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THE RELATION OF PYRUVIC ACID
to
CARBOHYDRATE METABOLISM

A Thesis Submitted to the Faculty of the
College of Arts and Sciences
of the
UNIVERSITY OF LOUISVILLE
Through Dr. A. W. Hemberger,
in Partial Fulfillment for the Degree
"Master of Science,"
by
Sylvester Louis Schulte

Louisville, Kentucky.

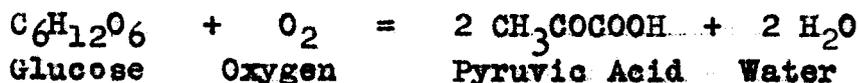
June, 1919.

THE RELATION OF PYRUVIC ACID

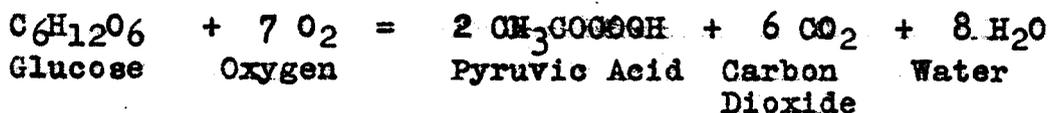
to

CARBOHYDRATE METABOLISM

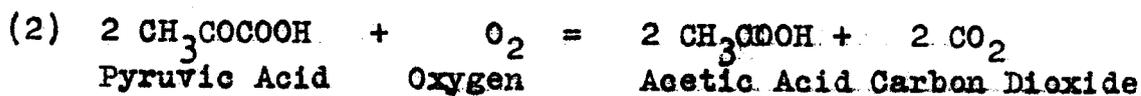
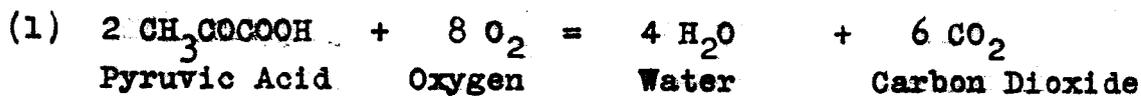
It is known that carbohydrates on oxidation break into fragments which later are oxidised, or reduced, according as the conditions of the bodily functioning may favor, and it is in this process or reaction of the carbohydrate molecule that the formation of pyruvic acid probably takes place¹. From this point the pyruvic acid molecule on being fully oxidised would give the products carbon dioxide and water, and partial oxidation would give carbon dioxide and other products :



or else this reaction might take place:



The pyruvic acid thus formed could be utilized to furnish energy as is shown by the following reactions:



I. Mathews : Physiological Chemistry, (text.) Second Edition P.30.

I. McCollum: Org. Chem. for Students of Med. and Biol. P.339.

The object of the following work was to attempt the proof that pyruvic acid is a product of the decomposition of carbohydrates and that it is an intermediate step in the body to produce energy by being oxidised, wholly or partially, to the compounds carbon dioxide and water.

A vast amount of work has been done on this subject, and on matters in direct relation to this subject:-

Hans Euler^I decomposed pyruvic acid by the action of ultra-violet rays, even in the absence of a catalyzer, into carbon dioxide, ethyl alcohol, and acetic acid.

C. Neuberg and L. Karczag^{II} showed pyruvic acid to be quite easily fermentable by yeast, the products obtained being carbon dioxide and acetaldehyde.

Paul Meyer^{III} states that "even in cases where the excretory power of the kidneys was not noticeably impaired injection of pyruvic acid caused no increased excretion of sugar"

A. I. Ringer^{IV} phlorizinized dogs and these dogs produced glucose from pyruvic acid in the diabetic organism, but sometimes the amounts of glucose were found to be much less than with similar amounts of alanine or lactic acid. Pyruvic acid, he said, is not an intermediary product in the change of

