CLXVIII. PYRUVIC ACID IN BACTERIAL METABOLISM.

WITH AN ACCOUNT OF THE METHODS USED FOR THE DETECTION AND DETERMINATION OF PYRUVIC ACID.

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QUASTEL [1925] showed that pyruvic acid was produced from a variety of substances by the action of *B. pyocyaneus*, and was apparently directly connected with the growth process of the bacteria. Previous work by Beijerinck and Folpmers [1916] had shown that this acid is formed from malic and fumaric acids under the influence of *B. fluorescens* and *B. pyocyaneus*. These experiments were carried out with cultures of growing bacteria, and it was thought of interest to study the production of pyruvic acid from substrates in the presence of a relatively large amount of already grown bacteria.

As this substance is very easily metabolised by bacteria, methods were adopted to prevent, if possible, its further breakdown. Quastel and Wooldridge [1927, 1, 2] had noted that if the suspensions of resting bacteria were treated with various reagents certain mechanisms could be entirely eliminated. The effect of nitrite on the dehydrogenation of lactic acid in the presence of nitrate had been noted earlier by Quastel, Stephenson and Whetham [1925]. With *M/20* nitrite pyruvic acid was formed in relatively large amounts. In the experiments to be mentioned the substrates, which were nearly all hydrogen donors, were oxidised in the presence of atmospheric oxygen. The oxygen used for the process can be very easily measured in the Barcroft differential manometer. The experiments are also of some interest as an attempt to study the effect of oxygen on fermentative processes. Use was made of the capacity of pyruvic acid to form an addition compound with sodium hydrogen sulphite. By the use of this compound very definite differences were noted in the metabolism of pyruvic acid by yeast and by *B. coli communis*.

The detection and determination of pyruvic acid. Much time was spent in finding a suitable specific method for the detection and estimation of this compound. It is felt that owing to its undoubted importance in carbohydrate metabolism, reference might be made to the methods which have been indicated. For the qualitative detection of pyruvic acid the most sensitive and specific test is that of Posternak [1927] in which the solution is treated with

an equal volume of concentrated hydrochloric acid and a few crystals of phloroglucinol; a positive reaction (red colour) is obtained with 1 in 5000 pyruvic acid and the test is thus superior to those described by Quastel [1924].

Of the methods which have been proposed for the quantitative determination of pyruvic acid [MacLean, 1913; Kayser, 1924; Simon and Piaux, 1924; Wieland, 1924; Krishna and Sreenivasaya, 1928], that of Wieland is, according to the writer's experience, the only one of use for small quantities. Better results can be obtained by utilising the capacity which pyruvic acid shares with many other compounds of forming addition compounds with sodium bisulphite. This method of bisulphite fixation has been applied by Friedemann, Cotonio and Shaffer [1927] to the determination of acetaldehyde from the oxidation of lactic acid in the quantitative determination of the latter. The method used here is essentially the same. Certain difficulties arise in the presence of other compounds which combine with bisulphite, but these may be surmounted. The method may be used to estimate acetaldehyde and pyruvic acid in a single sample.

EXPERIMENTAL.

If bisulphite has been used in the experiments it must be eliminated before the determination of pyruvic acid can be carried out, except for the methods depending on the nitroprusside reaction and the bisulphite fixation method. The bisulphite may be easily removed by hydrolysis with a small quantity of acid and boiling, or it may be titrated with iodine. The results obtained using Wieland's method and the bisulphite-binding capacity method will be given in some little detail.

Wieland's method.

The reagents consist of normal alkali, standard iodine and thiosulphate. Protein must be removed from the material to be analysed, as it interferes with the reaction; methylglyoxal, acetone, sugars, etc. must also be removed from the solution. To a convenient amount of the solution are added 5 cc. of normal alkali (more alkali if the solution was previously acid) and then a known volume of standard iodine solution. The amount of iodine added must be considerably in excess of that required for the transformation of the pyruvic acid into iodoform. The mixture is then left at room temperature for not more than 2 hours. Acid is added in excess and the iodine set free is titrated with standard thiosulphate.

1 cc. $N/10$ iodine combined = 1.46 mg. pyruvic acid.

To make the method more sensitive the iodine may be diluted to a suitable concentration. Concentrations of iodine below $N/100$ are however not satisfactory. Table I gives the results of an actual experiment.

