Alcohol Dehydrogenase Activity and Electron Transport in Living Yeast

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1. The excretion of H^+ ions, with practically equivalent uptake of K^+ ions (from 0.1M-potassium chloride), occurs during the aerobic oxidation of ethanol. 2. Acetaldehyde and acetic acid formed at the same time are quantitatively equal to the amount of ethanol oxidized. 3. A slow uptake of K^+ ions occurs during the oxidation of acetaldehyde and a more rapid uptake during the oxidation of D-glyceraldehyde 3-phosphate. 4. The anaerobic reduction of methylene blue is studied, and the inhibitory effect of K^+ and other inorganic cations on the system demonstrated. 5. The cation requirement for equal inhibitory effect is parallel with the reciprocals of the transport affinities for the 'physiological K-carrier' (as taken from Conway & Duggan, 1958). 6. The cation inhibition of methylene blue reduction is reversed by treatment of the yeast with Teepol or by freezing-and-thawing. 7. Azide is shown to inhibit the reduction of methylene blue with intact cells. The inhibition is partially reversed by Teepol treatment and completely by freezing-and-thawing.

It was shown by Conway & O'Malley (1946) that when yeast was fermented at room temperature (17–20°), in the ratio of 1 part of yeast to 0.6 part (w/v) of a fluid containing 5% glucose and 0.1M-potassium chloride, free H^+ ions were produced in the suspending fluid and K^+ ions were taken up in practically equivalent amounts. After 20 min. fermentation the excretion of H^+ ions amounted to 21 m-equiv./kg. and the uptake of K^+ ions was 28 m-equiv./kg. Rothstein & Enns (1946) showed that potassium uptake and acid excretion also occurred during the oxidation of ethanol, when yeast was suspended (1:20, w/v) in a fluid containing 0.01M-citrate, 0.033M-potassium chloride and 7% (v/v) ethanol.

In the experiments described here ethanol was used as a substrate, the conditions being a 1:20 (w/v) suspension in 0.1M-potassium chloride and 0.257M-ethanol bubbled with oxygen at 22°, and it was found that in 20 min. the excretion of H^+ ions was 25.6 m-equiv./kg. and the uptake of K^+ ions was 24.3 m-equiv./kg. Thus the excretion of H^+ ions and uptake of K^+ ions under these conditions were very similar to the findings of Conway & O'Malley (1946), although the ratio of yeast to suspending fluid was very different.

The excretion of H^+ ions and the uptake of K^+ ions are further investigated here in the presence of ethanol, and the results are considered in the light of equations expressing the actions of alcohol and aldehyde dehydrogenases:

and

There is developed in the paper the action of methylene blue as acceptor of electrons in the anaerobic oxidation of ethanol by a buffered suspension of yeast. The action of cations on this system is of special interest, as will appear.

A preliminary account of part of this work has appeared elsewhere (Ryan, 1965).

MATERIALS AND METHODS

Yeast. Fresh baker's yeast, as supplied by the Cork Yeast Co., was used in all the experiments. Before use the washed yeast was 'starved' by oxygenation of a 1:10 (w/v) suspension for 24 hr. at 22°.

Chemical determinations

Potassium. This was determined with a Beckman flame photometer.

Ethanol and acetic acid. These were determined by microdiffusion procedures (Conway, 1962).

Acetaldehyde. The extinction of the acetaldehyde-semicarbazide complex was determined with a Beckman DU spectrophotometer at wavelength 224 mμ. The concentration of acetaldehyde was calculated from a standard curve prepared as described by Burbridge, Hine & Schick (1950).

Excretion of hydrogen ions. Samples of the yeast suspensions (1:20, w/v) were centrifuged for 10 min. at 1000g.

Portions (5 ml.) of the supernatant fluids were evacuated for 20 min. in a vacuum desiccator, then titrated to pH 7.0 with 0.01 N-NaOH.

Oxygen uptake and carbon dioxide production. These were determined with the conventional manometric techniques by using 3 ml. samples of yeast suspension (usually 1:200, w/v) per flask and shaking at 120 cyc./min. at 22°. Concentrations are expressed throughout per kg. of centrifuged yeast (wet wt.).

