

## PERMEATION AND DIFFUSION OF K IONS IN FROG MUSCLE

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### ABSTRACT

The movements of tracer K and net changes of K have been measured in frog muscle. The quantities moving can be linearly related to the square root of the time after a delay of 4 to 30 minutes depending on the external K concentration. The slope of the uptake- $t^{1/2}$  line is increased when the external K concentration is raised. The  $Q_{10}$  of the uptake is about 1.9 per unit  $t^{1/2}$ . K uptake from 1 to 2 mM concentration is diminished by a factor of about 2 if strophanthin is applied. The output per unit  $t^{1/2}$  is increased by a factor of about 1.4 by strophanthin. Tetrabutylammonium substituted for 10 per cent of the Na in the medium causes a reversible slowing of K uptake and Na output. The rates of movement found in the tracer experiments can be used to calculate the net losses of K taking place in K-free or strophanthin-containing media. The results are interpreted on the basis of K movement being limited both by a resistive outer layer and by diffusion within a K-rich region. The internal diffusion constant is  $10^{-11}$  to  $10^{-10}$  cm.<sup>2</sup> sec.<sup>-1</sup> depending on the K concentration. The rate of movement of the K can be related to the electrochemical activity of the ion, the lability of the sites on which it is absorbed, and cation + anion pair diffusion within the cell. The surface resistance to K ions can be accounted for as the sum of a membrane resistance equal to that found by electrical methods and the resistance offered to the movement of K by an annulus sufficiently thick (ca. 3  $\mu$ ) to accommodate the cell Na at a density equal to the mean density of cation within the cell through which K diffuses with the same diffusion constant as holds in the K-rich region. Na movement, if assumed to take place by diffusion from the annulus with diffusion constant equal to that for K ions, has a rate which agrees well with observed values. The influence of strophanthin and tetrabutylammonium on the ion movements is interpreted as being the result of these agents causing an expansion of the outer non-selective region, normally occupied mainly by Na, at the expense of the inner K-rich region.

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Movement of tracer K between tissue cells and a solution does not follow a simple law. If the major barrier to movement were a thin membrane enclosing freely diffusible cellular K the process would follow a first order law; that is, the tracer content would be a rising or falling exponential function of time. The law is slightly modified by the delay before the ions reach the interior of the specimen when it is made up of a number of adherent cells as in the

sartorius (Harris and Burn (17)). However, to fit experimental observations it has usually been necessary to postulate at least two fractions of unequal exchange ability (Harris (15); Carey and Conway (3); McLennan (26); Schreiber (28)). The sum of the amounts of K in the fractions does not always equal the total tissue K. In order to relate uptake to output rates it was, for example necessary to suppose that as much as half the K of frog muscle did not exchange with tracer (Harris (15)). The amount which was found to participate in the exchange increased when the external K concentration was raised. It was necessary to invoke a non-exchangeable fraction because tracer output from a loaded muscle was not equal to the rate of uptake divided by the degree of dilution of tracer within the cell. A further difficulty met in giving tracer results a quantitative interpretation has been that the K movement is considerably slower than would be expected from the electrical conductance of the membrane (for figures see Keynes (25)).

The present paper is concerned with tracer K movement in frog muscle and its modification by certain agents. It will be shown that uptake and output can be related without assuming inexchangeable fractions. The tracer results can be shown to agree with the analytical results obtained when net changes are induced either by poisoning or by omission of K from the medium.

A factor which has not previously been taken into account in permeability studies is the rate of internal mixing. That the tracer fails to become uniformly mixed with the tissue K has been shown by Harris and Steinbach (20). If internal diffusion is slow compared with the rate of passage through the cell membrane diffusion will limit the rate of tracer movement and impose its own time course on the process. The ion movement will not measure exclusively a membrane property but will also depend on the internal diffusibility; it is on this basis that the results are interpreted. The membrane permeability and the internal diffusion constant have been evaluated under various conditions.

It is to be noticed that the diffusion equation involves a series of exponential terms so that the occurrence of diffusion-limited movement is kinetically indistinguishable from movement from several compartments each governed by a first order law.

#### *Methods*

*Tracer Experiments.*—The tracer experiments were made exclusively upon sartorii from *Rana temporaria*. The tracer K was obtained in the form of  $\text{KHCO}_3$ . The solution used in the experiments had the following composition: NaCl 90 mM,  $\text{NaHCO}_3$  30 mM,  $\text{CaCl}_2$  2 mM,  $\text{MgCl}_2$  1 mM, and KCl 2 mM or an amount specified in the text. It was made up freshly before use and bubbled with 95 per cent  $\text{O}_2$  + 5 per cent  $\text{CO}_2$  during the experiments.

Uptake of tracer K was followed as described by Harris (15). The muscle was immersed for a period in the tracer solution, then given a wash (1 minute) in normal mixture before being put beneath a Geiger counter for assay of the radioactivity.

After counting it was returned to the radioactive solution for further periods. At the end of the experiment the muscle was extracted in warm water. The activity in the extract was assayed and compared with that of a dilution of the soaking solution. The extract was also used for Na and K analysis.

The output of K from a previously loaded muscle was followed by passing the specimen through a series of small volumes (2 or 3 ml. each) of saline mixture with measured times of immersion. The amounts of tracer collected in successive portions of solution provide a more accurate measure of output than is obtained by measuring the radioactivity of the tissue. The quantity of tracer was adjusted so that measurements were made on samples with count rates of at least several hundred per minute. Sufficient counts were registered to provide a statistical accuracy of better than 3 per cent.

*Analysis.*—The muscle cations extracted in warm water were analysed using an Evans Electro-selenium Ltd. flame photometer. Readings were reproducible to within 2 parts in 100.

#### RESULTS

*Diffusion into a Cylinder with Resistive Coating.*—Before giving experimental results it will be useful to examine the characteristics of the time course of diffusion into a cylinder through a resistive coating<sup>1</sup> because it will be argued that ion movement into the muscle cell is controlled both by the rate of internal diffusion and by the resistance of a surface layer. The appropriate mathematical function is given in the Appendix; it was used to draw the curves in Fig. 1 which relate the average saturation of the cylinder to a parameter containing the square root of the time for a number of values of the ratio: radius  $\times$  surface permeability/diffusion constant. When the surface has a low resistance (*i.e.* high permeability), the curve is slightly convex away from the abscissa; with the product of radius and permeability/diffusibility equal to about 50 the line is nearly straight for the initial 60 per cent of the uptake; with lower values of the product there is an initial concavity followed by a straight part. The intercept of the straight part depends on the parameter (radius  $\times$  permeability/diffusibility) while the slope of the straight part depends mainly, but not entirely, upon the concentration applied and on the root of the diffusibility so long as conditions are fixed. When the permeability is low, the curve approximates to the form which a simple exponential function ( $1 - \exp(-kt)$ ) would have when plotted against  $t^{\frac{1}{2}}$ . The more important the diffusion is relative to the surface permeability the less well can a single exponential fit the combined process. The slope of the curve can be changed either by changing the diffusibility or by making the surface resistance vary with time.

*K Uptake.*—When the uptake of tracer K is plotted against  $t^{\frac{1}{2}}$  (Fig. 2) the curves resemble some of the family of Fig. 1. There is an initial concave portion

<sup>1</sup> A resistive region is one which impedes movement of the substance without having the capacity to accommodate an appreciable quantity of it.

lasting 4 to 30 minutes (2 to 5 units of  $t^{\frac{1}{2}}$ ) followed by a straight part. When uptake becomes sufficient to produce some 60 per cent of the possible exchange (top curve of Fig. 2) there is some sign of deviation from linearity just as there is in the theoretical curves of Fig. 1.

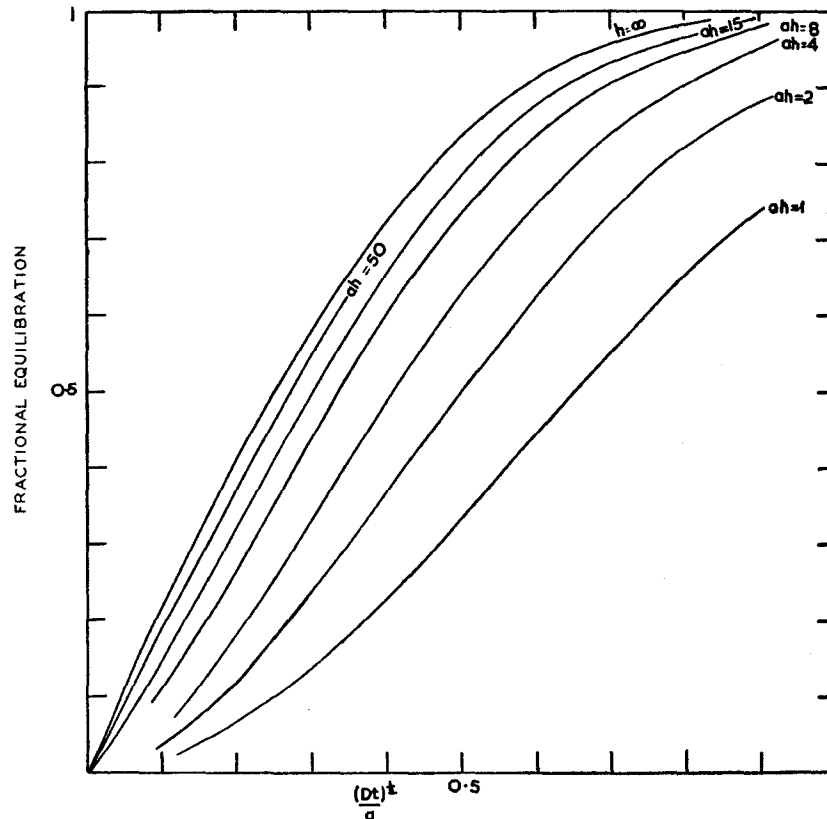


FIG. 1. Theoretical curves for the equilibration of a cylinder, having a resistive coating, with a constant concentration applied to the exterior. The radius is  $a$ , the diffusion constant  $D$ , and the ratio of surface permeability to internal diffusion constant is  $h$ . The curve for  $h = \infty$  is the usual diffusion curve for a cylinder. Note that abscissae are expressed as the parameter  $(Dt)^{\frac{1}{2}}/a$ .

