Ion Transport and Metabolism in Slices of Guinea-Pig Seminal-Vesicle Mucosa

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(*Received* 5 *January* 1959)

Lardy & Phillips (1943), and Blackshaw (1953) found that bull and ram spermatozoa show optimum motility *in vitro* only when at least 5–10 mM-K^+ ions are present in the medium. Potassium also stimulates the metabolism of spermatozoa, and Dott & Walton (1956) have shown that both glycolysis and respiration are needed for maximum motility. Potassium is therefore essential for the motility and metabolism of spermatozoa. It is present in the natural milieu for spermatozoa on ejaculation, namely seminal plasma, which is elaborated mainly by the seminal vesicles in the rat, guinea pig and bull (see Mann, 1954). As a preliminary to the study of the secretion of ions in seminal plasma, Breuer & Whittam (1957) tried to find out how the high potassium concentration was maintained in slices of seminal-vesicle mucosa of the guinea pig. They showed that anaerobiosis had little effect on the tissue sodium and potassium concentrations during incubation at 37°, but that inhibitors of glycolysis anaerobically or of respiration aerobically caused a loss of potassium and a gain of sodium. Metabolic energy from either respiration or anaerobic glycolysis was therefore needed to support the concentration gradients of sodium and potassium, which they suggested may depend upon a supply of adenosine triphosphate, the formation of which is common to both metabolic pathways. The electrical potential difference across the cell membranes is not known and it is not possible therefore to decide whether both potassium and sodium are actively transported. Thus the intracellular potassium concentration could be maintained either by a primary active transport of potassium or by a primary active outward movement of sodium, which would generate a potential difference and cause a passive inward movement of potassium. The present work has shown that, whether sodium or potassium or both ions are actively transported, there is a correlation between the retention of potassium and the exclusion of sodium, and the concentration of easily hydrolysable phosphate. Other experiments are described which show that a reduction in the sodium concentration of the medium caused a roughly proportional fall in the tissue sodium concentration, without the tissue potassium concentration being affected. Under these conditions the intracellular potassium is probably retained passively by non-diffusible anions.

METHODS

Materials and procedure. Guinea pigs weighing 400–600 g. were stunned and killed by bleeding from the carotid artery, and the seminal vesicles removed immediately. Slices of seminal-vesicle mucosa were made with a dry razor blade as described by Breuer & Whittam (1957). The slices of mucosa (30–50 mg.) were then placed in 3 ml. of medium (described below) in 15 ml. stoppered conical cups, which were shaken at 1°, 17°, 27° or 37° in a water bath. In anaerobic experiments, a stick of yellow phosphorus was placed in the centre well. After various periods, the slices were taken from the cups, blotted on Whatman no. 541 filter paper and weighed.

Media. The phosphate medium of Krebs & Henseleit (1932) was used as the standard medium. Low-sodium and sodium-free media were prepared by partially or completely substituting 0·154 M-choline chloride or 0·308 M-sucrose for the NaCl, and 10 mM-2-amino-2-hydroxymethylpropane-1:3-diol (tris) chloride buffer, pH 7·4, for the phosphate buffer. Unless otherwise stated, glucose (20 mM) and sodium L-glutamate (10 mM) were added to the media as substrates. Glutamate was added because Breuer & Whittam (1957) found that a higher potassium and a lower sodium concentration were maintained in the tissue in its presence.

Determination of sodium and potassium. The weighed tissue slices were placed in 5 ml. of N-HNO_3 and allowed to stand overnight (to leach the soluble salts from the tissue). Samples (1 ml.) of the acid extract of the tissue were diluted to 5 ml. for the flame-photometric estimation of Na and K with the flame-photometer attachment of the Beckman DU spectrophotometer.

Determination of chloride. Chloride was determined, on a 1 ml. sample of the HNO_3 extract of the tissue, by the potentiometric-titration method of Sanderson (1952).

Determination of easily hydrolysable phosphate. Mucosal slices (30–50 mg.) were placed in 1 ml. of 5% (w/v) trichloroacetic acid, broken up with a glass rod and allowed to stand at 4° during the night. The samples were then shaken and centrifuged. Samples (0·4 ml.) of the supernatant acid extract were taken for the estimation of inorganic phosphate by the method of Berenblum & Chain (1936). For the estimation of easily hydrolysable phosphate, 1 ml. of N-HCl was added to 0·2 ml. of the extract in

trichloroacetic acid, and the mixture heated for 7 min. in a boiling-water bath and the inorganic phosphate then estimated. The value for easily hydrolysable phosphate was obtained by subtraction of the inorganic phosphate concentration before acid hydrolysis from that found after heating for 7 min. at 100° in N-HCl. Easily hydrolysable phosphate is taken to originate mainly from adenosine triphosphate (ATP) and creatine phosphate.

