The Nature of the Cation Exchanges during Yeast Fermentation, with Formation of 0.02 N-H Ion

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The entrance of potassium into yeast during short period fermentation, and its subsequent release was shown by Pulver & Verzar (1940). A similar interchange was observed for bacteria (*Esch. coli*) by Leibowitz & Kupermintz (1942).

The 'ammonia-yeast' already described (Conway & Breen, 1945) offered a means of studying whether the movement of K was specific for this ion. In the course of such studies the further significance of these electrolyte exchanges was investigated and the occurrence was observed of a remarkable production of acid (0.02N) by ordinary bakers' yeast under special but comparatively simple conditions, suggested by the theory advanced to account for the cation movements.

METHODS

Procedure with the ordinary or K-yeast. In Pulver & Verzar's technique (1940) the yeast was first washed in tap water to get rid of any freely diffusible K; 1 part was suspended in 3 parts of 0.325% NaCl for 5 hr., and then to 50 ml. of this suspension 20 ml. of 2% glucose were added. In this way there was an external K value of about 20 mg./100 ml. immediately after adding the glucose. In the experiments here described, the yeast was likewise washed a few times either in tap water or in the standard solution to be investigated. Where an approximately stable K figure external to the yeast cells was required, it was found that this could be secured by washing the yeast only a few times with the required standard K solution. The volume and nature of the suspending fluid was varied with each group of experiments and is described accordingly.

Procedure with the ammonia-yeast. Ammonia-yeast was prepared as previously described (Conway & Breen, 1941, 1945). The freshly prepared ammonia-yeast was washed twice in 0.325% NaCl and then 1 part of yeast was suspended in 3 parts of the 0.325% NaCl for some hours, when the external NH_3 concentration was about 20 mg./100 ml. The yeast was prevented from settling by the constant bubbling of air through the suspension. Details as to the various fermenting mixtures are described as they arise.

Volume of the extracellular spaces. On the few occasions when this figure was required it was obtained either by a starch or an inulin method, in which the yeast originally suspended in a starch or inulin solution, was resuspended in tap water or saline and the starch or inulin content determined.

Total hydrogen ion concentration within the yeast cells. This was determined in a somewhat similar way to Dixon & Atkin's procedure (1913). The yeast was first pressed to exclude as much as possible of the extracellular fluid, this

being reduced from approximately 20% to 4% of the total volume. The friable yeast powder was then pressed into a cylinder, surrounded by cellophan, and frozen in liquid air. On removal and warming by exposure to air, the liquid mixture resulting was centrifuged and the pH of the free juice thus obtained was determined by the glass electrode.

The method used may give a somewhat lower pH than the true value. Probably the best method for total pH estimates in yeast is from a study of the bicarbonate- CO_2 system as applied to muscle by Conway & Fearon (1944), though this method is not very suitable for fermenting yeast. Using it for resting yeast suspended in its own volume of fluid having a composition similar to Ringer's solution with increased bicarbonate content (about 12 m.equiv./l.) and equilibrated with a gas mixture of 10% CO_2 and 90% oxygen, we obtained a value of approximately 5.8-5.9 as compared with a value of 5.5-5.6 by the freezing method. It may be noted that Mahdihassan (1930) obtained a value of 5.9-6.0 by the method of micro-injection of different indicators into yeast cells, and Brandt (1945) a value of 6.2 for resting yeast by a method of rapid heat fixation, this value being increased by subjecting the yeast to high CO_2 tensions, with constant external pH. Brandt (1945) gives an account of the literature in a very full treatment of the effect of CO_2 in baker's yeast. Here we were chiefly concerned in obtaining a maximum figure for the total internal acidity, and this the method described above appears to give, as shown by its comparison with other methods.

Analytical

Potassium. This was determined by a modification (Boyle, Conway, Kane & O'Reilly, 1941) of the method of Shohl & Bennett (1928).

Ammonia. Determined by a micro-diffusion method (Conway, 1939; Conway & O'Malley, 1942).

Sodium. This was determined by a modification (Boyle *et al.* 1941) of the method described by Salit (1932).