The similarity, though striking, may arise in part from extraneous causes which have to be examined. The uptake curves were plotted using readings made after a 1 minute wash, except in a few special experiments. The wash will remove a little tracer which will make the figures slightly too low. However, it can be seen that in 1 minute very little K moves into the tissue, and experiment shows that in 1 minute very little moves out. Carey and Conway (3)

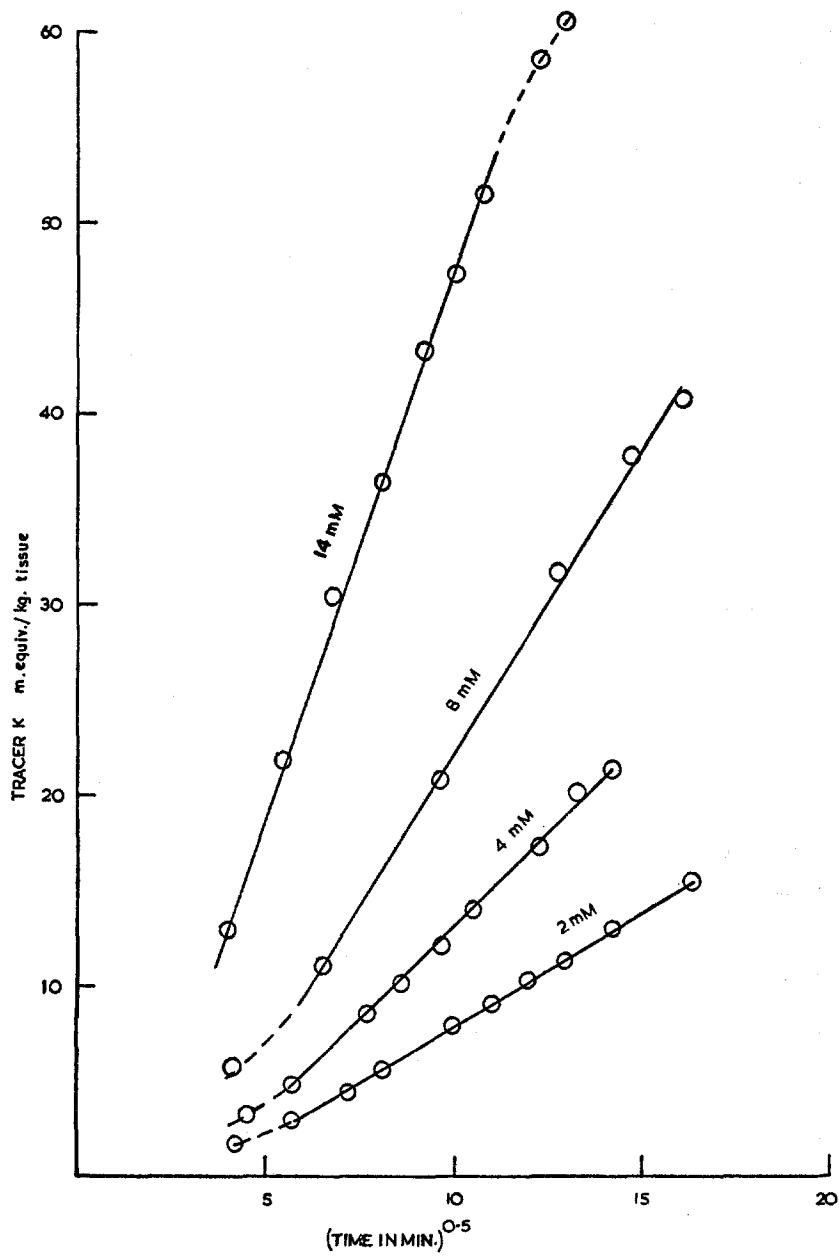


FIG. 2. Tracer potassium uptake from solutions of 2, 4, 8, and 14 mM K concentration plotted against  $(\text{time in minutes})^{1/2}$ . Temperature 20°. Na concentration 120 mM.

have also observed that the exact duration of the wash does not affect K uptake results, which also points to the loss in washing being negligible. A more serious cause of departure of the uptake process from simple kinetics is the delay introduced by extracellular diffusion through the tissue space when a muscle consisting of an appreciable thickness of packed fibers is examined. The mathematics of a diffusion process preceding a penetration of a resistive layer was previously examined (Harris and Burn (17)) but in the present paper it is considered that the intracellular diffusion is so much slower than the extracellular diffusion that the latter is relatively unimportant in this particular example. However, it remains true that it takes some 10 minutes for extracellular movement of K ions to permit restoration of excitability to a sartorius first rendered

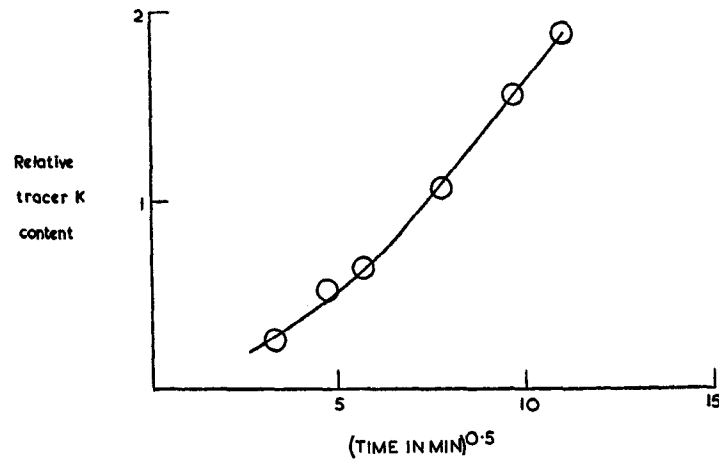


FIG. 2 A. Tracer K uptake by a toe muscle showing that the form of the curve resembles that found when using the sartorius muscle (Fig. 2).

inexcitable by immersion in 20 mM K (Csapo and Wilkie (7)). Hence, with the sartorius, a delay of up to 10 minutes may occur before uptake into the innermost cells takes place; this will contribute to the observed shift of the intercept of the straight part of the uptake- $t^{\frac{1}{2}}$  curve to the right. To exclude that extracellular diffusion is the sole source of the shift an experiment was made on a toe muscle (Fig. 2 A). It appears that uptake into this small muscle (mass 3 mg.) shows relatively as much delay before it becomes linearly related to  $t^{\frac{1}{2}}$  as when the sartorius is used. Since extracellular equilibration of the toe muscle is unlikely to take more than 1 minute it can be concluded that the initial concavity of the uptake- $t^{\frac{1}{2}}$  curve is due to a property of the cells rather than to extracellular diffusion. Results obtained experimentally (Table I) have been fitted to the theoretical curves of Fig. 1 without attempting to apply any correction for extracellular diffusion; presence of this source of error will tend to make estimates of permeability err on the low side.

*Tracer K Output.*—When tracer has been introduced into muscles by exposing them to tracer solutions or by injection into the frog the result of subsequent output experiments made on isolated preparations depends upon the conditions of loading (see for example Schreiber (29)). Exposure of frog sartorii for

TABLE I

*Constants for tracer K Uptake Determined Using Uptake- $t^{\frac{1}{2}}$  Plots*

Uptake ( $\mu\text{e/g}$ ) =  $A t^{\frac{1}{2}} - B$  for  $t^{\frac{1}{2}}$  greater than 5,  $t$  in minutes. Intercept  $\times A = B$ .

