9171

Dissimilation of Pyruvic Acid by *Clostridium butylicum.*

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Clostridium butylicum (Beijerinck) Donker is a member of the butyric acid-butyl alcohol group of bacteria forming large quantities of butyl alcohol; it is characterized by the production of isopropyl alcohol and in this manner is differentiated from *Cl. acetobutylicum,* the organism used in the butyl alcohol-acetone industry. Relatively little work has been done on the metabolism of *Cl. butylicum.* Van der Lek[1] studied the organism and determined the final products.

We have studied the intermediate reactions of cell-suspensions of this species which normally produces butyl and isopropyl alcohols, acetic and butyric acids, carbon dioxide and hydrogen with traces of 2,3-butylene glycol from the fermentation of glucose. Experiments were performed with the idea of determining: (a) the products formed by the action of the cell-suspension on pyruvic acid alone; (b) whether pyruvic acid donates hydrogen for the reduction of butyric acid; (c) whether formic and lactic acids donate hydrogen for the reduction of butyric acid. Pyruvic acid is shown to be an intermediate compound in the breakdown of glucose by *Cl. butylicum;* its behavior becomes of importance in formulating a scheme of dissimilation.

Pyruvic acid was determined by iodoform-titration as given by Wendell,[2] butyl alcohol by the method of Stahly, Osburn and Werkman,[3] butyric and acetic acids according to the method of Osburn, Wood and Werkman,[4] and lactic acid by the Friedmann, Cotonio and Shaffer[5] method. The mercuric oxide method was used for the gravimetric determination of formic acid. Carbon dioxide was absorbed in Bowen potash-bulbs and determined gravimetrically. Hydrogen was collected in bottles by water-displacement and estimated gravimetrically after burning in a combustion-chamber.

Ten liters of broth containing 0.5% yeast-extract, 1% glucose

1 Van der Lek, J. B. (1928), Onderzoekingen over de butylalkoholgisting. Thesis. Delft, Holland.

2 Wendell, W. B., *J. Biol. Chem.*, 1931, **94**, 717.

3 Stahly, G. L., Osburn, O. L., and Werkman, C. H., *Analyst.*, 1934, **59**, 319.

4 Osburn, O. L., Wood, H. G., and Werkman, C. H., *Ind. Eng. Chem. Anal. Ed.*, 1933, **5**, 247.

5 Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

and 0.1% K_2HPO_4 were inoculated with *Cl. butylicum*. After 24 hours' incubation the cells were concentrated to a relatively dry paste by means of the supercentrifuge. Approximately 2 gm. portions of this mass were used in experiments with test substances in the absence of glucose and a source of nitrogen. Under these conditions the bacteria do not multiply but the enzymes are active. The experiments were carried out in 500 cc. Erlenmeyer flasks connected with Bowen potash-bulbs and bottles for collecting the hydrogen. The flasks were made anaërobic by replacing the air with nitrogen, and incubated at 37°C.

Pyruvic acid was isolated and identified from 2% glucose-fermentations to which sodium bicarbonate was added to pH 7.0. When the glucose was completely fermented (72 hr.) the medium was cleared with trichloracetic acid and pyruvic acid precipitated as the 2,4-dinitrophenylhydrazone which melted at 212.5°C. Pyruvic acid was further identified as the *p*-nitrophenylhydrazone. We have been unable to isolate pyruvic acid from fermentations conducted in acid media.

Table I shows the results of 2 experiments: (a) pyruvic acid neu-

TABLE I.
Fermentation of Pyruvic Acid.

	Millimoles			
	a		b	
Product	Added	Recovered	Added	Recovered
Pyruvic acid	27.0	1.0	40.0	0.0
Acetic "	—	10.4	—	20.4
Butyric "	—	6.9	—	7.5
CO_2	—	25.7	—	41.5
H_2	—	5.4	—	11.4
Carbon	81.0	77.1	120.0	112.3

TABLE II.
Pyruvic Acid with Butyric Acid.

	Millimoles			
	a		b	
Product	Added	Recovered	Added	Recovered
Pyruvic acid	50.0	0.0	50.0	0.0
Butyric "	10.0	13.5	10.0	13.8
Acetic "	—	21.9	—	24.6
Butyl alcohol	—	9.1	—	13.0
CO_2	—	50.0	—	49.4
H_2	—	8.2	—	8.8
Carbon	190.0	184.2	190.0	205.8

tralized with $CaCO_3$ and the excess carbonate removed by filtration; (b) pyruvic acid adjusted to pH 6.6 with NaOH.

These data show that pyruvic acid was converted to acetic and butyric acids, carbon dioxide and hydrogen. It is of interest that butyric acid under these conditions of pH above 6.3 is not reduced by *Cl. butylicum* to butyl alcohol. The significance of this critical pH will be discussed in a subsequent communication. However, when the pH is less than 6.3, butyric acid is reduced to the alcohol. Table II shows the results from 2 experiments in which a mixture of pyruvic and butyric acids was adjusted to pH 4.8 with NaOH and K_2HPO_4. Although butyric acid was reduced to butyl alcohol, it is to be noted that the reduction was not complete. In experiments with mixtures of formic and butyric acids as well as with lactic and butyric acids no enzymatic activity was manifested. Practically the same molarity of these compounds added was recovered unaltered. It must be considered, however, that the above experimental conditions are somewhat removed from those which obtain in a normal fermentation.

Unpublished results obtained in our laboratory indicate that isopropyl alcohol as well as traces of acetylmethylcarbinol is produced from a mixture of pyruvic acid and acetone. Moreover, lactic and formic acids are formed from pyruvic acid when the pH is maintained slightly above 7.0 by addition of sodium bicarbonate.

Conclusions. Pyruvic acid is converted by cell-suspensions of *Cl. butylicum* into acetic and butyric acids, carbon dioxide and hydrogen. Hydrogen donated by pyruvic acid is available for the reduction of butyric acid to butyl alcohol at a suitable pH. Formic acid it not decarboxylated and lactic acid is not dehydrogenated.

9172

Effects of Androsterone and Testosterone on Oestrous Cycle of Rats.

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Ihrke and D'Amour[1] reported that injections of bull testis-tissue male hormone concentrates prepared by Gallagher and Koch resulted in the suppression of the oestrous cycle of female rats during

[1] Ihrke, I. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 289.