Chloride. A micro-diffusion method was used (Conway, 1939).

Keto-acids. The total keto-acid content was estimated first by Lu's method (1939) and later in accordance with Friedemann & Haugen's procedure (1943).

Phosphate. Inorganic and total P was determined by Berenblum & Chain's method (1938).

pH. The glass electrode was used throughout.

Fermentation rate. This was determined by a micro-diffusion procedure. 2 ml. of the fermentation mixture were placed in the outer chamber and standard NaOH in the centre of a number of Conway units. At varying times the NaOH in the centre was titrated after preliminary addition of a little saturated BaCl_2 . After a short time a linear relation is established between time and CO_2 absorption, which gives a measure of the rate of fermentation.

RESULTS

I. Cation absorption

(a) *Ammonium ion by ammonia-yeast.* This is shown by the following experiments. Ammonia-yeast was prepared (Conway & Breen, 1945) and suspended as described under Methods, so that

Fig. 1. Curves for the suspending fluid showing the absorption and subsequent release of NH_4 ions during fermentation of glucose by 'ammonia-yeast'. A, no glucose; B, with glucose.

the external ammonia content was approximately 20 mg./100 ml. (This increased slowly with the time of suspension.) To 30 vol. of this suspension 1 vol. of 40% (w/v) glucose in 0.2M- NaH_2PO_4 was added and mixed. A control sample without glucose was

followed by a rise in which the ammonia concentration increases beyond its original value. The control without glucose usually shows a rise throughout. When the values from the control curves are subtracted from that with glucose, the resulting curve (Conway & O'Malley, 1943) is very similar to those obtained by Pulver & Verzar (1940) in their experiments on K absorption and by Leibowitz & Kupermintz (1942) for bacterial K exchange. Fig. 1 summarizes an experiment in which the control curve rose only slightly, curve A being the control and curve B that with glucose. It will be seen that an essentially similar effect occurs with the ammonia-yeast and ammonium ion as with ordinary yeast and the K ion. Similar curves for ammonia-yeast were also obtained in repeat experiments on the same yeast.

It may be concluded, therefore, that the cation absorption in fermentation is not specific for potassium.

(b) *K absorption; effect of changing the external K.* The yeast was washed twice in a standard solution of KH_2PO_4 (made up in tap water). 1 vol. of the washed yeast was suspended in 2.5 vol. of the same standard KH_2PO_4 , and 5 vol. of 14% glucose were added to 100 vol. of this suspension. For control experiments 5 vol. water were added instead of the glucose solution. Figs. 2, A and B show the results obtained with and without glucose on the same yeast.

The following may be noted. (i) The K absorbed increases with the external K (the upper limit was not here determined, but is considered later for pure

Fig. 2. Changes in the K content of the suspending fluid with and without glucose. Conditions described in text. A, with glucose; B, without glucose.

similarly treated. Samples were taken at intervals and centrifuged at once, the supernatant fluid being analyzed for ammonia. After the addition of glucose the curve of the ammonia content of the supernatant fluid plotted against time shows a marked fall,

KCl solutions). (ii) With decrease of external K a point is reached (about 10 mg./100 ml.) when no K is absorbed during fermentation, and below which K increases in the external solution. (iii) The onset of fermentation would appear to increase the per-

meability to K, since in the initial absence of external K this ion emerges more rapidly, and to higher levels, when glucose is present than otherwise. After 3 hr. with glucose, K rose to 16 mg./100 ml. whereas without glucose it reached to only 7 mg./100 ml. of the clear fluid.

II. Changes associated with the cation absorption

Hydrogen-ion concentration. Increase of H^+ concentration during fermentation was observed in all the experiments. There are two points of importance connected with this increase.