(a) With varied external K concentration			
External K	A	Intercept on $t^{\frac{1}{2}}$ axis	B
<i>m.eq./liter</i>	$\mu\text{eq. } t^{-\frac{1}{2}}$		$\mu\text{eq./gm.}$
0.2	0.08	5.6	0.45
1.0	1.12	3.6	4.0 (2 experiments)
	0.80	3.1	2.5
1.5	0.93	1.6	1.5
	0.62	4.0	2.5 (2 experiments)
	0.65	3.8	2.5 (2 experiments)
	0.96	4.2	4.0
2.0	1.0	2.5	2.5
	1.16	2.6	3.0
	1.10	3.6	4.0
3.0	1.56	2.2	3.5 (2 experiments)
4.0	2.0	3.0	6.0
	1.75	2.8	5.0
	2.2	1.6	3.5
8.0	3.2	2.5	8.0
	3.1	1.9	6.0
10.0	4.25	Not measured	
14.0	5.2	1.5	8.0 (2 experiments)

(b) With varied temperature			
External K	A at 0°	A at 20°	$Q_{10}$ of A
1.0	0.28	0.93	1.82
1.7	0.25	0.95	1.95
2.0	0.43	1.20	1.77
		(at 18°)	

some hours to media with 8 to 10 mM K leads to nearly complete exchange of the tissue K (15). This has also been shown by the examination of successive water extracts (20). When an equilibrated muscle is used for an output experiment the uptake of ordinary K must, in the steady state, equal the output measured by the tracer. Hence the amounts of tracer collected during output should have the same time relation as the amounts of K which enter the muscle in an uptake experiment. Fig. 3 shows that the form of muscle tracer- $t^{\frac{1}{2}}$  curves

both at  $0^\circ$  and  $18^\circ$  resembles that of the uptake curves; the lines do not pass through the origin. This latter point serves as additional evidence that the short wash given before each reading during uptake does not give rise to the delay before the onset of the straight portion. No wash is given during output experiments. The lines of Fig. 3 have a steeper slope than those for uptake of K under comparable conditions. This can be attributed to the effect of the raised K concen-

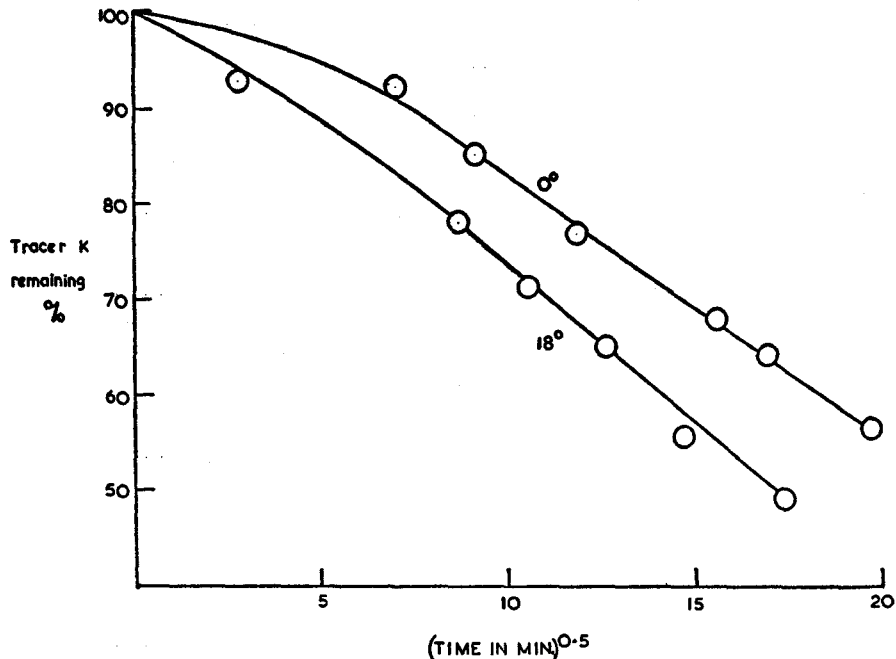


FIG. 3. The output of tracer K from muscles taken from a frog which had received an injection of tracer K on the previous day. Output was compared at  $0^\circ$  and  $18^\circ$ , using a medium with 2 mM K.

tration used in the loading solution. The muscles even at the end of the experiment had 110 to 120  $\mu\text{eq. K/gm.}$  while untreated muscles had about 90  $\mu\text{eq./gm.}$

On account of the possibility of changing the cells' properties by exposure to raised K during loading the relation between tracer output and K concentration is better studied by using muscles which have been loaded for a time in a tracer solution with K concentration equal to that later used during the output (15). Under these conditions the output does not follow a simple law because the degree of exchange of the tissue K is not uniform (20). It is, however, possible to apply the superposition method as used previously with the exponential formulation (15) provided that an equation to fit the uptake is available. The linear parts of the uptake curves of Fig. 2 can be fitted by the



simple relation: Uptake ( $Q$ ) =  $A t^{\frac{1}{2}} - B$  in which  $A$  and  $B$  are constants for a given experiment; the law holds for  $t^{\frac{1}{2}}$  greater than about 5 units. Then, by the superposition method, the amount of tracer in the tissue after loading for  $T$  minutes and unloading for  $t$  minutes will be:  $Q = A(T + t)^{\frac{1}{2}} - A t^{\frac{1}{2}}$  after an interval corresponding to the 5 units before the original relation held. Fig. 4 shows the lines for both loading and unloading in each of two experiments.

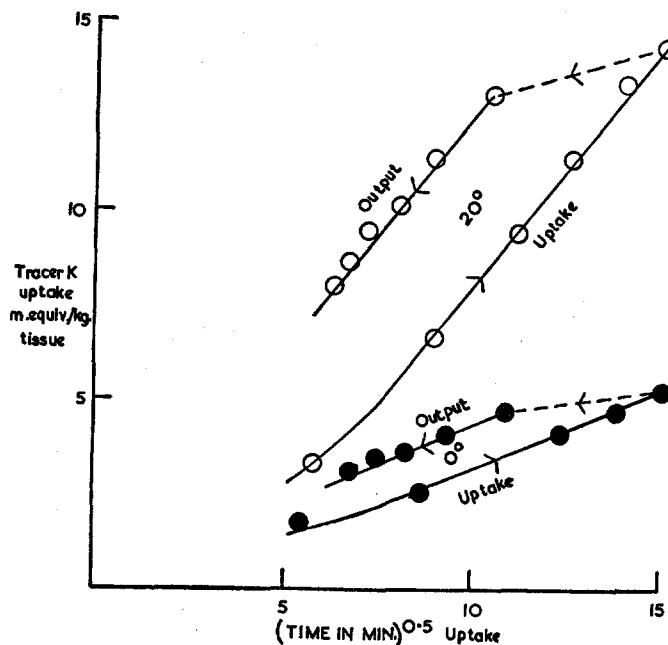


FIG. 4. Uptake of tracer K by paired muscles at two temperatures plotted against (time in minutes) $^{\frac{1}{2}}$ . At the end of the loading period in each case the tracer was allowed to escape to ordinary saline at the same temperature as the loading solution. The quantity of tracer remaining in the tissue is plotted against the value of  $(T + t)^{\frac{1}{2}} - t^{\frac{1}{2}}$  in which  $T$  is the time of loading and  $t$  is the time of unloading.

Uptake and output lines are parallel, the constant  $A$  being the same in each equation, while the output line passes through the origin, corresponding to there being no constant  $B$  in the output equation. Should conditions change, or the muscle deteriorate, the lines will not be parallel and may not be straight.

It will be clear why the form of the K output curve will depend upon the conditions of loading of the muscle. Only with fully loaded muscles will output follow the same law as uptake with sign reversed.

*The Effect of Strophanthin on K Movement.*—K entry into the muscle is slowed by strophanthin. When linear time plots were used it was not possible

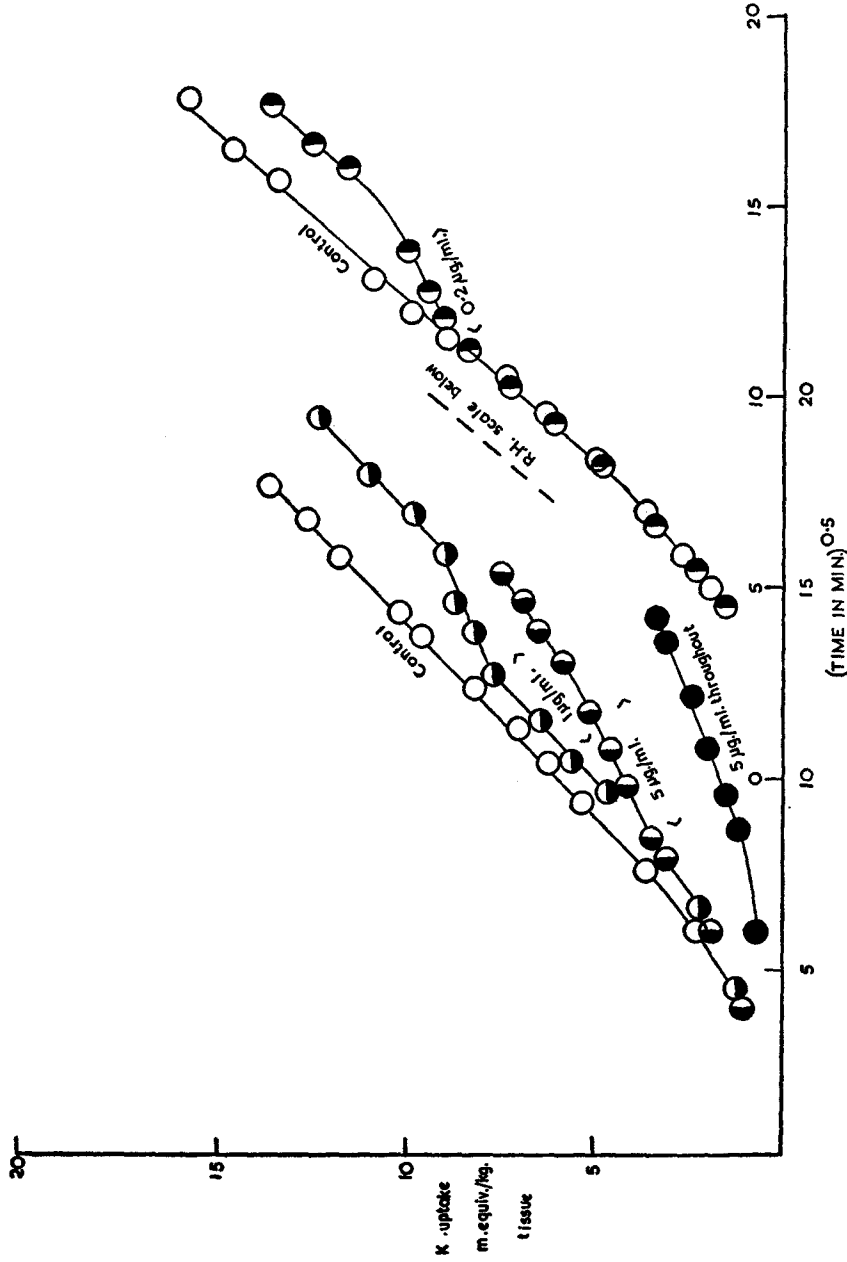


FIG. 5. The effect of strophanthin on tracer K uptake. The drug was added in the indicated concentration during the experiment. External K concentration 1.5mM. Temperature 20°. Note that the scale of the abscissa has been shifted to the right for the right-hand pair of curves.

