J. Physiol. (1953) 121, 629-637

CATION EXCHANGES IN SYMPATHETIC GANGLIA

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(*Received* 20 *April* 1953)

This study was undertaken with a view to determining whether any differences could be found between normal ganglia and those in which the post-ganglionic fibres had been sectioned and allowed to degenerate. Brown, McLennan & Pascoe (1952) have recently shown that in the latter there is a failure of ganglionic transmission. There have, however, been no previous investigations of the kinetics of sodium and potassium movements in ganglia, and it was necessary to study normal tissue fully before considering the abnormal ganglia. The present paper deals with the results obtained on normal ganglia and the paper which follows it (McLennan, 1953) with those on axotomized ganglia.

METHODS

The superior cervical ganglia of rats, rabbits, cats and dogs were used. The animals were anaesthetized, the ganglia excised as rapidly as possible, placed in a dish of saline medium and the capsule removed with scissors. They were then blotted with filter-paper, and the fresh weight determined with a small torsion balance. The saline medium used had the following composition (mM): Na 142, K 3, Ca 1, Mg 0·5, Cl 101, SO_4 0·5, bicarbonate 40, phosphate 3, glucose 10. Oxygen containing 5% CO_2 was passed through the fluid, giving a pH of 7·4 at 18° C. For experiments at 37° C the bicarbonate concentration was reduced to 20 mM and the Cl increased to 121 to give the same pH when equilibrated with 5% CO_2.

Radioactive K and Na were both obtained in the form of bicarbonate from A.E.R.E., Harwell. Isotonic solutions of K phosphate and NaCl were prepared directly from the bicarbonates by treatment with H_3PO_4 and HCl respectively. ^{42}KCl free from possible contamination by ^{24}Na was prepared by precipitating $KClO_4$ and igniting the precipitate. Radioactive saline media of the composition given above were prepared from these solutions.

To measure the rate of uptake of ^{42}K a whole ganglion was placed in the radioactive medium, the flow of gas ensuring adequate mixing. During this period the radioactivity of the tissue was determined at intervals. For this the ganglion was withdrawn and washed for exactly 1 min in non-radioactive saline in order to remove adhering radioactive fluid. It was then placed on a small nickel dish which could be slid under an end-window Geiger tube. The radioactivity was measured for two 1 min periods, after which the ganglion was returned to the solution.

At such time as the tissue radioactivity, after correction for blank, decay, and resolution time of the counter, had reached a steady level, the ganglion was transferred to non-radioactive medium, and the loss of radioactivity followed. During this phase of the experiment it was not necessary to wash the tissue before assay of the activity, since the bathing solution, which was changed from time to time, had a negligible activity.

At the end of the experiment the ganglion was dissolved in a few drops of nitric acid, water added, and an aliquot of this solution counted for radioactivity. The activity was compared with that of a dilution of the radioactive medium used in the first half of the experiment, so that it was possible to relate the number of counts/min of the tissue to μequiv of K derived from the solution.

The volume of medium used was always at least 500 times the volume of the ganglion, so that the specific activity of the K of the solution remained essentially unchanged during the course of an experiment. In a long experiment it was, however, changed at least once.

Na exchange was measured in an identical way.

Na and K analyses were carried out on aliquots of a solution obtained by heating to dryness the nitric acid solution of the ganglion, and taking up the residue in water. These analyses were performed with a flame photometer. Chloride estimations on fresh ganglia were done by a slightly modified Volhard method.

TABLE 1. Analyses of fresh ganglia

	Rats			Rabbits			Average (±S.D.)
K	122[1]	140[2]	118[3]				
	97[4]	81[5]	101				
	100	72	81				
	133	59	59	72			90±25
	59	69	98				
	83	83					
Na	70[1]	104[2]	82[3]				
	79[4]	68[5]		—			81±14
Cl	43	84		112	100	86	92±29
				128			

Values are given in μequiv/g.

The superscripts identify particular ganglia in which both Na and K were determined.

RESULTS

Na, K *and* Cl *contents of freshly excised ganglia*

Analyses of the total Na, K and Cl contents of the superior cervical ganglia of rats and rabbits are shown in Table 1. The high values found for Na and Cl, if these ions are assumed to be largely extracellular, indicate that the extracellular fluid in this tissue comprises 50–60% of the weight. Histological estimation of the fraction of the total volume of the ganglion occupied by cell bodies and processes has been made on three rabbit ganglia, the average being 33%, so that the extracellular space determined in this way is at least as much, if not more than that calculated from the chemical analyses. The extracellular space in other nervous tissue has been reported as 40% (Manery & Hastings, 1939). Dry-weight determinations have shown that solids comprise about 15% of the fresh weight of the ganglion, and therefore the intracellular K concentration may be as high as 250–350 μequiv/g of cell water. The superscripts used to identify particular ganglia in Table 1 show that high K is not invariably associated with low Na, i.e. with a particularly small extracellular space. Values which have been reported for the K contents of the superior cervical ganglia of dogs fall in the range 45–62 μequiv/g fresh tissue (Vogt, 1936).