As a test of the method four 2 cc. samples of the pyruvic acid solution were treated with (A) 8 cc. of buffer, (B) 2 cc. of bacterial suspension and 6 cc. buffer, (C) 2 cc. of $M/10$ NaHSO_3 and 6 cc. buffer, (D) 2 cc. bacterial suspension, 2 cc. $M/10$ NaHSO_3 , and 4 cc. buffer; the bacteria were precipitated in

Table I.

Pyruvic acid taken mg.	cc. <i>N</i> /10 I bound	cc. <i>N</i> /50 I bound	cc. <i>N</i> /100 I bound	Pyruvic acid found mg.
17.2	1.18	—	—	17.0
17.2	1.18	—	—	17.0
1.72	—	5.85	—	1.72
1.72	—	5.90	—	1.71
0.69	—	2.40	—	0.70
0.69	—	2.40	—	0.70
0.345	—	1.2	—	0.35
0.345	—	—	2.3	0.34
0.172	—	—	1.2	0.175

B and D. The sulphite in C was hydrolysed with acid. In D it was titrated with iodine. The results obtained are shown in Table II.

Table II.

Mixture	cc. of <i>N</i> /10 I bound	Pyruvic acid (mg.)
A	1.18	17.2
	1.18	17.2
B	1.18	17.2
	1.18	17.2
C	1.13	16.5
	1.18	17.2
D	1.18	17.2
	1.13	16.5

Pyruvic acid taken, 17.2 mg.

Estimation by bisulphite-binding capacity.

The method consists in titrating the excess sodium hydrogen sulphite with iodine and liberating the bound sulphite with sodium carbonate. The liberated sulphite is then titrated with standard iodine solution, 1 cc. of *N*/10 iodine being equivalent to 4.4 mg. pyruvic acid. If acetaldehyde or other bisulphite-binding substance is present the total bound sulphite is estimated. Acetaldehyde or any substance of a volatile nature may be distilled off after hydrolysis with acid and collected in bisulphite solution. The acetaldehyde may then be estimated in the distillate. The pyruvic acid in the original solution may be determined by difference and the result can be checked by neutralising the original solution (very carefully), adding fresh bisulphite solution and again titrating the bound sulphite. Pyruvic acid even in small amounts is quite stable in acid solution. For this method the use of ammonia-free water in the manipulations is absolutely necessary. Table III gives some results obtained; quantities as low as 0.2 mg. of pyruvic acid may be determined.

Table III.

Pyruvic acid taken mg.	cc. iodine for bound sulphite		Pyruvic acid found mg.
	<i>N</i> /10 I	<i>N</i> /100 I	
33	7.50	—	33
6.6	—	15	6.6

The identification of pyruvic acid.

Pyruvic acid is best identified as the *p*-nitrophenylhydrazone. Quastel [1924] and Klein and Fuchs [1929] give an interesting account of other derivatives of pyruvic acid that may be prepared and serve as a further means of identification of this substance. The use of these substances as fixatives to demonstrate the transitory formation of pyruvic acid in fermentation is also discussed in this paper.

The production of pyruvic acid by B. coli in aerobic oxidations.

The bacterial suspension used throughout these experiments was obtained from a strain of *B. coli communis* and was prepared as described by Cook and Stephenson [1928]. All these experiments were conducted at p_H 6.0. The CO_2 output was determined as described in another paper [Cook, 1930]. It is of some interest to compare the degree of oxidation obtained by methods in which the use of inhibitors and fixatives has been involved. Table IV gives the oxygen uptakes and CO_2 outputs obtained with the organism in the presence of 1 cc. of *M*/25 sodium bisulphite at p_H 6.0 and with the organism after being treated with *M*/25 sodium nitrite and then washed free of the solution as described by Quastel and Wooldridge [1927, 1]. This table should also be compared with a similar table given by Cook [1930] where values for the untreated organism and the organism treated with toluene are given.

Table IV.

1 cc. of <i>M</i> /100	Bacteria with 1 cc. <i>M</i> /25 $NaHSO_3$		Bacteria treated with $NaNO_2$	
	O_2	CO_2	O_2	CO_2
Glucose	1.09*	—	0.67*	—
Lactic acid	0.33*	0.37*	0.36*	—
Pyruvic acid	0	?	0	—
Formic acid	0.51	—	—	—
Acetic acid	0	0	0	—
Succinic acid	0.72*	0.54*	—	—
Fumaric acid	0.22*	—	—	—

The figures represent centimols of gas respired. These values have been calculated from the mm.³ of dry gas at N.T.P. respired for 1 cc. of *M*/100 solution of substrate and are the mean of several experiments. The sign 0 represents no appreciable oxidation, while blank spaces represent experiments not as yet done or which are variable or uncertain and await confirmation.

