37. PYRUVIC DEHYDROGENASE OF *BACTERIUM COLI*

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BACTERIUM COLI oxidizes pyruvate to CO_2 and water [Cook & Stephenson, 1928]; and in the presence of dinitrophenol or NaN_3 the oxidation is complete [Clifton & Logan, 1939]. Anaerobically, the main products of the breakdown of pyruvate are acetic, lactic, formic and succinic acids, ethyl alcohol, H_2 and CO_2 [Tikka, 1935]. The theories concerning the oxidation have been summarized by Mazza & Cimmine [1934]. More recent work appeared in two papers by Krebs [1937, 1, 2].

Cell-free extracts of *Bact. coli* prepared by crushing the cells in the Booth-Green mill were found to contain an enzyme system capable of oxidizing pyruvate. This paper reports the study of some of the properties of such extracts.

I. Methods

Pyruvic acid was estimated by the bisulphite fixation method of Clift & Cook [1932]. The cocarboxylase was prepared by Dr H. Weil-Malherbe, to whom the author is grateful.

The activity of the enzyme system was followed by measuring the O_2 uptake in Warburg manometers at 37° . Anaerobically, methylene blue acts as hydrogen acceptor for the reaction.

II. Preparation of the extracts

Bacterium coli-commune Escherich (National Collection of Type Cultures No. 86) was grown on agar with tryptic digest of casein, lactate and yeast water. Yeast water was added to ensure an adequate supply of substances required for synthesis of cocarboxylase [Silverman & Werkman, 1939]. After 24–30 hr. growth the cells were washed off, centrifuged and crushed for 2 hr. in the wet-crushing mill [Booth & Green, 1938]. The crushed cells were diluted with an equal volume of distilled water and centrifuged at 12,000 r.p.m. for 40 min. The clear yellow supernatant fluid was dialysed for 14 hr. against running water.

III. Carboxylase

The presence of carboxylase in the fresh undialysed extracts could not be shown under aerobic conditions. Similar extracts of crushed yeast cells showed high carboxylase activity under the same conditions. This confirms the conclusion of Cook [1930] that pyruvate is not decarboxylated by *Bact. coli* in the same way as by yeast.

IV. Components of the system

Lipmann [1939, 3 for summary] has obtained from *Lactobacillus delbrückii* (an anaerobe which does not oxidize acetate) phosphate extracts which oxidize pyruvate to acetate and CO_2 . The oxidizing system comprises at least the following: (1) a protein or proteins, (2) inorganic phosphate, (3) cocarboxylase, (4) a metal, Mg, Mn or Co and (5) flavin adenine dinucleotide.

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The dialysed extracts of *Bact. coli* resemble Lipmann's preparations in several respects, as indicated in Table 1.

Table 1. *Components of the oxidizing system*

	Additions to manometers (ml.)				
	1.0	1.0	1.0	1.0	1.0
Extract	—	0.9	0.9	0.9	0.9
<i>M</i> /30 phosphate pH 6.17	0.4	0.4	0.4	—	0.4
Coccarboxylase 100 µg./ml.	0.3	0.3	0.3	0.3	—
Manganese 100 µg./ml.	0.2	0.2	—	0.2	0.2
<i>M</i> pyruvate	1.3	0.4	0.6	0.8	0.7
Water	49.5	235.0	17.5	32.0	143.0
O ₂ uptake, µl./60 min.					

Phosphate, coccarboxylase and a metal are apparently involved in the oxidation of pyruvate by the dialysed extract. The degree of dependence upon added phosphate varied greatly in different preparations. Flavin adenine dinucleotide caused a small increase in the rate of oxidation. It was possible to treat the extract with ammonium sulphate up to 62 % of saturation and retain the active system in the precipitate, most of which dissolved in glass-distilled water to give an orange-yellow solution. Such treatment removed substrate, coccarboxylase and much of the metal component (Table 2).

