Relations between Potassium and Sodium Levels in Mammalian Muscle and Blood Plasma

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In a previous account of the question of ionic permeability of the muscle fibre membrane (Conway, 1946), two regulative mechanisms were considered: one, a differential and passive permeability whereby potassium and chloride entered freely, whereas ions such as sodium, magnesium, sulphate, etc. were excluded (or entered relatively very slowly), such permeability being determined by the ion size; the other, a possible mechanism whereby sodium ions if they entered very slowly and over a long period could be extruded by some active process.

For the first of these mechanisms the evidence already presented (Boyle & Conway, 1941; Conway, 1946) scarcely admits of any other interpretation in the case of isolated sartorius of the frog, and applies also to the proximal convoluted tubules of the frog's kidney (Conway, FitzGerald & MacDougald, 1946) and liver (Conway & Boyle, 1944). Wilde (1945) has shown mammalian muscle to be permeable to chloride, and many studies with isotopic potassium (e.g. Noonan, Fenn & Haege, 1941) have shown mammalian muscle to be freely permeable to potassium ions. Also, Shanes (1946) has presented evidence showing that spider crab nerve is freely permeable to potassium and chloride.

Concerning the second mechanism, there is no satisfactory evidence that sodium ions which have entered into skeletal muscle fibres are actively extruded. The small amount of the exchanges recorded with respect to the muscle tissue as a whole leaves it open to reasonable doubt as to the exchange of the sodium ions from *within* the fibres. An outstanding experiment of Heppel (1939), working in Fenn's laboratory, showed that when rats were fed on a diet with very low potassium content, sodium undoubtedly entered the muscle fibres, but experiments demonstrating the reversal of the process and its rate do not appear to have been carried out, though the restoration of the potassium levels was demonstrated (Heppel & Schmidt, 1938). The importance of this question for muscle physiology and the theory of ionic exchanges across the membranes of mammalian tissue cells led us to repeat these experiments, and to find whether extrusion of sodium occurred on returning the rats to a diet rich in potassium. Although our findings confirmed those of Heppel, the amount of sodium entering did not occur to the same extent, due possibly to the shorter time allowed on the low potassium diet; the duration of Heppel's experiments was about 45 days, ours lasting upwards of 30 days on the low potassium intake. We found an average increase from 53 to 80 mg. sodium/100 g. muscle after 30 days, 53 mg./100 g. being an average normal figure for the group of rats used in these experiments. After 24 days the sodium increased to *c.* 76 mg./100 g. The corresponding chloride changes left no doubt that much the greater fraction of this sodium had entered the fibres. The changes with respect to sodium on restoring such rats to a high potassium diet, as well as the concomitant potassium, chloride and water changes, are described below.

METHODS

Experimental. Young rats, averaging about 70 g. in weight, of the Wistar strain were used throughout. They were maintained on the low K diet for the required number of days, after which they were bled into small dry centrifuge tubes and the serum separated immediately and stored for analysis. For the muscle analyses, a small portion of the leg muscle (about 100 mg.) was removed into a weighed platinum crucible for subsequent ashing and Na and K determinations; larger amounts were required in the series in which muscle Cl analyses were carried out.

Diet. The low K diet consisted of the following (given as percentages by weight): glucose 52, casein 30, salad oil 10, cod-liver oil 1·6, wheat germ oil 0·7, 'ammonia' yeast (yeast in which all the K had been replaced by NH_4 ions (Conway & Breen, 1945)) 1·6, and salt mixture 4·1. The salt mixture was similar in composition to that used by Heppel (1939). The K content of this diet was 7–10 mg./100 g. and the average daily consumption of the diet/rat was about 10 g. The high K restoration diet contained (in g./100 g.): wheat flour 36, oat flour 36, casein 5, yeast 21, $CaCO_3$ 1, NaCl 1, with 0·17 KCl added (total K = 340 mg./100 g.). For a group of rats maintained for 28 hr. on the extra high K intake, a similar diet was used with KCl added to form 1 g. KCl/100 g. (total K 780 mg./100 g.).

