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MEMBRANE BARRIERS TO THE ENTRANCE OF SODIUM IONS INTO ISOLATED AMPHIBIAN SKELETAL MUSCLE

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SVMMARIVM — Si sartorius ranae musculus separatus per longum tempus (usque ad octo dies) immergitur in Ringer-Conwayanam solutionem kalio vel, ut dicitur, potassio carentem — cuius calor sit O°C — iones natrii in muscolares fibras tribus temporibus ingrediuntur, quorum aliud ad aliam membranae speciem attinet. Primum tempus quattuor circiter horis absolvitur; alterum triginta, eiusque stabilitas per duos dies plerumque permanet; tertium autem efficit ut in fibra musculari aquae saturatio natrio compleatur, kalium vero fere totum abeat.

When isolated frog sartorii muscles are immersed at room temperature in Ringer-Conway solution (with Na = 104 mM and K = 2.5 mM), there is a continuous relatively small entrance of Na ions and release of K ions. It was found that with the presence of frog blood plasma in the external fluid (2 parts of plasma to one part of the special Ringer fluid) the entrance of Na could be practically entirely inhibited to

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upwards of about one hour (CAREY and CONWAY, 1954). It was also shown by KERNAN (1962) that the membrane potential without the plasma was in the average 91 mV but with the frog plasma it reached, practically speaking, the full theoretical requirement from the equation:

$$E_m = \frac{RT}{F} \ln \frac{[K_1]}{[Ko]}$$

where E_m is the membrane potential and K_0 and K_i are the concentrations of K in the external solution and in the fibre water. From this it may be concluded that there is something in the physico-chemical structure of the plasma which inhibits the free entrance of Na ions and that in the free by resting *in vivo* condition there is a practically complete impermeability to the Na ion, and that this impermeability is manifested in the most external membrane which separates the fibre water from the external fibre. One may now consider the effect on the entrance of Na ions under conditions which have been much investigated here, namely, the immersion of sartorii in K free Ringer-Conway fluid at 0°C. This has been carried out with numerous results up to a time limit at first of 24 hrs. (CONWAY, HARRINGTON and MULLANEY, 1963). Among the results obtained may be mentioned the following. There is a relatively rapid entrance of Na^+ ions which phase reaches an end after about 4 hrs. This is referred to as the first phase of entrance. When the first phase is at an end, there appears a slow steady entrance of Na^+ up to the 24 hrs. examined. The rate of entrance in the second phase is much slower than in the first. It may be assumed that the second phase is occurring from the outset, and is additive to the first phase. The average Na content of the muscles due to the first phase is — approx. 40 m.eqs/kg. though this may vary between about 50 and 65 m.eqs/kg.

Fig. 1 shows a typical curve of entrance of Na into K free medium as described. It is also shown in Fig. 1 how the entrance of Na is inhibited by 5 or 10 mM K.