Experimental procedures

Aerobic suspensions. Samples of the 'starved' yeast were suspended (1:20, w/v) in the various media and bubbled with O₂ at 22°. Samples were removed at intervals and centrifuged for 10 min. at 1000g. When the yeast was required for potassium analysis the samples were washed twice in 20 vol. of distilled water.

Estimation of dehydrogenase activity. The yeast suspensions were contained in Pyrex tubes, 2 cm. x 14 cm., fitted with rubber stoppers and adapted so that N₂ could be bubbled through the suspensions. The tubes were immersed in a water bath at 22°. The yeast was suspended (1:100; 0.2 g./20 ml.) in a fluid containing 0.01 M-tris-HCl (pH 7.4)-0.257 M-ethanol-0.125 mM-methylene blue. Before the addition of the methylene blue the O₂ was expelled from the suspension by bubbling N₂ through it for 5 min. The time required for 90% decolorization of the methylene blue was determined by visual comparison with a standard solution. The standard solution was similarly prepared but contained boiled yeast instead of living cells and a concentration of 0.0125 mM-methylene blue.

In studying the effects of inhibitors etc. on the system the activity of the inhibited suspension was compared with that of a control suspension determined simultaneously. For comparative purposes the results are expressed as a percentage of the rate of reduction in the control suspensions.

RESULTS

Aerobic ethanol oxidation by yeast

Uptake of potassium ions and the excretion of hydrogen ions during the oxidation of ethanol. When the yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-potassium chloride and 0.257 M-

ethanol and bubbled with oxygen at 22°, K⁺ ions entered the cells and acid was excreted into the external medium. A study of the electrometric titration curves of the external fluid showed that the acid formed was largely free hydrochloric acid with small amounts of acetic acid. When the acetic acid was determined quantitatively, and subtracted from the total acid formed, the residual free H⁺ ion excretion was equivalent to the K⁺ ion uptake as shown in Table 1.

When the suspension was buffered at pH 7.4 with 0.1 M-tris hydrochloride the initial rate of K⁺ ion uptake was slower than in the unbuffered suspension but it proceeded linearly with time for the 40 min. duration of the experiments. The results in Table 1 show that the quantity of acetic acid excreted at pH 7.4 is considerably greater than in the unbuffered suspension.

Production of acetate and acetaldehyde during the oxidation of ethanol. When yeast oxidizes ethanol at pH 7.4 the acetaldehyde formed may be trapped by the addition of semicarbazide to the medium (Silhankova, 1963). In studying the formation of acetate and acetaldehyde the yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-semicarbazide (adjusted to pH 7.4 with tris), 0.1 M-potassium chloride, 0.04 M-ethanol and bubbled with oxygen at 22°. Samples were removed at intervals and centrifuged. The supernatant fluid was analysed for acetate, acetaldehyde and ethanol, and the yeast was analysed for potassium. The results from one such experiment are shown in Table 2. It may be seen that the total amount of acetate + acetaldehyde formed (expressed as m-moles/kg. of yeast) was approximately equal to the amount of ethanol oxidized for a period of about 40 min.

The presence of semicarbazide had no apparent effect on the rate of potassium uptake, as may be seen from a comparison of the figures in Table 2 with those of the buffered suspension shown in Table 1.

Oxygen consumption and carbon dioxide pro-

Table 1. *Uptake of potassium and the excretion of acid during ethanol oxidation*

The yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-KCl and 0.257 M-ethanol. The suspensions were oxygenated at 22°. The buffered suspension contained 0.1 M-tris-HCl, pH 7.4. Average values from three experiments in each series are shown.

Time (min.)	Unbuffered			Buffered at pH 7.4	
	Uptake of K ⁺ (m-equiv./kg.)	H ⁺ ion excreted* (m-equiv./kg.)	Acetic acid excreted (m-equiv./kg.)	Uptake of K ⁺ (m-equiv./kg.)	Acetic acid excreted (m-equiv./kg.)
10	17.5	18.6	0.3	5.5	6.4
20	24.3	25.6	6.9	9.8	28.4
30	26.5	27.9	9.4	16.0	32.8
40	29.7	33.4	13.1	22.6	40.2

* H⁺ ion excreted equals the total acid minus the acetic acid.

Table 2. *Production of acetate and acetaldehyde during the oxidation of ethanol*

The yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-semicarbazide (adjusted to pH 7.4 with tris) and 0.1 M-KCl and 0.04 M-ethanol. Suspensions were oxygenated at 22°.

