sulphate in the reaction mixture and to the temperature. Consequently, to obtain maximum recovery of acetaldehyde, the concentration of ceric sulphate should be such as to ensure only a slight excess at the end of the reaction. The rate of aeration should be the maximum possible. From our results it would appear that the concentration of ceric sulphate recommended by Long (1946) is somewhat too high.

In view of our findings it is difficult to understand how a quantitative conversion of lactic acid to acetaldehyde can be obtained by the procedure of Winnick (1942). This author recommends a mixture of 3 ml. sample and 1 ml. saturated ceric sulphate and the reaction is carried out in Conway vessels and thus relies upon diffusion to remove the acetaldehyde. Since the purity of his ceric sulphate is not stated we can only guess at the final concentration, but it would seem to be in the region of 0.2N. Likewise the modification of Winnick's procedure described by Conway (1950) is open to serious criticism in the light of the observations of both Long (1946) and ourselves. In this method the reaction is carried out in stoppered test tubes, under conditions somewhat similar to those used both by Long and ourselves to study the oxidation of acetaldehyde by ceric sulphate.

It is possible to remove the acetaldehyde formed from lactic acid, almost quantitatively, either by aeration or by steam distillation, and we have examined both procedures. We prefer the steam distillation method for the following reasons. The blanks are negligible save when using 0.00257N iodine; but even here they are small and are equivalent to about 0.1 μ mole lactic acid. The method is extremely rapid, one distillation taking little more than a minute to perform. The end point is sharper because the final volume in which the titration is carried out is only 30 ml. and this is particularly important when small amounts of lactic acid are to be estimated. The advantages of

this ceric sulphate method over the permanganate methods, described by Friedemann, Cotonio & Shaffer (1927), Friedemann & Kendall (1929), and Friedemann & Graeser (1933) are speed and the small volume in which the titration is carried out.

SUMMARY

1. The effect of ceric sulphate concentration and temperature on the oxidation of lactic acid by ceric sulphate have been investigated.
2. The effects of temperature and rate of aeration on the removal of acetaldehyde from solutions have been investigated.
3. Conditions for the estimation of lactic acid with ceric sulphate using the aeration method are described.
4. A new method for the estimation of lactic acid using ceric sulphate and the removal of acetaldehyde by steam distillation is described.

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Active Transport of Sodium Ions from the Yeast Cell

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It has been shown (Conway & Hingerty, 1948) that sodium ions which had entered muscle extensively in rats on a potassium-free diet were actively extruded on changing the animals to a potassium-rich diet. The mean half period of extrusion, even with the plasma potassium above the normal level within 24 hr. after the change, was about 3 days.

Very recently, Desmedt (1953) has described a more rapid net excretion of sodium ions from the isolated frog sartorius in which sodium ions had entered extensively after immersion in a potassium-free Ringer solution in the manner described by Steinbach (1951). To demonstrate the excretion, companion muscles which were similarly treated

were re-immersed in a fluid with composition similar to that of the inorganic content of frog blood plasma (Boyle & Conway, 1941).

In the present paper, active transport of sodium ions from the yeast cell is studied. A series of experiments is described which show the surprising fact that not only is yeast capable of actively and specifically extruding sodium ions but that the rate of excretion is much more rapid than for rat muscle, the half period (under suitable conditions) being about 35 min. at 37° or about 100 min. at room temperature. The yeast experiments have been carried out on a 'sodium-yeast' (Na-yeast) produced as described by Conway & Moore, 1954.

It is considered that the evidence presented is much in favour of active transport of sodium ions by a redox-pump mechanism (Conway, 1950*b*, 1952*a*) with direct utilization of electron energy.

A preliminary account of some of the results described here was presented at the Ciba Conference on Water Metabolism (Conway, 1952*b*).

METHODS

Isotope experiments

The isotopes of sodium and potassium used were ^{24}Na and ^{42}K supplied by the Atomic Energy Research Establishment, Harwell.

Before counting, the yeast samples were suspended in 5*N*-NaOH (1 ml./g. yeast). The activities of such suspensions were then measured by a Geiger-Müller tube (G.M. 4, Dynatron Radio Co.) the counts being registered on a Dynatron scaling unit, type Sc 200 (as supplied by the Dynatron Radio Co., Maidenhead). Corrections for decay were made from a curve or by using a control solution counted alternately with each sample.

Chemical determinations

Sodium and potassium. These were determined partly with the Barclay flame photometer and later with the Beckman flame photometer (Conway & Moore, 1954).

Bicarbonate and chloride ions. These were determined by a microdiffusion procedure (Conway, 1950*a*).

Experimental procedure

Preparation of the Na-yeast. Na-yeast was prepared by allowing washed baker's yeast to ferment for 2 hr. in 20 vol. of 0.2*M* sodium citrate plus 5% (w/v) glucose. After being washed twice in 20 vol. of tap water, the Na content of the sample lay, with few exceptions, within the range 50–80 m-moles Na/kg. centrifuged yeast. Concentrations are expressed throughout per kg. centrifuged yeast.