to decide whether or not the effect was reversible. Use of the  $t^{\frac{1}{2}}$  plots (Fig. 5) shows that the slope of the curve will return to its original value if the drug is withdrawn after application at concentrations of 1 or 0.2  $\mu\text{g./ml.}$  At the low concentrations there is a delay before the poison takes effect and the effect may persist for some time after withdrawal. Application of 5  $\mu\text{g./ml.}$  has an immediate action. In some experiments in which 2 or 5  $\mu\text{g./ml.}$  had been applied for 50 minutes or more, there was no recovery. Estimates of the slopes of the uptake curves during the action of strophanthin are collected in Table II. The amount taken up  $A$  over unit  $t^{\frac{1}{2}}$  interval is reduced by a factor of between 1.5 and 3.

TABLE II  
Effect of Strophanthin or Digoxin on the Constant  $A$  for  $K$  Uptake  
Uptake ( $\mu\text{e/g.}$ ) =  $A t^{\frac{1}{2}} - B$  for  $t^{\frac{1}{2}}$  greater than 5,  $t$  in minutes.

External $K$  <i>m. eq./liter</i>	$A$ before addition	$A$ after addition Drug Concentration in parentheses	Whether uptake increased after withdrawal of drug
1.0	Not measured	0.48 (5 $\mu\text{g./ml.}$ )	Yes to 0.71
1.0	1.12	0.53 (0.2 $\mu\text{g./ml.}$ )	Yes
1.5	0.96	0.46 (1 $\mu\text{g./ml.}$ )	Yes
1.5	0.65	0.45 (5 $\mu\text{g./ml.}$ )	Not in 2 hrs. (treated 70 min.)
1.5	0.65	0.19 (5 $\mu\text{g./ml.}$ )	Yes (treated 44 min.)
2.0	1.10	0.85 (2 $\mu\text{g./ml.}$ )	No (treated 2 hrs.)
3.0	1.56	0.62 (10 $\mu\text{g./ml. digoxin}$ )	No
4.0	1.75	1.08 (2 $\mu\text{g./ml.}$ )	Yes (after 34 min. treatment, no after 53 min. treatment)

When digoxin or strophanthin is applied during the course of the loss of tracer  $K$  from a loaded muscle the slope of the plot of quantity in the muscle against  $t^{\frac{1}{2}}$  or  $(T + t)^{\frac{1}{2}} - t^{\frac{1}{2}}$  (according to the conditions of loading) is increased. With lower concentrations there is a delay before the effect sets in. The slope becomes 1.2 to 1.4 times that holding before poisoning. An explanation of why uptake can become slower and output faster is given in the Discussion. As pointed out already the slope of the uptake line can be reduced if the surface resistance increases with time and it is to this that the slowing of uptake by strophanthin is ascribed.

*Tetrabutylammonium Ions.*—During some tests of the effect of different substances upon the action potential it was observed that substitution of 10 per cent of the  $\text{Na}$  of the saline mixture by the equivalent of tetrabutylammonium lengthened the duration. The substance has a profound effect on the electrical response of crustacean muscle (Fatt and Katz (12)). It was thought that there might be a change in the rates of ion movement even in the resting state and this proved to be the case. Tetrabutylammonium has a strophanthin-like action but the effect is more readily reversed than that of the latter substance. With

10 per cent substitution for Na the K uptake per unit  $t^{\frac{1}{2}}$  interval is reduced to 0.7 of the original value and after restoration of the normal saline  $A$  recovers to 1.0 to 1.1  $\times$  the original value (3 experiments). Substitution of 2 per cent of the Na led to an insignificant reduction of  $A$ . Output of tracer K was but slightly, if at all, affected. The net change in K content in  $2\frac{1}{2}$  hours of treatment with 10 per cent tetrabutylammonium was (with 2 mM K)  $-3.9 \mu\text{eq./gm. tissue}$  (by comparison of 3 pairs of muscles). The loss calculated from the difference  $(A - A')t^{\frac{1}{2}}$  with  $A = 1.1$  (from the 2 mM results in Table I) and  $A' = 0.7 \times 1.1 = 0.77$  is  $-4.1 \mu\text{eq./gm. in } 2\frac{1}{2} \text{ hours}$ .

TABLE III

*Effect of Digoxin and Strophanthin on the escape of Tracer K from Loaded Muscles*

The quantity collected in an interval  $t_1-t_2$  is divided by the difference:  $t_2^{\frac{1}{2}} - t_1^{\frac{1}{2}}$  for fully loaded muscles (e.g. treated 10 mM tracer K overnight) or by

$$(\sqrt{T+t_2} - \sqrt{t_2}) - (\sqrt{T+t_1} - \sqrt{t_1})$$

for muscles loaded time  $T$  in a solution of K concentration equal to that used for soak out. The quotient should be constant so long as the condition of the muscle remains unchanged. A fall of membrane potential will cause the quotient to rise.

Poison and concentration $\mu\text{g./ml.}$	Value of quotient, $\mu\text{eq. tracer K lost per unit } t^{\frac{1}{2}} \text{ interval}$		
	Before exposure	During exposure	After exposure
Digoxin 0.1	1.06, 1.20, 1.11	1.12, 1.19, 1.22	1.12, 1.39, 1.35
" 0.25	1.06, 1.32, 1.30	1.47, 1.27, 1.60	1.80, 1.89, 1.83
" 0.5	0.72, 0.99, 0.93	0.90, 1.00, 1.13	1.15, 1.40, 1.36
Strophanthin 0.1	1.29, 1.38, 1.58, 1.66	1.67, 1.66, 1.91	1.89, 1.96, 1.88
" 0.5	0.71, 0.86, 0.95	0.97, 0.99, 0.17	1.06, 1.34, 1.40
" 2	1.17, 1.06, 1.07	1.19, 1.35, 1.34, 1.43, 1.49, 1.54, 1.47	Not observed

The rate of output of tracer Na measured according to the method described previously (9) was reduced to 0.7 to 0.8 of its original value during application of tetrabutylammonium and rose again after restoration of the normal saline.

Tetrabutylammonium provides a further example of a substance which has similar effects on K uptake and Na output. Parallel tests made of tetraethylammonium showed no effect on ion movements.

*Net Changes of Cation Content.*—When muscles are stored in K-free saline or in the 2 m.eq./liter K mixture with the addition of 4  $\mu\text{g./ml.}$  strophanthin they gain Na and lose K. Compared with the changes taking place in control muscles stored in normal solution the test muscles lose about 0.63  $\mu\text{eq. K}$  and gain about 0.63  $\mu\text{eq. Na/gm. tissue per unit interval of } (t \text{ in minutes})^{\frac{1}{2}}$  (9). The inevitable scatter of points obtained each from a different group of muscles does not provide as convincing a demonstration of the applicability of the  $t^{\frac{1}{2}}$  relation as does the use of a single set of muscles to obtain a series of ob-

servations on the loss at different times. When a K-free solution is used, the amounts of K collected in successive portions of solution can be separately determined. Experiments made in this way provided the data for the lines plotted in Fig. 6. On the figure have been included the line corresponding to data given by Schatzmann and Witt (27) for K loss from a sartorius to K-free solution. The figure shows that net K losses measured from the initial K content of fresh muscle fit closely an equation of the form  $\delta K = At^{\frac{1}{2}} - B$ . Values of  $B$  are similar to those found in tracer experiments. The loss of K per unit interval of ( $t$  in minutes) $^{\frac{1}{2}}$  is 0.9 to 1.5  $\mu\text{eq./gm. tissue}$ . Strophanthin increases the loss, from 0.8 to 0.95 (using figures from Schatzmann and Witt (27)) or from 0.9 to 1.25 (Fig. 6 B), which agrees with the observation of the acceleration of tracer output (Table III). The values just quoted are total losses from the tissue, while the figure mentioned earlier (0.63  $\mu\text{eq./gm. per } t^{\frac{1}{2}}$ ) was additional to such loss as takes place from a muscle to the normal saline. The time course of the total loss, like that of tracer K output from a uniformly loaded muscle, involves the use of the constant  $B$ ; whereas comparative loss, being given by the difference between two losses, will only involve such small difference between the  $B$ 's as arises between paired sets of muscles.