I. Chemical Abstracts, 5, 17, (1911).

II. Ibid. 5, 23, (1911).

III. Ibid. 7, 14. (1913)

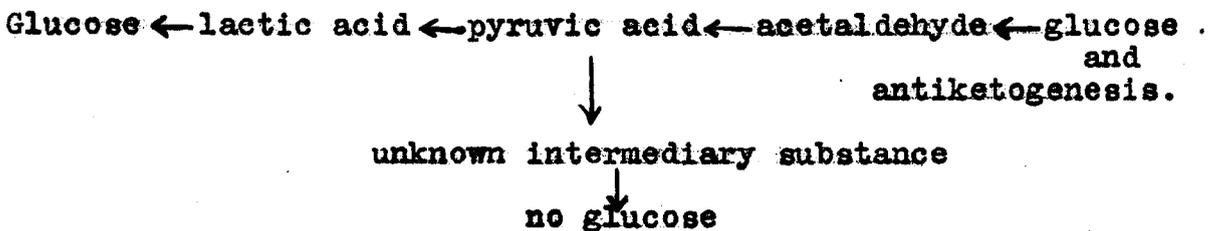
IV. Ibid. 7, 19. (1913)

alanine into lactic acid, and furthermore, alanine is not subjected to oxidative deaminization.

H. C. Dakin and N. W. Janney^I found that sodium pyruvate when given by mouth to phlorizinized dogs may cause an excretion of almost as much "extra glucose" as lactic acid itself, and apparently, glucose cannot be formed directly from pyruvic acid unless the conditions are favorable for the reduction of the acid to lactic acid.

I. L. Karczag and L. Moczar^{II} by the aid of bacteria grown in media containing pyruvic acid, obtained thru fermentation of the acid, fatty acids and gases, hydrogen being identified among the latter.

A. I. Ringer states that there are three paths of metabolism that pyruvic acid might follow:



This latter step may be the reaction of the oxidation of pyruvic acid to form carbon dioxide and water.

Carl Neuberg and Joh. Kerb^{IV} found no lactic acid in working with 22 kilograms of yeast on 1 kilogram of pyruvic acid in 10 liters of water.

I. Chemical Abstracts, 7, 19, (1913)

II. Ibid. 8, 6. (1914)

III. Ibid. 8, 11. (1914)

IV. Ibid. 8,13. (1914)

P. A. Levene and G. M. Meyer^I found that leucocytes and kidney tissue do not decompose pyruvic acid.

A. Fernbach and M. Schoen^{II} obtained pyruvic acid by fermentation of yeast.

Carl Neuberg^{III} found that putrefactive bacteria decompose potassium pyruvate and give sodium formate, sodium acetate, and gases containing 88.9 % carbon dioxide, 7.5% hydrogen, and 0.75% methane.

Max Oppenheimer^{IV} by use of juice of yeast, obtained fermentation of pyruvic acid with active evolution of carbon dioxide.

Marie Hensel^V in research on acetylation processes in the animal organism, found that pyruvic acid is decomposed, with acetic acid as an intermediate step. Her results show, also, that the body stores the substance yielding acetic acid since the amount of acetyl derivative is larger in the after-period than during the main part of the experiment.

L. Karczag and L. Moczar^{VI} state that sugar-decomposing bacteria also decompose pyruvic acid.

I. Chemical Abstracts, 8, 15. (1914)

II. Ibid. 8, 20. (1914).

III. Ibid. 9, 4. (1915).

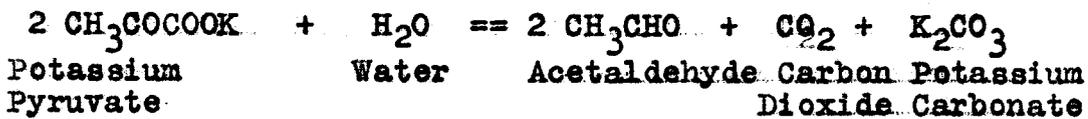
IV. Ibid. 9, 17. (1915).

V. Ibid. 9, 12. (1915).

VI. Ibid. 9, 21. (1915).

L. Karczag and E. Schiff^I found that pyruvic acid is decomposed by *B. Coli* thru a number of intermediate steps, almost quantitatively, into gaseous products- 90% hydrogen and 10% carbon dioxide. No formic acid, nor acetic acid was found. Formic acid and glycollic acid are fermentable by some bacteria which ferment pyruvic acid, and so, these two acids are considered as intermediate products in the fermentation of pyruvic acid.

V. I. Pallodin, E. I. Lovchinovskii, and A. I. Alekseyev^{II} demonstrated the fact that methylene blue inhibits the decomposition of potassium pyruvate by old plants, and that the theoretical requirements of the equation



do not hold, but, that parallel with the action of carboxylase the potassium carbonate is broken down.