(a) *The change in pH is synchronous with the K absorption and subsequent release.* This is shown in Fig. 3. For these experiments fresh baker's yeast

Fig. 3. Curves of K, pH, and inorganic P content of suspending fluid and of keto-acids in the yeast during short period fermentation. The ordinates on left express units of pH, also inorganic P (mg./100 ml.) in suspending fluid and keto bodies in the yeast (mg./100 g.).

washed twice in tap water, 1 part, was suspended in 4 parts of $M/300$ KH_2PO_4 for 4 hr. It was kept in suspension by bubbling air through the mixture. To 1 vol. of this suspension 0.02 vol. of 30% glucose was added. At various times samples were removed, immersed in crushed ice and centrifuged as quickly as possible. The various analyses were carried out on the supernatant fluid.

(b) *The K absorbed is largely accounted for as an exchange with H^+ ions released into unbuffered mixtures.* The relations of K absorption to H^+ ion release are shown in Table 1, which gives also the nature of the fermenting mixtures. It appears evident that the entrance of H^+ ion into the suspending fluid increases with the concentration of KCl up to a certain level, and that a pH as low as 1.78 is reached (a pH of 1.55 has been recorded in one experiment). The concentration of free hydrogen ion (as deduced from the pH, with activity corrections) is about 80% of that of the absorbed potassium. As shown below some K enters with Cl, and allowing for this,

the increase in H^+ ion would appear about equal to the absorbed K. (A preliminary fermentation is mentioned in Table 1. This was found to increase the acidity subsequently developed in the second fermentation in the presence of KCl.)

Table 1. H^+ and K^+ exchanges with different K^+ concentration in unbuffered suspending fluids

(Fresh baker's yeast was washed twice in tap water; then suspended in 4 parts of 0.6% glucose and fermented for 2½ hr. 50 g. lots of the centrifuged yeast were taken and each washed twice in the KCl solution ($M/200$, $M/50$, $M/25$, $M/10$, $M/2$) then 25 ml. of the KCl solution and 5 ml. 30% glucose were added.)

K in centrifuged fluid		pH of the centrifuged fluid after 20 min.	Increase of free H^+ conc. (allowing for activity correction (mm./l.))	Decrease of potassium conc. (mm.)
At zero time (mg./100 ml.)	After 20 min. (mg./100 ml.)			
17	9	2.62	3	2
80	39	2.18	8	10
147	88	2.00	11.5	15
330	234	1.77	20	25
1760	1650	1.78	21	28

(c) *With sufficient glucose for the continuance of the fermentation the external H^+ and K-ion concentrations reach in about 30 min. a steady state which may*

Fig. 4. pH curves of fermenting mixtures with different glucose concentrations at zero time.

continue for hours. This is shown for the H^+ -ion concentration in Fig. 4, the conditions being the

same as for the experiments referred to in the previous section, but without a preliminary fermentation before the addition of K^+ .

Keto-acids. The fermenting mixtures were the same as described for the hydrogen-ion changes (§ (a)). The results are shown in Fig. 3 and may be seen that the increase in keto-acid content also synchronizes with the K absorption, though this in itself is not a definite indication that keto-acids are the source of the hydrogen ions extruded.

Sodium. While K freely enters during fermentation, we have found that the Na ion does not enter, confirming the observations of Pulver & Verzar (1940). While it is true that in some experiments with low Na and K there was evidence of Na entering, though much more slowly than K, yet with high Na and conditions similar to those when K is absorbed, no appreciable Na disappears from the external fluid. This is shown for two experiments in Table 2.

Table 2. *Effect of fermentation on the Na^+ and H^+ concentrations of suspending fluid containing initially only NaCl and glucose*

(50 g. fresh baker's yeast washed twice in $N/20$ NaCl 25 ml. $N/20$ NaCl plus 5 ml. 30% glucose were then added.)

At zero time		After 30 min.	
Na (mg./100 ml.)	pH	Na (mg./100 ml.)	pH
95.8	4.36	95.5	2.96
95.8	4.00	97.8	2.85

It is of interest to note that not only is no appreciable Na absorbed, but the fall in pH is considerably less than if KCl were used, when the pH figure would be approximately 1.7–2.00. Hence a marked rise in hydrogen-ion content is dependent on the K absorption.