Time (min.)	Acetate formed (m-moles/kg.)	Acetaldehyde formed (m-moles/kg.)	Acetate + acetaldehyde (m-moles/kg.)	Ethanol oxidized (m-moles/kg.)	Uptake of K ⁺ (m-moles/kg.)
10	13	66	79	100	5.7
20	35	162	197	195	11.7
30	52	248	300	295	17.7
40	82	298	380	392	25.5
60	102	348	450	585	38.1

Table 3. *Oxygen consumption and carbon dioxide production during the oxidation of ethanol*

Each flask contained 3 ml. of a suspension of yeast (1:200, w/v) in 0.1 M-KCl and 0.257 M-ethanol at 22°. R.Q., respiratory quotient.

Time (min.)	Uptake of O ₂ (m-moles/kg.)	CO ₂ production (m-moles/kg.)	R.Q.
10	48	2	0.04
20	103	9	0.09
30	153	20	0.13
40	229	29	0.13
50	297	37	0.13
60	372	51	0.13

duction during the oxidation of ethanol. When the uptake of K⁺ ions was studied in an unbuffered suspension with ethanol the amount of acetic acid excreted was relatively small compared with that at pH 7.4. Although it is probable that some acetate accumulates inside the cells under these conditions its rate of oxidation is very slow, as may be deduced from the measurement of R.Q. shown in Table 3.

Uptake of potassium during the oxidation of acetaldehyde. Low concentrations of acetaldehyde were readily metabolized by the yeast. A study of the q_{O_2} of the yeast at varying concentrations of acetaldehyde showed that the maximum rate of oxidation occurred when the concentration of acetaldehyde was decreased to about 0.035 M. In studying the effect of acetaldehyde on potassium uptake the yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-potassium chloride and 0.035 M-acetaldehyde and bubbled with oxygen at 22°. The rate of uptake of potassium was considerably slower than that which took place in an unbuffered suspension of ethanol, as shown in Fig. 1.

Uptake of potassium during the oxidation of D-glyceraldehyde 3-phosphate. Preliminary experiments showed that the yeast readily oxidized D-glyceraldehyde 3-phosphate. A maximum q_{O_2} was reached at a substrate concentration 0.4 mM.

Fig. 1. Relative rates of entrance of K⁺ ions into yeast with various substrates. The yeast was suspended (1:20, w/v) in 0.1 M-KCl and bubbled with oxygen at 22°. The substrates were: (2.7 mM) D-glyceraldehyde 3-phosphate (●); 0.257 M-ethanol (▲); 0.035 M-acetaldehyde (■). The yeast contained approx. 90 m-moles/kg. at zero time. In the absence of added substrate there was no net entry of K⁺ ions.

This remained constant with further increases in substrate concentration up to 2.7 mM (the highest concentration tested).

The rate of uptake of potassium by yeast suspended (1:20, w/v) in 0.1 M-potassium chloride and 2.7 mM-D-glyceraldehyde 3-phosphate and bubbled with oxygen at 22° is shown in Fig. 1. It may be seen that the rate of uptake of potassium is considerably faster under these conditions than with acetaldehyde as a substrate. In the initial stages the rate of uptake exceeds that observed in the presence of ethanol.

Effect of phenazine methosulphate on potassium uptake during ethanol oxidation. The effect of phenazine methosulphate on the uptake of potassium from a buffered solution of ethanol is shown in Table 4. The experimental conditions were the

Table 4. *Effect of phenazine methosulphate on the uptake of potassium during ethanol oxidation*

The yeast was suspended (1:20, w/v) in a fluid containing 0.1 M-KCl and 0.257 M-ethanol and 0.1 M-tris-HCl at pH 7.4. Suspensions were oxygenated at 22° for a period of 40 min.

Concn. of phenazine methosulphate (m-mole/l.)	Uptake of K ⁺ (m-moles/kg.)	Inhibition (%)
0.00	33.2	—
0.025	28.3	15
0.038	20.7	38
0.050	17.5	47
0.100	13.8	58
0.200	13.8	58

same as those described above for uptake of potassium at pH 7.4. It may be seen that low concentrations of the substance have a marked effect on the uptake of potassium and 50% inhibition is reached at a concentration 0.057 mM. Further increases in the concentration of phenazine methosulphate up to 0.2 mM only increased the inhibition to about 58%.