Edgar J. Witzemann^{III} proved that with potassium permanganate it is possible to obtain every degree of velocity of oxidation of acetone, from zero to almost instantaneous oxidation by suitably varying the alkalinity, and pyruvic acid is represented as one stage of the oxidation. On further oxidation of the pyruvic acid by this method carbon dioxide and acetic acid are obtained.

I. Chemical Abstracts, 9, 21. (1915)

II. Ibid., 10, 22. (1916)

III. Ibid., 12, 3. (1918)

This method approximates the method of the body in oxidising its material, and so, if pyruvic acid yields carbon dioxide by the method above, then the author thinks pyruvic acid ought to yield carbon dioxide, and thus, also energy in the body.

L. Karczag^I found that rats and guinea pigs show a high rate of tolerance to pyruvic acid, and that injection of the acid produces neither hyperglucemia nor excretion in the urine of dextrose, lactic acid, volatile fatty acids nor of pyruvic acid, but the urine contains large amounts of carbonates and is alkaline. He showed, also, that certain bacteria bring about fermentation of pyruvic acid only when grown on bouillion, but not on simpler media.

The results of the injection of pyruvic acid in the experiment above of L. Karczag seem to show that all the pyruvic acid must be oxidised completely, or to a great extent, to carbon dioxide in order that an excess of carbonates may be secured in the urine and none of the other products mentioned above being obtained.

Carl Neuberg^{II} has shown that pyruvic acid during fermentation by yeast yields carbon dioxide and acetaldehyde, and, that during yeast fermentation this latter substance is readily reduced to ethyl alcohol, and in this connection, Carl Neuberg and Elsa Reinfurth^{III} found that acetaldehyde is produced in the

I. Chemical Abstracts, 12, 4. (1918)

II. Ibid., 13, 4. (1919)

III. Ibid., 13, 4. (1919)

ordinary yeast fermentation of sugars, so that it seems that pyruvic acid and sugar follow parallel courses in the metabolism of the body.

P. Mazé¹ states that bacteria in acting on lactic acid, oxidise it to pyruvic acid.

- E X P E R I M E N T A L -

In order to establish the proof of the author's theories advanced above the following experiments were performed.

The whole amount of pyruvic acid used in all of the experiments stated below was made in separate portions as follows: 20 grams of tartaric acid were pulverized and dried in the electric oven for two hours at 50° C. 30 grams of sodium bisulphate were fused, pulverized, and dried in a dessicator for six hours. The tartaric acid and sodium bisulphate were then well mixed and quickly put into a 250 cc. distilling flask, and immediately distilled, the heat being applied to the flask evenly and during more than one half hour. The distillate obtained was then redistilled under reduced pressure, about 717 millimeters pressure being maintained during the distillation. Three fractions were taken: the first distilled from 0° to 115°, the second from 115° to 130°, and the third from 130° to 150°.

1. Chemical Abstracts, 13, 8. (1919).

8.

This last fraction was retained as pure pyruvic acid and weighed 4 grams, thus representing a yield of 35% of theoretical results. This method, used as stated above, is a modification of that used by A. Wohl and R. Maag. I

Pyruvic acid with ammonia gas dried over solid sodium hydroxide gave a sticky jelly-like greenish-gold substance which seemed to be iminopropionic acid, $\text{CH}_3\text{C}=\text{NHCOOH}$, which later solidified to a yellow mass. This imino-acid with dilute hydrochloric acid gave a white crystalline solid, probably the hydrochloride of iminopropionic acid, $\text{CH}_3\text{C}=\text{NH}_2\text{ClCOOH}$. The probable imino-acid itself with dried hydrogen chloride gave a peach-colored substance, a solid, soluble in water, ether, and ethyl alcohol, but insoluble in acetone. The melting point of this probable hydrochloride of iminopropionic acid, $\text{CH}_3\text{CNH}_2\text{ClCOOH}$, is 110°C .

Pyruvic acid with barium chloride solution gave the barium salt of the acid, $(\text{CH}_3\text{COCOO})_2\text{Ba}$, which was as soluble as the acid itself, and so, was hard to isolate, for on evaporation a brown, semi-solid mass was obtained.

The silver salt of pyruvic acid was made, and it was insoluble in water. It formed leaf-like fluffy crystals which melted at 107° and decomposed somewhat at 95° . No analysis was made, but its behavior indicated that the substance was, undoubtedly, the silver salt of pyruvic acid.

I. Chemical Abstracts, 4, 20. (1910)

9.