Table 2. *Components of the precipitated system after dialysis*

	Additions to manometers (ml.)				
	1.0	1.0	1.0	1.0	1.0
Enzyme	—	1.5	1.5	1.5	1.5
<i>M</i> /15 phosphate pH 6.17	0.2	0.2	0.2	—	0.2
Coccarboxylase 100 µg./ml.	0.3	0.3	0.3	0.3	—
Manganese 100 µg./ml.	0.2	0.2	—	0.2	0.2
<i>M</i> pyruvate	1.5	—	0.2	0.2	0.2
Water	141.0	138.5	0	3.5	39.0
O ₂ uptake, µl./45 min.					

(1) *Protein*. By fractional precipitation of the extract it was found that the precipitate obtained by the addition of ammonium sulphate up to 40 % saturation contained very little activity. This fraction was not completely soluble when re-suspended in water and dialysed. It contained a very active lactic dehydrogenase. The precipitate obtained between 40 and 62 % saturation with ammonium sulphate contained most of the pyruvic enzyme. This fraction re-dissolved in glass-distilled water to give a greenish yellow solution which became slightly opalescent after dialysis. This fraction also contained some of the lactic dehydrogenase. This separation was not always complete with every preparation. However, it was observed that while the 40–62 % fraction was always more active, its activity could be greatly increased by the addition of some of the 0–40 % fraction. The combined activity was considerably more than the activity of the two fractions separately (Table 3). The factor present in the 0–40 % fraction responsible for this phenomenon was non-dialysable and heat-labile. In one instance both fractions were inactive before mixing.

As it seems likely that this system is similar to that of *Lactobac. delbrückii*, the effect may be due to the partial separation of two enzymes. The reaction, as Lipmann pictures it, involves two coenzymes or prosthetic groups which have so far been considered as owing their biological activity to their union with specific proteins. In the case of the pyruvic dehydrogenase, the question arises whether coccarboxylase and flavin adenine dinucleotide have a specific protein

Table 3. *Activities of the fractions*

	Additions to manometers (ml.)						
	0.5	0	0.5	0.5	0.5	0.5	0.5
Fraction 0-40 %	—	1.0	1.0	1.0	1.0	1.0	1.0
Fraction 40-62 %	1.0	1.0	1.0	1.0	1.0	—	1.0
<i>M</i> /20 phosphate pH 6.17	0.2	0.2	0.2	0.2	0.2	0.2	—
<i>M</i> pyruvate	0.2	0.2	0.2	0.2	—	0.2	0.2
Coccarboxylase 100 μ g./ml.	0.3	0.3	0.3	—	0.3	0.3	0.3
Manganese 100 μ g./ml.	1.0	0.5	—	0.3	0.2	1.0	0.2
Water	14.5	172.0	353.0	52.5	9.0	370.0	8.0
O ₂ uptake, μ l./40 min.							

each, or one protein for the two groups. If there are two, the accelerating effect obtained after mixing the two fractions may be due to the fact that one of the proteins is almost completely absent from one of the fractions.

(2) *Inorganic phosphate*. The necessity for this component of the system could be demonstrated for certain only with enzyme extract after dialysis and before precipitation with ammonium sulphate (Table 1). Ammonium sulphate of B.D.H. A.R. standard was used throughout. Attempts to obtain preparations showing no oxidation unless phosphate was added were unsuccessful. Extensive dialysis inactivates the enzyme; so also does heating to 55-60°, which was a useful method for removing inactive protein from bacterial extracts containing soluble dehydrogenases [Still, 1940, 1].

Arsenate was found to replace phosphate in facilitating the oxidation of pyruvate by the dialysed extract (Table 4). This recalls the fact that arsenate will replace the phosphate required for the oxidation of 3-phosphoglyceraldehyde to 3-phosphoglyceric acid [Still, 1940, 2].

Table 4. *Effect of arsenate and phosphate*

Manometers contained 1.0 ml. dialysed extract, 20 μ g. coccarboxylase, 30 μ g. Mn and 0.2 ml. *M* pyruvate. Total volume 3.2 ml.