Chemical. Na and K were determined by colorimetric modifications (Boyle, Conway, Kane & O'Reilly, 1941) of the methods of Salit (1932) and of Shohl & Bennett (1928) respectively, after ashing as described by Fenn & Cobb (1934). Serum Cl was determined by a microdiffusion method (Conway, 1939; 1947) and muscle Cl by a similar method after a preliminary diffusion into isotonic Na_2SO_4. The water content was found by drying overnight at 105° prior to ashing for Na and K determinations.

RESULTS

The mean results for the first series are shown in Table 1. It will be seen that an increase of Na in skeletal muscle occurs as in the experiments of Heppel. The increase is at first rapid and then more gradual, reaching approximately 36·5 mmol./kg. after 26 days. Prolongation of the dietary to 46 days, as for Heppel's rats, would probably give figures not far different from his. For the individual results there is a rather wide variability. Thus, after the 26th day, the three rats examined had 46·6, 34·4 and 28·7 mmol. Na/kg. Similar variability may be seen in the results of Heppel, who gives the pooled analyses from 2 or 3 rats; after 46 days the values ranged from 64·4 to 44·8 mmol. Na/kg. and the range for single rats would no doubt be larger.

before. For this group, the 1 % NaCl was replaced by a salt mixture similar to Heppel's. The rats were maintained on this low K diet for 27 days, then 15 were taken and the leg muscles and serum analyzed. The remaining rats were placed on the K-rich diet (containing 340 mg. K/100 g.) of which they partook avidly. Table 2 shows that the muscle Na falls slowly to normal over a period of about 16 days, and then slightly below normal. After 24 hr., though the plasma K is well above the normal value and the muscle K restored, there is no significant change in the muscle Na. The same holds after the second 24 hr., the plasma K being now as high as 6·9 mmol./l. It is only after 3 days that a significant decrease is observed. After 6 days the muscle Na is near to the normal average value and after 16 days slightly below this level.

Table 1. *Effect of low* K *diet on muscle* Na, K, Cl *and water content*

(Mean values are given.)

No. of rats	Days on low K diet	Muscle Na (mmol./kg.)	Muscle K (mmol./kg.)	Muscle Cl (mmol./kg.)	Muscle water (g./kg.)	Plasma Cl (mmol./kg.)
10	0	22·8	100·5	12·4	765	117·5
2	4	25·4	86·8	10·7	757	119
2	8	31·4	72·8	13·0	775	117
2	12	34·4	75·3	14·4	770	116
2	16	34·5	81·6	15·2	763	117
3	26	36·5	75·4	15·2	760	122

Table 2. *Changes of muscle and serum* Na *and* K *on transfer from low* K *diet to high* K *diet*

No. of rats	Days on low K diet	Muscle Na (mmol./kg.)	Muscle K (mmol./kg.)	Serum Na (mmol./kg.)	Serum K (mmol./kg.)	Muscle water (g./kg.)
10	0	22·8	100·5	141	4·9	765
15	27	33·2	82·4	142	3·6	760
	Days after change to high K diet					
6	1	33·5	102·6	143	5·5	—
5	2	33·1	101·0	146	6·9	—
5	3	26·5	98·2	148	7·6	—
5	4	26·5	100·7	146	8·0	—
5	6	23·9	105·1	144	8·7	—
5	16	21·7	102·0	144	9·0	—

The mean increase in the muscle Na was from 22·8 to 36·5 mmol./kg. whereas the Cl increased from 12·4 to 15·2 or an increase of 2·8 mmol./kg. Only a small fraction of the Na increase could therefore be interpreted as due to change in the interspaces between the muscle fibres; in short, the Na must have largely entered the fibres.

Another point of interest from Table 1 is that there is no immediate relation between the rise of Na in muscle and fall of K. After 26 days the K reduction is about 25 mmol. whereas the Na increase is only about 14. After 16 days the fall in K is 19 whereas the Na increase is only 12 mmol./kg.