An asterisk denotes that pyruvic acid is formed.

As has been pointed out in another paper by the writer [Cook, 1930], while the amount of oxygen taken up by pyruvate in the presence of the untreated organism corresponds to one atom of oxygen less than that used in the oxidation of lactate, the amount of CO_2 is approximately the same. This would perhaps indicate that all the lactate passes through pyruvate, the latter then being oxidised. It must be noted however that when the toluene-treated organism or the organism treated with sodium nitrite or in the presence of sodium hydrogen sulphite is used, the amount of oxygen taken up does not correspond to the theoretical amount according to the equation

The amount of oxygen for such a reaction should be theoretically 0.5 centimols in the above experiments, but, as will be seen, it is 0.38 centimols for the toluene-treated organism and 0.36 and 0.33 for the nitrite-treated organism and organism in the presence of sulphite respectively. Also some CO_2 is given off in the case of the organism in the presence of sulphite. The possibility of secondary reactions is at present under consideration.

The fermentation of glucose by B. coli.

It is interesting to consider how far the results obtained in the aerobic oxidation of glucose by *B. coli* under conditions where no growth is to be observed may be applied to the process of fermentation where active growth is taking place. *B. coli* will break down glucose under aerobic conditions in a fermentation experiment to lactic acid, etc. These substances are known to be oxidised by oxygen in the presence of *B. coli* from the experiments of Cook and Stephenson [1928]. It seems certain that the production of these substances and their subsequent removal is a question of the relative velocities concerned, the acid products accumulating too rapidly under the conditions of the fermentation experiments to be removed subsequently by the slower process of oxidation. Care must therefore be taken in interpreting these experiments, as the conditions of the experiment were conducive to the maximum amount of oxidation. Low concentrations of substrates were used with good aeration and efficient means of removing the CO_2 as fast as it formed, all tending towards this result.

Harden [1901] was the first to isolate the products of decomposition of glucose by *B. coli communis*. He suggested that the following formula represented the reaction:

Grey and Young [1921] investigated the fermentation under aerobic and anaerobic conditions and with bacteria that had been grown anaerobically and aerobically. They found that, where the immediate past history of the organism had been anaerobic, under anaerobic conditions little or no lactic acid was produced, the yield of succinic acid was diminished, but acetic acid was produced in large amounts. The introduction of oxygen during the fermentation increased the yield of acetic, lactic and succinic acids, but decreased the amounts of hydrogen, CO_2 and formic acid, while it had little influence on the yield of alcohol¹.

Aubel [1926] has studied very carefully this same fermentation but in a glucose-inorganic medium under aerobic and anaerobic conditions. These

¹ In this connection it is interesting to note that Cook and Stephenson [1928] have shown that as regards oxidations by molecular oxygen in the presence of *B. coli* the degree of oxidation is much the same whether the organism has been grown under the strictest anaerobic conditions or aerobically.

results are quite in contrast to those of Grey and Young. The presence of pyruvic acid and the absence of succinic acid are to be noted. In Aubel's experiments the introduction of oxygen increases the amount of pyruvic acid, formic acid and CO_2 , while the amounts of alcohol, and of acetic and lactic acids are decreased.

These striking differences may perhaps be interpreted as being due to the totally different conditions under which the various authors performed their experiments. In the experiments of Grey and Young the glucose was inoculated with heavy suspensions of already grown bacteria, while in Aubel's experiments a process of normal growth took place. It is clear that some differences in reaction to the medium may be explained by this fact, as also that other factors enter the process, *e.g.* the strain of bacteria used. Certain strains of *B. coli* produce fermentation products in quite different proportions, and certain strains do not produce succinic acid while other strains produce it in large quantities [Virtanen, 1927]. The results of Grey and Young show that great differences may exist between the yields of any given substance with the same strain of bacteria, according to the conditions. Such considerations must be taken into account, but they do not affect the general argument.