Measurement of respiration and anaerobic glycolysis. The consumption of O_2 and liberation of CO_2 by the mucosa was measured in Warburg manometers at 1°, 17°, 27° and 37° in the usual way. The results are expressed as Q_{O_2} and Q_{CO_2} (μl./mg. dry wt./hr.).

Water content of tissue. The tissue water was taken to be equal to the loss of weight on drying the tissue overnight at 105°.

RESULTS

Concentrations of potassium and easily hydrolysable phosphate in tissue slices

Previous experiments showed that anaerobiosis did not prevent the retention of potassium and the exclusion of sodium by slices of seminal-vesicle mucosa, although cooling and inhibitors of respiration and glycolysis did prevent this (Breuer & Whittam, 1957). A maintenance of concentration gradients in the same tissue, under both aerobic and anaerobic conditions, eliminates any mechanism of cation transport depending on consumption of O_2 and electron transport in the cytochrome system. An alternative possibility is that easily hydrolysable phosphate (e.g. ATP or creatine phosphate) might support active cation transport, both aerobically and anaerobically, by energizing a carrier mechanism. In this case, low and high potassium concentrations would be expected with low and high concentrations of easily hydrolysable phosphate respectively, irrespective of whether potassium is actively transported inwards directly or follows as a consequence of active sodium transport outwards. This expectation has been tested by measuring the concentrations of easily hydrolysable phosphate in slices containing either low or high concentrations of potassium. These were obtained by incubation at 1° or 37°, and in the presence or absence of metabolic inhibitors.

To obtain enough tissue for incubation under the six conditions shown in Table 1 the tissue from two animals was required. Slices were immersed in the standard medium at room temperature until they had all been made (about 10 min.), and then transferred to the conical cups for gassing and incubation. Analysis of the tissue immediately after slicing showed that the potassium concentration fell by 21 μmoles/g. of whole tissue, and that the sodium concentration rose by a similar amount, during the immersion in the medium (Table 1). The reason for these changes is not clear, but they may be partly due to a loss of potassium and a gain of sodium by damaged cells on the outside of the slices. This explanation requires that about 18% of the cells would be damaged. It is also suggested by the similar loss of potassium that occurred after immersion in 0·308 M-sucrose solution (see Table 4). This loss was then about the same after 2 min. (22 μmoles/g.) as after 45 min. (27 μmoles/g.), as would be expected from an equilibration of damaged cells with the bathing solution. For this reason, the concentrations found after incubation have been compared with the values for the tissue after immersion in the medium at room temperature and not with those for freshly prepared slices.

Table 1. *Concentrations of easily hydrolysable phosphate and of potassium and sodium in slices of seminal-vesicle mucosa after incubation* in vitro *for 45 min.*

20 mM-Glucose and 10 mM-sodium glutamate were added to the medium, pH 7·4. Mean values of at least two results are given. When added, the concentration of 2:4-dinitrophenol (DNP) was 0·2 mM and that of sodium iodoacetate (IAA) was 1 mM.

			Concentrations (μmoles/g. of whole tissue)				Change during incubation (μmoles/g. of whole tissue)		
			Easily hydrolysable phosphate	K	Na	Na + K	Easily hydrolysable phosphate	K	Na
Tissue after dry-slicing ...			—	109	35	144	—	—	—
Tissue before incubation ...			8·9	88	67	155	—	—	—
Tissue after incubation									
Temp.	Gas phase	Inhibitors added							
37°	O_2	None	8·1	84	75	159	−0·8	−4	+8
37	N_2	None	8·8	75	85	160	−0·1	−13	+18
37	O_2	DNP + IAA	5·4	48	108	156	−3·5	−40	+41
37	N_2	IAA	4·7	50	104	154	−4·2	−38	+37
1	O_2	None	5·9	39	113	152	−3·0	−49	+46
1	N_2	None	5·7	35	115	150	−3·2	−53	+48

The results of incubation for 45 min. at 37° show that the concentration of easily hydrolysable phosphate (8·9 μmoles/g.) remained approximately constant under both aerobic (8·1 μmoles/g.) and anaerobic (8·8 μmoles/g.) conditions (Table 1). After aerobic incubation, the tissue potassium concentration was practically unchanged (84 μmoles/g.), whereas anaerobically it fell 15%, from 88 to 75 μmoles/g. The addition of 0·2 mM-2:4-dinitrophenol plus mM-sodium iodoacetate aerobically and of iodoacetate anaerobically caused respective losses of 39 and 47% in the easily hydrolysable phosphate concentration, and of 45 and 42% in the potassium concentration. Under these conditions, the sodium concentration increased by an amount approximately equivalent to the loss of potassium. After either aerobic or anaerobic incubation at 1°, the loss of easily hydrolysable phosphate was 35% and that of potassium about 60%, and, again, the loss of potassium was balanced by an equivalent gain of sodium. The percentage losses of easily hydrolysable phosphate and of potassium at 1° are therefore only roughly correlated, possibly because cooling retarded the rate of hydrolysis of phosphate esters without markedly changing the rate of loss of potassium. The absence of major changes in the concentrations of easily hydrolysable phosphate and of sodium and potassium under both aerobic and anaerobic conditions at 37°, and the comparable losses of easily hydrolysable phosphate and potassium, and the gains of sodium, produced by metabolic inhibitors, suggest that the retention of potassium and the exclusion of sodium are probably facilitated by labile phosphate esters.