In the second series of experiments conducted very shortly after the first and on similar rats, a group of 50 rats was placed on the low K diet as

It was of interest to find if very high levels of K, rapidly produced, would increase the rate of Na return from the muscle. Some rats of the above group after the 27th day were fed on a diet with the K content raised by further KCl addition to 780 mg./kg. The rats ate considerable amounts of this food and after 28 hr. the plasma K was as high as 9·8 mmol./kg. The mean Na content of the muscles of these rats was 28·2 mmol./kg., which was still much higher than the mean 22·8 of the normal group, but lower than the corresponding 33·5 of the rats having 5·5 mmol. K/l. of plasma. It would appear, therefore, that for mammalian muscle very high plasma K had some influence in hastening the return of muscle Na from mammalian muscle.

With amphibian muscle at room temperature or in the cold, no evidence of the return of muscle Na, much increased by previous immersions in K-free Ringer, could be obtained when using high external K.

DISCUSSION

From the above results the following conclusions may be drawn.

(1) Mammalian muscle has the power of slowly extruding Na from within the muscle fibres.

(2) The extrusion of the Na is comparatively very slow compared with the rates of K entrance. Thus, from the work of Noonan *et al.* (1941), labelled K enters to half its equilibrium value in about 45 min. The return of the muscle Na when the plasma K is increased beyond the normal level becomes noticeable after about 3 days, and after about 1 day when the K is raised considerably.

(3) The process of the restoration cannot be interpreted as an antagonism between Na and K or a competition for acidic groups on the non-diffusible constituents, since almost immediately the muscle K was restored to its old level and the plasma K became even higher than normal, whereas for 48 hr. in the above experiments no sign of Na decrease occurred. The explanation must take account of the relative rates of passage of the ions to and from the fibres, and of a membrane alteration produced by very low K levels.

An interpretation of the ionic permeability of the muscle-fibre membrane in relation to the experimental findings

This is essentially that already put forward (Conway, 1946) in which two mechanisms controlling the normal K and Na entrance are postulated. The first, a permeability such that while K and Cl ions enter rapidly, Na enters comparatively very slowly, possibly many hundreds of times more slowly than K. The second, a slow, active extrusion of the very slowly entering Na ions. This interpretation is not inconsistent with the previous treatment of the physiological significance of inorganic levels in the internal medium (Conway, 1945).

It is further considered that when the plasma K falls below the normal level, the membrane is placed under increasing strain owing to the very high potential gradient across it. This strain may be likened to the dielectric strain of a condenser. As a result, Na ions enter the muscle more rapidly than usual. The question of such a change of permeability arising out of potential gradients across the membrane increased above a critical value has also been considered by Wilbrandt (1947) in a recent review. If we now consider an extrusion mechanism which, up to a point, returns Na ions to the plasma at a rate largely proportional to their concentration, then a steady state will develop with each level of plasma K. At normal K levels, the Na content of the muscle will be low, and when the plasma K falls to about half its normal level it will be relatively high. Similarly, with high plasma K the muscle Na, though low in concentration, will fall still lower, and this fall may be measurable. On such a view the Na extrusion rate need not alter, but only the entrance rate; at the same time the extrusion rate may alter considerably with the increased permeability of the membrane. The fact that in the restoration process the lowering of the high Na content of the muscle fibres is slow compared with the rate indicated by Heppel's experiments with radioactive Na, suggests either a marked reduction of the exit rate of Na on increasing the plasma K or a slow restoration of the membrane abnormality. It is also possible that the rate of mixing with the labelled ions does not measure the rate of ion transport (Ussing, 1947).

The entrance rate of sodium into the fibres in the low plasma potassium experiments of Heppel, compared with the normal entrance rate of potassium

Heppel found that radioactive Na interchanged freely with the Na which had entered the fibres. The figures suggested an even faster entrance rate than the normal K value, and was so referred to by Krogh (1946). This suggestion of a faster rate than K, however, is not borne out by closer examination, for it must be considered that Na is entering from a high external Na level into a relatively low concentration, whereas on the other hand K is entering from a very low external level into a high concentration. When these facts are taken into account, Na may be shown to enter the fibres under the abnormal conditions at only about one thirtieth the rate of the normal K entrance, as derived from the data of Noonan *et al.* (1941). How the K rate is affected by the abnormally low plasma K has not been shown, but we may suppose that it does not remain unaltered and is almost certainly increased, and possibly very much increased.