Determination of the 'active' sodium excretion. The freshly prepared Na-yeast was washed twice in 20 vol. of tap water and suspended 1:20 in the various solutions investigated. A stoppered bottle of about 500 ml. capacity was half filled with the yeast suspension and then placed in a shaker and samples were removed at intervals with a pipette. The samples were centrifuged at once and the centrifuged yeast was washed twice in about 20 vol. of tap water (which con-

tained only 3 parts Na and 0.5 part K per million) before determining the content of Na etc. or radioactivity when isotopes were used. The centrifuged yeast sample was not washed when 0.1*M*-KCl or tap water was used as the suspending fluid, and the Na content only was required, as the Na in the fluid interspace between the cells is negligible. Such experiments were carried out at room temperature unless otherwise stated.

Occasionally also even when 20 vol. of a suspending fluid containing 0.1*M*-NaCl were used the centrifuged sample was not washed but an allowance made for the Na space of the yeast cell, into which Na and K ions freely diffuse, altogether a correction amounting to 33% by volume of the centrifuged yeast (Conway & Downey, 1950).

Experiments with inhibitors. Inhibitors such as cyanide or iodoacetic acid were introduced as their appropriate neutral salts in the suspending solution, to make 0.002*M* strength. With metal inhibitors (Hg, Pb, etc.) the suspending fluid consisted of 0.02*M*-NaCl plus 0.025*M*-KCl including small measured amounts of the inhibitor used. After centrifuging a sample of the suspension, the centrifuged yeast was washed twice with 0.1*M*-NaCl, and allowance made in the subsequent analyses for the passive Na entrance into the 33% region as considered above.

Determination of oxygen uptake. This was carried out with conventional manometric technique using 1 or 2 ml. of the Na-yeast suspension (1 in 20 usually) with 0.2 ml. *M*-KOH (with small strip of filter paper dipping in) in the central chamber for absorbing the CO₂ and shaking at 120 cye./min. Readings were begun after 15 min. equilibration. Simultaneous determinations of the Na excretion from the yeast were made, the suspensions being shaken in a bath at the same temperature.

RESULTS

Demonstration of the active extrusion of sodium ions from yeast

Portions of twice-washed Na-yeast were suspended in 20 vol. of the following fluids: (a) 0.1*M*-NaCl, (b) tap water, (c) 0.1*M*-NaCl + 0.1*M*-KCl, (d) 0.1*M*-KCl. After varying times, samples of the suspensions were removed, centrifuged, and the yeast was analysed for sodium.

The Na content of the yeast at zero time was approximately 60 m-moles/kg. of centrifuged yeast. In Fig. 1 it will be seen that there was no net excretion of Na ions from the Na-yeast immersed in 0.1*M*-NaCl in 20 hr. There was, however, a net excretion into water, which came practically to an end after 3–4 hr. When 0.1*M*-KCl was present in the suspending fluid as well as 0.1*M*-NaCl there was a rapid excretion of Na ions, the rate differing little if any from that when 0.1*M*-KCl was present alone.

Ions exchanging with or accompanying the excreted sodium ions

When the Na ions were excreted into a medium containing 0.1*M*-KCl, the K ions taken up by the yeast were very similar in quantity to the Na ions excreted (Table 1). There was practically no loss of

K from the Na-yeast during the excretion of Na ions into tap water. In fact there was a slight increase after 24 hr., but this was probably within the total sampling error.

When the Na excretion occurs into water it must be either in exchange for H ions or accompanied by HCO_3 ions or by organic acid anions. Exchanges with H ions did not occur to any appreciable extent, as the pH of the originally unbuffered suspension remained on the average at about 6.2.

Fig. 1. The curves show the excretion rate of Na at room temperature into 20 vol. of various suspending fluid. Curve A, 0.1M-NaCl; curve B, tap water; curve C, 0.1M-NaCl plus 0.1M-KCl (crosses) and 0.1M-KCl (circles). The yeast contained approximately 60 m-moles/l. at zero time.

Table 1. Changes of Na and K content of Na-yeast preparations on suspending in 0.1M-KCl and in water

At zero time Na^+ content = 64.9 m-moles/kg. yeast, K^+ content = 84.7 m-moles/kg. yeast.