K *uptake and output* in vitro

If an isolated ganglion is incubated in a saline medium containing radioactive ^{42}K, the radioactivity of the tissue gradually increases until a steady value is attained, usually after about 4 hr. Values obtained in one experiment for the quantity B (see below) were: 22·5% of the tissue K exchanged after 4 hr, 25·2% after 5 hr, 26·0% after 6 hr and 26·0% after 7 hr. If the tissue is then transferred to a non-radioactive medium of similar chemical composition, the loss of radioactivity from the ganglion can be followed. Fig. 1 indicates a typical experiment of this type.

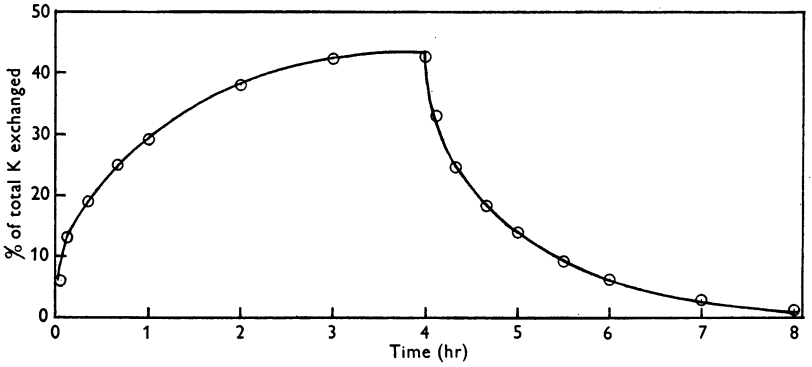

Fig. 1. The exchange of ^{42}K in a rat ganglion. Temperature 17° C. After 4 hr incubation in radioactive saline medium, the ganglion was permanently transferred to non-radioactive saline.

It can be seen that there is a rapid phase, lasting about 20 min, succeeded by a slower phase. Empirically, an equation can be fitted to the uptake curve of the form

$$*K = A[1-\exp(at)] + B[1-\exp(-bt)],$$

and the time course of the loss of radioactivity from the tissue after transfer to an inactive solution at time t_1 is given by Harris (1953):

$$*K = A[1-\exp(-at)] + B[1-\exp(-bt)] - A\{1-\exp[-a(t-t_1)]\} - B\{1-\exp[-b(t-t_1)]\}.$$

The constants for these equations have been determined from a number of experimental curves, and the values are shown in Table 2. A represents the amount of K which exchanges rapidly (i.e. with a time constant 'a'), and is found to be 2–4 times the expected extracellular K in rat ganglia, but about the expected value in ganglia from rabbits.

The quantity B denotes that part of the tissue K which exchanges more slowly, the rate constant 'b' being about 0·7 hr^{-1} for rats and 0·22 hr^{-1} for rabbits at 17° C. In these experiments, where the ganglia have been incubated

in media having a low K concentration, it will be noted that the sum of A and B is usually of the order of 45% of the total K; that is, that a considerable part of the tissue K either exchanges very slowly or is inaccessible to the ^{42}K of the soaking solution. These results are similar to those reported for the exchange of frog muscle K by Harris (1953).

The experiments which have so far been described were all performed at room temperature. When incubation was carried out at 37° C the ganglia usually failed to maintain their K content for a sufficient time to permit the degree of exchange to be measured. In only two cases has it been possible to

TABLE 2. K influx in ganglia

Parameters of the equation $^*K = A[1 - \exp(-at)] + B[1 - \exp(-bt)]$. A and B as percentage of total K; 'a' and 'b' in hr^{-1}.