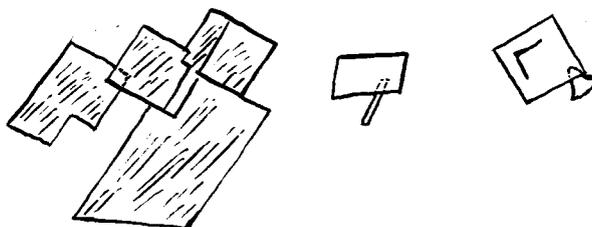
The following procedure was tried: 0.5 gram of phenylhydrazene hydrochloride, 1.5 grams of sodium acetate, and 0.3 gram of pyruvic acid were put into 15 cc. of distilled water and warmed for ten minutes. The flocculent precipitate which first formed later dissolved and only some decomposition products of the phenylhydrazone remained. The latter were filtered off, and the filtrate heated for thirty minutes on the water-bath. The solution was then allowed to cool for two hours and beautiful needle-like crystals appeared. These crystals were dried on filter papers and then recrystallized from hot water. They had a melting point of 171° , and from their form, and comparison to other phenylhydrazone crystals they appeared to be the phenylhydrazone of pyruvic acid.



Phenylhydrazone of Pyruvic Acid (recrystallised from water)

The following plate form of crystals (1) was obtained on recrystallising the crude crystals above from ethyl alcohol.

(1)



Phenylhydrazone of Pyruvic Acid (recrystallised from alcohol)

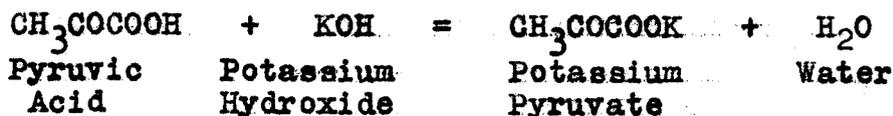
Pyruvic acid with very dilute sulphuric acid gave no

product of value due to incompleteness of the reaction.

A solution of pyruvic acid with the addition of metallic sodium gave on evaporation a white residue which charred on a platinum wire and left a white residue which was very soluble in water and ethyl alcohol and insoluble in ether and acetone.

The Grignard Reaction of ethyl magnesium iodide, C_2H_5MgI , plus pyruvic acid was tried and a liquid of a dark red color was obtained. This liquid had no constant boiling point but boiled from 75° to 83° . It was soluble in water, ethyl alcohol, ether, carbon disulphide, chloroform, and acetone.

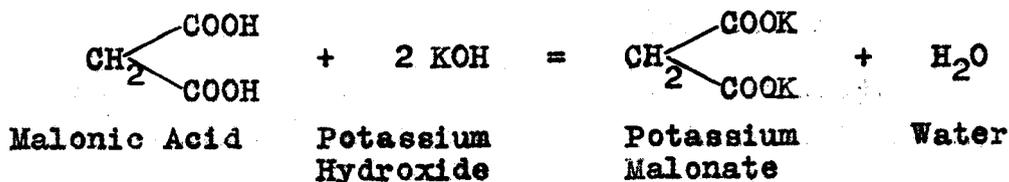
Pyruvic acid with concentrated nitric acid gave on evaporation a white solid which melted at 98° and was soluble in water and ethyl alcohol, the former solution decolorising potassium permanganate. This white solid on titration with potassium hydroxide showed that 0.0646 grams of the white solid required 0.0555 grams of potassium hydroxide for neutralisation, using phenolphthalein as indicator, and so, if the reaction:



took place here then the following figures would represent the weight requirements of the constituents in the reaction:

88 : 56 :: 0.0646 : X, where X = 0.0411 gram of potassium hydroxide, but 0.0555 gram of the hydroxide was used, and so, the reaction shown above does not hold true, and neither does

the following reactions:

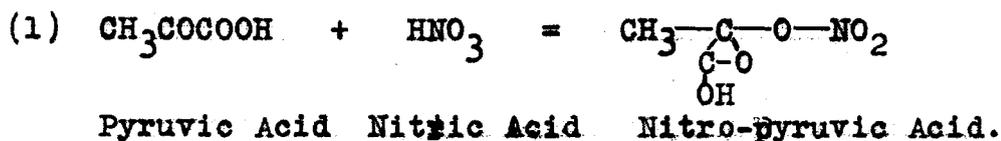


104 : 112 :: 0.0646 : X, where X = 0.0689 gram potassium hydroxide, whereas only 0.0555 gram was used.

