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SODIUM EXCHANGES IN CARDIAC MUSCLE

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The observation of McDowall & Zayat (1952, 1953) that sodium and potassium changes in the environment materially affect the activity of the isolated rat ventricle, makes it desirable to study the problem quantitatively. The present work deals with sodium, potassium and water exchanges under varying environmental conditions. Special attention has been paid to changes in the sodium environment, as from the work referred to this appears to be more important than other ions.

METHODS

The method is similar to that of Feigen, Masuoka, Thienes, Saunders & Sutherland (1952) but simplified by McDowall & Zayat (1952) who used a broader strip of ventricle suspended in a bath after the manner of Bülbring (1946) for the rat diaphragm. Rats weighing between 150 and 200 g were used throughout, no differentiation between the sexes being made. The time interval between killing the animal and placing the preparation in the organ-bath was kept constant at 3 min. The maximum thickness of the ventricle after being slightly stretched in the stimulating electrode was 0·5 mm $\pm$ 0·05 (10), and when allowed to soak without stretch 0·7 mm $\pm$ 0·07 (10). (The $\pm$ sign indicates the standard deviation about the mean, and the figure in brackets the number of observations. These have the same meaning subsequently.)

The compositions of the various solutions used are given below in m-mole/l.

(1) *Normal Krebs* (Krebs & Henseleit, 1932). Na^+ 145, K^+ 5·9, Ca^{2+} 2·6, Mg^{2+} 1·2, Cl^- 130, HCO_3^- 25, $H_2PO_4^-$ 1·2, SO_4^{2-} 1·2.

(2) *Low sodium Krebs.* Na^+ 93, Cl^- 83·5, tonicity maintained with sucrose. Other ions as for normal Krebs.

(3) *High sodium Krebs.* Na^+ 177, Cl^- 158. Other ions as for normal Krebs.

Aeration was with 5% CO_2 and 95% O_2 mixture. Anoxia was produced with 5% CO_2 and 95% N_2 mixture. The pH was 7·4 at 33° C in all solutions.

All experiments were carried out at 33° C, at which temperature the preparation will continue to give maximal responses to electrical stimulation for many hours longer than required by the experiment.

For sodium and potassium estimations the following procedure was used. Muscles after removal from the bath were dried with blotting paper, weighed in glass tubes, dried for 24 hr at 120° C, re-weighed after cooling in a desiccator (to obtain the total water content), and then dissolved in 1 ml. 50% (v/v) nitric acid in a boiling water-bath. This solution was diluted appropriately with distilled water for estimation of sodium and potassium by flame photometry.

For the calculation of extracellular water the inulin space was determined. For the *in vitro* estimations the bath medium was prepared to contain 1% (w/v) of inulin. Preliminary work had

shown that equilibration of inulin between bath and muscle was maximal in 2 hr, and this time factor was kept constant. Inulin in muscle was determined by the procedure described by Ross & Mokotoff (1951) and in Krebs (and in plasma) by the method of Roe, Epstein & Goldstein (1949). Muscle blanks for *in vitro* preparations were uniformly negative. The volume of extracellular fluid in the tissue was calculated from its inulin content and the bath inulin concentration.

For the *in vivo* estimation of sodium and potassium concentration in terms of cell water it was necessary to estimate the *in vivo* inulin space. Rats of known weight were anaesthetized by an intraperitoneal injection of 1 ml. of a 25% (w/v) urethane solution. Both renal pedicles were then ligated, and an injection of a 22% (w/v) solution of inulin given into one external jugular vein in the proportion of 0·5 ml. per 100 g body weight as described by Ledingham (1953). After 3 hr blood was collected from a carotid artery and the heart muscle dissected in the usual way. Inulin was measured as described for the *in vitro* preparations. Muscle blanks are required for the *in vivo* estimations. Plasma blanks are negative. The amount of blood remaining in the ventricle was found in initial trials to be less than 0·5% of the tissue volume and is therefore negligible. The method of calculating the *in vivo* space was as used for the soaking experiments.

Calculations of sodium and potassium per litre of intracellular water were made by deducting the amount of ion in the extracellular phase from the total amount in 1 kg wet weight, and correcting the remaining intracellular ion to the amount that would be occupied by 1 l. of cell water, as described by Boyle & Conway (1941).

The preparation used here satisfies the usual criteria for adequate oxygenation. In the normal stretched preparation the muscle thickness is 0·5 mm (Feigen *et al.* 1952) which, theoretically, allows diffusion of oxygen. This is supported by the fact that the preparation will respond to stimulation for 36 hr with very little deterioration, while during the first hour there is an actual improvement in its contraction height. This would certainly not occur if any anoxia was present for it will be seen that anoxia, even when quite short-lived, produces a progressive deterioration which rapidly leads to death of the preparation.