The relative rates as indicated above may be derived as follows: The rate (dQ_{mr}/dt) at which labelled K after injection accumulates in a muscle fibre may be expressed by the equation

$$\frac{dQ_{mr}}{dt} = (P_p C_{pr} - P_m C_{mr}) \times \text{surface}, \qquad (1)$$

where P_r and P_m are the entrance and exit rates respectively of K for the muscle fibre (per unit concentration and unit surface) under the given conditions, and C_{pr} and C_{mr} are labelled K concentrations in plasma and muscle respectively. For the relatively short half period for equilibration the potential across the membrane may be taken as

constant. Also, it may be noted that it has been shown (Boyle & Conway, 1941; Hill & Kupalow, 1930) that all, or practically all, the K in the muscle fibre is ionized and not bound to protein, so that the difference between P_r and P_m may be held to reflect only the effect of the potential difference within and without the fibre. To bring these P_r and P_m values more into relation with permeability figures as ordinarily expressed, and to Fick diffusion constants, it may be assumed that the diffusion from the outer to the inner medium occurs into zero concentration, and similarly for the inner to outer diffusion. In the steady state dQ_{mr}/dt is zero.

If the labelled K in the plasma is constant or nearly so during the half period of equilibration, it may be deduced from equation 1 that

$$P_p = \frac{0{\cdot}276r}{t_{0{\cdot}5}} \times \frac{C_m}{C_p}, \qquad (2)$$

where r is the radius of the fibre (0·8 of which is present as 'fibre water') and $t_{0{\cdot}5}$ is the half period of equilibration. Writing a similar equation for labelled Na under the same conditions and dividing we obtain

$$P_p/P'_p = \frac{t'_{0{\cdot}5}}{t_{0{\cdot}5}} \times \frac{C'_p}{C'_m} \times \frac{C_m}{C_p} \qquad (3)$$

in which the dashed symbols represent the values for Na. If we compare the entrance rate of labelled Na in Heppel's experiments with the entrance rate of labelled K into normal rats, then the concentration ratio of K in the fibre to K in the plasma is practically the same (in Heppel's data) as the normal ratio, and the potential difference also may thus be taken as the same. In such experiments of Heppel, the Na very probably enters at a more rapid rate than for normal fibres, seeing that Na has entered the fibres to a considerable extent, but if we suppose it to enter at the same rate, then the conditions of comparison would be the same or very similar.

Allowing for the interspace Na, it appears from Heppel's data that the half period of exchange of the labelled Na is approximately 15 min. Throughout this period the serum counts for the labelled Na do not change appreciably with time. From Noonan, Fenn & Haege's data the half period of the labelled K entrance in normal rats is of the order of 30–60 min. 45 min. may be taken as probably not far from the real figure, and it would appear from the data as a whole that there was no considerable fall in the labelled K in the plasma, the radioactive material being injected mainly intraperitoneally and for some observations, subcutaneously. It may be assumed then that the ratio of the half periods for the labelled Na equilibration and that of labelled K in such experiments is about three to one, so that

$$P_p/P'_p = 82/3 = 27,$$

i.e. the entrance rate of Na ions into the muscle under strain with the abnormally low plasma K is only about one thirtieth the rate of the entrance of K ions into the muscle under normal conditions. It may therefore be concluded that the entrance rate of Na ions into mammalian muscle in the living animal, and under conditions of membrane strain, is considerably less than the entrance rate of K ions, and for muscle under normal conditions it may be only a very minute fraction.

It would seem rational to suppose that a regulating system for the muscle fibre which would allow K ions to pass freely, but exclude Na ions without energy expenditure, would be more advantageous than one requiring energy for the extrusion of the Na ions which freely entered. The formation of a membrane by the cell or muscle fibre which would achieve this *perfectly*, and at 37°, is perhaps impossible, but it would appear that a close approximation to it can be achieved, whereby the energy requirement for Na extrusion approaches near to zero. It may also be added that in numerous experiments we have been unable to detect any extrusion rate of Na ions from the frog's sartorius in the cold.