Time of suspension (hr.)	Change in content (m-moles/kg. centrifuged yeast)			
	In 0.1 M-KCl		In water	
	Na	K	Na	K
0.5	-20.6	+25.3	- 7.9	-0.3
1	-36.8	+39.8	-14.0	-0.8
3.25	-40.9	+41.2	-19.8	+1.0
19	-47.8	+41.8	-23.4	+3.7

Estimations of the total CO_2 of the suspending fluid were made just after suspending and for different Na-yeasts after 60, 90 and 120 min. The increase of HCO_3 ions was calculated from the pH value and the total carbon dioxide and is shown in Table 2. Organic acid anions are considered to make up the remaining balance and their excretion in approximately such amount was confirmed by a study of electrometric titration curves. It is possible that the bicarbonate appearing in the suspending fluid was only indirectly formed following small exchanges of Na for H ions and restoration of the pH value by carbon dioxide but a considerable amount of bicarbonate was present within the cells of the freshly prepared and washed Na-yeasts. Thus there was a total acid-labile carbon dioxide content of 27.2 m-moles/kg. in the Na-yeast at zero time and only 15.0 m-moles/kg. centrifuged yeast after 2 hr. suspension. The Na content of this yeast changed from 55.4 to 47.3 m-moles/kg. over this period.

The nature of the organic acid anions was not determined but they were probably mostly succinate ions. Succinic acid is freely excreted by fermenting yeast (Conway & Brady, 1947).

The specificity of the sodium-excreting mechanism

Na-yeasts containing ^{42}K were prepared by fermenting for a short time in ^{42}K -labelled 0.1M-KCl containing some glucose, a companion sample being treated in unlabelled KCl. The samples were then washed in water, centrifuged and Na-yeast prepared from them in the usual manner.

On suspending samples of each of these yeasts in unlabelled and labelled 0.1M-KCl and measuring the counts of the centrifuged and washed yeast it was found that in 3 hr. there was practically no movement of K ions out of the cell (2-3 m-moles/kg.) but there was a considerable movement (approximately 45 m-moles) into the cell (Table 3). Table 4 shows that when Na-yeast was excreting Na ions into 0.1M-NaCl plus 0.1M-KCl, the external Na being labelled, there was practically no entrance of labelled Na, i.e. the movement of the Na ions was in the opposite direction to the K ions.

Table 2. Anions accompanying the Na excretion from Na-yeast into tap water

All Na, K, HCO_3 and organic anion values are given as m-equiv./kg. centrifuged yeast.

Time (min.)	Na content of yeast at zero time	Na excretion	pH of suspending fluid	Increase	
				HCO_3^-	Organic anions
60	49	9.2	6.2	3.1	6.1
90	45	9.8	6.4	7.9	1.9
120	60	13.6	6.1	4.1	9.5

Table 3. *Passage of labelled K ions to and from the cells of a Na-yeast suspended in 0.1 M-KCl at room temperature*

The counts of the yeast and the external fluid were made under similar conditions as described in Methods.

Time after suspending (min.)	Relative counts		
	Na-yeast with unlabelled K, labelled external K		
	Na-yeast with labelled K, unlabelled external K. Counts after 5 min. taken as 100	Counts as percentages of external counts for similar vols.	Percentage ratio of specific counts for yeast K to that for the external fluid
5	100	10.5	13
180	97	45.0	38

Table 4. *Passage of labelled Na into a Na-yeast immersed in a solution containing 0.1 M-NaCl plus 0.1 M-KCl, the external Na ions being labelled*

Time (min.)	Na counts in yeast as percentage of external counts under similar conditions of counting	Na content of yeast as determined by the flame photometer (m-moles/kg. centrifuged yeast)
0	0.8	55.2
60	0.8	32.1
150	0.7	17.3

Fig. 2. Percentage Na excretion from a Na-yeast after 90 min. at room temperature (1 in 20 suspensions). Curve A (dots), excretion into 0.1 M-KCl with varying amounts of NaCl; curve B (crosses), excretion into 0.1 M-NaCl with varying amounts of KCl.

The effect on the sodium excretion of varying the sodium and potassium content of the suspending fluid

The effect on the active excretion of varying the K and Na ion concentrations in the suspending fluid was studied in a series of experiments. It will be seen from Fig. 2 that when the suspending fluid was 0.1 M-NaCl there was no net excretion of Na ions, but very small additions of KCl caused a marked excretion. When the external KCl concentration was 25 m-moles/l., the effect of K was maximal.

Fig. 3. Percentage excretion from a Na-yeast at 0, 17 and 37°. The suspending medium contained 0.1 M-KCl (1 in 20 suspensions).

When the external K concentration was maintained at 100 m-moles/l. and the Na concentration varied, the level of Na concentration had to reach about 420 m-moles/l. to reduce the excretion to one-half the maximum value (Fig. 2).

Effect of temperature and of pH on the excretion of sodium from sodium-yeast

The effect of temperature on the Na excretion into 0.1 M-KCl was studied (only to a small extent) by comparing the excretion rates at 0, 17 and 37° (Fig. 3). The excretion rate was very slow at 0° and a scarcely appreciable quantity of sodium was lost. More detailed data would be required for the determination of the activation energies for the overall process which are likely to differ over various temperature ranges; also owing to the complexity of the process involved they would be unlikely to provide useful information.