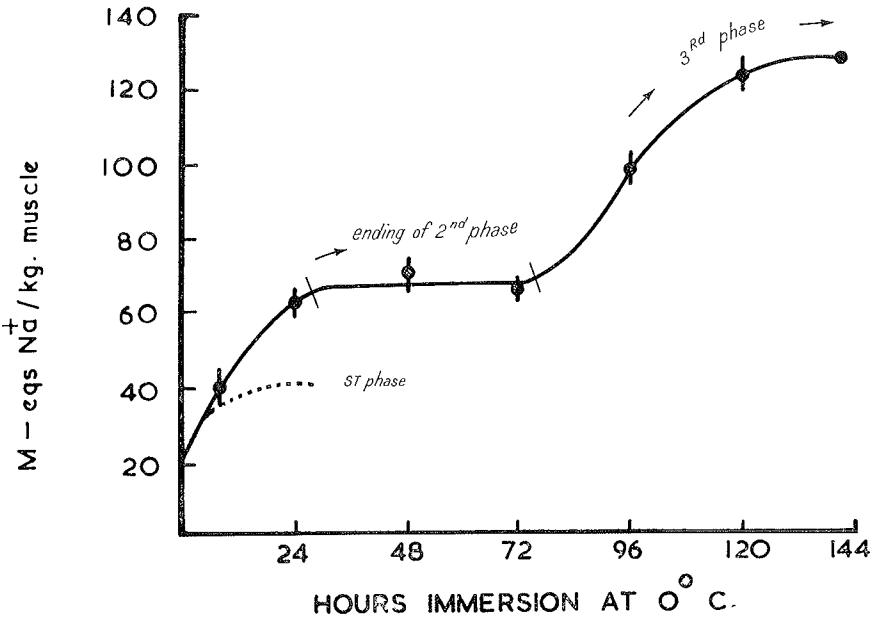

Fig. 1 — Net sodium entrance into isolated frog sartorii incubated in modified Ringer-Conway fluids containing 120 mM-Na and zero K (●) (curve A); 120 mM-Na and 5 mM-K (K) (curve B); 120 mM-Na and 10 mM-K (O) (Curve C). Each point on the curves represents the mean of 4-11 experiments.

Results of long period immersions at 0°C in K-free Ringer Conway fluid

Resulting from the observations of the 24 hours period of immersion, it was decided to extend the duration of the immersion period to upwards of 120 hours or more (Conway and

HARRINGTON, 1964 unpublished experiments). The results are striking. The second phase comes to an end after about 32 hours and from that time onward to about 70 hrs. the mean value of Na in the muscle remains virtually unchanged. The mean curve of entrance develops a plateau over the period.

After this, there begins a marked increase which extends the third stage of entrance of Na and this brings the muscle Na to the same level as the external sodium. This rate of increase of muscle Na in the third phase varies from one group of muscles to another. It may be comparatively rapid or more long drawn out.

Fig. 2 illustrates the mean values of a large number of observations in one set of experiments.

THE SIGNIFICANCE OF THE PHASES OF SODIUM ION ENTRANCE

The first phase. As mentioned above, the isolated sartorius of the frog allows Na^+ to enter at room temperature even though the composition of the external fluid is as close as possible to the inorganic composition of the blood plasma.

When the temperature of the immersion fluid is reduced to about O°C Na^+ entrance is much reduced but not eliminated.

The fact that the incorporation of frog blood plasma into the external fluid (it was introduced in the proportion of 2 to 1) inhibits the Na^+ entrance even at room temperature indicates that some constituent of the plasma is affecting the permeability of the outer cell membrane, and is necessary for the maintenance of normal permeability in the resting fibre. The volume of fibre water affected in the first phase may be taken as that between the external fibre membrane and the myofibrils.

The Second phase. In the second phase there occurs an exchange between the K content of some special region of the myofibrils and the external Na. The exchange is practically

