which is contrary to most evidence, or (b) some nerve fibres participate in the ionic movements, in which case the observed rates of movement will depend to some extent on the presence or absence of the epineurium and on the tightness of packing of the fibres as well as on the penetration into the fibres themselves.

It seems from the present experiments that the exchangeable K is situated in a compartment in which are found a constant total number of alkali ions, since cooling leads to a roughly equivalent loss of exchangeable K to gain of Na. On the other hand, the release of inexchangeable K does not require an equivalent net gain of Na, so that this K may be associated with some negatively charged group which is also lost at the low temperature. It is not considered that at 0° the rate constant governing the efflux of all of the exchangeable K is increased; but that, a large proportion of this K being lost, a small amount in some more accessible compartment remains, this small amount being present also but largely concealed at the higher temperature.

SUMMARY

1. The contents and rates of turnover of the sodium and potassium of rabbit nerve have been studied at different temperatures.

2. The sodium content rises on storage at 0° , whereas at 18° or 37° the values are similar to those found in fresh nerve. This rise is associated with a decreased rate of efflux of sodium.

3. The sodium of the tissue can be equilibrated with labelled sodium, whereas the nerve potassium will only reach a total turnover of 36 % at 18° in saline solutions. Complete turnover can be obtained by the use of isotonic potassium phosphate solutions.

4. The rate of entry of potassium is diminished by cooling to 0° , whereas the rate of efflux is accelerated. There is thus a considerable net loss of potassium. Half of this net loss is from the fraction which will turnover at 18° , and half from the fraction which does not.

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REFERENCES

- Aebi, H. (1953). *Biochem. J.* **54**, xi.
 Domingo, W. R. & Klyne, W. (1949). *Biochem. J.* **45**, 400.
 Harris, E. J. (1953). *J. Physiol.* **120**, 246-253.
 Harris, E. J. & McLennan, H. (1953). *J. Physiol.* **121**, 629.
 Hodgkin, A. L. & Keynes, R. D. (1953). *J. Physiol.* **120**, 15P.
 Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
 Shanes, A. M. & Berman, M. D. (1953). *J. cell. comp. Physiol.* **41**, 419.
 Whittam, R. & Davies, R. E. (1953). *Biochem. J.* **54**, vii.

The Amino Acid Composition of some Insect Viruses and their Characteristic Inclusion-body Proteins*

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The majority of known insect virus diseases are characterized by the production of immense numbers of inclusion bodies. These are of two main types: polyhedra, each of which contains large numbers of virus particles, and oval 'capsules', each of which contains a single virus particle. These inclusions vary in size and shape with the virus and host insect (Bergold, 1953). After dissolving them in very weak alkali, about 1.5-5% of the weight is recoverable as infectious virus. The remainder consists principally of protein which is homogeneous in the ultracentrifuge, and is of molecular weight varying from 275 000 to 375 000, depending on the host (Bergold, 1947).

There have been few previous analyses of the amino acids of insect viruses or their inclusion-body proteins. Desnuelle, Chang & Fromageot (1943), and Desnuelle & Chang (1943, 1945) reported a partial quantitative analysis of what was thought to be the virus of *Bombyx mori* L. (silkworm), but which later proved to be the non-infectious polyhedral protein of this virus (Bergold, 1953). More recently, Yoshitake, Aruga & Suzuki (1950) made a qualitative analysis of the *Bombyx* polyhedra, and Wellington (1951) made a qualitative comparison of the amino acids of both *Bombyx* and *Cacoecia murinana* Hb. viruses and their respective inclusion-body proteins. In addition, a comparative quantitative study of the proteins of the silkworm virus, its membranes, and its polyhedra has just been concluded (Bergold & Wellington, 1954).

The quantitative paper chromatographic method (Wellington, 1952, 1953) used in this last study is sufficiently sensitive and accurate to permit analysis of minute amounts of virus material for comparison of the results with those of other workers who have

analysed the viruses for other constituents. For example, Wyatt (1952) studied the deoxypentose nucleic acids (DNA) of a number of insect viruses and, although he found no general parallelism between DNA composition and biological relationship, he did find a discontinuous distribution of molar ratios of adenine plus thymine: guanine plus cytosine among the viruses. It seemed worth while to determine whether variations in amino acid composition would bear any relationship to this distribution. It was also of interest to know whether insect viruses would exhibit variations in amino acid composition similar to those reported by Knight (1947) for different strains of tobacco mosaic virus. Qualitative work such as that reported earlier (Wellington, 1951) has never shown any such differences.

In addition to clarifying such relationships, it was hoped that quantitative analyses might also show both the relationship of the virus to its specific inclusion protein, and the interrelation of the various inclusion-body proteins.

MATERIALS AND METHODS

Preparation of the viruses. Viruses were isolated from purified inclusion bodies by dissolving these in very weak Na_2CO_3 followed by centrifuging, as described by Bergold (1947, 1953).

No attempt was made to isolate the protein fractions of the viruses for two reasons, firstly because the amount of material available was so small, and secondly because Wyatt (personal communication) had not succeeded in separating the nucleic acid fraction of *Porthetria dispar* (L.) virus from the protein except by partial hydrolysis of the protein. Therefore it was assumed that the protein-nucleic acid linkage was of a similar firm type for all the insect viruses. In addition the virus preparations all contained virus membranes of the type recently described for the virus of *Bombyx mori* (L.) by Bergold & Wellington (1954).

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