Chloride. The chloride change on fermentation was investigated with approximately $N/100$ KCl as the suspending fluid; the yeast was also previously washed in $N/100$ KCl. The volumes were 100 parts yeast, 150 parts $N/100$ KCl and 5 parts 35% glucose. Under these conditions there was a marked potassium absorption after 30 min. (2.8 m.equiv./l. suspending fluid), but very little change in Cl (only 0.1 m.equiv./l.) in the same period (as shown in Table 3). The experiment was repeated with approximately $N/10$ KCl, and smaller volumes of external fluid, the mixture being 1 vol. yeast, 0.5 vol. KCl and 0.0% glucose. Here, again, considerable K was absorbed—32.7 m.equiv./l. of suspending fluid after 30 min., and at the same time the Cl absorbed was only 5.4 m.equiv./l. (the fall in Cl became more marked at 60 min., as shown in Table 3).

Table 3. *Effect of fermentation on Cl absorptions relative to K*

Time (min.)	Change in conc. in suspending fluid (m.equiv./l.)		pH	Volume conditions
	K	Cl		
	Initial conc. in suspending fluid of K and Cl = 9.7 m.equiv./l.			
0	0.0	0.0	4.4	100g. yeast, 150ml.
15	- 1.5	- 0.6	3.1	suspending fluid,
30	- 2.8	- 0.1	2.8	5 ml. 35% glucose
60	- 2.2	- 0.3	3.0	
Initial conc. in suspending fluid of K and Cl = 88 m.equiv./l.				
0	0.0	0.0	4.25	1 part yeast, 0.5
15	- 9.7	- 0.3	2.63	parts suspending
30	- 32.7	- 5.4	1.97	fluid, 0.1 part 30%
60	- 31.4	- 10.2	1.93	glucose

The volumes of the suspending fluid were checked throughout the course of the fermentation by centrifuging samples in capillaries and measuring the lengths of the columns of clear fluid and yeast. There was no appreciable change.

The results show, therefore, that only a relatively small proportion of Cl is absorbed relative to the K ion, but that some is absorbed appears evident.

Phosphate. It was at first considered (Conway & O'Malley, 1943) that the explanation of the K absorption during fermentation might lie in the associated absorption of inorganic phosphate, which esterified with glucose or its derivatives and converted into a non-diffusible anion, would hold the K electrostatically. It was found, however, that inorganic P absorption would account for only a small fraction of the K absorption. Also, very considerable K absorption could occur in the absence of phosphate, as already shown.

In Fig. 3 is shown the typical course of the inorganic P concentration after adding glucose. The conditions were the same as for the hydrogen-ion and keto-acid curves of Fig. 3.

III. H and K exchanges with different K and H concentrations outside the yeast cells

From the previous sections it would appear that during fermentation and above a certain external K level, K ions move across the cell membrane in exchange for H ions. At one external K concentration there is no exchange and below it K ions emerge from the yeast cells. With sufficient sugar outside the yeast cells a steady state of the relations can be maintained for a relatively long period. This suggests a movement of K and H ions to some relation representing zero energy change of small transfers of a kind which would be ordinarily termed an equilibrium relation, but which must be thought

of as occurring here with a steady state of organic reactions and their relatively large energy changes. In an approach to a quantitative expression of the K and H exchanges, the following picture of the conditions may be assumed. In some region of the cell, which may be regarded as a surface, an organic acid is being formed, and in a steady state is disappearing as fast as it is formed. A steady concentration of the acid is then present at the surface and may be assumed to be in relatively higher concentration than in the surrounding fluid. The acid is ionized and forms free H ions (attached to the surface) and organic acid anions. An increase or decrease of the external K concentration now causes an exchange of K and H ions, and the transfer may be, at least initially, considered as occurring in accordance with the Donnan membrane principle, a steady state of H concentration at the membrane behaving like a fixed concentration. We may write then,

$$\frac{H_0/V_0}{\alpha_1(H_1 - X)/V_1} = \frac{(K_0 - X)/V_0}{\alpha_2(K_1 + X)/V_1}, \quad (1)$$

where H_1 = conc. of H ions in the 'acid' region of the yeast, expressed as m.equiv., corresponding to 1 l. external fluid.

K_1 = m.equiv. K ions, similarly to H_1 (before the transfer or movement of K).