	O ₂ uptake μ l./60 min.
No phosphate	26
0.4 ml. <i>M</i> /5 phosphate	121
0.4 ml. <i>M</i> /5 arsenate	107

Lipmann has shown that when pyruvate is oxidized by dried cells of *Lactobac. delbrückii* in the presence of adenylic acid, there is a simultaneous phosphorylation of the adenylic acid. Depending largely upon the amount of adenylypyrophosphatase present in the cells and its degree of inhibition by NaF, the accumulation of adenylypyrophosphate could be demonstrated in the reaction mixture [Lipmann, 1939, 1].

Using the dialysed extract of crushed cells of *Bact. coli* no conclusive evidence could be adduced for the accumulation of adenylypyrophosphate, even in presence of *M*/32 NaF (Table 5). It was found in these experiments that the incubation of the extract with adenylic acid caused a considerable increase in inorganic phosphate whether or not pyruvate had been oxidized at the same time. In the absence of adenylic acid, incubation of the extract with or without pyruvate produced only a small increase in inorganic phosphate. *Bact. coli* rapidly dephosphorylates and then deaminates adenylic acid [Lutwak-Mann, 1936]. Both NaF and adenylic acid caused some inhibition of the rate of oxidation of pyruvate.

After precipitation with ammonium sulphate, the O₂ uptake of the fraction precipitated between 40 and 62 % saturation was considerably increased by the

Table 5. *Oxidation and phosphorylation by dialysed extract*

	Additions to manometers (ml.)			
	1.0	1.0	1.0	1.0
Extract	1.0	1.0	1.0	1.0
<i>M</i> /5 phosphate pH 6.17	0.3	0.3	0.3	0.3
Coccarboxylase 100 µg./ml.	0.4	0.4	0.4	0.4
Manganese 100 µg./ml.	0.3	0.3	0.3	0.3
<i>M</i> pyruvate	—	0.4	0.4	—
<i>M</i> NaF	0.1	0.1	0.1	0.1
0.25 <i>M</i> adenylic acid	—	—	0.3	0.3
Water	0.7	0.3	—	0.4
O ₂ uptake, µl./90 min.	15.0	563.0	426.0	27.5
Initial				
Inorganic P, mg.	1.81	2.02	1.90	3.02
Hydrolysable P, mg.	0.05	0.01	0	0.06

Phosphate removed by 7 minutes' boiling with *N* HCl was considered as having been derived from adenylypyrophosphate.

addition of inorganic phosphate (Table 6). Using this fraction it was possible to show the formation of adenylypyrophosphate during the oxidation of pyruvate (Table 7). Presumably the adenylypyrophosphatase was largely removed or inactivated during fractionation with ammonium sulphate. The oxidation of pyruvate by this fraction was not inhibited by NaF (Table 6).

Table 6. *Components of the active fraction*

	Additions to manometers (ml.)					
	1.0	1.0	1.0	1.0	1.0	1.0
Fraction 40–62 %	1.0	1.0	1.0	1.0	1.0	1.0
<i>M</i> /5 phosphate pH 6.17	0.5	—	0.5	0.5	0.5	0.5
Coccarboxylase 100 µg./ml.	0.4	0.4	0.4	—	0.4	0.4
Manganese 100 µg./ml.	0.3	0.3	0.3	0.3	—	0.3
<i>M</i> NaF	0.1	0.1	0.1	0.1	0.1	—
0.8 <i>M</i> pyruvate	0.5	0.5	—	0.5	0.5	0.5
Water	0.4	0.9	0.9	0.8	0.7	0.5
O ₂ uptake, µl./60 min.	145.0	37.0	0	11.0	16.0	156.0