Animal	Tissue weight (mg)	Temp. (° C)	Length of incubation (hr)	A	a	B	b
Rat	1·8	12·5	5	7	—	37	0·38
	1·7	12·5	3	4	—	34	0·46
	1·0	16	5	14	9	46	0·56
	1·2	17	4	8	10	32	0·72
	1·3	17	4	12	12	25	0·70
	1·5	17	4	12	9	31	0·79
	1·5	37	2	15	—	38	1·70
	1·2	37	3	12	—	41	1·72
Rabbit	7·6	17	4	3	6	47	0·24
	8·2	18	6	1	5	45	0·12
	8·2	18	8	3	7·5	34	0·12
	10·4	18	4	3	5·5	31	0·29
	5·3	19	3	6	8·5	32	0·32
Dog	120	17·5	7	12	4	51	0·12

complete the experiment, when the rate constant 'b' for rats' ganglia was 1·7 hr^{-1} instead of 0·7 hr^{-1}. Although a steady state was more rapidly attained, the fraction of the total K which exchanged did not appear to be increased. At 12·5° C 'b' becomes 0·42 hr^{-1}. These values would correspond to an activation energy for the process of K transfer of about 10,000 cal/mole. Similar activation energies have been obtained by Raker, Taylor, Wheeler & Hastings (1950) and Solomon (1952) for K entry into erythrocytes.

Further evidence in support of the finding that some of the tissue K fails to exchange with the K of a saline medium is given by measuring the rate of efflux of ^{42}K when the ganglion is transferred to a non-radioactive medium. Table 3 shows values for various ganglia of the lower rate constant ('b') which governs the efflux of ^{42}K once the fast-moving fraction has left the tissue. It will be seen that the agreement between these values and those given in Table 2 is excellent. It might be argued that the apparent equilibrium during the soaking-in phase of the experiment is a result of a balance being struck between the influx of the isotope and an efflux of ganglion K so high that the lower specific activity of the cellular K was compensated by the

greater total efflux. This situation has been described for nerve by Keynes (1951). However, it would require in our experiments that the efflux should be so much higher than the influx that a *net* loss of 15% of the total K per hour would occur. As in fact the greatest rate of net loss of K which has been observed is only 3% per hour, the rate constants which have been determined for ^{42}K exchange cannot apply to the total tissue K. That the equilibrium is not due to a gradual decrease in the rate of influx of K has been shown by an experiment in which one of a pair of ganglia was incubated for $3\frac{1}{2}$ hr in non-radioactive medium and then transferred to the radioactive solution. The rate of K influx and the degree of exchange of the tissue K was the same in this ganglion as in the other which was exposed to the radioactive medium from the beginning.

TABLE 3. Rate constants for K efflux

Average values of 'k' at 17° C for the equation $-d{*K}/dt = k{*K}$, where *K is the concentration of radioactive K in the tissue. Number of determinations in parentheses.

Animal	'k' (hr^{-1})
Rat	0·68 (7)
Rabbit	0·23 (3)
Cat	0·17 (1)
Dog	0·13 (1)

Incubation in isotonic K *phosphate*

If a ganglion which has been incubated in saline medium with ^{42}K until the radioactivity of the tissue has reached a steady state is then transferred to an isotonic solution of radioactive K phosphate, there is a further rise in the activity of the tissue until a new steady state is reached after 30–60 min (Fig. 2). This new level of activity corresponds to complete exchange of the tissue K, as shown in Table 4. As in frog muscle (Harris, 1952), some net gain of K has probably occurred as a result of the K phosphate, and the analytical levels determined at the end of the experiment are somewhat higher than normal. Thus the degrees of exchange attained after exposure to the saline medium but before exposure to the phosphate which have been calculated from these analytical values may be too low.

Na *efflux*

Incubation of ganglia in saline medium containing ^{24}Na as a tracer results in a rapid, very large increase in the radioactivity of the tissue. The amount of isotope which is taken up corresponds to complete exchange of the analytically determined Na, 91 ± 24 μequiv/g (S.D., six determinations) calculated from ^{24}Na uptake, compared with 81 ± 14 μequiv/g found in fresh tissue (Table 1). Since the Na of the extracellular space takes part in this exchange, the inaccuracies introduced by washing have made measurements of the rate of uptake of ^{24}Na impracticable. However, the rate of efflux of tracer to an