H_0, K_0 = conc. of external H and K ions (m.equiv./l.).

V_0, V_1 = external volume and volume of the 'acid' region; V_0 may be taken as 1 l.

X = m.equiv. K ions absorbed (per litre of external fluid).

α_1 and α_2 represent activity factors, which inserted as above convert the ratio of concentrations into ratios of activities.

In the further treatment two special conditions will be dealt with.

The external solution buffered. From equation (1), and assuming that α_1 and α_2 are approximately equal, we may derive

$$2X = (H_0 + H_1 + K_0) - \sqrt{[(H_0 + H_1 + K_0)^2 - 4(K_0H_1 - H_0K_1)]}. \quad (1)$$

This equation has been studied with a K_0 value of 4.6 throughout. The mean data were obtained for three sets of experiments in which the pH was varied from 1.8 to 5.0. In these experiments 1 part of yeast was suspended in 3 parts of N/200 KCl. M/50 sodium acetate was used for buffering with varying HCl additions. In some experiments the pH was changed merely by adding HCl or NaOH in small quantities.

The pH did not remain throughout quite constant (but this was not essential), and the pH of the 30 min. period when the maximum pH change was usually reached was taken. The concentration of glucose in the external fluid at zero time was 0.6 %.

With a K_0 of 4.6 equation (1) becomes

$$2X = (4.6 + H_0 + H_1) - \sqrt{[(4.6 + H_0 + H_1)^2 + 4(H_0K_1 - 4.6H_1)]}. \quad (2)$$

When X is zero, from equation (2),

$$H_0K_1 = 4.6H_1.$$

From Fig. 5 it will be seen that the curve through the points above and below the line of $X=0$, cuts this line at approximately 2.38 pH or

$$4.2K_1 = 4.6H_1 \text{ and } H_1 = 0.9K_1.$$

Hence

$$2X = (0.9K_1 + 4.6 + H_0) - \sqrt{[(0.9K_1 + 4.6 + H_0)^2 + 4(H_0K_1 - 4.14K_1)]}. \quad (3)$$

Putting in values of H_0 a series of curves may be obtained for different K_1 values. The solid line in

Fig. 5. K absorption from suspending fluid (m.equiv./l.) at different pH levels. Observations taken 30 min. from beginning of fermentation. Conditions described in text. The various curves are theoretical curves from equations for different K_1 values.

Fig. 5 corresponds to a K_1 value of 5.0, H_1 being then 4.5. The equation describing the data is

$$2X = (9.1 + H_0) - \sqrt{[(9.1 + H_0)^2 + 20H_0 - 82.8]}. \quad (4)$$

The external solution unbuffered. The conditions here were the same as for Table 1, 1 vol. of yeast being suspended in 0.6 vol. fluid containing only KCl and glucose.

With the fluid unbuffered any additions of H ion to it may be assumed to change the pH just like

equivalent additions of strong acid. Hence $H_0 = X$, and from equation (1), again assuming the activity factors to be approximately equal,

$$X = \frac{K_0 H_1}{K_1 + K_0 + H_1} \quad (5)$$

As K_0 is increased X approaches H_1 , so that H_1 can be determined in this way. For the series of experiments in Table 1, X appears as 21 m.equiv./l. of the external fluid. This figure largely agrees with that obtained for the previous group of buffered experiments when we consider that for unit volume of yeast five times the volume of suspending fluid was used compared with those under consideration.

Fig. 6 shows a series of curves with different K_0 values and $H_0 = 21$. It will be seen that when $K_1 = 10$, the curve describes very well the experi-

Fig. 6.

Fig. 6. K_0 is the initial potassium concentration of the suspending fluid (m. equiv./l.), and X that of the hydrogen ion liberated per litre of suspending fluid. The curves are from equations with different K_1 values.

Fig. 7. Curves showing the effect of carbohydrate inhibitors (m/200) on the K absorption.

mental points. From the previous experiments (buffered solutions) the K_1 value of 25 might have been anticipated, but the yeast for the unbuffered experiments had been not only well washed but also passed through a preliminary fermentation stage with only glucose present in the suspending fluid.