Anaerobic oxidation of ethanol with methylene blue as hydrogen acceptor

Reduction of methylene blue by 'starved' yeast. In the absence of added substrate the rate of reduction of methylene blue by 'starved' yeast was very slow and with the procedure described in the Materials and Methods section a time of 120–140 min. was required for 90% decolorization. When 0.257 M-ethanol (1.5%, v/v) was present in the suspending fluid 90% decolorization was achieved in 5–10 min., representing a rate of reduction of 135–270 m-equiv. of methylene blue/kg. of yeast/hr. A figure of 182 ± 49 (s.d.) m-equiv./kg./hr. was calculated from determinations on 17 different samples of yeast. Although there was considerable day-to-day variation in the yeast preparations, the rate of reduction for any one sample remained relatively constant for several hours after the overnight oxygenation. The rate of reduction was constant for ethanol concentrations between 0.01 and 0.6 M.

Effect of cations on the rate of reduction of methylene blue. The various cations were added to the suspending fluids as chlorides. The results are expressed as a percentage of the rate of reduction in control suspensions (no cation added) determined simultaneously. Fig. 2 shows the effect of K⁺ ions and Na⁺ ions on the rate of reduction of the methylene blue. Low concentrations of K⁺ ions have a marked effect on the reducing mechanism and, as may be seen, about 50% inhibition was

Fig. 2. Effect of Na⁺ ions (○) and K⁺ ions (●) on the anaerobic reduction of methylene blue. The yeast was suspended (1:100, w/v) in 0.01 M-tris-HCl, pH 7.4, and 0.257 M-ethanol and 0.125 mM-methylene blue.

Table 5. *Effect of various cations on the rate of reduction of methylene blue*

The yeast was suspended (1:100, w/v) in a fluid containing 0.257 M-ethanol and 0.125 mM-methylene blue and 0.01 M-tris-HCl at pH 7.4. The suspensions were bubbled with nitrogen gas at 22°. The reciprocals of transport affinities were calculated from the data of Conway & Duggan (1958).

Cation species	Concn. at 50% inhibition (m-moles/l.)	Relative concn. at 50% inhibition (K=1)	Reciprocals of transport affinities
K ⁺	0.4	1	1
Rb ⁺	1.1	2.8	2.4
Cs ⁺	3.9	9.8	14.0
Na ⁺	8.0	20.0	26.0
Li ⁺	13.6	34.0	200.0

achieved at a concentration of 0.4 m-mole of K⁺/l. Sodium ions, on the other hand, were much less effective and a concentration of 8 m-moles/l. was required to produce 50% inhibition.

The effects of Rb⁺, Cs⁺ and Li⁺ ions were also investigated and the concentration of each ion at 50% inhibition was calculated. The results are shown in Table 5 and a comparison is made between the relative concentrations of the various cations at 50% inhibition and the reciprocals of the transport affinities of these cations during glucose fermentation, as determined by Conway & Duggan (1958).

Fig. 3. Effect of K⁺ ions on the anaerobic reduction of methylene blue with Teepol-treated cells (●) and frozen-and-thawed cells (○). Suspensions were 1:100 (w/v) in 0.01 M-tris-HCl, pH 7.4, and 0.257 M-ethanol and 0.125 mM-methylene blue. Controls contained intact cells.

Fig. 4. Effect of sodium azide on the anaerobic reduction of methylene blue: intact cells (●); Teepol-treated cells (○); frozen-and-thawed cells (Δ). Suspensions were 1:100 (w/v) in 0.01 M-tris-HCl, pH 7.4, and 0.257 M-ethanol and 0.125 mM-methylene blue. Controls contained intact cells (no azide).

Table 6. Effect of phenazine methosulphate on the rate of reduction of methylene blue

The yeast was suspended (1:100, w/v) in a fluid containing 0.257 M-ethanol and 0.125 mM-methylene blue and bubbled with nitrogen gas at 22°. The control suspension had no added K⁺ ions or phenazine methosulphate.