The products of fermentation. That the hydrogen and CO_2 in these experiments is derived from formic acid would seem to be conclusively proved by the experiments of Pakes and Jollyman [1901], Penfold [1911], Harden and Penfold [1912], Grey [1914] and Stickland [1929]. Aubel [1926] has pointed out that the hydrogen may also be regarded as being formed by the decomposition of the glucose to pyruvic acid, the CO_2 being formed by the decarboxylation of the latter. Such a step, as will be shown later, is extremely unlikely in this fermentation.

The presence of acetic acid and alcohol in approximately equimolecular proportions seems to indicate that their origin is in a common substance, which would from analogy with the yeast fermentation be acetaldehyde. The presence of this substance in the fermentation is undoubted. The origin of the acetaldehyde will be considered in some detail later.

Grey and Young point out that the proportion of alcohol to acetic acid has a wider range under anaerobic than aerobic conditions. They attribute this to the oxygen inhibiting the mechanism of autoreduction which is responsible for the production of alcohol. Assuming from analogy with the yeast fermentation that the acetic acid and alcohol are formed from the same parent substance, acetaldehyde, certain interesting relations may be observed. Thus if we calculate the theoretical values of acetic acid which should be formed from the alcohol present we note that though this is formed in considerably larger quantities under aerobic conditions, it is also present in amounts greater than the theoretical yield from the amount of alcohol found under anaerobic conditions. This seems to indicate some other mechanism which also produces acetic acid. It may be argued that the acetic acid is produced by the direct oxidation of acetaldehyde with reduction of another compound. This would decrease

the yield of alcohol, but even under aerobic conditions it remains little altered.

The presence of pyruvic acid in Aubel's experiments may help us to interpret this production of acetic acid. Neuberg [1914] has shown that by the action of mixed putrefactive bacteria on α -keto-acids, formic acid and a fatty acid with one carbon atom less than the original acid are produced. Thus pyruvic acid may be pictured as decomposing thus:

It will be seen that here we have indication of a process quite different from that which occurs in the decomposition of pyruvic acid by yeast. It is to be noted that CO_2 , hydrogen and acetic acid are to be found in the decomposition products of pyruvic acid by *B. coli* [see particularly Karczag and Moczar, 1913].

Aubel's results are clearly in favour of this view. If we suppose that the alcohol and acetic acid are formed from acetaldehyde derived from the pyruvic acid we should expect that the yields of alcohol and acetic acid would be much higher under aerobic conditions where the production of pyruvic acid is at its maximum. We find however that while both these are reduced in amount, the quantity of acetic acid produced is still in excess of that which should be formed from the amount of alcohol present. The formic acid however is produced in much greater amount than would correspond to the acetic acid produced; an extra origin for the formic acid is thus indicated.

The formation of pyruvic acid. There is no doubt that pyruvic acid is formed from glucose in bacterial fermentations. It may however be formed indirectly from other substances resulting from the primary decomposition of the glucose [Beijerinck and Folpmers, 1916; Quastel, 1925]. These results have been confirmed for *B. coli* (see Table IV). In the fermentation of glucose by *B. coli*, lactic acid is produced in large quantities. Can we then regard the pyruvic acid as being formed by the oxidation of the lactic acid? The oxidation of lactic acid to pyruvic acid has been effected by numerous species of bacteria [Mazé and Ruot, 1916; Mazé, 1918].

It has been shown that this transformation may easily be brought about by *B. coli* in the presence of a suitable hydrogen acceptor such as oxygen, and where the reaction is arrested at the pyruvic acid stage by means of a fixative for the pyruvic acid, or under conditions where the cell processes are so affected that further metabolism of the pyruvic acid is stopped.

Mixtures were made up as shown in Table V. The solutions were aerated

Table V.

	M/10 lactate	M/5 NaHSO ₃	Bacterial suspension	Buffer pH 6
1	50 cc.	50 cc.	50 cc.	350 cc.
2	20	20	20	75
3	5	5	5	10
4	2	2	2	4

in a current of air for the times indicated. The pyruvic acid was then estimated. Table VI gives the results.

Table VI.

Experiment	Time (hours)	mg. pyruvic acid per 100 cc. solution
1	8	28.52
2	7½	31.00
3 (a)	8	28.00
(b)	48	27.00
4 (a)	8	(21.00)
(b)	8	27.20

88 mg. of pyruvic acid correspond to complete oxidation of lactic acid to pyruvic acid.

Sodium pyruvate was then set up and aerated in the presence of sodium bisulphite with *B. coli*. Table VII indicates the results obtained.

Table VII.