The effect of varying the pH of the external fluid between 4.0 and 7.0 was investigated. The suspending fluid was 0.1 M-KCl + 0.1 M-NaCl and the pH was varied by including different amounts of HCl on the acid side and of NaHCO₃ when studying pH effects a little above the neutral level. It was

decided to use HCl and NaHCO₃ to avoid any possible complications from organic buffering. It was found that practically no effect was produced by a change of pH from 4.0 to 6.0, but at 7.0 a fall of about 15% occurred.

Effect of azide on excretion of sodium into 0.1M sodium chloride. Though Na-yeast suspended in 0.1M-NaCl did not excrete any net quantity of Na ions, considerable exchange could be demonstrated if the Na-yeast was suspended in labelled NaCl (Table 5). The efflux and influx must be equivalent and from the efflux data, half of the Na ions originally in the yeast had crossed the membrane in 116 min. Sodium azide (0.002M) reduced this efflux rate to about one-half, half the Na ions now passing across in about 260 min., but it almost abolished the influx. The effect of azide under these conditions was, therefore, to cause a net outward excretion of Na ions into 0.1M-NaCl, which up to about the first 2 hr. was of the same magnitude as the net excretion into tap water.

Effect of azide on the excretion of sodium into 0.1M potassium chloride and into tap water

When the Na-yeast was suspended in 0.1M-KCl there was a rapid excretion of sodium ions. In the presence of 0.002M azide this was decreased but the residual excretion was of the same magnitude as the excretion into tap water, or as the excretion into 0.1M-NaCl with azide (Fig. 4). Fig. 4 also shows that the excretion into tap water was not affected by azide.

Table 5. *Exchange of labelled Na between Na-yeast and 0.1M-NaCl*

In studying the entrance of Na into the Na-yeast, only the external Na was labelled, and for the excretion of Na only the internal Na was labelled.

The figures for the entrance rate are obtained from the expression $\frac{100 \times (\text{counts per g. yeast})}{\text{counts per ml. exit fluid}} - 33$.

The figure 33 represents the interspace value plus that of the outer region into which the Na ions can diffuse freely (Conway & Downey, 1950) and 100 represents the m.-equiv. Na ions externally.

The figures for the excretion rate are obtained from the expression $-\frac{60 \times (\text{counts per g. yeast})}{\text{counts per g. yeast at zero time}}$. Here 60 represents the Na concentration as m.-equiv./kg. centrifuged yeast at zero time.

Time (min.)	Without azide		With azide (0.002M)	
	Entrance of labelled Na	Excretion of labelled Na	Entrance of labelled Na	Excretion of labelled Na
60	—	19.8	0.6	13.8
120	—	30.6	2.3	20.4
180	36.7	37.8	3.5	25.2
210	39.2	39.6	4.3	26.2

Under all conditions studied azide blocked the active uptake of K and Na ions. This inhibition of K ion uptake during fermentation has been shown in a previous communication (Conway, Brady & Carton, 1950). The effect on the influx of K ions into ordinary resting yeast suspended in 0.1M-KCl and shaken with air or oxygen is shown in a later section of the present paper. The following experiments illustrate this azide effect with Na-yeast.

Effect of azide (0.002M) on the uptake of potassium by a sodium-yeast suspended in 0.1M potassium chloride

These experiments were carried out as for the previous group, the K content of the yeast with and without azide being determined after different immersion times. The results are shown in Table 6. It will be seen that after 60 min. immersion the net uptake of K has been reduced from 27.0 to 3.4 m-moles/kg. or nearly eliminated; between 20 and 60 min. no measurable uptake of K occurred.

Fig. 4. Percentage excretion of Na from a Na-yeast into tap water and 0.1M-KCl with and without azide (0.002M) (1 in 20 suspensions). Curve A, into tap water (circles); into tap water plus azide (0.002M) (dots) and into 0.1M-KCl plus azide (0.002M) (crosses); curve B, into 0.1M-KCl.

Table 6. *Effect of azide (0.002M) on the K uptake and Na excretion of Na-yeast*

Na and K values in yeast are given as m-equiv./kg. of the centrifuged material. Samples of Na-yeast were suspended (1 in 20) in the various fluids at 18°.

Time of suspension (min.)	Without azide		With azide	
	Na content	K content	Na content	K content
0	59.4	97.0	59.4	97.2
20	48.8	105.4	49.0	100.6
40	42.2	117.0	47.0	100.0
60	32.0	124.0	43.0	100.6

Table 7. *Effect of 2:4-dinitrophenol (0.002M) on the Na excretion and K uptake of Na-yeast*

Na and K values in yeast are given as m-equiv./kg. of the centrifuged material. Samples of Na-yeast were suspended (1 part in 20) in the various fluids at 18°.