These quantitative relations of K and H are again dealt with in the Discussion.

IV. Effect of carbohydrate inhibitors on the K absorption

It would appear that the nature of the potassium absorption consists very largely in a direct exchange of K and H ions and that only a small fraction enters accompanied by anions. The question arises as to the immediate source of the H ions. To examine this a number of inhibitors were tried. The effect of fluoride, moniodoacetic acid, cyanide and arsenate are shown in Fig. 7. In these experiments the yeast was washed twice in m/200 KH_2PO_4 , and then made up one in four in this solution; 50 ml. vol. were taken and to each 5 ml. of the inhibitor solution (m/10) was added and the suspension aerated for 30 min. 2 ml. of 18% glucose was then added at zero time.

Fig. 7.

It will be seen that the external K after 30 min. action of moniodoacetic acid and fluoride was somewhat higher than for the control, or when the other inhibitors were present, showing that iodoacetate and fluoride caused a greater release of K from the yeast, but in the subsequent fermentation they very largely inhibited the absorption of K as compared with the control experiment. Arsenic and cyanide markedly increased the K absorption.

The great effect of monoiodoacetic acid and fluoride indicates that the H-ion formation causing the K interchange is entirely or mainly located at or below the stage of phosphopyruvic acid formation in the progressive breakdown of carbohydrate. The source of the H ions will be dealt with in a subsequent communication.

DISCUSSION

It is clear from the foregoing experiments that the K absorption and release during short period fermentation as noted by Pulver & Verzar (1940) does not turn on some special quality of the ion other than its size or shape, since the ammonium ion behaves similarly with an ammonia-yeast in which all the K has been replaced by ammonia.

The rapid absorption of K is essentially an interchange with H ions, as shown by the practically quantitative relations of K absorbed and H extruded, when the solution is unbuffered initially. The production of high acidity (pH values of the order of 1.7 and 1.55 are recorded) is of special interest in suggesting a way in which the high acidity of such fluids as gastric juice is produced. The quantitative relations of the K and H interchanges are usefully interpreted by the assumption that the movement is towards a position of zero free energy with respect to the apparent osmotic and electrical work done in the transfers. This is only another way of saying that the Donnan principle appears to apply to these electrolyte exchanges in a steady state of the fermentation, in which, of course, there is much energy liberation but of a kind not directly linked with the absorption of potassium and release of H ions.

In a fuller interpretation, however, the following difficulty arises. If in some acidic region of the yeast cell the ratio of K- and H-ion concentrations becomes the same as the external value, it would be expected that this ratio should also apply to the K and H ions throughout the yeast cell. But the concentration of K in the yeast cell as a whole is of the order of 100mm. or more, and the H-ion concentration is relatively very small (pH of 5.5-5.6 resting and 5.8-6.2 in fermenting yeast measured as described under Methods). The following points may therefore be considered.

(a) The free K-ion concentration in the yeast cell may be far lower than the total concentration of K, though probably much higher than the free H-ion concentration; yet its mobilization into the acid region may so deplete the amount remaining that the ratio of the K- and H-ion concentrations comes to be about the same as the external ratio. In this picture the K mobilization from within may be assumed to be only a fraction of that from without.

However, we may apply this principle quantitatively, the free K-ion concentration in the yeast cell could only be a small fraction of the total concentration.

(b) The acidic region may be separated from the remainder of the cell by a special membrane; and if some enzymes in the yeast cell work best at a much lower pH than that registered for the whole cell, this would seem a reasonable hypothesis. This membrane may be considered impermeable or only very slowly permeable to cations.

(c) If, during the transference of one of the ions K^+ or H^+ from the acidic region to the remainder of the yeast cell, work is done additional to the apparent osmotic or electrical work arising out of the concentration differences, then for such *internal* relations the Donnan principle cannot apply.

Concerning explanation (a) it is improbable that the free K concentration is so much lower than the total K. In such cellular material as muscle fibre, for example, there is a similar total K which is all or practically all ionized (Boyle & Conway, 1941). The total exchange of K by ammonia in the production of ammonia yeast (Conway & Breen, 1945), shows that at least the K cannot be bound by reactions specific for potassium.