Effect of temperature on the tissue ionic concentration

To find whether a reduction of the temperature of incubation would cause movements of tissue sodium, potassium and chloride to levels which might be correlated with the metabolic energy supplies, as shown by the Q_{O_2} and $Q^{N_2}_{CO_2}$ values, slices were incubated at 37°, 27°, 17° and 1° under both aerobic and anaerobic conditions. The results are shown in Table 2. The sum of the concentrations of sodium plus potassium was approximately constant at about 160 μmoles/g., irrespective of the temperature of incubation, which shows that any losses of potassium were balanced by equivalent amounts of sodium. The Q_{O_2} and $Q^{N_2}_{CO_2}$ values were each about 7 after incubation at 37°, and fell by 52 and 34% respectively when the temperature was reduced from 37° to 27°. The ionic concentrations changed by only 7–14% on reducing the temperature from 37° to 27°, and are not therefore directly related to the metabolic quotients. The energy available from metabolism at 27° was probably sufficient to meet the demands of the active transport processes. At 17°, the Q_{O_2} and $Q^{N_2}_{CO_2}$ values were approximately only 20 and 30% of those at 37°. The reduced energy supply then probably limited the active movements of ions, and accounts for the lower concentration of potassium (58·5–61·0 μmoles/g.), and the higher concentrations of sodium (103–106 μmoles/g.) and chloride (68·0–74·5 μmoles/g.) found aerobically and anaerobically. At 1°, the loss of tissue potassium and the gains of sodium and chloride were even greater than at 17°, and the O_2 consumption and CO_2 output were immeasurably small with the Warburg apparatus.

The intracellular concentrations of sodium and potassium have also been calculated, a value of 13 ml./100 g. of tissue being used to correct for the ions in the extracellular space. This was the inulin space previously found under both aerobic and anaerobic conditions at 37° (Breuer & Whittam, 1957), and it has been assumed also to represent the extracellular space at the lower temperatures. The intracellular potassium concentrations are likely to be accurate since the correction for extracellular potassium was small. The sodium concentration, on the other hand, can be considered as only approximate in view of the large correction for extracellular sodium and the likelihood of small variations in the inulin space. Fig. 1 shows the results of aerobic incubation and Fig. 2 those of anaerobic incubation. Both graphs show that whereas the concentrations depended on the temperature of

Table 2. *Effect of the temperature of incubation on the potassium, sodium and chloride concentrations, and the metabolic quotients of seminal-vesicle mucosa*

Slices of mucosa (about 30–60 mg.) were shaken for 1 hr. in 3 ml. of phosphate or bicarbonate medium containing 20 mM-glucose and 10 mM-glutamate, pH 7·4.

Gas phase		O_2				N_2+CO_2 (95:5)			
Temp.		37°	27°	17°	1°	37°	27°	17°	1°
Q_{O_2} or $Q^{N_2}_{CO_2}$		6·7	3·2	1·4	<0·2	7·3	4·8	2·4	<0·2
Ionic concentrations (μmoles/g. of whole tissue)	K^+	87·0	79·5	58·5	40·0	74·5	69·0	61·0	35·0
	Na^+	69·0	79·0	106·0	113·5	85·0	92·0	103·0	115·0
	Cl^-	61·5	66·0	74·5	—	44·5	52·0	68·0	—
	$Na^+ + K^+$	158·0	158·5	164·5	153·5	159·5	161·0	164·0	150·0

incubation they were not simply related to the rates of respiration or of anaerobic glycolysis. A lower potassium concentration, at temperatures below 37°, was accompanied by a higher sodium concentration, and the sum of the two concentrations was approximately constant (148–165 μmoles/g. of cells). A similar dependence of chloride concentrations on temperature was shown in slices of guinea-pig-kidney cortex by Whittam (1956), without a simple relationship between concentration and metabolic rate.

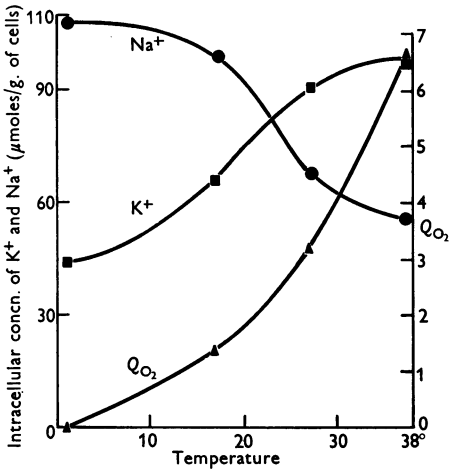

Fig. 1. Effect of the temperature of incubation on the intracellular sodium and potassium concentrations and the Q_{O_2} of seminal-vesicle mucosa. Slices were incubated for 45 min. in phosphate standard medium.