Concn. of phenazine methosulphate (m-mole/l.)	Rate of reduction (% of control)		Inhibition (%)
	No K ⁺ present	0.4 m-mole of K ⁺ /l.	
0	100	51	49
0.025	116	58	50
0.038	112	73	35
0.050	112	82	27
0.100	112	104	7
0.200	104	104	0

Effect of (a) Teepol and (b) freezing-and-thawing on the rate of reduction of methylene blue. In these experiments the yeast was suspended in the usual manner with the addition of 15.0 mg. of Teepol/l. of medium, and allowed to stand for a period of 20 min. at 22°. Such suspensions reduced methylene blue in the presence of ethanol at approximately the same rate as untreated suspensions of the same yeast. The addition of K⁺ ions to the Teepol-treated suspensions had only slight inhibitory effect, as shown in Fig. 3.

When the yeast was frozen-and-thawed three times, with liquid nitrogen, its subsequent ability to reduce methylene blue was only about 33% of the control samples (intact cells). This treatment abolished the inhibitory effect of K⁺ ions, as shown in Fig. 3.

Effect of azide on the rate of reduction of methylene blue. The effect of varying concentrations of azide on the system was studied with (a) intact cells, (b) Teepol-treated cells and (c) frozen-and-thawed cells. The same procedure was followed as in studying the effect of K⁺ ions on the system. Azide was added in the form of sodium azide and equivalent amounts of Na⁺ ions were added to the controls as sodium chloride. The results (Fig. 4) show that with intact cells a 50% inhibition in the rate of reduction is achieved at a concentration of 0.8 m-mole of azide/l. In the Teepol-treated suspensions a concentration of 2.7 m-moles/l. is required to produce the same effect, whereas in the frozen-and-thawed suspensions azide concentrations up to 5 m-moles/l. had no effect.

Effect of phenazine methosulphate on the reduction of methylene blue in the presence of potassium ions. When the yeast was suspended in varying concentrations of phenazine methosulphate ranging from 0.025 to 0.2 mM, the rate of reduction of the methylene blue was slightly faster than in the

controls. The inhibitory effect of 0.4 m-mole of K^+ ions/l. on the system was reduced progressively as the concentration of phenazine methosulphate increased. The results shown in Table 6 are expressed as a percentage of the rate of reduction in control suspensions with no K^+ ions or phenazine methosulphate added.

DISCUSSION

The excretion of H^+ ions and the uptake of K^+ ions, that occurs when yeast is fermented in glucose plus potassium chloride, has been studied extensively and a detailed account of the literature may be found in the reviews of Conway (1954) and Rothstein (1955). Conway (1951, 1959) has based his redox-pump theory for cation transport largely on evidence derived from this system. Conway's theory proposes that hydrogen atoms are transferred from a substrate, by means of a dehydrogenase system, to a redox carrier bound to the cell membrane. The carrier in turn liberates free H^+ ions at the outer surface of the membrane and retains the electrons. Potassium ions present in the external medium can bind to the reduced carrier and return with it to the inner surface of the membrane, where re-oxidation of the carrier liberates them into the internal medium. According to Rothstein (1955), the potassium-hydrogen exchanges are dependent on glycolytic enzymes located at the cell surface. The experiments described in this paper are considered to support the views of both Conway and Rothstein.

The present results show that the excretion of H^+ ions and the uptake of K^+ ions that takes place when yeast is fermented in glucose plus potassium chloride also occurs extensively during the oxidation of ethanol by 'starved' yeast. A rapid uptake of potassium was also shown to occur during the oxidation of D-glyceraldehyde 3-phosphate. Although considerable amounts of H^+ ions were excreted here also, quantitative determination was not possible owing to the presence of the substrate. The fact that rapid cation movements can occur during the oxidation of these substrates supports Rothstein's view that the glycolytic enzymes play an essential role in the transport system. Further evidence in support of this may be derived from the fact that the oxidation of ethanol proceeds only as far as acetate in the 40 min. duration of these experiments. Hence the free H^+ ions formed may be considered as arising from reactions involving either alcohol or aldehyde dehydrogenase. Also, since only a slow uptake of potassium was shown to occur during the oxidation of acetaldehyde, it appears probable that the action of alcohol dehydrogenase provides the immediate source of H^+ ions during the oxidation of ethanol. Attempts to relate

the rate of ethanol oxidation and potassium entry have, however, so far proved unsuccessful. The slow potassium uptake that does occur during the oxidation of acetaldehyde may also be associated with the direct oxidation of the substrate by surface enzymes. Alternatively the conversion of acetaldehyde into ethanol, by the action of aldehyde mutase, may provide an essential link with the glycolytic enzymes.