Table 7. *Oxidation and phosphorylation by active fraction*

	Additions to manometers (ml.)			
	1.0	1.0	1.0	1.0
Fraction 40–62 %	1.0	1.0	1.0	1.0
<i>M</i> /5 phosphate pH 6.17	0.3	0.3	0.3	—
Coccarboxylase 100 µg./ml.	0.4	0.4	0.4	0.4
Manganese 100 µg./ml.	0.3	0.3	0.3	0.3
<i>M</i> NaF	0.1	0.1	0.1	0.1
0.8 <i>M</i> pyruvate	0.5	0.5	—	0.5
0.25 <i>M</i> adenylic acid	—	0.6	0.6	—
Water	0.6	—	0.5	0.9
O ₂ uptake, µl./160 min.	340.0	489.0	0	87.5
Initial				
Inorganic P, mg.	2.08	2.59	2.75	0.34
Hydrolysable P, mg.	0	0.14	0	0

(3) *Coccarboxylase*. Table 8 shows the response of the dialysed extract to the addition of coccarboxylase. No special precautions were taken to incubate the enzyme and coenzyme together before the reaction for any time longer than the equilibrium period of the manometers.

Tables 1, 2 and 3 show that most of the coccarboxylase can be removed by dialysis and practically all of it by precipitation of the enzyme with ammonium

Table 8. *Effect of cocarboxylase on oxidation of pyruvate*

Manometers contained 1.0 ml. dialysed extract, 1.6 ml. *M*/100 phosphate pH 6.17, and 0.2 ml. *M* pyruvate. Total volume 3.2 ml.

Cocarboxylase $\mu\text{g.}/3.2 \text{ ml.}$	$\mu\text{l. O}_2/41 \text{ min.}$
—	10
2.5	86
5.0	143
7.5	171
10.0	181
15.0	184
20.0	191

sulphate and subsequent dialysis. The fact that reactivation of the enzyme by recombination with cocarboxylase takes place after dialysis indicates a point of difference between this enzyme and carboxylase. Green *et al.* [1940] have shown that once cocarboxylase has been dialysed from carboxylase, subsequent addition of cocarboxylase does not restore the lost activity.

(4) *Metal component.* Addition of Mn, Mg or Co to the dialysed extract increased the rate of oxidation of pyruvate in the presence of cocarboxylase. The increase was more marked if the enzyme had been precipitated with ammonium sulphate and dialysed, or incubated for some time and dialysed again (Table 9). Precipitation with ammonium sulphate at an acid reaction was found to be necessary in order to remove the metal component from the pyruvic dehydrogenase system of *Lactobac. delbrückii* [Lipmann, 1939, 3]. Tables 1, 2, 3 and 6 show the partial removal of this component when the extracts are precipitated.

Table 9. *Effect of metals*

The dialysed extract was incubated at 37° for 45 min., diluted once and dialysed once again for 2 hr. against distilled water. Manometers contained 1.0 ml. enzyme, 0.5 ml. *M*/5 phosphate, 0.2 ml. *M* pyruvate and 20 $\mu\text{g.}$ cocarboxylase. Total volume 3.2 ml.

	$\mu\text{l. O}_2/30 \text{ min.}$
No addition	0
Manganese, 30 $\mu\text{g.}$	64.5
Magnesium, 30 $\mu\text{g.}$	60.0
Cobalt, 30 $\mu\text{g.}$	15.0

(5) *Flavin component.* Addition of flavin adenine dinucleotide (F-A-D) to the dialysed extract increased the rate of oxidation of pyruvate by about 30 %. In view of the participation of F-A-D in the oxidation of pyruvate by extracts of *Lactobac. delbrückii* [Lipmann, 1939, 2], attempts were made to split reversibly the enzyme from *Bact. coli*. The dialysed extract was half saturated with ammonium sulphate at pH 3.0 and at 0°. Little of the resulting precipitate was soluble when re-suspended in water. The suspension, when tested under the standard conditions, did not oxidize pyruvate in the presence or absence of F-A-D. While the above procedure was followed by Lipmann [1939, 2] for phosphate extracts of *Lactobac. delbrückii*, less drastic treatment would seem to be more suitable for the extracts of *Bact. coli*.