An attempt was made to estimate the loss to normal saline by comparing the mean analysis of groups of 6 muscles after storage with the analysis of 6 paired muscles made directly after dissection. Fig. 7 shows that the results for K loss can be linearly related to  $t^{\frac{1}{2}}$ . The regression equation is:  $\delta K = 0.5 - 0.46t^{\frac{1}{2}}$  ( $t$  in minutes). Na gain includes a rapid uptake of about 5  $\mu\text{eq./gm.}$  and a slow component equivalent to K loss. The regression equation is  $\delta Na = 5 + 0.49t^{\frac{1}{2}}$ . Values of  $\sigma_D$  are high (1.0) and the 95 per cent confidence limits are about 2.8  $\mu\text{eq.}$  each side of the lines so it is justified to regard Na gain as equal to K loss + 5  $\mu\text{eq./gm.}$  The best lines are then  $\delta K = -0.46 t^{\frac{1}{2}}$  and  $\delta Na = 5 + 0.46 t^{\frac{1}{2}}$ . The 5  $\mu\text{eq.}$  of Na which the muscles gain within 20 minutes agrees with Carey and Conway's observation of a rapid gain of 7 to 8  $\mu\text{eq. Na/gm.}$  in a bicarbonate medium. Similar experiments made by Shaw and Simon (29) on toad muscle can be fitted by the relation  $\delta K$  or  $\delta Na = 1.2 t^{\frac{1}{2}}$ .

By combining the results for K loss to normal saline just mentioned with the previous data (9) for loss to K-free solution relative to the loss of 2 mM K solution one finds that the total K loss to K-free solution will be about 1.1  $\mu\text{eq./gm. per unit interval of } t^{\frac{1}{2}}$ . This value is close to the more precise figures found in the experiments of Fig. 6 A. Similarly the loss to strophanthin in 2 mM K comes to 1.1  $\mu\text{eq./gm. per } t^{\frac{1}{2}}$  interval (from Fig. 7 and (9)). The latter value is not far from that expected from the tracer experiments. Uptake in 2 mM K with the drug is about 0.6  $\mu\text{eq./gm. per } t^{\frac{1}{2}}$  (Table II), output will be about  $1.4 \times 1.1 = 1.54 \mu\text{eq./gm. per } t^{\frac{1}{2}}$  (Table III) so net loss expected will be 0.94  $\mu\text{eq./gm. per } t^{\frac{1}{2}}$ .

Values for the constant  $A$  relating K movement to  $t^{\frac{1}{2}}$  in some of the experiments on net change of K content are collected in Table IV.

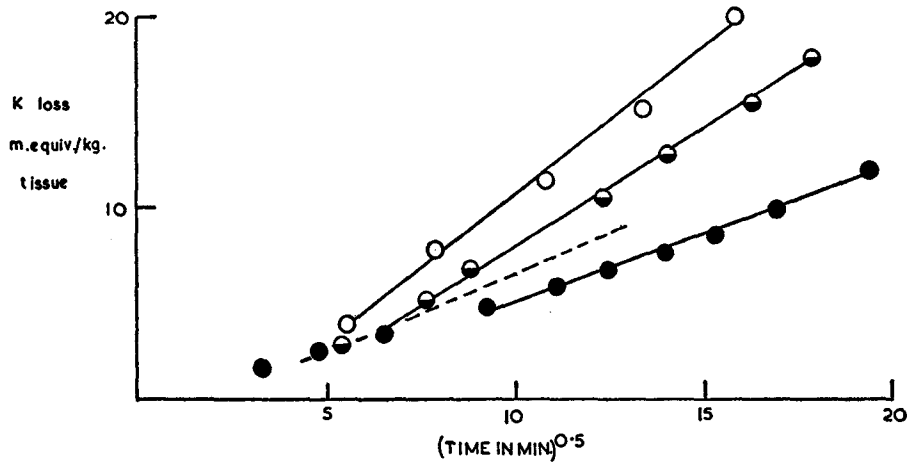


FIG. 6 A. The net loss of K from small muscles measured by collecting the K in portions of initially K-free solution. The three lines with points indicated were obtained from three different sets of muscles in the present work, the dotted line is replotted from figures given by Schatzmann and Witt (27).

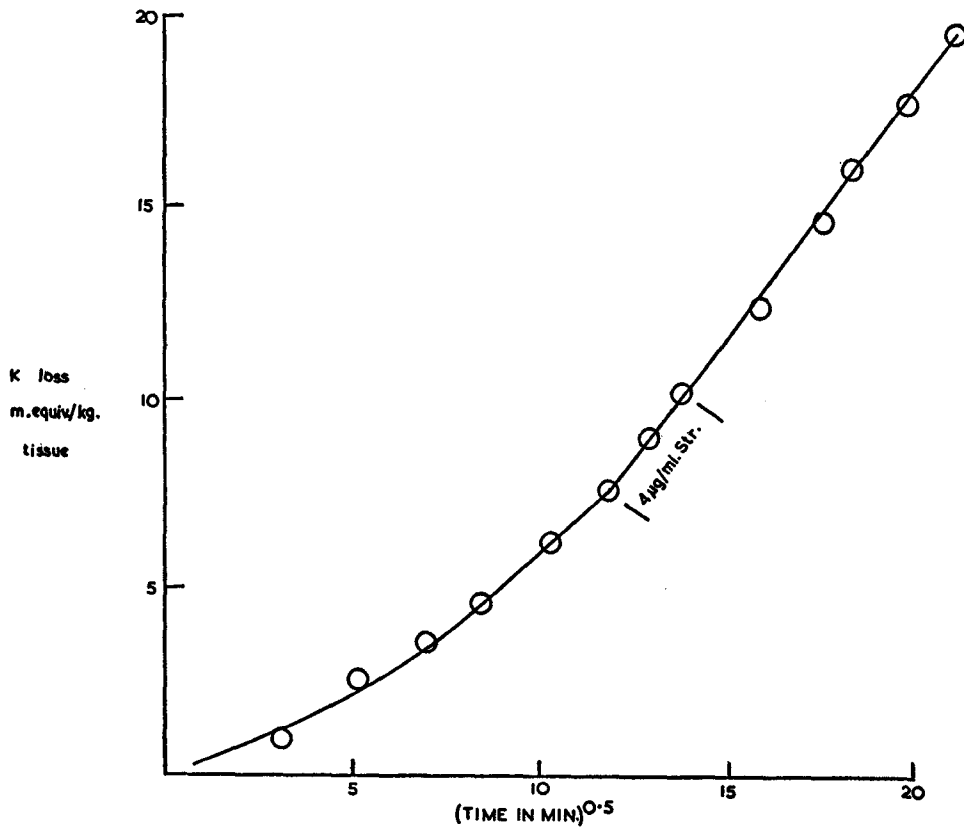


FIG. 6 B. The net loss of K from small muscles to portions of initially K-free solution. Over the interval shown 4 µg/ml. strophanthin was present.

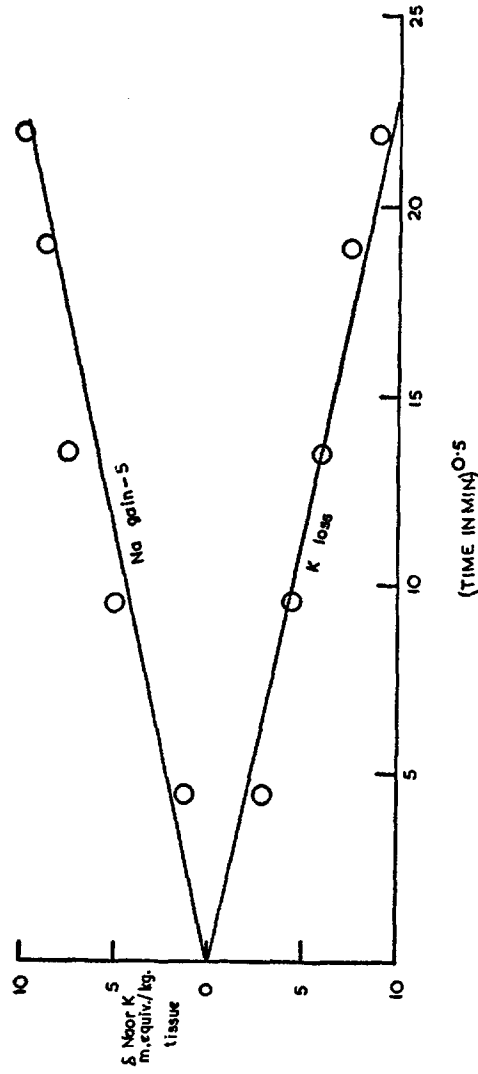


FIG. 7. Loss of K and gain of Na by muscles stored at 20°C. in saline mixture with 2 mM K. The changes are measured between groups of six muscles which have been stored and the six paired muscles which are freshly dissected. Note that a constant value, 5  $\mu\text{eq./gm.}$ , has been subtracted from all the Na gains. The lines are drawn for best fit assuming that the slopes should be equal and correspond to a change of 0.46  $\mu\text{eq./gm.}$  tissue per unit interval of  $t^{\frac{1}{2}}$ .