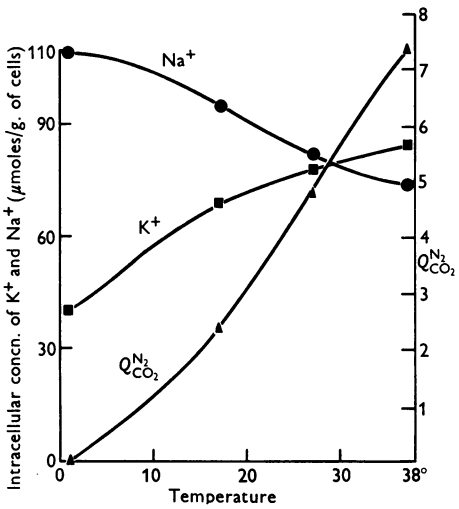

Fig. 2. Effect of the temperature of incubation on the intracellular sodium and potassium concentrations and the $Q^{N_2}_{CO_2}$ of seminal-vesicle mucosa. Slices were incubated for 45 min. in bicarbonate standard medium.

Effects of lowering the sodium concentration of the medium

Tissue sodium concentration. The concentration gradient of sodium between cells and medium in a sodium-free solution, made isotonic with either a non-penetrating electrolyte or non-electrolyte, will favour a movement of sodium from cells to medium instead of from medium into cells as in the medium of Krebs & Henseleit (1932). The potassium concentration of the tissue in the absence of external penetrating cations may be due to a Donnan equilibrium or to potassium movements either directly or indirectly dependent upon metabolism. To find the effect of the sodium concentration of the medium on the tissue sodium and potassium concentrations, slices of seminal-vesicle mucosa have been incubated aerobically at 37° in media in which sodium chloride had been partially or completely replaced with choline chloride or sucrose. Choline choloride and sucrose were chosen as replacements for sodium chloride as examples of a relatively non-penetrating electrolyte and non-electrolyte. Although the extent of their penetration into slices of seminal-vesicle mucosa was not determined, it seemed unlikely from results with other cells that this would be appreciable during incubation times of 45 min.

The results showed that the tissue sodium concentration was proportional to that of the medium over the range 20–120 mM, and that it was constant (about 65 μmoles/g. of whole tissue) when the medium contained 120 and 155 mM-Na^+ ions (Fig. 3). In sodium-free solution, the tissue sodium concentration was not significantly different from zero. Similar results were found after incubation in

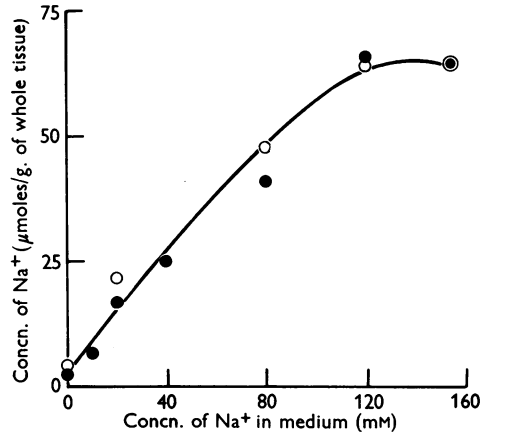

Fig. 3. Effect of sodium concentration in the medium on the sodium concentration of slices of seminal-vesicle mucosa after aerobic incubation for 45 min. at 37°. ●, Medium containing sucrose; ○, medium containing choline chloride.

the sucrose or choline chloride media. The results in Fig. 3 show that the cell membranes were permeable to Na^+ ions, which migrated from cells to medium in the low-sodium and sodium-free solutions. The lower tissue concentrations found at higher external sodium concentrations (120 and 155 mM) must have been due to the active extrusion of sodium from the cells.

To find the rate at which tissue sodium leaked into sodium-free media, the tissue was analysed for sodium after incubation for various times. Fig. 4 shows that the tissue sodium concentration fell to the same extent in both the choline chloride and sucrose media. This fall represents a loss of sodium from extra- and intra-cellular fluid, although a clear resolution into two components is not possible from the curve. The graph shows, however, a rapid initial fall gradually merging into a slower fall, as would be expected from a more rapid loss from extracellular fluid than from within the cells.

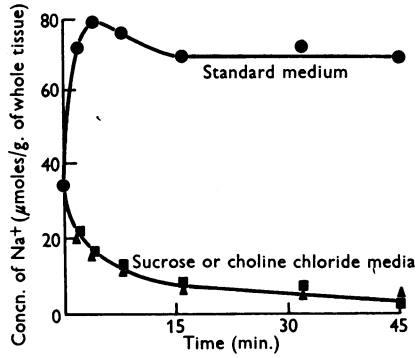

Fig. 4. Effect on the sodium concentration in slices of seminal-vesicle mucosa of aerobic incubation at 37° in standard medium (●) or in sodium-free sucrose (■) or choline chloride (▲) media.