V. Reaction with molecular oxygen

pH. The optimum pH for the oxidation of pyruvate by the dialysed extract is about 6.2. Fig. 1 shows the relation between pH and the rate of O₂ uptake in *M*/8.9 phosphate. Actually the O₂ uptake is quite appreciable in the absence

of added buffer. Substitution of citrate or acetate buffer markedly inhibits the reaction. This effect of citrate may be due to its combination with the metal component.

Fig. 1. Effect of pH on oxidation of pyruvate. Warburg manometers contained 1.0 ml. dialysed extract, 0.2 ml. cocarboxylase 100 μ g./ml., 0.2 ml. *M* pyruvate, 1.8 ml. *M*/5 phosphate buffer. Total volume 3.2 ml.

Substrate. A considerable concentration of pyruvate is required to saturate the enzyme (Table 10), and it was found that pyruvate remained in the reaction mixture even after 3–4 hr. incubation.

Table 10. *Effect of concentration of pyruvate*

Manometers contained 1.0 ml. dialysed extract, 1.6 ml. *M*/15 phosphate pH 6.17, 20 μ g. cocarboxylase. Total volume 3.2 ml.

Concentration of pyruvate m-Mol./l.	μ l. O ₂ /25 min.
—	0
3.0	24.5
6.0	37.5
12.0	62.5
25.0	85.0
62.0	122.5
125.0	119.5

Phenyl pyruvate, at a concentration of *M*/80, was not oxidized under the same conditions.

Oxygen equivalent. The O₂ uptake per molecule of pyruvate oxidized is the equivalent of one atom of oxygen per molecule of pyruvate (Fig. 2). This result corresponds to the reaction (1):

Fig. 3 shows the O₂ uptake and CO₂ output during the oxidation of pyruvate, measured simultaneously by the indirect method of Warburg. As the residual pyruvate was not determined in this instance, the percentage of theoretical O₂ uptake and CO₂ output was greater than is indicated in the figure. It will be seen that the average R.Q. over 2 hr. was 0.45 against 0.50 which would be

required by (1). Fig. 3 also shows the anaerobic CO₂ output over the same period. This did not reach one-quarter of the aerobic CO₂ output, which fact would seem to eliminate the possibility that pyruvate is not oxidized directly but is first dismutated according to (2):

and that the subsequent O₂ uptake is due to direct oxidation of lactate.

Fig. 2.

Fig. 3.

Fig. 2. Oxidation of pyruvate (A) and acetaldehyde (B). A, Warburg manometers contained 1.5 ml. enzyme (40–62% fraction), 0.7 ml. *M*/5 phosphate buffer pH 6.17, 0.4 ml. cocarboxylase 100 μg./ml., 0.3 ml. Mn 100 μg./ml., 0.3 ml. 0.07 *M* pyruvate. Total volume 3.2 ml. Blank without substrate subtracted. B, as above but containing 0.3 ml. *M* acetaldehyde in place of pyruvate.

Fig. 3. Oxidation and dismutation (C) of pyruvate. Warburg manometers contained 1.7 ml. enzyme (40–62% fraction), 0.6 ml. *M*/5 phosphate buffer pH 6.17, 0.4 ml. cocarboxylase 100 μg./ml., 0.3 ml. Mn 100 μg./ml., 0.2 ml. 0.07 *M* pyruvate. Total volume 3.2 ml. A, CO₂ output. B, O₂ uptake. C, CO₂ output. In C, the gas space contained N₂ instead of air.

Krebs [1937, 2], using intact cells of *Bact. coli*, found that under neutral or slightly acid conditions pyruvate was broken down mainly according to (3):

This reaction had been suggested previously by Tikka [1935] to account for the acetic and formic acids produced in *Bact. coli* fermentation. Under more acid conditions and in the presence of 1 atmosphere of CO₂, the process approximated more closely to (2), which Krebs found applicable to the breakdown of pyruvate by cocci and animal tissues. Barron & Lyman [1939] discuss these views in so far as they relate to certain cocci.