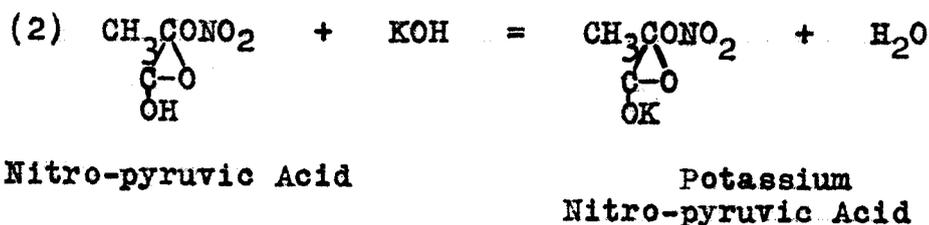
Pyruvic acid itself required for neutralisation of 0.0866 gram pyruvic acid 0.053105 gram potassium hydroxide, using phenolphthalein as indicator, theoretical results calling for 0.0563 gram potassium hydroxide for the amount used above.

The titration above seems to indicate that the nitric acid product of pyruvic acid contains a nitro group (NO_2), and so, the product was reduced with zinc and hydrochloric acid, and the amine formed seemed to be alanine for it responded to the test for alanine.

The possibilities of the reaction of concentrated nitric acid on pyruvic acid are represented below:



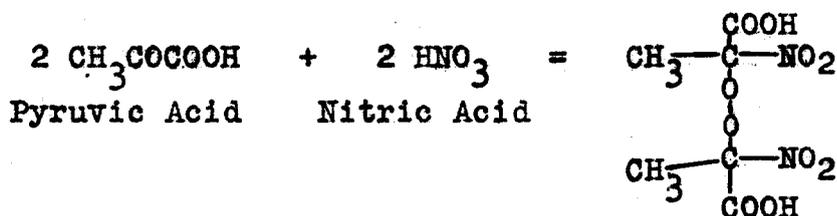
If it be considered that this compound was formed, on calculating the following equation:



the results obtained are expressed thus:

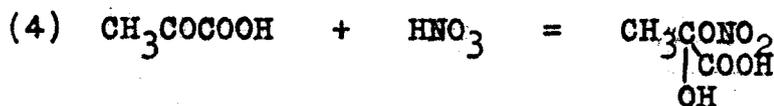
134 : 56 :: 0.0646 : X and X = 0.054 gram potassium hydroxide. It was found that actually 0.0555 gram potassium hydroxide was required. From these results the formula above (1) probably represents the compound formed on treating pyruvic acid with concentrated nitric acid.

(3)



Di-nitro-di-pyruvic Acid

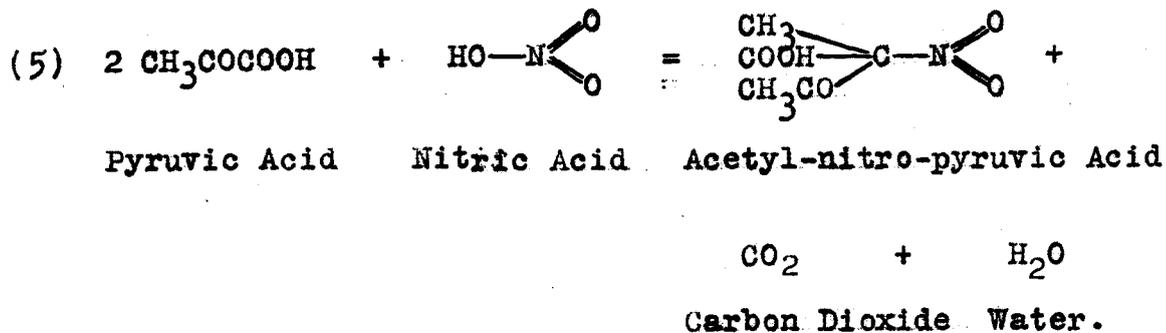
This formula gives the same quantitative results with potassium hydroxide as the one above. (Formula (1)).



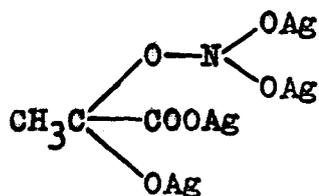
Pyruvic Acid Nitric Acid Hydroxy-
Nitro-pyruvic Acid.

This formula (4) agrees well with the titration above with potassium hydroxide.