Time of suspension (min.)	NaCl (0.002M) in tap water	2:4-Dinitrophenol (0.002M) in tap water	KCl (0.1M)		2:4-Dinitrophenol (0.002M in 0.1M-KCl)	
	Na content	Na content	Na content	K content	Na content	K content
0	54.4	54.4	54.2	68.5	54.2	68.5
30	45.3	44.4	37.4	87.0	37.2	71.8
60	42.2	41.2	24.4	92.2	30.0	76.4
90	39.5	39.6	15.8	102.0	28.3	76.4

Table 8. *The effect of cyanide and anoxia on the Na and K content of yeast suspended in tap water and in 0.1M-KCl*

The washed Na-yeast was suspended in 20 times its volume of fluid at 18° for 90 min. Na-yeast immediately after suspending had 62 m-moles Na⁺/kg. and 78 m-moles K⁺/kg. of centrifuged yeast.

Conditions	Na changes (m-moles/kg. centrifuged yeast)		K changes (m-moles/kg. centrifuged yeast)	
	In tap water	In 0.1M-KCl	In tap water	In 0.1M-KCl
No inhibitor, oxygen present	-13.7	-38.0	-0.8	+33.3
No inhibitor, oxygen absent	-3.8	-8.6	-1.7	-0.3
Cyanide (0.002M) and oxygen present	-5.0	-13.6	-0.0	-1.1

Effect of 2:4-dinitrophenol on the excretion of sodium ions

Samples of Na-yeast were suspended in tap water (1:20) containing 0.002M 2:4-dinitrophenol, the pH of which had been adjusted to 6.0 with NaOH, the suspending fluid in the control mixture containing 0.002M-NaCl.

The results shown in Table 7 indicate that like 0.002M azide, 2:4-dinitrophenol had no effect on the Na excretion when no KCl was present in the suspending fluid.

When 0.1M-KCl was present in the suspending fluid and the excretion of Na ions in such a suspension, without inhibitor, was much increased thereby, then the presence of 0.002M 2:4-dinitrophenol caused a marked inhibition. This was not so marked as with 0.002M azide, the Na excretion not being reduced to the level of that into tap water; this might be expected to occur with a higher dinitrophenol concentration.

Effect of cyanide and oxygen lack on the excretion of sodium ions from sodium-yeast

The inhibition produced by cyanide and anoxia is shown in Table 8. Whether the Na excretion was occurring into tap water or into 0.1M-KCl, cyanide (0.002M) and anoxia inhibited the excretion very largely. The uptake of K was completely inhibited by cyanide and by anoxia.

The active transport of Na ions in non-fermenting

yeasts is thus very largely, if not entirely, dependent on electron transference to oxygen. It may be noted here that where the effect of oxygen lack was being studied complete absence of oxygen from the yeast suspensions was probably rare.

The effect of cyanide and azide on the potassium-carrier in ordinary resting yeast

Conway & Moore (1952) showed that the influx of labelled K was extremely slow into gently stirred resting yeast. If the yeast was shaken with air or oxygen the entrance rate was very much more rapid, but this increased rate was inhibited by cyanide and azide. In the present experiments yeast samples from the yeast factory were washed, and suspended in 20 vol. 0.1M-KCl labelled with ⁴²K, with and without sodium azide (0.002M) or sodium cyanide (0.002M). The suspensions were well shaken with air or oxygen throughout. Samples were removed at intervals for estimations of ⁴²K in the yeast. The samples were centrifuged and counts were made in each case on the centrifuged yeast suspended in an equal volume of water. Fig. 5 shows the effect of the inhibitors. The points give the counts observed minus the count at 5 min. They represent, therefore, the entrance of ⁴²K into the yeast cells (from 5 min. onward) apart from the interspaces and the outer cell region into which K and Cl ions diffuse freely (Conway & Downey, 1950). It is obvious that an active carriage of K ions was

proceeding in the resting yeast which was shaken with air or oxygen, and that this was inhibited by azide and cyanide.

The effect of metallic ions on the active excretion of sodium from sodium-yeast

Sodium-yeast was suspended in a solution containing 0.1M-NaCl, 0.025M-KCl and various concentrations of the metallic salt. A control in which no metallic ions were included was set up at the

Fig. 5. Relative rates of entrance of K into untreated yeast (1 in 20 suspensions) in labelled 0.1M-KCl with and without inhibitors. Curve A, without inhibitors; curve B, with azide (0.002M) in the suspending fluid; curve C, with cyanide. The points give the counts for the yeast, after subtraction of the 5 min. values.

same time, and the suspensions shaken for 60–90 min. Samples were then centrifuged and the yeast was washed twice quickly with 20 vol. of 0.1M-NaCl, allowance being made for interspace and outer region (altogether 33%) in calculating the Na inside the yeast membrane. The results are summarized in Table 9.