VI. Product of oxidation

Acetic acid was detected by its reaction with lanthanum nitrate [Krüger & Tschirch, 1930]. The absence of propionic acid was considered a reasonable assumption under the conditions of the experiments.

Acetic acid was estimated by the method of Friedemann [1938] owing to the presence of residual pyruvate which is to some extent volatile in steam. The following were incubated for 4 hr. in a Krebs vessel, O₂ being bubbled through

the mixture: 12 ml. enzyme solution (40–62 % fraction of the extract), 600 μ g. cocarboxylase, 400 μ g. Mn, 8 ml. *M*/5 phosphate pH 6.17 and 59.8 mg. pyruvic acid brought to pH 6.17, the total volume being 22 ml. After the incubation the mixture was made up to 25 ml. and the residual pyruvate and the acetic acid formed were estimated. Two distillations were made; one, a steam distillation in the presence of tungstate and MgSO_4 and then a redistillation in the presence of tungstate, MgSO_4 and HgSO_4 . This procedure was followed in order to exclude lactic, pyruvic or formic acid from the final titration. A satisfactory recovery of the acid was obtained; thus, 24.94 mg. pyruvic acid disappeared and 17.38 mg. acetic acid was recovered. The amount calculated from reaction (1) would be 17.0 mg.

If similar experiments were carried out and the reaction mixture was deproteinized by heating with dilute H_2SO_4 , residual pyruvic acid could be demonstrated as the 2:4-dinitrophenylhydrazone. In addition the mixture contained a substance giving a 2:4-dinitrophenylhydrazine derivative insoluble in NaHCO_3 , and presumably not acidic. This question has not been further investigated, but the matter recalls the observations of Quastel & Wooldridge [1929] on the products of fermentation of pyruvate by *Bact. coli*.

VII. Oxidation of acetaldehyde

The extract oxidized acetaldehyde under the same conditions as it oxidized pyruvate; i.e. without the addition of coenzyme I or methylene blue. However, as will be seen in Fig. 2, the rate of oxidation was small. This does not support the view that acetaldehyde as such is an intermediate in the oxidation of pyruvate to acetate. During the oxidation of pyruvate it was not possible to detect the presence of acetaldehyde using Rimini's reaction [Bernhauer, 1936]. Acetate is not oxidized by the preparation. The ability to oxidize acetaldehyde disappears after heating to 55° [Still, 1940, 1].

VIII. Anaerobic breakdown of pyruvate

Anaerobically, the intact cells liberated CO_2 from pyruvate, oxaloacetate and acetoacetate, but not from mesoxalate or α -ketoglutarate. These experiments were carried out in N_2 and phosphate buffer at pH 6.0. In NaHCO_3 - CO_2 buffer pyruvate was also decomposed, the optimum pH being about 7.2. The anaerobic reaction has not been sufficiently studied to justify any statement as to the relative importance of reactions (2) and (3) under these conditions.

Fresh undialysed extract of the crushed cells decomposed pyruvate in NaHCO_3 - CO_2 buffer at pH 7.2 at a rate which was increased by the addition of cocarboxylase. The same extract also liberated CO_2 from pyruvate in N_2 and phosphate at pH 6.0, and the latter reaction could be increased by addition of cocarboxylase (Table 11). Adenylic acid and coenzyme I were without effect.

Table 11. *Effect of cocarboxylase on anaerobic decomposition*

Manometers contained 1.0 ml. undialysed extract, 0.2 ml. *M* pyruvate, 1.5 ml. *M*/15 phosphate pH 6.0. Total volume 3.2 ml. N_2 in the gas space.