Fig. 4 also shows that after about 30–45 min., the tissue sodium had become experimentally indistinguishable from zero. In contrast with the loss of tissue sodium in sodium-free media, during incubation in the medium containing 160 mM-Na^+ ion, the tissue sodium concentration increased almost twofold in the first 2 min. and then stayed approximately constant (70 μmoles/g. of whole tissue) for the remainder of the 45 min. incubation (Fig. 4).

Tissue potassium concentration. Results in Table 3 show that a partial or complete replacement of sodium with sucrose in the medium had no significant effect on the potassium concentration of incubated slices, which was between 81 and 86 μmoles/g. of whole tissue irrespective of the sodium concentration of the medium. After incubation in the sodium-free choline chloride medium, the concentration was 72·5 μmoles/g. of whole tissue, which is lower than that found in tissue from the standard medium. The difference of 13 μmoles/g. (85·5 − 72·5), with S.E. 2·8, is derived from mean values from a number of experiments in which there was some scatter, and is significant ($P < 0.01$). It is probably due to an entry of choline into the cells in exchange for potassium, for Maizels & Remington (1958) have recently shown that choline partially displaces potassium in renal-cortex slices, and it cannot be regarded as a completely non-penetrating cation. No attempt has been made to identify the anion which must leave the cells with sodium during incubation in sodium-free sucrose medium although chloride seems the most likely. In choline chloride medium, a loss of intracellular chloride is most unlikely, however, in view of the high external concentration, and sufficient choline may penetrate to balance the loss of sodium and the small loss of potassium.

Table 3. *Effect of the replacement of sodium chloride in the medium with either sucrose or choline chloride on the potassium concentration, the water content and Q_{O_2} of slices of seminal-vesicle mucosa*

About 30–60 mg. of tissue was shaken for 45 min. at 37° in 3 ml. of medium, pH 7·4, containing 20 mM-glucose, 10 mM-glutamate and 5 mM-K^+ ion. Gas phase was O_2. Mean values and S.E. are given, and the figures in parentheses are the number of observations.

Compound with which NaCl was replaced	Concn. of sodium in medium (mM)	Q_{O_2}	Concn. of potassium in tissue (μmoles/g. of whole tissue)	Water content (%, w/w)
Choline chloride	0	4·0±0·4 (5)	72·5±2·1 (20)	79·1±0·7 (12)
	5	—	71·5±1·1 (6)	79·0±0·1 (10)
	20	—	76·0±1·8 (8)	80·2±0·3 (12)
	40	—	75·0±1·8 (8)	79·8±0·1 (12)
	80	5·9±0·4 (6)	81·5±1·2 (8)	80·4±0·3 (12)
	120	—	78·5±0·9 (14)	80·7±0·4 (10)
Sucrose	0	3·7±0·2 (10)	81·0±1·4 (8)	79·3±1·0 (6)
	20	—	85·0±0·8 (6)	79·4±0·2 (8)
	80	5·2±0·2 (9)	83·5±1·6 (8)	79·5±0·5 (8)
	120	—	83·0±0·9 (8)	81·2±0·1 (8)
Control in standard medium	155	6·4±0·1 (36)	85·5±1·9 (16)	80·5±0·4 (10)

Table 4. *Sodium and potassium concentrations in slices of seminal-vesicle mucosa after incubation at 37° in standard medium or in sodium-free media*

Slices of tissue (about 50 mg.) were incubated in media, pH 7·4, containing 10 mM-tris buffer, 10 mM-glutamate, 20 mM-glucose and 5 mM-K^+ ion. Gas phase was O_2. Mean values are given ± S.E. and the figures in parentheses are the number of observations. Abnormal media are named after the principal solute.

	Concn. of potassium (μmoles/g. of whole tissue)		
Medium ...	Standard	Choline chloride	Sucrose
Time of incubation (min.)			
0	108·7±1·5 (15)	108·7±1·5 (15)	108·7±1·5 (15)
2	81·9±1·8 (6)	85·6±3·7 (8)	86·7±2·0 (10)
4	77·2±2·1 (6)	85·4±1·7 (8)	81·7±2·5 (10)
8	81·7±4·0 (6)	79·2±2·9 (8)	83·5±2·7 (10)
16	86·7±3·4 (8)	75·5±1·0 (8)	81·4±3·0 (6)
32	87·8±4·0 (12)	72·4±1·5 (8)	81·3±3·2 (6)
45	83·9±1·6 (28)	72·5±2·1 (20)	81·2±1·4 (8)

After incubation in either the standard medium or in sodium-free media, the tissue lost potassium (about 20–30 μmoles/g. of whole tissue) in the first 2–4 min. (Table 4). This loss occurred in both phosphate and bicarbonate media, and is probably due to a loss of potassium from damaged cells on the outside of the slice. Table 4 shows that the tissue potassium concentration was not significantly different after incubation in the sodium-free sucrose medium and in the standard medium (81·2 and 83·9 μmoles/g. of whole tissue), although it was significantly lower in choline chloride medium (72·5 μmoles/g. of whole tissue) (see also Table 3).