*Miscellaneous Data for Calculation of K Movement.*—To relate K movement to the K concentration with due regard to the electrical forces arising from the internal-external potential difference, data will be needed for the internal K concentration of fresh muscle and for membrane potentials.

TABLE IV  
Values of *A* Relating Net Movements of K to *t*<sup>2</sup> Found in Analytical Experiments

Solution	$\mu\text{eq./gm. per } t^2 \text{ interval}$ <sup>A</sup>	Method
K-free	0.65, 1.14, 1.55	Direct analysis of portions of solution initially K-free
K-free	0.80	Direct analysis of portions of solution initially K-free, from data given by Schatzmann and Witt (27)
K-free	1.1	Comparisons between groups of control and test muscles (Fig. 7 + Edwards and Harris (9))
K-free + strophanthin	0.95 (0.8 before strophanthin)	By direct analysis of portions of solution, from data given by Schatzmann and Witt (27)
K-free + strophanthin	1.25 (0.9 before strophanthin)	By direct analysis (Fig. 6 B)
2 mM K	0.46	Comparisons between groups of muscles stored or freshly dissected (Fig. 7)
2 mM K + strophanthin	1.1	Comparison between groups of muscles (Fig. 7 + Edwards and Harris (9)).

To estimate the K concentration in the cell water it is necessary to know the K content, the extracellular space, and the proportion of dry matter in the tissue. Analyses of fresh muscles gave the following results:

Muscle	K content. $\mu\text{eq./gm}$ (means of 6 $\pm$ s.d.)
Sartorius	90.8 $\pm$ 6.3
Semitendinosus	91.4 $\pm$ 5.2
Ileofibularis	84.9 $\pm$ 5.2

The dry matter after water extraction was 17.8 per cent (mean of 6). If an estimate of the weight of salts extracted is made for 20  $\mu\text{eq. NaCl}$  and 100  $\mu\text{eq. K}_2\text{HPO}_4$ , total dry matter comes to 19.1 per cent which compares with direct determinations of 20.0 per cent (mean of 6) for *Rana temporaria* and 19.2 per cent for *Leptodactylus* muscle (Harris and Martins-Ferreira (18)). Extracellular space determined with inulin or sucrose in 11 pairs of sartorii was 19.0 ml./100 gm. (9). If the internal K concentration is calculated for 19 per cent dry matter, 18.4 per cent by weight (equivalent to 19 per cent by volume) of extracellular fluid, and 91  $\mu\text{eq./K/gm. tissue}$  the result is 145.5  $\mu\text{eq./gm. cell water}$  (cf. Adrian (1) who finds 139  $\mu\text{eq./gm. cell water}$ ).

The resting potential of muscles after 3 to 5 hours' storage in the 2 mM K solution was measured. Values, the means of 10 to 12 penetrations, were 85  $\pm$  2.5, 86  $\pm$  3.5, 89  $\pm$  5, ( $\pm$  s.d.) mv. In the strophanthin solution there was deter-



oration of the surface fibers, some of which had potentials of 40 to 60 mv., but the inner fibers had consistently the value found for the control muscles. While some of the K loss seen in strophanthin must be attributed to loss from the outer fibers it is unlikely that these fibers alone could contribute sufficient K to account for the extra output.

*The Effect of Strophanthin and Tetrabutylammonium on the Resting Heat Production.*—It was thought that two agents which diminish the rates of uptake of K and of output of Na might act by interfering with the metabolic turnover of the sites which adsorb the cations. This might be reflected in a changed resting heat rate. Measurements of resting heat were kindly made by Mr. V. Howarth of the Physiology Department, University College, London, using the apparatus described by Howarth and Hill (21). Comparative readings of the rate of heat production were made at intervals when the muscle was exposed to normal Ringer's solution and again after either strophanthin (2  $\mu$ g./ml.) or tetrabutylammonium (12 mM) substituted for equivalent Na was present in the solution. The following results were obtained.

*Relative Values of Resting Heat of Sartorius Muscles before and after Exposure to Strophanthin (2 $\mu$ g./ml.) or Tetrabutylammonium (12mM Replacing Na)*

Solution	Time of exposure	Relative heat rate
		<i>per cent</i>
1. Normal	125 min.	100
“	170 “	97
Strophanthin	30 “ after 170 min. in normal	101
“	75 “ “ “ “ “ “	96
2. Normal	135 min.	100
“	190 “	105.5
“	210 “	104
Strophanthin	15 “ after 210 min. in normal	102
“	50 “ “ “ “ “ “	100
3. Normal	110 min.	100
“	180 “	84.5
“	225 “	84.5
Tetrabutylammonium	30 “ after 225 min. in normal	146
“	75 “ “ “ “ “ “	216.5
“	120 “ “ “ “ “ “	207.5
“	150 “ “ “ “ “ “	200
4. Normal	145 min.	100
“	180 “	98.5
Tetrabutylammonium	30 “ after 180 min. in normal	108
“	65 “ “ “ “ “ “	184
“	105 “ “ “ “ “ “	235
“	195 “ “ “ “ “ “	240

Evidently strophanthin has no effect on the resting heat while tetrabutylammonium can double it. These results provide no support for the ion movements being necessarily correlated with the metabolic process responsible for the heat production.

#### DISCUSSION

The principal argument for the hypothesis that the movement of K into (or out of) muscle depends both upon the permeability of a surface layer and on the internal diffusibility rests upon the resemblance between the experimental uptake and output results and those calculated for a combined permeation-diffusion process. The results cannot be fitted by a simple model with resistive membrane enclosing a volume in which most of the muscle K mixes with the normal diffusion constant. With the normal aqueous diffusion constant ( $1.6 \times 10^{-5} \text{ cm.}^2 \text{ sec.}^{-1}$ ) mixing would be very rapid, *e.g.* in an  $80 \mu$  diameter cell equilibration would be 90 per cent complete in less than 1 second. From the upper curve of Fig. 2 it will be seen that the  $t^{1/2}$  relation holds up to as high a fractional saturation (*ca.* 60 per cent) as is to be expected from the theoretical curves of Fig. 1. This shows that all the tissue K shares the diffusion property. How then is it possible to impose a low rate of mixing upon the K? It has been suggested (Ling (25); Shaw and Simon (29); Harris and Steinbach (20)) that K ions are held by adsorption on anionic sites. Movement between sites would not be as rapid a process as free diffusion because an ion could only move into an adjacent site if room was made for it; the probability of thermal agitation simultaneously imparting enough energy to two adjacent ions to permit their interchange could be very low, so that diffusion would take place slowly. If one imagines that the cell is filled with a matrix of adsorption sites between which the cations move it is possible to explain many of the observations.

The figures in sections (a) and (b) of Table I show that the quantity of K moving per  $t^{1/2}$  interval rises as the external K concentration is increased. Passive diffusion of a cation at concentration  $c_e$  into a volume held at potential  $-E$  with respect to the exterior will take place as if the external concentration is  $c_e \exp(eE/kT)$ ; this neglects the effect of interionic attraction on the activity but changes the "chemical" concentration to the "electrochemical" concentration referred to the interior. Formally one could suppose that the electrical discontinuity existed just outside the region into which diffusion was occurring and that a Donnan distribution was set up such that  $E = kT/e \ln c_e'/c_e$  in which  $c_e'$  is the concentration just inside the discontinuity ( $= c_e \exp(eE/kT)$ ). Diffusion from this raised concentration then takes place into the region. A steady state or equilibrium is reached when the electrochemical activities are equal on the two sides of the electrical discontinuity. Here it has arbitrarily been chosen to apply the electrical factor to the external concentration so that the internal concentration (of K) is unmodified by the electrical force. The site of the electrical discontinuity is not likely to be wholly at the membrane

because changes either in the nature of the adsorbing sites, or of the ions held on them will set up an internal potential difference.

To apply the diffusion-permeation equation given in the appendix the uptake into the cell is regarded as related to  $c_o \exp(eE/kT)$  for K ions; that is, the muscle K is supposed to be within the negative region whose potential is measured by the microelectrode method. Uptake may vary as  $K_o$  changes not

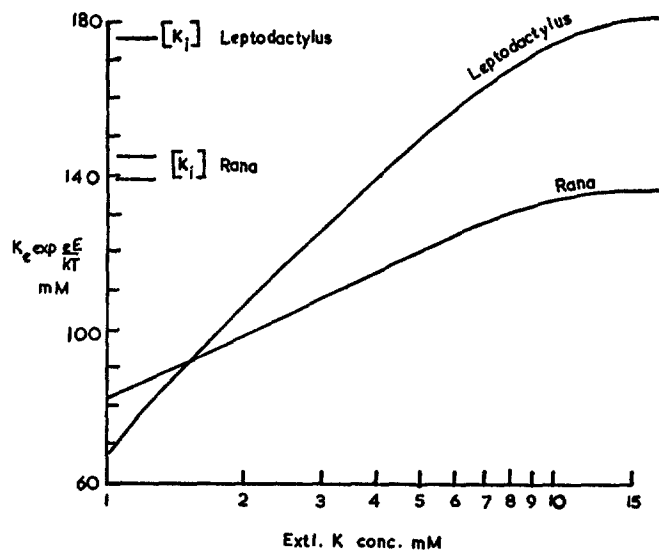


FIG. 8. The electrochemical activity of the external K ( $K_o$ ) referred to the interior of the muscle as a function of the external K concentration. The values are calculated from the product  $K_o \exp eE/kT$  in which  $E$  is the membrane potential (from Harris and Martins-Ferreira (18) for *Leptodactylus* and from Adrian (1) for *Rana*). The reduction of activity due to the Debye-Hückel effect has not been included because approximately the same factor applies to both internal and external K (Adrian (1)). The level of internal K concentration ( $K_i$ ) is indicated. The amount by which this level exceeds the electrochemical activity of the external K is supposed to be a measure of the rate of net K leak from the muscle.

only because  $K_o$  and  $E$  alter but also because the lability of the adsorbing sites changes. Increase of external K concentration makes not only K but also Na more readily transferred from site to site (for acceleration of Na movement by K see Keynes (24); Edwards and Harris (9)).