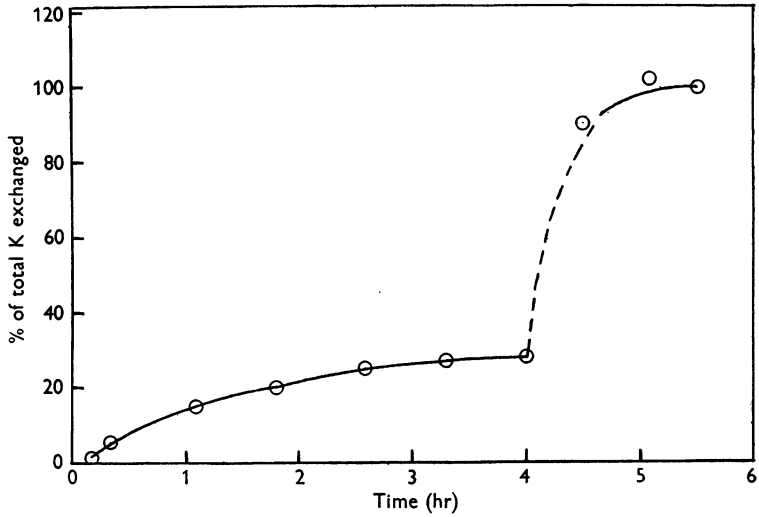

Fig. 2. Effect of incubation in isotonic K phosphate. Rabbit ganglion, temperature 19° C. The ganglion was incubated for 4 hr in radioactive saline medium, and then transferred to the K phosphate.

TABLE 4. Effect of incubation in isotonic K phosphate solution

Animal	Final analytical value (μequiv K/g)	Exchange attained in saline, % of analysis	Time of incubation in saline (hr)	Exchange attained in phosphate, % of analysis
Rat	72	36	5	101
	94	37	3	100
	113	42	4	97
Rabbit	113	35	4	104

inactive solution has been determined, since here there is no necessity to wash the tissue before counting (see Methods). Fig. 3 illustrates the curves obtained for Na efflux for ganglia of rats and rabbits.

The shapes of these curves admit of two alternative explanations: the efflux of Na is either governed by a pure diffusion process with a diffusion constant very much less than in free solution, or several different processes with unequal time constants are taking place at the same time. If a diffusion process is assumed, the values for the diffusion constant which can be calculated from these curves are, for rat ganglia, $0{\cdot}6 \times 10^{-7}$ and $1{\cdot}0 \times 10^{-7}$ cm^2/sec, depending whether the ganglion is considered as a sphere or as a cylinder. The corresponding values obtained for rabbit ganglia are $2{\cdot}4 \times 10^{-7}$ and $4{\cdot}6 \times 10^{-7}$ cm^2/sec respectively, while the value for the diffusion constant of Na in free solution is $11{\cdot}6 \times 10^{-6}$ cm^2/sec at 18° C. Thus, even if all the tissue Na is considered to be contained in the extracellular space, diffusion of Na from the bathing solution into this space is considerably impeded.

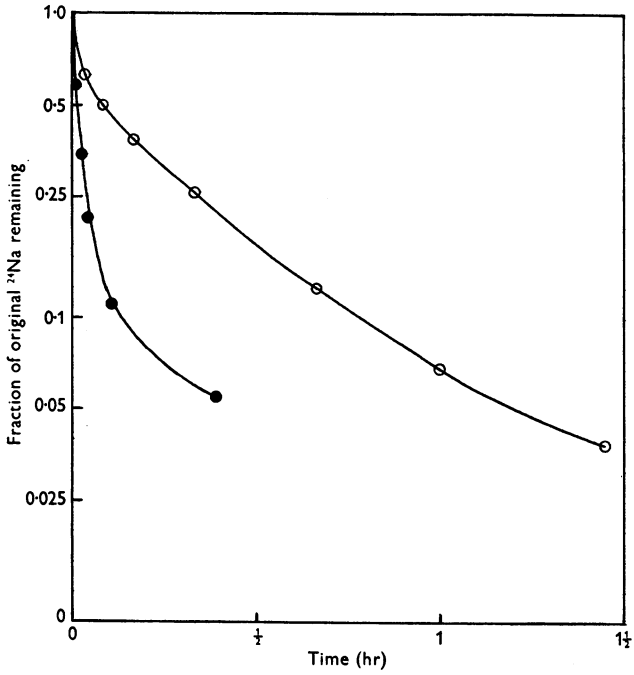

Fig. 3. Na efflux from ganglia. ●—●, rat ganglion, 17° C. ⊖—⊖, rabbit ganglion, 16° C.

DISCUSSION

Our results for the time course of K exchange in sympathetic ganglia show that a first-order equation does not describe the process, as it would if one had to deal with a cell bounded by a membrane setting the limit to the rate of movement of the ion. It is not surprising that a structure so complicated as the ganglion should behave in a complex way, and it proves difficult to say *a priori* what model to use to imitate it. The following considerations must be taken into account:

(1) If there is a freely accessible extracellular space, there will be rapid movement of those quantities of both Na and K which are dissolved in the medium surrounding the cells. It has been shown, however, that the rate of Na efflux is only of the order of 1/50th of the rate of diffusion of the ion in free solution, and a similar relationship holds for that part of the K which undergoes rapid exchange. Thus the extracellular fluid, although of composition similar to the bathing solution, may be contained within a system whose geometry imposes a diffusion constant less than that expected from the linear dimensions of the tissue, making the time course of its equilibration slower than might have been expected.