The experiments on the rapid reduction of methylene blue by yeast in the presence of ethanol are of special interest in this paper. The fact that methylene blue does not readily penetrate the membranes of intact yeast cells is generally recognized and made use of in vital-staining techniques. In further support, Silhankova (1959) has shown that the rate of reduction of methylene blue by fermenting yeast is increased in the presence of the cationic detergent Ajatin. She has interpreted her findings on the basis of permeability changes due to the presence of the detergent.

In the experiments described here, when the 'starved' yeast was suspended anaerobically in the presence of ethanol, a rapid reduction of methylene blue occurred. Since the dye does not penetrate the membrane the process of reduction must take place at the outer surface of the cell. The rate of reduction of methylene blue was strongly inhibited by the presence of K^+ ions in the medium. Other cations also inhibited the system but to lesser degrees. The relative concentrations of the various cations at 50% inhibition in the rate of reduction were determined. The figures obtained were shown to be similar to the reciprocals of the transport affinities of the carrier for these cations, as determined by Conway & Duggan (1958). Considering the difference in the manner of arriving at such data, the evidence strongly supports the view that the physiological potassium carrier is involved in the reduction of methylene blue.

The results are interpreted on the basis that the physiological potassium carrier is reduced in the membrane by the alcohol dehydrogenase system and, in the absence of external K^+ ions, passes both electrons and H^+ ions to the methylene blue. Potassium or other cations may be expected to compete with the methylene blue for the reduced form of the carrier, thus accounting for the inhibitory effect of these cations on the system. The degree of such inhibition goes in accordance with the transport affinity of the carrier for a particular ion species. Since no alternative electron acceptors are available under these conditions, no inward movement of cations can occur.

Armstrong (1957) and Silhankova (1959) have shown that cationic detergents have a cytolytic effect on yeast cells. The presence of Teepol in the system may be expected therefore to increase the

permeability of the membrane to methylene blue, thus abolishing the necessity for a carrier link with the alcohol dehydrogenase system. This would account for the fact that K^+ ions no longer inhibited the rate of reduction with Teepol-treated cells. A similar effect was shown with frozen-and-thawed cells, although here the rate of reduction was considerably slower than with intact cells.

The inhibitory effect of azide on the production of H^+ ions during fermentation was shown by Conway, Brady & Carton (1950). Foulkes (1956) made a more detailed study of the azide effect and concluded that the immediate action of this substance was on the potassium carrier. The fact that azide strongly inhibited the rate of reduction of methylene blue by intact cells is taken as further evidence that the potassium carrier is involved in this system. Although Teepol treatment failed to abolish the effect of azide completely, considerably higher concentrations were required to produce the same degree of inhibition as with intact cells. Azide had no effect on the slower rate of reduction that took place in the presence of frozen-and-thawed cells.

The uptake of potassium during the oxidation of ethanol was considerably lowered in the presence of phenazine methosulphate. According to Hunter (1963), this substance functions by removing electrons near the substrate level and passing them directly to the final acceptor. The inhibitory effect on potassium uptake is interpreted on the basis that, in the presence of phenazine methosulphate, the electrons are largely carried directly to oxygen, thus by-passing the potassium carrier. It was also shown that the inhibitory effect of K^+ ions on the rate of reduction of methylene blue was progressively removed as the concentration of phenazine methosulphate was increased. It is probable that the substance again functions as a direct link between the dehydrogenase system and methylene blue, hence eliminating the necessity for a carrier-mediated reduction.

In conclusion it may be stated that the evidence indicates that the potassium-hydrogen ion-trans-

port system depends on the presence of a redox-type carrier present in the cell membrane. Although direct evidence of the nature of this carrier is not available, according to Conway & Gaffney (1966) a cytochrome-like substance could function in this manner. Mitchell (1962) has proposed a theory involving dehydrogenase systems for the production of H^+ ions by *Micrococcus lysodeikticus*. Such a scheme appears essentially similar to that visualized in the yeast cell.

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