SUMMARY

1. Sodium which had entered the muscle fibres of rats, after a period of potassium deprivation, was slowly extruded when the rats were placed on a diet rich in potassium. The mean half period of extrusion, even with the plasma potassium above the normal level after 24 hr., was about 3 days.

2. The muscle potassium, which had fallen to levels of 75–82 mmol./kg. in the average, was fully restored to normal values (about 100 mmol./kg.) after 1 day on the high potassium diet, and did not appreciably change from this when the plasma concentration reached 9·0 mmol./kg.

3. Considerable variability was shown by individual rats in the amounts of sodium which had entered the muscle fibres during potassium deprivation.

4. Examination of the data of Heppel (1940) for the rate of mixing of labelled ions with the sodium which had entered the fibres during the low potassium period shows that the true entrance rate of potassium/unit concentration into such fibres is about 30 times as great as that of sodium.

5. The relative rate of passage of potassium ions into the normal fibres may be far greater than this, as evidenced by the comparatively very slow extrusion of muscle sodium, and it is also possible, as suggested by Ussing (1947), that the rate of mixing of the labelled ions may give values which are too high for ion transport.

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The Microbiological Degradation of Steroids

4. FISSION OF THE STEROID MOLECULE

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During recent years the presence of an enzyme system capable of effecting the oxidation of hydroxysteroids to the corresponding keto derivatives has been clearly demonstrated in a number of bacterial species. The investigations of the Italian schools of Ercoli and Arnaudi have resulted in the isolation from yeast of several new organisms, *Flavobacterium dehydrogenans* (Arnaudi, 1939), *F. androstenedionicum* (Ercoli & Molina, 1944), and *F. carbonilicum* (Molina & Ercoli, 1944), which, in general, oxidize the nuclear hydroxyl groups of steroid compounds devoid of the 17 side chain. Zimmermann & May (1944) obtained similar oxidations by the action of pseudodiphtheria bacilli. In the bile acid series stepwise oxidation of the hydroxyl groups has been achieved using *Alcaligenes faecalis* (Schmidt, Hughes, Green & Cooper, 1942), and more recently, *Escherichia coli* (Schmidt & Hughes, 1944). The independent investigations of the flora of soils by Tak (1942), and by the present author (Turfitt, 1943, 1944*a*), have shown that the cholesterol-decomposing organisms fall essentially within the genus *Proactinomyces*. These organisms are of particular interest in the breakdown of steroids since their oxidizing action is not, as reported for other organisms, inhibited completely by the presence of the intact side chain. Their place in the economy of nature is discussed later.

Whilst this primary oxidation of steroid hydroxyl groups is firmly substantiated, actual breakdown of the steroid molecules, yielding distinct chemical fission products, has not hitherto been recorded. Evidence of a secondary action of this nature has, however, been obtained during large-scale work on the oxidation of cholesterol to cholest-4-en-3-one (Turfitt, 1944*b*). With cholesterol as the sole carbon source, energy for growth must inevitably, in view of the non-autotrophic character of *Proactinomyces*, have resulted from utilization of the sterol carbon. During the course of the work certain small fractions were obtained which undoubtedly represented products of degradation, but attempts to isolate definite compounds were unsuccessful. A detailed account of the various methods by which information has been gained as to the course of the degradation process is given in the present publication.

EXPERIMENTAL

Foci of bacterial attack

Fission of the steroid skeleton by bacterial oxidation might be expected, by analogy with chemical methods, to occur primarily at one of several susceptible points, viz. OH groups, double bonds, or the side chain. With a view to determining whether the presence or absence of each of these points exerts any material effect upon the bacterial oxidation, a series of compounds covering different combinations of OH, double bond and side chain has been subjected to the oxidizing action of *P. erythropolis*.

In each experiment, performed in triplicate, 50 mg. finely powdered, sterile compound were introduced with aseptic