Further work is necessary to produce evidence adequate for deciding between the other two explanations.

The present investigation shows that the membrane of the yeast cell, during fermentation at least, has a permeability for inorganic electrolytes similar to that of muscle (Conway & Boyle, 1939; Boyle & Conway, 1941). (It may be mentioned that it is also similar to that of the liver and proximal convoluted tubule cells of the frog, according to unpublished experiments made in this laboratory.) The permeability is such that K and small anions can freely pass through the membrane, but Na and the larger anions are either debarred or pass very slowly. The slow passage of Na would involve a special energy-expending mechanism for extruding it. The facts so far established do not appear to warrant the assumption of such a mechanism for muscle fibres, liver or yeast cells (cf. Conway, 1945).

The question is of such general importance that the following may be added. The isolated sartorius muscle of the frog has been shown to accumulate KCl in large amounts over some hours (Boyle & Conway, 1941), while during 48 hr. or more (in the cold) there is no appreciable increase of Na within the fibres, when the external Na concentration is approximately that of Ringer's solution. There can be no question here of a competition between K and Na for acidic points within the cell, or any similar idea, for the K and Cl ions are entering together. Hence, if Na ions enter they must be extruded back against a gradient, a process neces-

sarily requiring the expenditure of energy. But this energy expenditure would be of a kind quite unaffected by cyanide and occur at a temperature at or close to 0° (as may be shown), the muscle fibres under these conditions being, if anything, more efficient in keeping out Na ions over long periods (provided that there is always enough external K to maintain that in the muscle, and for isolated tissue this external K is much higher than the ordinary blood level). It may be urged that the idea of active Na extrusion from muscle fibres receives support from Heppel's experiments on rats (1939, 1940), fed with a K-free diet. In such experiments the muscle Na increases markedly, and it exchanges freely with the radioactive isotope, even more rapidly than the corresponding interchanges observed between radioactive K and muscle K. If such a fact were interpreted as indicating that Na entered the normal tissue as freely (or even more freely) than K, application of this principle to the isolated sartorius of the frog would require that the active extrusion of Na from 1 kg. muscle tissue would demand a minimal energy expenditure greater than that necessary for the whole normal excretion of urea by an average human subject. (This calculation may be made from the data supplied by Conway & Moore (1945), and Conway & Boyle (1941).) Such a free entrance of Na into muscle therefore appears to be untenable, and this conclusion is further strengthened by the fact that cyanide does not affect the cation exchange.

From these considerations, Heppel's results are seen to provide strong evidence that when the K ratio (and thus the potential) across the membrane of the muscle fibre reaches a certain level, an abnormal permeability develops in at least some of the fibres; the differential permeability as between K and Na is lost, Na freely enters, and can then, of course, freely exchange with its isotope. A similar result follows (as we have shown in unpublished experiments) with the isolated sartorius when this is subjected to low external concentrations of K.

Also, it has been shown (Conway & Moore, 1945) that KBr or KNO_3 enter the osmotically active muscle fibres at much slower rates than KCl, and that no measurable amount of K_2SO_4 enters in some hours at room temperature. The anions therefore exhibit a graded series in which the rates of entrance diminish to zero, and it will be allowed that no special mechanism for extruding Br, NO_3 or SO_4 is at all likely. The cations also show a graded series of rates, $\text{K} > \text{Rb} > \text{Cs} > \text{NH}_4$ (without CO_2 in the case of NH_4), and finally Na, this last entering in the cold at a rate too low to be measurable in 48 hr. (provided there is enough external K to maintain the K levels).

Consideration of these facts concerning Na permeability in muscle makes it evident that only two alternative explanations remain: either the membrane is absolutely impermeable to Na, or that there is a very slow entrance and extrusion, the latter requiring very little energy expenditure. Similar observations will be shown elsewhere to apply to yeast cells. At present, however, there is no definite evidence for such Na extrusion from muscle fibres, liver cells, etc., although, of course, the active passage of Na through cells does occur in certain tissues specialized for this purpose, such as the distal convoluted tubules of the frog.