Several of the heavy metals, such as uranium, mercury, gold and silver, are powerful inhibitors of the process of Na secretion and are effective in one to a few parts per million. Other heavy metals such as lead, platinum and iron have comparatively little effect. When the results obtained are studied in relation to the number of electrons in the two outermost orbits of the metals, an interesting relation appears, which may be expressed in the following statements:

(a) A metal will cause marked inhibition if the second outermost orbit contains 18 electrons and the outermost contains not more than two electrons.

(b) The inhibition produced by the metals in group *a* increases with the magnitude of the atomic number.

Uranium holds an exceptional position in so far as it is among the most powerful inhibitors but does not come into group *a*. At the same time it may be noted that uranium was added as uranyl acetate, so that the uranyl ion and not the uranium ion was present in the mixture.

It will be seen from Table 9 that when the reciprocal number of the concentration (expressed as

Table 9. *Inhibition of the Na excretion (in exchange for K) by heavy metals*

	Atomic number	Concentration causing 50% inhibition (m-moles/litre)	Reciprocals of such milli-molarities	Electrons in outer orbits
Metals with two outermost orbits containing 18 and 0–2 electrons				
Hg	80	0.74×10^{-2}	135	18, 2
Au	79	0.91×10^{-2}	110	18, 1
Ag	47	1.98×10^{-2}	50	18, 1
Pd	46	2.75×10^{-2}	36	18, 0
Cu	29	15.0×10^{-2}	6.7	18, 1
Zn	30	107.1×10^{-2}	0.9	18, 2
Metals with the two outer orbits containing 18 and 4 electrons				
Pb	82	410×10^{-2}	0.24	18, 4
Metals with second outermost orbits containing less than 18 electrons				
Pt	78	71.7×10^{-2}	1.4	17, 1
Ba	56	>100	<0.01	8, 2
Ni	28	>10	<0.1	16, 2
Co	27	>10	<0.1	15, 2
Fe	26	1.52	0.7	14, 2
Mn	25	>10	<0.1	13, 2
Cr	24	>4	<0.25	13, 1
Ca	20	>100	<0.01	8, 2
Al	13	>1	<1.0	10, 3
Mg	12	>100	<0.01	8, 2

Uranium with an atomic number of 92 is an apparent exception to the above grouping, as its two outermost orbits contain 12 and 2 electrons and the molarity for 50% inhibition was found to be 0.92×10^{-6} . The uranium, however, was added as the uranyl ion as contained in uranyl acetate. Sn showed no inhibition in the strengths used, but for solubility reasons the value could not be determined.

m-moles/l.) producing 50% inhibition exceeds 2.0, the metal has 18 electrons in its second outermost electron ring and not more than 2 electrons in its outermost. Palladium is listed for convenience as having no electron in the outer ring. Lead with 18 and 4 electrons in the outer rings has very little inhibiting action; the reciprocal number being 0.24. Zinc with 18 and 2 electrons in the outer rings and a reciprocal number of 0.9 has a smaller effect than would be anticipated. Yet this value is higher than that of the other metals of similar atomic number. (The reciprocal number of aluminium with atomic number of 13 is less than 1.0.) Platinum with the very high atomic number of 78 has a reciprocal number of only 1.4, only slightly exceeding that of zinc.

It may be concluded that the two rules given above are well borne out by the results, the most notable exception being uranium.

The relation of the oxygen consumed to sodium ions actively excreted from the sodium yeast suspended in 0.1 M potassium chloride

The present experiments, though adequate for their purpose in demonstrating a significant relation between oxygen utilization and Na excretion, may be considered as preliminary studies in this field.

Sodium-yeast was suspended 1 in 20 in 0.1 M-KCl; 1.0 ml. was immediately introduced into the Warburg apparatus for measurement of the oxygen uptake at room temperature; a larger sample of the suspension was placed in a flask and shaken well in a bath at room temperature for measurement of the Na excretion. The ratio of m-moles Na ions excreted to m-moles oxygen taken up was calculated for the period 20–80 min. after making the suspension.

The early experiments appeared to indicate that at least near the beginning of the Na excretion there was a limiting value of 4.0. On much further experimentation it was found that the oxygen consumption of the Na-yeast depended to a surprising extent on the previous history of the yeast, and could vary by as much as 5–10 times. With the higher oxygen consumption levels the ratio was much reduced at all periods of the excretion. For this reason we are now not inclined to attach much significance to the earlier results.

DISCUSSION

The various facts given in the Results section are well interpreted by the 'redox pump' theory, which is chiefly concerned with the manner in which the energy released in electron transference may be directly used for the performance of osmotic work (Conway, 1951, 1953).

In such a theory the carriers of the cations are considered to be respiratory enzymes in the reduced state. When oxidized the carriers release the ions, transferring electrons simultaneously to systems of higher redox potential. The nature of the energy transfer in performing osmotic work of such a kind has already been discussed (Conway, 1951, 1953). In the process by which the Na ions are excreted the carrier receives either H atoms or electrons, then attaches Na ions, and presents the Na-carrying group to another system, the electrons being transferred thereto and the Na ions released. This second system now picks up K ions and the process is reversed, the K ions being introduced into the cells and electrons transferred to a system with higher redox potential and ultimately to oxygen. If no K⁺ is present outside the cells, then Na⁺ is excreted at a slower rate with accompanying anions, the slower rate arising from the greater difficulty of bringing out anions than bringing K⁺ into the cell.