Almost the only possibility of forming a tertiary nitro group, ($\equiv\text{C}-\text{NO}_2$), as the product above of pyruvic acid with concentrated nitric acid seems to require, since it gave no tests for primary and secondary nitro groups, is by the following reaction:



The silver salt of the nitric acid product of pyruvic acid was prepared. It was found to be insoluble in water, but soluble in nitric acid solution. The amount of silver in the salt was found to be 75.32%, and so, the following formula could be ascribed to the silver salt:

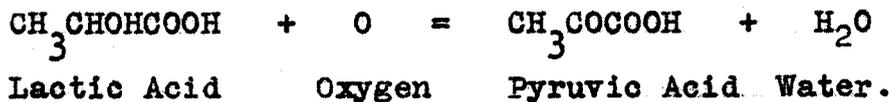


Tetra-silver-nitro-pyruvic Acid

The work below was done to show the close relation of pyruvic acid to lactic acid.

Two cubic centimeters of lactic acid and some 1% neutral solution of potassium permanganate were heated slightly. Extraction of the product with ether and evaporation on a water bath left a thick syrup which on treating with ammoniacal silver nitrate gave a silver mirror. Lactic acid itself was taken with ammoniacal silver nitrate and gave no reduction at all, the product in the first case corresponding to pyruvic acid thus:

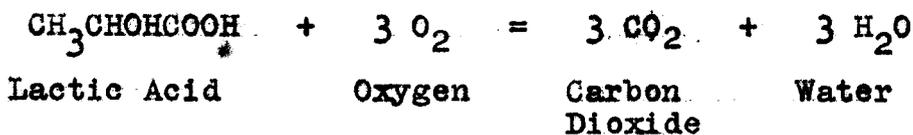
14.



Confirmation of the test above was shown by the red color obtained with the neutralised product of the oxidation of lactic acid, plus ferric chloride, the color being with ferric chloride as the reagent, the test for neutralised pyruvic acid.

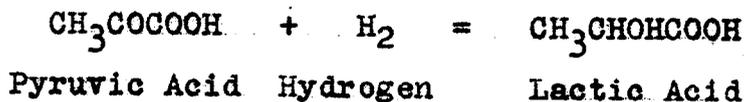
Lactic acid and dilute nitric acid were heated, evaporated, and the residue, after neutralisation, was treated with ferric chloride, and a positive test for pyruvic acid was obtained.

Concentrated nitric acid will carry on the oxidation of lactic acid too strongly, so that only carbon dioxide and water can be obtained. Such strong oxidations do not take place in the body.



Pyruvic acid was reduced with zinc and hydrochloric acid to lactic acid as shown by the test which when ferric chloride was added to the neutral solution of the reduction product a yellow color, the test for lactic acid with ferric chloride was obtained.

Pyruvic acid reduced with metallic sodium gave lactic acid as found by the ferric chloride test.



The Fermentation of Pyruvic Acid

The fermentation of pyruvic acid was done with the ordinary Fleischmann's compressed yeast at 40° for one day and the following products identified:

Carbon dioxide, found by using lime-water test.

Ethyl Alcohol, tested by the acetate test.

Acetaldehyde, tested with Tollen's Reagent.

Acetic Acid, tested by odor, and by formation of ethyl acetate with ethyl alcohol.

0.538 gram of pyruvic acid was fermented for five days at room temperature with yeast, and 0.0031214 gram of carbon dioxide was obtained from acid and yeast together. A blank fermentation of yeast of the same weight as that above gave 0.001128 gram of carbon dioxide.

(a) 1.4610 grams of pyruvic acid and 0.3298 gram of yeast were fermented for five days, (one-half day at 40°), and 0.0086 gram of barium sulphate was obtained from the barium carbonate formed by the carbon dioxide of the fermentation. A blank on 0.3298 gram of yeast, the same weight as in the original test, gave 0.0066 gram of barium sulphate.

0.0086 gram of barium sulphate from yeast and pyruvic acid.

0.0066 gram of barium sulphate from yeast alone.

0.0020 gram of barium sulphate from pyruvic acid alone.

0.002 times 0.188, the factor for the ratio of carbon dioxide to barium sulphate $\text{CO}_2/\text{BaSO}_4$, gives 0.000376 gram of carbon dioxide that was obtained from the pyruvic acid alone.

These results show that 0.051% of the pyruvic acid was fermented.