RESULTS

Concentrations of intracellular sodium and potassium for soaked muscles under varying environmental conditions are shown in Table 1, the effects of partial anoxia in soaked and stimulated muscles in Fig. 1, and a summary of total water contents and inulin spaces in Table 2.

Exchanges in normal Krebs's solution. It is seen from Table 1 that when the tissue is simply soaked for 6 hr in Krebs's solution at 33° C containing 143 m-equiv. of sodium and 5·9 m-equiv of potassium per litre, there occurs an uptake of sodium and loss of potassium which is most rapid during the first 2 hr but thereafter slow. If the sodium in the bath is now reduced to 93 mM, the osmotic pressure of the solution being maintained by sucrose, the intracellular sodium now rapidly falls to below the level of that in the bath. This may be considered indicative for the existence of a sodium extrusion mechanism and forms a useful measure of 'pump' activity. There is no change in the inulin space as a result of soaking.

Exchanges in high and low sodium Krebs. If the muscles are soaked in low-sodium Krebs from the beginning, a smaller uptake of sodium occurs, while the uptake of sodium is increased by soaking the muscle in a modified Krebs's solution containing 177 mM-sodium; the uptake of sodium is increased in rate and in amount but there is unexpectedly no increased loss of potassium. There

is, however, a loss of intracellular water. To allow comparison between high and low sodium solutions to be made, the osmotic pressure of the low sodium solution was made equal to that of the high sodium solution by adding sucrose. The inulin space is not affected by changes in the sodium content of the solution.

TABLE 1. Intracellular Na and K concentrations

In vivo: Na_{cfw} 27·7±1·6 (8) K_{cfw} 141·0±4·1 (8)

		2 hr	4 hr	6 hr	6 hr normal Krebs + ½ hr low Na Krebs
Oxygenated Krebs, T=33° soaked	Na_{cfw}	64·0±5·6 (10)	71·5±4·3 (12)	80·5±7·7 (12)	62·5±6 (6)
	K_{cfw}	125·7±5·5 (10)	115±14 (12)	110±11·7 (12)	111±6 (6)
Stretched muscles 33°, O_2 Krebs	Na_{cfw}	64±2·1 (4)	—	84±5·2 (6)	—
	K_{cfw}	140±10·0 (4)	—	127±8·5 (6)	—
Low-sodium Krebs, 33° soaked*	Na_{cfw}	56·5±4·9 (6)	55·7±4·5 (6)	61·2±3·9 (6)	—
		146±5·5 (6)	137±6 (6)	118±5·3 (6)	—
High-sodium Krebs, 33° soaked	Na_{cfw}	76±5·7 (6)	84±4·6 (12)	88±4·9 (10)	—
		133±8 (6)	129±4·9 (12)	119±6·2 (10)	—
High potassium	Na_{cfw}	—	69·0±5·1 (6)	—	—
	K_{cfw}	—	104±6·4 (6)	—	—

* Low sodium balanced to osmolarity of high-sodium Krebs.
Na_{cfw} = Na concentration in m-mole/kg fibre water.
K_{cfw} = K concentration in m-mole/kg fibre water.

TABLE 2. Total water content and inulin space of muscles

In vivo: $\frac{[H_2O]\ ec(i)}{[H_2O]\ (T)}\ \frac{206\pm30\ (8)}{772\pm2·0\ (8)}$

		2 hr	4 hr	6 hr
O_2 Krebs	$[H_2O]$ ec(i)	227±37 (10)	238±29 (10)	—
	$[H_2O]$ (T)	784±9·6 (10)	780±9·0 (12)	781±10 (12)
Stretched muscles	$[H_2O]$ ec(i)	314±28 (10)	—	331±18 (10)
	$[H_2O]$ (T)	783±10 (4)	—	781±9 (10)
Stimulated muscles	Rate 12 $[H_2O]$ (T)	787±2·0 (4)	—	—
	Rate 36 $[H_2O]$ ec(i)	302±30 (10)	—	—
	$[H_2O]$ (T)	791±5·0 (4)	—	—
	Rate 100 $[H_2O]$ (T)	786±3·0 (4)	—	—
*Low-sodium Krebs	$[H_2O]$ (T)	762±110 (6)	754±11·5 (6)	758±1·0 (6)
High-sodium Krebs	$[H_2O]$ ec(i)	237±16 (10)	—	—
	$[H_2O]$ (T)	774±3·0 (6)	766±2·0 (12)	767±3·0 (10)
Anoxic Krebs	$[H_2O]$ ec(i)	207±31 (10)	205±28 (10)	—
	$[H_2O]$ (T)	795±5·0 (7)	804±8·5 (7)	813±6·0 (13)

* Balanced to osmolarity of high Na Krebs.
H_2O ec(i) = extracellular water in ml./kg wet weight, determined by inulin space.
H_2O (T) = total water in g/kg wet wt.