Evidence for two carriers

The existence of two different carriers or carrier processes, one bringing out Na⁺ and the other carrying in K⁺, is strongly supported by the evidence, apart from the question of the carriers being redox systems. Such evidence turns on the specificity of carriage of Na⁺ outward and of K⁺ inward, and also on the different action of some inhibitors. Thus Na⁺ is excreted accompanied by anions, when Na⁺ in yeast is suspended in tap water (Table 2). At the same time no appreciable amount of K⁺ is excreted, though the K⁺ content of the yeast cell may be greater than the Na⁺ content (Table 1); also, using labelled ions, it has been shown that when Na⁺ is being excreted in exchange for K⁺ there is no appreciable efflux of K⁺ (Table 3). The Na⁺ carrier is thus specific for Na⁺ to a high degree compared with K⁺. The reverse is true for the active uptake of K⁺ into a Na⁺ yeast, since Na⁺ must be present in about 20 times the concentration of K⁺ before it is taken up at an equal rate (Fig. 2, Table 4).

From this the conclusion may be drawn that there are two separate carriers or carrier-processes, one of which may be termed for convenience the Na⁺ carrier and the other the K⁺ carrier (though it is to be understood that when the external Na⁺ content greatly exceeds the K⁺ value, it will be taken in freely on the K⁺ carrier).

This conclusion is strongly supported by the effect of inhibitors. When the Na-yeast is excreting Na⁺ into tap water, azide (0.002 M) has no effect on the rate of Na⁺ excretion (Fig. 4). If the Na-yeast excretes Na⁺ into 0.1 M-KCl, it does so at about 2–3 times the rate into tap water (Fig. 1) the Na⁺ exchanging for K⁺ (Table 1). With such conditions azide (0.002 M) has a considerable inhibiting effect

on the Na^+ excretion. But the fact that by isotopic studies it appears that the uptake of K^+ is practically completely inhibited, and that the Na^+ excretion returns to the same level as excretion into tap water, shows that the inhibition has been exercised on the K^+ carrier.

In this connexion it is of much interest to consider the condition where the Na-yeast is suspended in 0.1 M sodium chloride. While no net excretion of Na^+ occurs over many hours, from isotopic studies it is shown that Na^+ is being freely transported in both directions. If azide (0.002 M) is included, the influx of Na^+ is almost entirely inhibited, and there results a net excretion of Na^+ similar in amount to the excretion into tap water (Table 5 and Figs. 1 and 4). Here it would appear that without any K^+ outside, the Na^+ is brought in on the K^+ carrier which is inhibited by azide and now Na^+ is excreted (accompanied by anions) as if into tap water or into 0.1 M potassium chloride with azide included (Fig. 4).

Evidence that the carriers are respiratory enzymes rather than substances using phosphate bond energy directly for the transport energy

(a) The excretion of Na ions from a Na-yeast whether K ions are present or not outside is inhibited by cyanide (0.002 M) or by anoxia (Table 7).

(b) After the uptake and release of Na ions, the succeeding uptake and release of K ions by another and quite distinct system is not well accounted for by phosphate bond energy but is very well interpreted by electron transference across redox systems.

(c) Azide (0.002 M) has no effect on the specific Na ion carrier, but azide abolishes the new formation of adenosine triphosphate or of organically bound phosphate (Spiegelman, Kamen & Sussman, 1948; Conway & McGovern, unpublished observations). Similarly, 2,4-dinitrophenol (0.002 M) does not affect the Na carrier, or the Na excretion into tap water, and it is generally considered to uncouple phosphorylation from respiration without inhibiting the latter.

The fact that 2,4-dinitrophenol (0.002 M) inhibits the K carrier does not necessarily imply that this carrier uses phosphate bond energy directly. Phosphorylation may be required at some stage of the electron transference in which the K carrier is involved.

(d) Calcium or magnesium ions even at a concentration of 0.1 M have no effect on the active excretion of Na^+ and the uptake of K^+ . But Ca^{2+} and Mg^{2+} would displace Na^+ and K^+ from such polyphosphate carriers *in vitro*.

Connected with this, it has recently been shown here (Conway & Beary, unpublished observations) that Mg^{2+} is steadily taken up by yeast suspended in

20 times its volume of a solution containing 0.05 M magnesium chloride + 5 % (w/v) glucose and stirred by bubbling with oxygen, the mixture being maintained at a pH of 6.2 by small additions of 0.1 N sodium hydroxide. The uptake of Mg^{2+} is inhibited by K^+ in the external fluid. Practically complete inhibition is obtained by a K^+ concentration as low as 0.01 M potassium.