Rate of respiration. The rate of respiration of the tissue in standard medium at 37° of 6·4 μl./mg. dry wt./hr. was reduced by 19% by a lowering of sodium concentration of the medium to 80 mM (Q_{O_2} 5·2–5·9), and by 42% in the sodium-free media (Q_{O_2} 3·7–4·0). Significant differences were not found between the choline chloride and sucrose media. [The tissue-water content (79–81%) was not changed by incubation in the low-sodium media (Table 3).]

Effect of metabolic inhibitors. The addition of 2:4-dinitrophenol to slices of seminal-vesicle mucosa respiring in standard medium caused a loss of potassium, which was balanced by an uptake of sodium, and this was part of the evidence indicating a dependence of ionic concentrations on metabolism (Breuer & Whittam, 1957). The question now arises whether the potassium concentration found after incubation in sodium-free media is also dependent on metabolism, whether directly or indirectly, or whether it is due to a Donnan effect arising from the presence of non-diffusible anions. Fig. 4 shows that, after incubation in sodium-free, sucrose or choline chloride media, the tissue sodium concentration approached zero, and Table 4 that the potassium concentration was approximately equal to that after incubation in standard medium. If retention of potassium in the cells in sodium-free media is a result of a Donnan equilibrium, interference with metabolism should not affect it, but if it is due to active transport, then metabolic inhibitors would be expected to lower the concentration.

Slices of mucosa have therefore been incubated in the standard medium, and in the sodium-free sucrose and choline chloride media, in the presence of 2:4-dinitrophenol and iodoacetate, which were added to inhibit both respiration and glycolysis. The inhibitors added to the standard medium caused a lowering of the potassium concentration by 24 μmoles/g. of whole tissue anaerobically, and by 28 μmoles/g. of whole tissue aerobically, compared with the control slices, which contained about 75 μmoles/g. of whole tissue (Table 5). In contrast, the inhibitors caused only a small loss of tissue potassium (4–13 μmoles/g. of whole tissue) in the sucrose or choline choloride media, both aerobically and anaerobically. The media contained 10 mM-glucose and 10 mM-L-glutamate, and it seemed possible that this small loss might be due to a movement of glutamate accompanied by potassium.

Effect of glucose and glutamate. To test this possibility, slices were incubated aerobically in each medium without the addition of glucose and glutamate. The potassium concentration was then 70 μmoles/g. of whole tissue after incubation in the sucrose medium, and 57 μmoles/g. of whole tissue after incubation in the choline chloride medium (Table 5). The values are 13 μmoles/g. lower than those found after incubation with glucose and glutamate. These effects are almost identical with those found after the addition of 2:4-dinitrophenol plus iodoacetate in the presence of glucose and

glutamate, and show that about 10–13 μmoles of potassium/g. of whole tissue, of the concentration found after incubation in sodium-free media containing glucose and glutamate, was due to the presence of these compounds and to the availability of metabolic energy. The results are compatible with an active uptake of 10–13 μmoles/g. of glutamate accompanied by potassium, which was abolished by 2:4-dinitrophenol plus iodoacetate.

In contrast with the small effects of glucose and glutamate on the tissue potassium concentrations in sodium-free media, a large difference of 31 μmoles of potassium/g. of whole tissue was found in tissue incubated in the standard medium, with and without glucose and glutamate (Table 5). Again, this difference is similar to that found by adding 2:4-dinitrophenol plus iodoacetate (28 μmoles/g.). If an active movement of glutamate accompanied by potassium occurs to the same extent as in the tissue incubated in the sodium-free media, it would explain only about 40 % of the effects in the standard medium. The greater effects in tissue incubated in the standard medium are undoubtedly due to the presence of external sodium, which was able to replace internal potassium in metabolically deficient tissues.

Effect of cooling. To test further whether a high tissue potassium concentration could be maintained in the absence of metabolism, slices were incubated at 1°, since cooling should cause a loss of potassium maintained by a coupling to metabolism, but not when the potassium concentration is due to a Donnan equilibrium. Table 5 shows that the tissue potassium concentration was 40 μmoles/g. of whole tissue after incubation at 1° in standard medium, whereas that from sucrose medium was 76 μmoles/g. of whole tissue, and that from choline chloride medium 56 μmoles/g. of whole tissue.