Exchanges with high potassium. The addition of potassium to the bath in an amount which normally increases the mechanical response does not produce any changes different from those in normal Krebs's solution.

The effect of increased tension. The study of sodium and potassium exchanges with stimulated muscles is complicated by an increase in the inulin space when

the muscle is stretched as is usual between clamp and lever. This increase in inulin space appears to be a direct consequence of stretching *per se*, as stretched non-stimulated muscles also show this increase (Table 2). Total water content remains the same in stimulated muscles, stretched controls, and soaked muscles (Table 2). This means that the extracellular water has increased at the expense of the intracellular water, and because of the small fraction of extracellular potassium, the shift of water out of the cell has the effect of significantly raising intracellular potassium level ($t = 3{\cdot}18$, $0{\cdot}001 < P < 0{\cdot}01$). This can be seen from the figures below, where muscles stimulated at rates of 12, 36 and 100 per min, for 2 hr are compared with stretched controls and unstretched controls. Sodium levels in terms of concentration in m-mole/kg intracellular water do not differ in unstretched and stretched controls. The maintenance of a similar concentration of sodium with reduced intracellular water means that sodium has been extruded by stretching the muscle (also Harris, 1954).

Effect of stimulation. There is no difference between the sodium content of stretched control muscles and that of those stretched and stimulated by maximal stimuli at various rates. Small differences were, however, found between the control potassium and the lowest potassium in the series at rate 100 per min and are possibly significant ($t = 3{\cdot}42$, $0{\cdot}01 < P < 0{\cdot}02$), suggesting that higher rates of stimulation may cause increased potassium loss:

	Stretched controls	Unstretched controls	Rate of stimulation per min		
			12	36	100
Na_{cfw}	64·1±2·1 (4)	64·0±5·6 (10)	63·3±3·7 (4)	61·1±3·8 (4)	60±3·6 (4)
K_{cfw}	140±10·0 (4)	125·7±5·5 (10)	135±8·4 (4)	124±8·3 (4)	115±8·3 (4)

Effects of anoxia. If muscles soaked for 1½ hr to allow stabilization are subjected to anoxia they take up sodium rapidly, and this is increased if they are stimulated with a maximal stimulus at a frequency of 12 per min at the same time (Fig. 1). If they are subjected to the anoxia for only half an hour, when oxygen is re-introduced they at first recover their sodium extruding mechanism, but this fails again after several hours. If stimulation is continued during the anoxic period the period of temporary recovery is absent. Even when the anoxia has been of short duration the sodium extruding mechanism is apparently damaged for low sodium for 1 hr at the end of a 6 hr period does not lower the intracellular sodium below that of the external medium, as in the case of muscles continuously oxygenated. The anoxia does not affect the inulin space (Table 2).

DISCUSSION

The observation that isolated frog muscle will take up sodium and lose potassium when placed in physiological media has long been known. Earlier workers, influenced no doubt by current views that sodium did not penetrate cell membranes, attributed the increase in sodium to expansion of the extra-

cellular space when measured as chloride (Fenn, Cobb & Marsh, 1934). That the increase is intracellular is confirmed by Creese (1954) using rat diaphragm. He comments on the relative immobility of potassium and the inadequacy of sodium extrusion under stable conditions.