(e) That the K carrier is of a redox kind is shown also by the following experiment. Redox dyes in small concentration (M/10 000) in the suspending fluid (1:20) with the yeast fermenting anaerobically can alter markedly the external redox potential as determined by a platinum electrode in the suspension (Conway, 1953). With dyes of different characteristic potentials the external potential can be considerably lowered or increased above the value without the dyes. Complete inhibition can be produced when the potential is lowered by about 60 mv, and corresponding stimulation can occur when the potential is raised. There is in fact a linear relation between the relative change of the K^+ transport and the redox potential. (Such experiments were described at the Bangor Congress on Active Transport, 1953, and it is hoped to describe them in more detail with Mr Kernan in a subsequent paper.) While the inhibition of the K carrier system by 2,4-dinitrophenol points to a phosphorylation requirement in the active transport this of course need be only indirect.

The evidence appears strongly in favour of respiratory ferment carriers and the direct utilization of electron energy as in the 'redox pump', though the question of the specificity of the carriers implies closer linkage than that given by long range electrostatic forces. The complex may be considered somewhat in the nature of the linkage of an enzyme with its substrate.

SUMMARY

1. The excretion of Na ions from a 'sodium-yeast' (containing about 60 m-moles/kg.) into 0.1 M-KCl is nearly 3 times faster than into water and there is a nearly quantitative exchange of K for Na ions. When Na ions are excreted into water they are accompanied by bicarbonate and organic acid anions and the excretion ceases when about half the Na has been excreted. There is no net excretion of Na ions into 0.1 M-NaCl.

2. When a sodium-yeast is suspended in 1:20 of 0.1 M-NaCl, no net excretion of Na^+ occurs over many hours. If suspensions are made with a series of solutions containing 0.1 M-NaCl and various concentrations of KCl, the excretion of Na^+ increases with the K^+ concentration of the suspending fluid, but this effect is maximal at a K^+ concentration of 0.04 M-KCl.

3. When Na^+ is excreted under similar conditions into 0.1M-KCl with NaCl ranging from zero to 1.0M, little reduction in the rate of Na^+ excretion occurs up to an external Na^+ value of 0.2M. At an external value of 0.43M the Na excretion is approximately 50% that at zero Na^+ concentration.

4. The existence of two separate carriers for Na (outwards) and for K (inwards) is shown by the relative specificity of the transport in each case and the different action of inhibitors. Where Na ions (0.1M) without any K ions are present in the suspending fluid Na ions can be carried inwards on what may be regarded as normally the K carrier.

5. The Na carrier excreting Na is inhibited by cyanide (0.002M) and anoxia; it is not inhibited by azide (0.002M) or 2:4-dinitrophenol (0.002M).

6. The K carrier involved in the uptake of K^+ (or of Na^+ if no K^+ is present outside, and Na^+ is present in quantity) is inhibited by anoxia, and by cyanide, azide and 2:4-dinitrophenol at concentration of 0.002M.

7. The results are shown to support strongly the theory of a 'redox pump' for the biological performance of osmotic work.

8. Various metallic ions can greatly inhibit the active excretion of Na ions into 0.1M-KCl. Inhibition depends on the magnitude of the atomic number and on the presence of 18 electrons in the second

outermost orbit, and not more than two in the outermost.

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Studies of Sebum

5. THE COMPOSITION OF SOME SEBUM-LIKE MATERIALS OF HUMAN ORIGIN

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Apart from true sebum or surface-skin lipid there exist a number of materials which are alleged to originate from true or modified sebaceous glands. Some of these materials such as cerumen or ear wax, smegma and vernix caseosa are normal in origin while others, for instance dermoid cyst lipid, are pathological. The latter material is of interest. Dermoid cysts frequently grow to relatively enormous size in the ovaries; the cysts are hollow and are lined with what appears to be epidermal tissue. The cyst is usually filled with a mixture of water, fatty material and hair. The fatty material has long been thought to be sebum (Sotnitchewsky, 1880), and while it has been investigated by numerous workers, no direct comparison has been made between its composition and that of sebum.

In order to obtain information about the origin of these sebum-like materials, ovarian dermoid cyst

lipid, ear wax, vernix caseosa and epidermal cyst lipid have been analysed and the results are compared with those obtained with sebum (MacKenna, Wheatley & Wormald, 1950, 1952). Preliminary reports of part of this work have already been published (Wheatley, 1951, 1953a).

EXPERIMENTAL

Materials

Fatty material was obtained from two ovarian dermoid cysts and was found to contain little water. It dissolved readily in CHCl_3 , and the lipid which remained after filtering and evaporating this CHCl_3 solution to dryness was analysed. Ear wax was obtained from European patients of this hospital and contained much dirt and extraneous material. The lipid was extracted from both single samples and bulked specimens by refluxing the material with CHCl_3 .