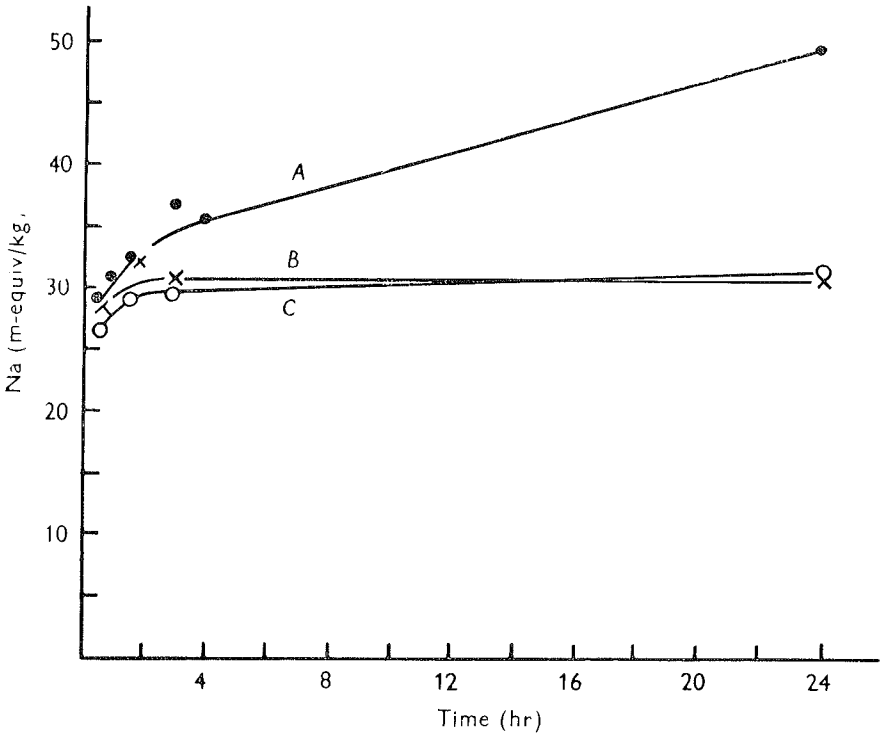

FIG. 2 — The diagram gives the mean results of Na entrance into frog sartorii immersed for long periods in K-free Ringer fluid at O°C. Each dot on the diagram represents the average of a number of results and the small vertical lines through the dots indicate the magnitude of the standard deviation of the means. It will be noted that over a period of nearly three days after the second phase of entrance of Na, there exists a steady state before the third phase begins and this third phase represents about 50% of the total fibre water.

linear up to about 24 hrs. after the initial immersion, and then rapidly tails off.

Having reached a steady state which is maintained for the next two days (or occasionally three or even four days), it is to be noted that the mean level of Na in the muscle fibre is approximately 70 m.eqs/kg. There has been a total increase

(if one observes Fig. 1) in the muscle Na of approximately 70-23 (=47 m.eqs./kg.). This increase is accommodated in a special region, between the outer membrane and the myofibrils and some special part of each myofibril. The remaining space, which may be regarded as the part of the myofibril from which the external Na^+ ion is excluded under the conditions. This remaining space is relatively large and may be presumed to be protected by a membrane which is completely impermeable to the Na^+ ion over the time observed.

The Third phase. After some two to days there sets in a marked increase in the Na^+ content of the muscle, until it reaches what may be expected when the Na in the whole fibre water is in equilibrium with that in the external fluid.

During this phase it is obvious that some additional membrane has deteriorated in permeability, or broken down, and finally the Na^+ of the external fluid has fully penetrated throught the fibre water.

Simultaneous determinations of the K content of the muscle were made, and the sum of the Na and K content remain about the same.

After 166 hours immersion the median Na content in a series (five determinations) was found to be 110 m.eq.Na/kg. and 6.9 m.eqs.K/kg.

The behaviour of the sodium pump and the phases of Na entrance

To test the activity of the sodium pump during the phases of entry described, the muscles after the immersion at 0°C were re-immersed at room temperature in Ringer-Conway fluid containing 104 mM Na and 10 mM K.

Restoration after the first phase

After the first phase — in which there was full entrance after about 4 hours in K free fluid — there was no appreciable restoration.

In the permeability breakdown of the first membrane inhibiting the entrance of Na, the pores of entrance appear much enlarged as not only does Na^+ enter but also such substances as sucrose, raffinose and other such substances. The *loss* of the permeability takes some time and as mentioned is not complete until after about four hours. But when this loss of permeability occurs, it remains, so that for example, labelled Na, having entered, is lost from the first phase as quickly as if there were no barrier, or as if there were the freest diffusion to and from the external fluid. (Conway and Clarke, 1964, unpublished experiments). It will be shown below how in fact the permeability of the first membrane can be restored).

Restoration in the Second Phase

Contrary to what happens in the first phase and confirmed in numerous experiments made here, re-immersion in Ringer-Conway fluid (containing 104 m.eqs. Na and 10 m.eqs.K per litre) causes a pronounced excretion of Na and uptake of K, the whole occurring over 2 hrs. but mostly after the first hour of the re-immersion. How the possibility of the excretion of Na, here is related to the critical energy barrier is discussed at length elsewhere (Conway, 1960); as well as the manner in which the critical energy barrier can be greatly modified by insulin and lactate (Kernan, 1962).