	μ l. CO_2 /60 min.
Without pyruvate	4.5
With pyruvate	60.0
With pyruvate + 20 μ g. cocarboxylase	89.0

This anaerobic reaction under acid conditions was not marked, and after dialysis, or treatment with ammonium sulphate and subsequent dialysis, it was

considerably diminished. Activity was not restored by the addition of riboflavin, which has been found to be essential for the dismutation of pyruvate by air-dried cells or phosphate extracts of *Lactobac. delbrückii* anaerobically under acid conditions [Lipmann, 1939, 3]. This result suggests that the reaction occurring in the extract of *Bact. coli* is probably not dismutation by reaction (2). However, the absence of a dismutation mechanism in cells grown and maintained aerobically, may be quite normal.

IX. Oxidation of lactate

In view of the probability that dismutation of pyruvate is brought about by the system, pyruvic dehydrogenase—carrier—lactic dehydrogenase, the occurrence of a very active lactic dehydrogenase in the cell-free extracts of *Bact. coli* is significant. The enzyme used in the experiments of Table 12 was the fraction of the extract precipitated by 40% saturation with ammonium sulphate and then dialysed. The results in Table 12 agree with those obtained by Stephenson [1928] working with a soluble lactic dehydrogenase prepared from autolysed cells of *Bact. coli*. The enzyme does not require coenzyme I, is not inhibited by the product of the oxidation and requires a carrier in order to react with molecular O_2 . This enzyme seems to be identical with the lactic dehydrogenase of yeast recently obtained in a very pure state by Dixon & Zervas [1939].

Table 12. Oxidation of lactate

	Additions to manometers (ml.)				
	0.5	0.5	0.5	0.5	0.5
Enzyme, 0-40% fraction	1.5	1.5	1.5	1.5	1.5
M/10 phosphate pH 7.3	0.1	—	0.1	0.1	0.1
2M lactate	0.1	0.1	—	0.1	0.1
Coenzyme I (27% pure) 10 mg./ml.	0.5	0.5	0.5	—	0.5
Methylene blue 0.5%	0.2	0.2	0.2	0.2	—
2M NaCN	0.3	0.4	0.4	0.8	0.5
Water					
O_2 uptake, μ l./21 min.	222.0	25.0	238.0	19.0	250.0

DISCUSSION

From the results of this investigation it would seem probable that *Bact. coli* contains a pyruvic oxidizing system very similar to the pyruvic dehydrogenase of *Lactobac. delbrückii*. Until the enzyme is reversibly split or other evidence for the participation of flavin adenine dinucleotide in the oxidation is obtained, complete identity remains in doubt. Under anaerobic conditions, the 'hydroclastic' reaction (3) is probably much more important than dismutation (2), but this has not been fully investigated as yet.

No attempts have been made to isolate phosphorylated compounds intermediate between pyruvic acid and acetic acid. The removal of phosphorylated substances already in the enzyme preparation presents some difficulties. In this connexion it is interesting that Silverman & Werkman [1940] recently found that the conversion of pyruvic acid into acetyl methyl carbinol by extracts of *Aerobacter aerogenes* also required the presence of inorganic phosphate. Virtanen & Karström [1931] first observed that inorganic phosphate was required for the oxidation of pyruvic acid by bacteria.

SUMMARY

1. Cell-free extracts of *Bact. coli* do not contain carboxylase, but oxidize pyruvic acid to acetic acid and CO₂.
2. This pyruvic dehydrogenase system has been shown to comprise at least (a) a protein or proteins, (b) inorganic phosphate, (c) cocarboxylase and (d) a metal, possibly manganese or magnesium. Flavin adenine dinucleotide may also be a component of the system.
3. The oxidation of pyruvic acid in the presence of adenylic acid is probably coupled with the phosphorylation of the latter substance.
4. The extracts also decompose pyruvic acid under anaerobic conditions.
5. Acetaldehyde is oxidized only slowly, and could not be detected during the oxidation of pyruvic acid.
6. The extracts oxidize lactate rapidly in the presence of a carrier.

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