SUMMARY

1. The absorption of potassium and its subsequent release by yeast in short period fermentation (Pulver & Verzar, 1940) is simulated by the absorption and release of ammonium ion into ammonia-yeast (Conway & O'Malley, 1943; Conway & Breen, 1945).

2. When the external potassium is changed prior to such fermentation there is found to be one concentration above which potassium is absorbed and below which potassium is released. This concentration for the conditions investigated is in the region of 10 mg. K^+ /100 ml.

3. The hydrogen-ion concentration of an unbuffered suspending solution follows closely the changes of potassium in fermentation, and when the external K is raised to about $n/10$ to $n/2$ the acidity produced can reach a pH of 1.78 or less (with 1 part of yeast to 0.6 of suspending fluid containing 5% glucose). The free H ion produced under the described conditions accounts by exchange for about 80% of the K^+ absorbed, and the remainder of the absorbed K^+ appears to enter in association with Cl^- .

4. The exchanges of H and K ions are very well described quantitatively by simple equations which are based on a movement of the inorganic ions to reach a Donnan relation in a steady state of the fermentation process between some region of the cell and the external fluid.

5. The effect of inhibitors shows that the main source of the acidity lies at or below the level of phosphopyruvic acid in the breakdown of carbohydrate.

6. Keto-acid formation shows a parallelism with H-ion formation but quantitatively keto-acid formation is inadequate to account for the H ion produced.

7. The investigation shows incidentally that during fermentation the membrane of the yeast cell becomes more permeable to K^+ ; also that, during fermentation at least, it is permeable to Cl^- but it is impermeable or very slowly permeable to Na^+ .

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The Metabolism and Functioning of Vitamin-like Compounds

1. AMMONIA FORMATION FROM GLUTAMINE BY HAEMOLYTIC STREPTOCOCCI; ITS RECIPROCAL CONNEXION WITH GLYCOLYSIS

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Nutritional studies have led to the identification of many substances which are essential for normal growth or behaviour of living organisms. In certain but not in all cases, the need for such substances has been found to be connected with their reactions in particular component systems of the organism. The commonest biochemical method of localizing vitamin action has followed Peters's (1936) observation that the metabolism of pyruvate, by intact animals or by tissues separated from them, was retarded during deficiency of aneurin but restored after its addition. This method, involving the successive induction and making good of a nutritional deficiency, has not always been found applicable. This is understandable for, to take as an example the case quoted above, stimulation by aneurin depended on circumstances which included: deficiency in aneurin being compatible with life of the organism, and means being found to produce deficient tissues; the deficient tissues being able to synthesize the added vitamin to the functioning coenzyme; and other components in the system using the coenzyme not being proportionately reduced in deficiency and remaining capable of reacting with the newly synthesized coenzyme.

Investigations of a different type (McIlwain & Hughes, 1944) gave an instance of a relationship which might be regarded as the inverse of that

above, and used as a supplementary or complementary method for localizing vitamin action. In this case, the addition of pantothenate to suspensions of bacteria which needed pantothenate in growth was not at first found to initiate or accelerate any process except its own inactivation; but the inactivation was connected with bacterial metabolism in that it occurred only during the progress of a reaction such as glycolysis. Evidence was obtained that this might be due to participation of pantothenate in glycolysis. The effect was thus in some ways comparable to the connexion found between aneurin requirement and carbohydrate intake in animals, and also to certain linked reactions in intermediary metabolism. The investigations of Lwoff & Lwoff (1937), Morel (1941) and of Winzler, Burk & du Vigneaud (1944) are also in many ways analogous.

In the present series an attempt is made to find how general and significant are such reactions and how their occurrence in non-proliferating cells may be connected with the physiological functioning of the substance, for example, in growth. In its relationship to β -haemolytic streptococci, for some strains of which it is a growth-essential (McIlwain, Fildes, Gladstone & Knight, 1939), glutamine has now been found to offer an example of both methods of connexion between a vitamin-like substance and