Experiment	Time (hours)	mg. pyruvic acid per 100 cc. solution
1 (a)	8	37.4
(b)	48	28.2
2 (a)	6	58.0
(b)	7½	44.5
(c)	23	33.5
(d)	48	22.5

Aubel [1926] has suggested that the breakdown of glucose by *B. coli* consists of two processes—(1) the production of lactic acid, (2) the production of pyruvic acid with the liberation of hydrogen. That pyruvic acid is produced by the decomposition of glucose in the presence of a fixative for the pyruvic acid and under conditions of free oxygen access is shown by the following experiments. *M*/100 glucose when oxidised in the presence of sodium bisulphite takes up an amount of oxygen corresponding to two atoms of oxygen per mol. of glucose, corresponding to the equation

(Table IV). This would correspond to Aubel's process for the production of pyruvic acid. It is noteworthy that in this case the reaction is set only in the one channel, no lactic acid being formed.

As regards the hydrogen production, it is curious that no hydrogen collects in a Durham tube when glucose is fermented with *B. coli* in the presence of sodium bisulphite although pyruvic acid is produced in large amounts. This interesting problem is also under further investigation.

In the case of oxidations by *B. coli* in the presence of sodium bisulphite the pyruvic acid is fixed as the bisulphite compound, the reaction being arrested there.

Although secondary reactions may well be taking place, as evidenced by the amounts of pyruvic acid which are formed by the oxidation of lactic acid being below the theoretical value (see Tables V and VI), very little acetaldehyde is formed. Neuberg and Reinfurth [1920, 2] have shown that in the case

of yeast the pyruvate-bisulphite compound is decomposed by carboxylase to acetaldehyde and CO_2 .

The action of zymin on pyruvate in the presence of bisulphite was investigated. Fig. 1 represents the CO_2 output from 1 cc. of $M/50$ pyruvate in the presence of 1 cc. $M/20$ sodium hydrogen sulphite.

It will be seen that the amount of CO_2 evolved corresponds to decarboxylation to acetaldehyde. The contents of the Barcroft cups were withdrawn and the zymin removed by centrifuging. The acetaldehyde was then estimated by the method of Friedemann, Cotonio and Shaffer [1927]. The following results were obtained: iodine corresponding to bound sulphite = 1.98 cc. of $M/50$ iodine (mean of six experiments) = 0.871 mg. acetaldehyde. Theory from 1 cc. $M/50$ pyruvate = 0.880 mg.

Fig. 1. Carbon dioxide output from 1 cc. $M/50$ pyruvate and 1 cc. $M/20$ NaHSO_3 at p_H 6.0 and 40° in the presence of zymin and in the presence of *B. coli*. I corresponds to the theoretical value for decarboxylation of the pyruvate to acetaldehyde.

This inability of *B. coli* to convert the pyruvate-bisulphite compound to acetaldehyde seems to indicate that in fermentations with *B. coli* the acetaldehyde has another origin than pyruvic acid. "Dimedon" [Neuberg and Reinfurth, 1920, 1] was also used as a fixative for acetaldehyde. It was thought that the sulphite might alter the normal course of reaction by combining with the pyruvate. Dimedon does not combine with pyruvic acid. It was found however in these experiments that although the pyruvic acid disappeared the quantities of acetaldehyde present were small in comparison with those found in experiments in which bisulphite was used.

The origin of the acetaldehyde. The presence of acetaldehyde in the decomposition of glucose by *B. coli* is undoubted. Grey [1913] first indicated its presence. Neuberg and Nord [1919] obtained large quantities by fixation with sodium sulphite. Nagai [1923] and de Graaff and Le Fèvre [1925] have also shown its production by fixation methods in the decomposition by bacteria of numerous organic compounds of biological interest. The latter workers

show that acetaldehyde is formed in the decomposition of pyruvic acid by organisms of the coli-typhosus group. They do not appear however to have estimated the acetaldehyde formed and in a subsequent experiment on the fermentation of pyruvic acid they found that it was broken down to acetic acid, hydrogen and CO_2 , in accordance with the following reaction:

This they attribute to the action of carboxylase in the same manner as for yeast. The fact that hydrogen is formed is difficult to explain by this action. It may readily be accounted for as arising from formic acid. Whetham [1927] has collected considerable evidence in favour of the view that pyruvic acid ferments to acetic acid and formic acid, the latter then decomposing into hydrogen and CO_2 . It should be pointed out that the hydrogen so formed may well be able to bring about reduction. The formation of lactic acid in fermenting solutions of pyruvic acid may be explained by this action [Aubel, 1924].