(2) The total of Na and K per g of ganglion exceeds the total concentration of these ions in the bathing solution. It is necessary to try to decide whether some cells contain high concentrations of both Na and K, or whether an important degree of adsorption of these ions on the external surfaces of the cells takes place. This can only be done by the technique of radio-autography.

(3) The extent to which the total tissue K will exchange with the K of the saline medium which bathes it is of the order of 45% in periods up to 7 hr. The major part of this K exchanges with a time course obeying an exponential equation, and could represent the rate of penetration of K through a restrictive membrane, although the diffusion curve after sufficient time also approaches an exponential form. Thus the lack of complete exchange might well be due to the limitations imposed by diffusion within the ganglion cells or in the extracellular space. In this connexion it is to be noted that the time required for 80% saturation of a sphere is six times that required for 40% saturation, so that, other things being equal, the larger ganglia of the dog and the rabbit would be expected to show a slower exchange of both extracellular and intracellular K. However, if the cessation of the exchange process at the 40–50% level quoted in Table 2 were due to failure of the tissue metabolism, it would be a surprising coincidence that all the ganglia examined should fail so completely at the same degree of turnover. The effect of K phosphate solutions in causing complete exchange might be due to the greatly increased number of ^{42}K ions diffusing into the tissue, which would thereby increase the rate of turnover within the cells, but from the results of the present experiments it is impossible to decide finally between permeability into the cells and diffusion into the recesses of the ganglion and within the cells themselves as the factor limiting the rate and amount of exchange of K.

A possible explanation of the fact that only one-half of the total K will exchange with the K of saline medium may be found by considering the structure of the tissue. If the volume of the cell bodies and of the nerve fibres in a ganglion is approximately the same, then clearly exchange in the latter is likely to be more rapid than in the cells themselves. It may be that the cells are only very slowly permeable to K, and that the turnover measured in these experiments is that of the K of non-cellular components of the tissue.

SUMMARY

1. Analyses of freshly excised sympathetic ganglia for their Na, K and Cl contents have been carried out. The total base in the tissue is greater than that in plasma, and the apparent extracellular space is unusually high (50–60%).

2. The exchange of the K of ganglia has been investigated with radioactive ^{42}K as a tracer. Following a rapid turnover of some 10% of the total K, in saline medium a further 35% only exchanges with first-order kinetics. The

exchange can be made complete by subsequent incubation of the tissue in isotonic K phosphate solution.

3. The rate of Na turnover has been followed with radioactive ^{24}Na. On the assumption that all the Na is contained in the extracellular space, the rate of diffusion of this ion into and within the tissue has been calculated to be only 1/50th of that in free solution.

The expenses of this work have been covered by a grant from the Government Grants Committee of the Royal Society.

REFERENCES

BROWN, G. L., MCLENNAN, H. & PASCOE, J. E. (1952). Failure of ganglionic transmission after post-ganglionic nerve section. *J. Physiol.* **117**, 28*P*.

HARRIS, E. J. (1952). The exchangeability of the potassium of frog muscle, studied in phosphate media. *J. Physiol.* **117**, 278–288.

HARRIS, E. J. (1953). The exchange of frog muscle potassium. *J. Physiol.* **120**, 246–253.

KEYNES, R. D. (1951). The ionic movements during nervous activity. *J. Physiol.* **114**, 119–150.

MCLENNAN, H. (1953). Potassium exchange in ganglia after postganglionic nerve section. *J. Physiol.* **121**, 638–640.

MANERY, J. F. & HASTINGS, A. B. (1939). The distribution of electrolytes in mammalian tissues. *J. biol. Chem.* **127**, 657–675.

RAKER, J. W., TAYLOR, I. M., WHEELER, J. M. & HASTINGS, A. B. (1950). Rates of potassium exchange of the human erythrocyte. *J. gen. Physiol.* **33**, 691–702.

SOLOMON, A. K. (1952). The permeability of the human erythrocyte to sodium and potassium. *J. gen. Physiol.* **36**, 57–110.

VOGT, M. (1936). Potassium changes in the stimulated superior cervical ganglion. *J. Physiol.* **86**, 258–263.