The increase of diffusibility of the internal K cannot be attributed solely to a rise of the electrochemical activity because this quantity only increases by about 1.5 times as the external K is made more concentrated (see Fig. 8). It is necessary to suppose that the diffusion constant has become higher. This could be the result of several factors: (a) when the cell is depolarised by adding K to solutions containing penetrating anions the anions enter (Boyle and Con-

way (2)) and there will then be a possibility of cation + anion pair diffusion independent of intersite movement; and (b) addition of K may speed up a chemical process causing adsorption-desorption to go on (as suggested recently (9) in relation to Na movement). It at present seems necessary to invoke both explanations because K uptake is accelerated by raised K concentration whether penetrating Cl or relatively non-penetrating phosphate is present (Harris (15)), but the uptake is faster from the solution with Cl than from the one with phosphate (an example of the Cl solution causing a faster uptake than a sulfate solution has been given by Carey and Conway (3), Fig. 5).

It is possible to show that the data obtained from the  $t^{\frac{1}{2}}$  plots can be used to relate tracer data to results of analytical experiments without going to the length of extracting the diffusion and permeability constants (which is done later). It will be convenient to use the following quantities: (a) the internal K concentration, assumed 140 mM (from page 184); (b) the electrochemical activity ( $\mu'$ ) of the external K referred to the interior (from Fig. 8); (c) the slope  $A$  of the line relating uptake to  $t^{\frac{1}{2}}$  (from Tables I to III); (d) the factor (called  $F$  in the following table) by which ion movements are accelerated by the external K present and referred to the rate in K-free solution; the value is taken from the results of measurements of rate of Na output. The ratio  $F\mu'/A$  for tracer uptake, or  $F(K_i - \mu')/A$  for a net change will be shown to remain constant, at about 120, under various conditions.

External K	Relative rate of Na output = $F$	$E$	$\mu'$	Tracer uptake			Net changes		
				$F\mu'$	Observed $A$ for tracer K uptake	Ratio	$F(K_i - \mu')$	Observed $A$ for net change of K content	Ratio
<i>mM</i>		<i>mv.</i>	<i>m.eq./liter</i>						
0	1	—	—	—	—	—	140	0.65–1.15*	122–215
2	1.4	97‡	97	136	1.1§	124	1.4 (140–97) = 60.	0.46	130
4	2¶	84‡	116	232	2.0§	116	2 (140–116) = 48	Not measured	
2 + strophanthin	1¶	89	71	71	0.5–0.8**	142–90	1.4 × 140–71 = 125§§	1.1 ‡‡	114

\* From Fig. 6 A.

‡ From Adrian (1).

§ From Table I.

|| From Fig. 7.

¶ From reference 9.

\*\* From Table II.

‡‡ From reference 9 and Fig. 7.

§§ From Tables II and III, strophanthin reduces uptake to the K-free value, but accelerates output.

From the curves in Fig. 8 it is possible to estimate whether a muscle with a given resting potential ( $E$ ) and internal K concentration ( $K_i$ ) will gain or lose K when in a certain external concentration ( $K_o$ ). When  $K_o \exp (eE/kT)$  is less than  $K_i$  there will be a net loss; this is found in ordinary saline (Fig. 7 and Boyle and Conway (2); Fenn and Cobb (13)). The potential is insufficient to maintain the internal K. This raises the question of how the muscle maintains its K content *in vivo*. Does the structure which retains K fail *in vitro* because some chemical is lacking from the medium? In this connection the observation of Carey and Conway (3) that isolated muscles rapidly gain Na from artificial media but not from blood may be suggestive. Trials of potential metabolites (Harris (15)) showed little effect on K retention and the question remains unanswered. Increasing the K in the medium can lead to some gain of K by the tissue, but this may later be followed by a loss (Fenn and Cobb (13)); this could be the result of a gradual fall of the resting potential. The curves in Fig. 8 predict a maintenance of  $K_i$  when  $K_o = 10$  to  $15$  mM, but this is only true so long as the potential is at the level which has been used to calculate  $\mu'$ .

*Diffusion Constants and Surface Permeabilities.*—These were calculated from the slopes and intercepts given in Table I together with the electrochemical activity ( $\mu'$ ) of the external K, referred to the cell interior, taken from Fig. 8. Calculations were made for a cell of  $40 \mu$  radius. The method used is based on the simple diffusion equation for entry into an infinite extension; practical cases approximate to this when the degree of equilibration is low (Eggleton, Eggleton, and Hill (11); Hill (22)). One sets: Uptake (per unit mass) = area per unit mass  $\cdot 2c_o \exp eE/kT (D_i t / \pi)^{1/2}$ . For a  $40 \mu$  radius fiber the area per unit mass of cells is close to  $530 \text{ cm}^2$  but as 18 per cent of the muscle mass is extracellular there will be only about  $435 \text{ cm}^2$  of surface exposed per gm. tissue. Sources of variation of the rate other than the electrochemical activity of the K have not been explicitly inserted in the equation. From the slopes, and assuming the above surface/volume ratio, the following approximate values for  $D_i$  are obtained:  $K_o = 2 \text{ mM}$   $D_i = 0.9 \times 10^{-11} \text{ cm}^2 \text{ sec}^{-1}$ ,  $K_o = 4 \text{ mM}$   $D_i = 2 \times 10^{-11} \text{ cm}^2 \text{ sec}^{-1}$ ,  $K_o = 8 \text{ mM}$   $D_i = 4.2 \times 10^{-11} \text{ cm}^2 \text{ sec}^{-1}$ ,  $K_o = 14 \text{ mM}$   $D_i = 10^{-10} \text{ cm}^2 \text{ sec}^{-1}$ . With these values the observed intercepts on the abscissa of the linear part of the uptake curve were transformed to the quantities  $(Dt_i)^{1/2}/a$  ( $a =$  cell radius,  $0.004 \text{ cm}$ ). By comparison with the intercepts in Fig. 1 estimates of the values of  $ah$  were made. Using these values, which were between 7 and 11, the figures for  $D_i$  were corrected for the fact that the surface resistance causes some diminution in slope (for example with  $ah = 8$  the slope is about  $1/1.2$  of the slope for infinite permeability and so the true value of  $D_i$  is about  $1.2^2$  times the value found from the slope). Final figures for  $D_i$  and  $H_i$  for four values of  $K_o$  are given in Table V.

TABLE V  
*Diffusion and Permeability Constants for K*

External K	Diffusion constant (observed)	Permeability constant (observed)	Permeability constant (calculated)
<i>mM</i>	<i>cm<sup>2</sup> sec.<sup>-1</sup></i>	<i>cm. sec.<sup>-1</sup></i>	<i>cm. sec.<sup>-1</sup></i>
2	$1.3 \times 10^{-11}$	$3.5 \times 10^{-8}$	$3.9 \times 10^{-8}$
4	$2.9 \times 10^{-11}$	$6.5 \times 10^{-8}$	$8.3 \times 10^{-8}$
8	$6.3 \times 10^{-11}$	$11 \times 10^{-8}$	$15 \times 10^{-8}$
14	$15 \times 10^{-11}$	$29 \times 10^{-8}$	$25 \times 10^{-8}$

The permeability has been calculated (last column) for a membrane of constant permeability ( $50 \times 10^{-8}$  cm. sec.<sup>-1</sup>) in series with a region  $3 \mu$  thick through which the K ions diffuse with the diffusion constant appropriate to the external K concentration.