The production of the acetaldehyde in the fermentation of pyruvic acid may be explained in various ways. It is quite possible that sufficient material is present in the cell to form small quantities of acetaldehyde. Neuberg and Kerb [1912] noted that acetaldehyde is formed as a product of autofermentation of yeast. With pyruvic acid and bacteria sufficient pyruvic acid may be synthesised to glucose to break down later to form acetaldehyde. It has been found that a small quantity of acetaldehyde is always formed in the oxidation of lactic acid to pyruvic acid by oxygen in the presence of *B. coli*. This however seems to depend not on the amount of pyruvic acid present, but on the amount of bacteria. Using different concentrations of pyruvate approximately the same quantity of acetaldehyde is always formed. This is shown by the following experiment.

Mixtures were made up as shown in Table VIII. The quantities given represent cc. of material used.

Table VIII.

	M/10 lactate	M/10 pyruvate	M/5 NaHSO_3	Bacterial suspension	Buffer p_{H} 6	Condition of experiment
1	—	—	2	2	6	Aerobic
2	—	—	2	2	6	Anaerobic
3	50	—	50	50	350	Aerobic
4	2	—	2	2	4	"
5	—	2	2	2	4	"
6	—	2	2	2	4	Anaerobic

At the times indicated in Table IX the mixtures were acidified and the acetaldehyde distilled into sodium bisulphite and then estimated by the method of Friedemann, Cotonio and Shaffer [1927].

A more sensitive test by which the presence of acetaldehyde can be shown and in which there is no necessity to fix the acetaldehyde and thereby perhaps alter the normal course of reaction is the detection of acetylmethylcarbinol (acetoin). This is formed, by yeast at least, by the condensation of two molecules

Table IX.

Experiment	Duration of experiment (hours)	mg. acetaldehyde per 100 cc. solution	cc. bacterial suspension per 100 cc. solution	cc. <i>M</i> /100 lactate or pyruvate per 100 cc. solution
1	8	Reaction + + + not estimated	20	20
2	8	0	20	20
3	8	1.80	10	10
4 (a)	8	1.05	20	20
(b)	48	2.40	20	20
5 (a)	8	1.25	20	20
(b)	48	2.00	20	20
6	24	0	40	20

Note. 10 cc. of *M*/100 lactate and pyruvate solutions contain 90 mg. lactic acid and 88 mg. pyruvic acid respectively.

of acetaldehyde under the influence of the enzyme carboligase [Neuberg and Hirsch, 1921]. Acetoin can be detected in very small amounts. It has been found that acetoin may be detected in small amounts in the fermentation of pyruvic acid. Its presence may be accounted for in a similar manner to that indicated before. The presence of acetoin as an indicator of acetaldehyde formation has been noted in the decomposition of the sugars and glycerol by various species of bacteria, but in the cases of lactic and pyruvic acids this compound is found to be present only in small quantities or not at all. We must therefore look for another source for the acetaldehyde formed in the decomposition of glucose by *B. coli*. It may be suggested that this is formed from the first breakdown product of the hexose molecule. This may be possibly methylglyoxal. The action may be represented thus:

Lusk [1925] has indicated that such a reaction may occur in the breakdown of carbohydrate by muscle under aerobic conditions. De Graaff and Le Fèvre [1925] have however shown that no acetaldehyde is formed in the fermentation of methylglyoxal by organisms of the coli-typhosus group in the presence of bisulphite.

The alternative primary decomposition compounds of glucose however, *viz.* dihydroxacetone and glyceraldehyde, yield acetaldehyde and carbon dioxide and hydrogen, as do glycol and glycerol.

A study of the bacterial metabolism of these compounds is at present under investigation.

SUMMARY.

1. The methods of determination of pyruvic acid are discussed.
2. A method of determination depending on the bisulphite-binding capacity of pyruvic acid is described.
3. Pyruvic acid is produced in the aerobic oxidation by *B. coli* of various substrates, if the reaction is checked by the use of fixatives for pyruvic acid.
4. Pyruvic acid (as pyruvate) is not decarboxylated by *B. coli*.
5. Evidence is brought to show that pyruvic acid is broken down by *B. coli* to a mixture of formic and acetic acids.

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