In the total average of experiments the loading with Na up to 2 hours was to reach an Na concentration of 59 m.eqs./kg. and after re-immersion at room temperature etc. it was re-

duced to 40 m.eqs./kg. occasionally to 35 m.eqs./kg. but not lower. In other words restoration of the first stage did not occur.

It may now be mentioned that the restoration of the first phase, and the permeability of its membrane can be brought about by the incorporation of frog plasma as shown by various experiments in this laboratory (CONWAY and LIDDANE, 1964, unpublished experiments). This will be dealt with elsewhere.

In the restoration of the second phase it is to be remembered that the Na^+ ion had penetrated into only a fraction (about one half) of the substance of the muscle fibril; but into this half it had entered fully and replaced the K content.

RESTORATION IN THE THIRD PHASE

The incidence of the 3rd phase may be looked for after about four to six days of the K free immersion.

In Fig. 1 the Na content rose to a level of about 124 to 128 m:eqs./kg. in 5 to 6 days. The muscles in this series showed a rather marked increase of water. In another series without marked water uptake the Na^+ content reached a median value of 108 m:eqs./kg. (10 results over the period 114 hrs. to 185 hours).

At such levels of Na content, with the K content much reduced (to about 23 m.eqs./kg. in the latter series) the Na pump is still quite active. Thus in the second series commented upon above the muscle content over the region 114 hours to 186 hours (10 analyses carried out) were 102.6 ± 4.2 m.eqs./kg. and after the re-immersion it was 70.4 ± 4.3. Thus there was a very substantial reduction in the Na content, and that this was due to an active process is evident from the action of ouabain. In a special series with ouabain (10^{-5} M) there was no secretion of Na.

DISCUSSION

The long period immersions of sartorii at 0°C in K-free Ringer-Conway fluid have brought to light some interesting findings with respect to Na^+ ion entrance. It becomes obvious that there are at least three membranes operative in inhibiting Na entrance. The most delicate or the one most easily affected is the most external, and at room temperature and immersion in a Ringer fluid simulating as closely as possible the inorganic composition of the internal medium the Na ion enters and K ion emerges.

When immersions are made at 0°C in K-free fluid (Na = 120 mM) Na enters as already described, and so also do a number of other substances such as sucrose, raffinose and no doubt many others, into the same spatial volume, which one may assume lies between the external membrane and the substance of the fibrils.

After the initial four hour immersions the permeability of the first membrane can be altogether, or in large part, restored by the inclusion of frog plasma in the 104/10 restoration fluid at room temperature.

The second membrane when the immersion remains as before, allows Na to enter slowly in exchange for K, but there seems to be none of the same free permeability resulting in the breakdown of the first membrane.

Also one has the striking fact that in the second stage of entrance only about one half of the fibril space is affected and the impermeability of the remaining half of the fibril remains for days.

After the third stage is complete or nearly so the Na pump is found to work, and to work well seeing that it can bring about an excretion of about 32 (104. - 70.4) m.eqs./Na/kg. But the reduction to 70.4 does not go appreciably lower than this. This could be interpreted as follows. The sodium pump

is not a normal equipment of that fraction of the fibril that remains impermeable to Na. When there is a total breakdown of the Na-K permeability, one half of the fibril remains ineffective for active Na transport, which the other half still retains, so that although effective Na transport or secretion occurs it is only down to a level of about 70 m.eqs.Na/kg.

REFERENCES

CAREY M.J. and E.J. CONWAY, « J. Physiol. », *125*, 232 (1964).

CONWAY D., HARRINGTON M.G. and MULLANEY M., « J. Physiol. Lond. », *165*, 246 (1963).

CONWAY E.J., « Nature », *187*, No. 4735 (1960).

KERNAN R.P., « Nature », *193*, 986-287 (1962).

— « J. Physiol. Lond. », *162*, 129 (1962).