Na output, if treated as an exponential process, has a rate constant of 1-1.5 hr.<sup>-1</sup> However, it seems likely that in muscle the Na movement is limited by diffusion as well as by the membrane permeability (see Harris and Prankerd (19) for the argument that Na movement in the human erythrocyte involves slow diffusion). It has been proposed that the Na of the muscle cell is present in an outer region (Conway and Carey (5); Edwards and Harris (9)). If this is correct it has two important consequences: (a) the ion will not have so long a diffusion path as the K so it will exchange more rapidly and (b) the presence of a region containing Na through which K has to diffuse will contribute to the "surface" resistance offered to K movement. Trial calculations were made using the hypothesis that both ions have the same diffusion constants inside the cell and that the resistance offered by the membrane is low, the figure chosen being  $50 \times 10^{-8}$  cm. sec.<sup>-1</sup>, which exceeds somewhat the highest K permeability measured but corresponds to electrical resistance (about 5000 ohm cm.<sup>2</sup>: Katz (23)), if the simplest relation between flux and resistance is used, together with an effective concentration of 100 mM. If one assigns an annulus to hold the cellular Na at the same mean concentration as that found for total cation within the whole  $40 \mu$  radius cell the annulus would have thickness  $3 \mu$  (for Na content 15 m.eq./kg. tissue). How long would a  $3 \mu$  thickness take to reach half-equilibration with tracer Na if the *D* and membrane permeability were the same as K? (The membrane permeability is in fact unimportant since it is so high.) The time comes to about 22 minutes regarding the annulus as a thin sheet exposed on one side. Most experimental observations of Na movement have been plotted as exponentials. An initial non-exponential part would not have been noticeable because it is obscured by the presence of a fast fraction, all of which has previously been assumed to be extracellular Na. To make comparisons it is more useful to calculate the half-time of decay of the exponential part of the diffusion process from the annulus. The result is close to 33 minutes. This time is in agreement with many measured half-times for

Na turnover in 2 mM K solution (Edwards and Harris (9)) and would correspond to a rate constant of  $1.25 \text{ hr.}^{-1}$ . The increase of  $D$  as external K is increased will account for the observed acceleration of Na output when external K is raised. Returning to the effect of an outer Na region on K movement; the resistance offered by the  $3 \mu$  layer is obtained as a permeability ( $= 1/\text{resistance}$ ) by division of the appropriate diffusion constant by the thickness. The value obtained varies with  $K_o$  because the diffusion constant varies. When the figure is added to the constant chosen for the permeability of the membrane itself the figures in the third column of Table V are obtained. They are close to experimental estimates of the K permeability as could be expected if the hypothesis of the outer Na layer is true because the over-all procedure of extracting the permeability constant is inaccurate.

It will be of interest to know the result of reducing the Na content of the outer region by putting the cells in solutions of reduced Na concentration. After soaking muscles in 30 mM  $\text{NaHCO}_3$ -sucrose mixture (with 2 mM each KCl and  $\text{CaCl}_2$ ) for 2 hours, cations and extracellular spaces were measured (for method see reference 9). The results for the cellular Na content in m.eq./kg. tissue in control and test pairs were: 21.9, 12.1; 22.3, 11.3; 25.5, 6.7; 23.5, 9.1. Cellular Na falls to one-half to one-third of the original value, but analyses for K showed only small changes (see also Shaw, Simon, Johnstone, and Holman (31)) so it is likely that the Na has left the muscle along with chloride or phosphate or been partly replaced by Ca, for which there is some recent evidence (Harris, unpublished data). Uptake of tracer K from the normal and the reduced Na medium when plotted against  $t^{\frac{1}{2}}$  showed no effect when the medium was changed so it can be concluded that the mere reduction of Na content has not altered the properties of the system which controls K movement.

Some arguments which may be raised against the hypothesis of slow internal diffusion may be examined. It has been questioned whether the distribution of surface/volume ratios which hold in a bundle of fibers of varying sizes might be the origin of the anomalies seen when K movement is plotted as a logarithmic process (Carey and Conway (3); Creese, Neil, and Stephenson (6)). If one computes the respective weighted mean of the degree of exchange attained at each of the two times in a system having a distributed surface/volume ratio as given by the latter authors (their Fig. 4), the results prove to be very close to the figures computed for fibers all of size equal to the group next greater than the most frequent value. The weighting to take account of the proportion by mass of the whole specimen tends to make the larger fibers relatively more important than their proportion by number. If a logarithmic relation between K movement and time held for each individual fiber the result would still be very nearly true when observing bundles having a radius variation of a factor of 2. According to the logarithmic law the amount moving does not

fall off with time as rapidly as found experimentally and as can be fitted by the equation for permeation-internal diffusion.

The internal electrical resistance of the muscle fiber is only about two to three times the resistance of an equal column of saline solution (Katz (24)). Whether slow diffusion caused by ions becoming trapped on sites is necessarily associated with a high resistance is doubtful; the result would depend on whether a new ion entering the locality of a site will facilitate the movement of the occupying ion out of the site. Ionophoresis of K along muscle takes place with nearly normal mobility (Harris (16)), but this, like the low resistance, may be explicable by the electric field setting the whole pattern of ions moving, which can have a different energy demand to an interchange between ions occupying adjacent sites as must take place in the process of diffusion. A further possibility in muscle is that longitudinal movement is easier than radial movement.

The application of the diffusion hypothesis to the red cell is described in the following paper (Harris and Pranker (19)). Trials of the applicability of the uptake- $t^{\frac{1}{2}}$  relation have also been made upon K entry into frog nerve, cat muscle, frog heart muscle; and, using other authors' results, to K movement in the squid axon. In all these examples a linear relation of the sort described in this paper has been found. In addition the relation proves to fit observations of the uptake of tracer Ca by frog muscle. The argument used to justify the diffusion hypothesis in the present paper is based on fitting the kinetics of ion movement and may be dismissed as fortuitous. However, other authors (Ling (25); Shaw, Simon, and Johnstone (30); Shaw, Simon, Johnstone, and Holman (31)) on quite other grounds have been led to reject the simple membrane theory and both Ling and Shaw and Simon (29) have proposed that the cations are held by adsorption. The possibility of a Na-rich region surrounding a K-rich region gives the possibility of variation of Na/K ratio in the cell without there necessarily being changes of resting potential or active membrane potential; Shaw *et al.* (30) have shown that there is no correlation between the ionic composition and the potentials. The measured resting potential will be the sum of a Donnan potential set up between the solution and the Na-rich region and a diffusion potential between Na-rich and K-rich regions. While the values should not depend upon the thickness of the Na region the rate of change of potential arising from ion movement will reflect the resistance met. Loss of K from the cell will be more difficult when the Na region is thicker and so recovery of internal negativity after a displacement will be slower for the high Na muscle than in the normal, as found by Desmedt (8).

If the part of the structure within the cell which forms the K-rich region is regarded as impenetrable by Na ions as long as the sites maintain their integrity, the magnitude of the diffusion potential between the K-rich region and the Na-rich region will be given by  $RT/F \ln K_i/K_e'$  in which  $K_i$  and  $K_e'$  are



the K concentrations holding in the two regions. Since the amount of potassium in the Na-rich region will depend upon both the external K concentration and on the amount of leak from the K-rich region, it follows that the contribution to the resting potential made by the diffusion potential will not depend simply upon external K concentration. It would be expected that at high external K levels sufficient K enters the Na-rich region to swamp the effect of the leak so the potential will then vary as  $\ln K_i/K_o$ ; as  $K_o$  is reduced the amount provided by the outward leak becomes more important and sets an upper value to the contribution of the diffusion potential to the total resting potential.

The results of the application of strophanthin or tetrabutylammonium on the ion movements and on the resting metabolism do not support the idea of a metabolically activated "pump" for the ions. Other authors (Ling (25); Shaw and Simon (29)) have also pointed to the divergence between the ability of the cell to maintain an unbalance of Na and K between inside and outside and the metabolism. It seems likely that the agents affect ion movement by making the relative proportions of the Na-rich region and the K-rich region change with time. A gradual expansion of the Na region caused by poisoning will make it increasingly difficult for K ions to reach the inner K-rich region so (a) K uptake will be slower than into the unpoisoned muscle. At the same time Na entering the poisoned muscle will displace K instead of moving out so (b) Na output will be reduced, (c) K output can be accelerated, (d) net Na gain will be equal to net K loss. (b and d were described by Edwards and Harris (9); c is shown by Table III.)

It is a pleasure to thank Mrs. V. Bolingbroke for help with the strophanthin experiments and Dr. C. Edwards for help with the tetrabutylammonium experiments. Part of the expenses were met from a grant from the Government Grants Committee of the Royal Society. A Medical Research Council grant for scientific assistance was received during part of the work.

#### APPENDIX

##### *The Time Course of Equilibration by Diffusion of a Cylinder Having a Resistive Coating*

When diffusion takes place into a cylinder of radius  $a$  and with a resistive coating of permeability ( $H$ ) enclosing the interior in which the diffusion constant is  $D$ , it can be shown from the appropriate solution of the diffusion equation (for which see e.g. Carslaw and Jaeger (4), p. 275) that the mean internal concentration  $C_i$  at time  $t$  is related to  $D$  and  $H$  by:

$$\frac{C_i}{C_o} = 1 - 4 \sum_1^{\infty} \frac{\exp(-D\rho_n^2 t)}{\left(1 + \frac{\rho_n^2}{a^2 h^2}\right) \rho_n^2}$$

in which  $\rho_1, \rho_2, \dots$  are the roots of  $\rho J_1(\rho) = ahJ_0(\rho)$ , and  $h = H/D$ . The function has been plotted in Fig. 1 for unit  $C_e$  and variable parameters  $ah$  and  $(Dt)^{1/2}/a$ . When permeability is so low that internal diffusion no longer sets a limit to the rate of movement, the equation reduces to

$$\frac{C_i}{C_e} = 1 - \exp(-2Dht/a) = 1 - \exp(-2Ht/a)$$

which is the usual "permeability"-limited uptake equation.

Inspection of the curves will show that when there is an appreciable surface resistance there is a portion which is nearly straight. The slope of the straight part gives an approximate measure of the diffusion constant which is made to appear too low as a result of the surface resistance. The position of the intercept of the straight part of the curve gives a measure of the surface permeability/diffusion constant ratio.

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