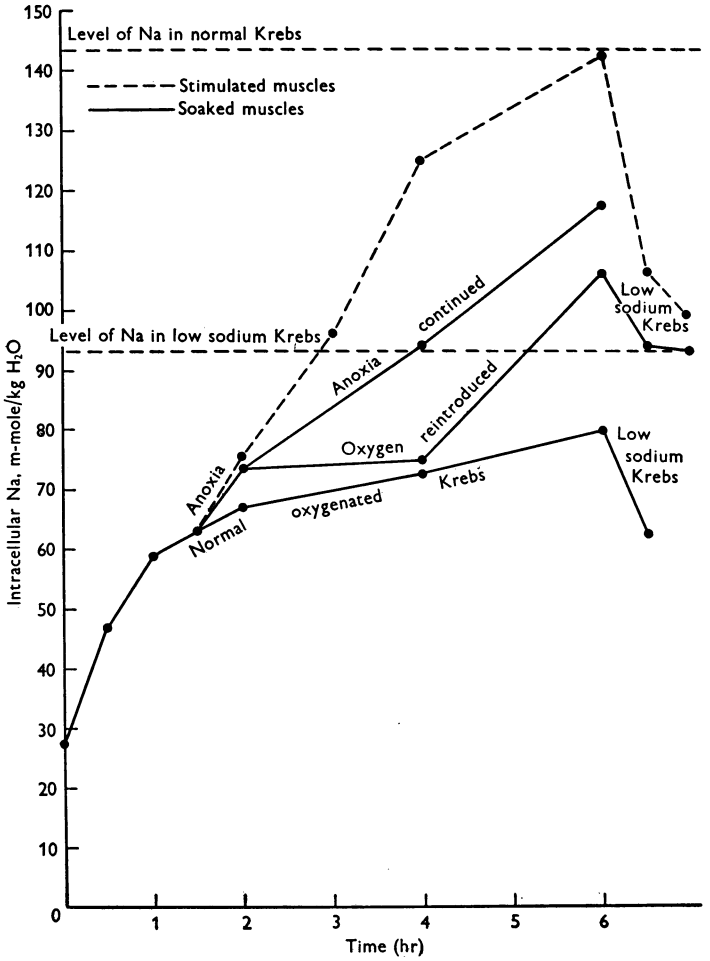

Fig. 1. Effect of anoxia on intracellular sodium concentration of resting and stimulated muscles. In normal Krebs's solution until 6 hr, then in low-sodium Krebs.

In the work described here the slow loss of potassium in stretched muscles can be seen to be due to movement of water from the intracellular phase to the extracellular phase. As previously discussed, the uptake of sodium without loss of potassium does not therefore oppose the view that sodium and potassium ions are exchanged quantitatively.

The observation that stretching frog muscle increases sodium extrusion has been made by Harris (1954), who correlated sodium extrusion with the temporary increase in heat production noted by Feng (1932). This increase in sodium extrusion may contribute to the efficiency of stretched muscle, as it has been shown by McDowall & Zayat (1953) that the efficiency of cardiac muscle is related to its sodium content.

Anoxia has been shown to increase greatly the uptake of sodium and loss of potassium. This has been observed in diseased hearts by Alexander, Boyle, Iseri, McCaughey & Myers (1950) and others, and demonstrated in the rat diaphragm by Creese (1954).

It is apparent that anoxia damages the mechanism for sodium extrusion, for when later the preparation is placed in a solution containing low sodium, the sodium content of the muscle is much reduced but does not return below the level of that of the external medium, thus differing from the case of continuously oxygenated muscle. It is interesting to see that such muscles may contract even when the internal sodium extrusion mechanism is not fully functioning. It is also clear that the rapid failure of the preparation during complete anoxia is not solely due to sodium uptake or potassium loss, for higher amounts of muscle sodium can be tolerated if the increase is obtained slowly by simply soaking the muscle in Krebs's solution. No doubt there is some adaptation to increased internal sodium, indeed such an adaptation is seen if sodium chloride is simply added to the bath.

The late failure of the muscle in the presence of oxygen after a short period of anoxia has been shown to be associated with an increased sodium uptake and this still further supports the view that even short periods of anoxia, from which the preparation recovers temporarily, damage the mechanism for sodium extrusion.

The finding that stimulation of oxygenated muscle does not increase sodium uptake is in agreement with the work of Fenn & Cobb (1936) who found an uptake of sodium only when stimulation was carried to the point of fatigue. It may, however, be that normal muscle can extrude sodium very rapidly between the contractions. The loss of potassium at stimulation frequencies producing exhaustion has been observed by Mitchell & Wilson (1921) in perfused frog muscle, and in ventricular fibrillation by Kehar & Hooker (1935). Normal amounts of potassium with moderate stimulation have been found in frog muscle by Mond & Netter (1930) and Mitchell & Wilson (1921).

SUMMARY

1. The rat ventricle preparation has been shown to take up sodium and lose potassium if placed in a bath of normal Krebs's solution. Stretching the muscle increases sodium extrusion.

2. Anoxia greatly increases the uptake of sodium and also produces sustained damage to the sodium extrusion mechanism.

3. Stimulation of anoxic muscle increases the sodium uptake, but not that of normal muscles which can extrude sodium efficiently.

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