The results in Table 5 therefore show that a high tissue potassium concentration could be maintained during incubation in sucrose or choline chloride media in the virtual absence of metabolic energy. In standard medium, only a low tissue potassium concentration was then found since the intracellular potassium could be readily replaced by external Na^+ ions (see Table 1). In the absence of external Na^+ ions, owing to a replacement with sucrose or choline, internal potassium was therefore retained passively, probably by a Donnan equilibrium.

DISCUSSION

Easily hydrolysable phosphate and potassium and sodium concentrations

The main result of the incubation of slices of seminal-vesicle mucosa of the guinea pig in medium at 37° is that, under both aerobic and anaerobic conditions, the concentrations of easily hydrolysable phosphate (taken to be mainly ATP) and of potassium underwent only small changes. The easily hydrolysable phosphate concentration would not be expected to be as high anaerobically as aerobically, because the rates of oxygen uptake and carbon dioxide output were the same, with Q_{O_2} and $Q^{N_2}_{CO_2}$ values about 7. Now, by assuming a phosphorylation quotient (P/O ratio) of about 3, it can be shown that for the production of ATP a Q_{O_2} of 1 is approximately equivalent to a $Q^{N_2}_{CO_2}$ of 6 (see Warburg, 1956). It follows therefore that the $Q^{N_2}_{CO_2}$ was only about one-sixth of that required to

Table 5. *Effect of cooling, the addition of glucose and glutamate and the addition of 2:4-dinitrophenol plus iodoacetate on the potassium concentration of seminal-vesicle mucosa after incubation in standard medium or in sodium-free sucrose or choline chloride media*

About 30–40 mg. of tissue was shaken for 45 min. in 4 ml. of solution in 15 ml. conical flasks containing 10 mM-tris buffer, pH 7·4. Concentrations were glucose, 20 mM; L-glutamic acid, 10 mM; 2:4-dinitrophenol (DNP), 0·2 mM and iodoacetate (IAA), 1 mM.

			Concn. of potassium (μmoles/g. of whole tissue)			Loss of potassium (μmoles/g. of whole tissue)		
			With glucose + glutamate					
Medium	Gas phase	Temp.	No DNP +IAA (*a*)	With DNP +IAA (*b*)	No glucose and glutamate (*c*)	DNP + IAA [(*a*) − (*b*)]	Glucose + glutamate omitted [(*a*) − (*c*)]	Cooling
Standard	O_2	37°	76	48	45	28	31	36
	O_2	1	40	—	—	—	—	
	N_2	37	74	50	41	24	33	—
Sucrose	O_2	37	83	72	70	11	13	7
	O_2	1	76	—	—	—	—	
	N_2	37	76	72	—	4	—	—
Choline chloride	O_2	37	70	60	57	10	13	14
	O_2	1	56	—	—	—	—	
	N_2	37	73	60	—	13	—	—

produce ATP at the same rate anaerobically as aerobically. The similar concentrations found under these conditions suggest that, for the synthesis to keep pace with the loss of ATP, the rate of hydrolysis anaerobically must be but one-sixth of the rate aerobically.

An increase in the efflux of sodium from squid giant axons poisoned with cyanide, on the injection of ATP (Caldwell & Keynes, 1957; Keynes, 1958; Caldwell, 1958), is the most direct evidence for a linkage of ATP with the active transport of a cation (sodium), and a little less so are the parallel falls in ATP concentration and potassium influx in glucose-free, human erythrocytes (Whittam, 1958). These are examples of cells which are respectively predominantly aerobic and exclusively anaerobic in their metabolism. The seminal-vesicle mucosa, on the other hand, maintains its potassium by energy from either metabolic pathway and, in this respect, resembles frog skin, which can actively transport sodium under anaerobic conditions at about 60–80 % of the rate under aerobic conditions (Leaf & Renshaw, 1957). It seems likely that ATP provided by either respiration or glycolysis is utilized by the ion-transport mechanism in these different cells and tissues.

Whatever unknown factors control the rate of hydrolysis of ATP, the energy available from anaerobic glycolysis was sufficient to support a potassium concentration almost as high anaerobically as aerobically. Losses of easily hydrolysable phosphate and of potassium, and a gain of sodium, occurred when metabolic inhibitors (2:4-dinitrophenol plus iodoacetate) were added at 37°, and on cooling to 1°. The maintenance of the concentrations of easily hydrolysable phosphate and of sodium and potassium both aerobically and anaerobically at 37°, and the rough parallelism between the net losses of potassium and labile phosphate, suggest that the active transport of sodium, and possibly of potassium, probably depends on labile phosphate esters. These results do not distinguish between whether both sodium and potassium are subject to primary active-transport processes or whether potassium is distributed passively as a consequence of active sodium transport. In either case, however, labile phosphate esters seem to be associated with the primary active process.