(b) 1.2838 grams of pyruvic acid and 0.3298 gram of yeast were fermented in exactly the same way as that above (a) and 0.0086 gram of barium sulphate obtained as above. A blank on 0.3298 gram of yeast, the same weight as was used in the original test, gave 0.0070 gram of barium sulphate.

0.0086 gram of barium sulphate from yeast and pyruvic acid.

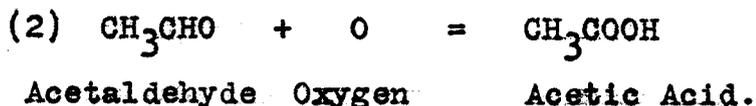
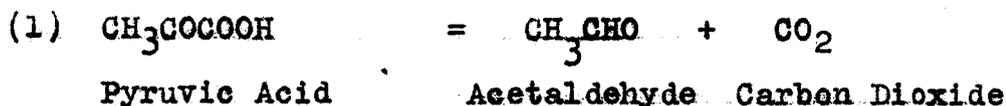
0.0070 gram of barium sulphate from yeast alone.

0.0016 gram of barium sulphate from pyruvic acid alone.

0.0016 times 0.188, the factor used above in test (a), gives 0.0003008 gram of carbon dioxide got from the pyruvic acid alone.

These results show that 0.0475% of the pyruvic acid was fermented.

The reactions of the fermentation of pyruvic acid are as follows:¹



0.1 gram of glucose was fermented together with the old product of (a) test above, using the same yeast, the same conditions prevailing as those in (b) test above, and 0.0017296 gram of carbon dioxide was obtained.

I. C. Neuberg, Biochem. Zeitsch. 1911, 31, 170; 32, 323.

The Fermentation of Glucose

The fermentation to completion of glucose gave:

Carbon dioxide, tested as above.

Ethyl Alcohol, tested as above.

Amyl Alcohol, tested by the odor, and by the formation
of amyl acetate with acetic acid.

Glycerol, tested by the borax and phenolphthalein
test.

5 grams of glucose were fermented at 40° for three hours and a part of the solution was then tested for its components and the following things were found;

Carbon dioxide, tested as above.

Ethyl Alcohol, tested as above.

Glycerol, tested as above.

After 19 hours fermentation of the glucose above the following substances were recognised:

Carbon dioxide, tested as above.

Ethyl Alcohol, tested as above.

Amyl Alcohol, tested as above.

Glycerol, tested as above.

The Oxidation of Pyruvic Acid

1.2244 grams of pyruvic acid on oxidising for four hours at 70° with 3% potassium permanganate solution gave a product which required 0.015 gram of potassium hydroxide for neutralisation.

1.2244 gram of pyruvic acid itself required 0.7185 gram of potassium hydroxide for neutralisation, and so, it seems that all but 2% of the pyruvic acid was oxidised completely to carbon dioxide and water.

In connection with these experiments just stated 0.3452 gram of acetic acid required 0.3112 gram of potassium hydroxide for neutralisation, and 0.2992 gram of lactic acid required 0.1331 gram of potassium hydroxide for neutralisation, and so, the oxidation product of pyruvic acid with potassium permanganate above could not be acetic acid nor lactic acid for the quantitative results obtained do not agree with such an assumption.

0.1908 gram of pyruvic acid was oxidised with 0.5% potassium permanganate solution, but as the oxidation proceeded too rapidly 0.25% permanganate solution was used till the oxidation had proceeded for ten hours at a constant temperature of 70° . On titration of the resulting product it was found that 0.04085 gram of potassium hydroxide was required for neutralisation.

0.2161 gram of pyruvic acid on oxidation with nitric acid (1.11%) gave an unsaturated compound.

S U M M A R Y

From the results obtained in the experimental processes cited there can be no doubt that pyruvic acid in most cases is oxidised immediately on its formation to carbon dioxide and water with the liberation of energy.

The exact processes which the body uses in metabolism are quite unknown to science, but by ascertaining how substances act outside the body it is possible to form theories concerning their actions in the body, and then, by continued experiments based on the theories formed the processes of the body can be approximated and results can be obtained which are entirely satisfactory so far as their utility is concerned.

The results obtained, then, in the experiments stated above confirm the belief that pyruvic acid is one of the products of carbohydrate decomposition in the body, and also, that pyruvic acid is further utilized by the body in furnishing energy by its direct transformation to carbon dioxide and water.