Under the conditions of incubation when the concentration of labile phosphate fell, the possibility has to be considered that the orthophosphate produced might leave the cells accompanied by potassium and thus account for the fall in potassium concentration. This possibility can be discounted because the loss of potassium was from three to ten times greater than could be accounted for by a loss of monobasic orthophosphate accompanied by potassium. (The discrepancy would be even greater if account is taken of the mixture of mono- and di-basic orthophosphate more likely to be present.) The loss of potassium and gain of sodium therefore seem to be due to a failure of active transport, which is correlated with hydrolysis of labile phosphate.

Tissues seem to fall into three classes from the way they depend on metabolic energy for the active transport of ions. First, there are those tissues and cells which are predominantly aerobic in their metabolism, and which require respiratory energy for the active transport of ions. Examples are chicken erythrocytes (Maizels, 1954), rat diaphragm (Creese, 1954) and skeletal muscle (McLennan, 1955), guinea-pig brain and kidney cortex, retina (Krebs, Eggleston & Terner, 1951; Mudge, 1951; Whittam & Davies, 1953) and squid giant axons (Hodgkin & Keynes, 1955). Secondly, cells which can transport ions anaerobically and do not possess a respiratory pathway are human erythrocytes (Harris, 1941; Maizels, 1949). Thirdly, frog skin (Leaf & Renshaw, 1957), seminal-vesicle mucosa (Breuer & Whittam, 1957), duck erythrocytes (Tosteson & Johnson, 1957) and ascites tumour cells (Maizels, Remington & Truscoe, 1958) possess the ability to transport cations actively both aerobically and anaerobically, thus combining in the same cells the features of the first two types.

Potassium retention in the absence of medium in the sodium

The finding that the replacement of sodium chloride in the medium with either sucrose or choline chloride caused almost a complete loss of tissue sodium shows that the cells in slices of seminal-vesicle mucosa are permeable to sodium. The retention of tissue potassium during incubation in the sodium-free media was largely unaffected by cooling, the addition of metabolic inhibitors and the deprivation of substrates (glucose and glutamate), although these factors caused a large loss of potassium from tissue incubated in standard medium. This result suggests that non-diffusible, intracellular anions set up a Donnan equilibrium, which probably accounts for the high tissue potassium found after incubation in the sodium-free sucrose and choline chloride media.

SUMMARY

1. Conditions of incubation are described under which slices of seminal-vesicle mucosa of the guinea pig retained most of their potassium in the absence of oxygen and of sodium in the medium. A comparison has been made between the ionic concentrations, the concentrations of easily hydrolysable phosphate and the rates of respiration and anaerobic glycolysis.

2. The tissue maintained its initial concentration of easily hydrolysable phosphate, and retained most of its potassium during both aerobic and anaerobic incubation for 45 min. at 37° in standard medium containing sodium chloride. The addition of 2:4-dinitrophenol and iodoacetate or a reduction of the temperature caused a loss of easily hydrolysable phosphate and of potassium, and a gain of sodium.

3. The rough correlation between the maintenance of the concentrations of sodium and potassium, and of easily hydrolysable phosphate, is compatible with a dependence of active cation transport on labile phosphate esters such as adenosine triphosphate.

4. Lowering the temperature of incubation from 37° to 27° did not affect the concentrations of sodium, potassium and chloride found in the tissue after both aerobic and anaerobic incubation, although Q_{O_2} and $Q_{CO_2}^{N_2}$ were reduced by about 50%. Incubation at 17° and 1°, however, caused a loss of potassium, gains of sodium and chloride and a further fall in the metabolic quotients. It appears that the energy supply was sufficient for the active transport of ions at 27° but not at 17° and 0°.

5. Tissue slices incubated in media in which sodium chloride had been partially or completely replaced by either choline chloride or sucrose contained sodium in a concentration roughly proportional to that of the medium. Their potassium concentration, however, remained unchanged in sucrose medium and fell only about 10 μmoles/g. of whole tissue in choline chloride medium. Incubation in sodium-free media caused a fall in Q_{O_2} from 6·4 in standard medium to about 4 in the sucrose or choline chloride media.

6. The tissue potassium concentration after incubation in sodium-free media was lowered only between 4 and 14 μmoles/g. of whole tissue by a reduction of temperature from 37° to 1°, or an addition of 2:4-dinitrophenol plus iodoacetate or an omission of glucose and glutamate (which were usually added to the medium). In contrast, these factors caused a large loss of potassium (24–36 μmoles/g. of whole tissue), which was balanced by a gain of sodium, after incubation in standard medium.

7. The results suggest that potassium can be retained passively, by a Donnan equilibrium created by non-diffusible internal anions, in slices of seminal-vesicle mucosa in the absence of metabolic energy, provided that sodium in the medium is replaced by sucrose or choline chloride. Potassium retention by the tissue in standard medium, however, depended on metabolic energy, because in its absence potassium was replaced by sodium.

We wish to thank Professor Sir Hans Krebs, F.R.S., for his helpful comments on the manuscript. The work was supported by grants from the Rockefeller Foundation (in Oxford) and the Deutsche Forschungsgemeinschaft (in Bonn).

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