XIX. THE DECOMPOSITION OF LACTIC ACID BY KILLED YEAST.

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According to the theory that the first stages both of anaerobic respiration and of alcoholic fermentation consist of a series of oxidations and reductions at the expense of water [Palladin, 1912], it must be concluded that amongst the first products of decomposition of glucose must be placed organic acids. These acids must undergo further anaerobic oxidation in which reductase participates. Palladin and Mlle Lovchinovskaya [1914] therefore investigated the action of a hydrogen acceptor (methylene blue) on the decomposition of some organic acids—supposed to be present among the intermediate products of the decomposition of glucose—by killed yeast. Of the acids investigated, gluconic and lactic caused the evolution of a greater quantity of carbon dioxide in the presence of methylene blue, than in its absence. The action of reductase consists in removing the hydrogen from one substance and transferring it to another. The substance which yields the hydrogen is called the oxidisable substance, or reductor ($R \cdot H_2$), whereas the substance receiving the hydrogen is called the hydrogen acceptor, or oxidator (A):

If we take the case in which reduction takes place with the participation of water, the hydrogen of the water will join the hydrogen acceptor, and the oxygen will join the oxidisable substance and either remove from it hydrogen with the formation of water, or produce some other oxidising reaction.

The oxidations produced by reductase by means of removing the hydrogen may be accompanied by the evolution of carbon dioxide. Thus, Bredig and Sommer [1910] have shown that formic acid is decomposed into carbon dioxide and hydrogen in the presence of a catalyst and methylene blue:

The carbon dioxide produced in the decomposition of gluconic and lactic acids also owes its production to reductase. Having put off for some time the detailed investigation of gluconic acid, we turned to the study of the decomposition of lactic acid. The results of this research form the contents of the present paper.

We started from the supposition that in the presence of the hydrogen acceptor (A) lactic acid first produces pyruvic acid under the influence of reductase:

The pyruvic acid formed is then decomposed into carbon dioxide and acetaldehyde under the influence of carboxylase:

Beilstein and Wiegand [1885], thinking lactic acid to be a secondary alcohol and pyruvic acid to be the ketone corresponding to it,

obtained pyruvic acid by oxidising calcium lactate with potassium manganate. Wieland [1912] subsequently found that when palladium black acts on lactic acid, pyruvic acid is also produced. We worked with hefanol and zymin which were considerably weakened by rather long preservation. During the first half of our work we used methylene blue as a hydrogen acceptor, and in the second half acetaldehyde *in statu nascendi*.

I. THE RATIO OF THE QUANTITY OF ACETALDEHYDE FORMED TO THE QUANTITY OF CARBON DIOXIDE EVOLVED IN PRESENCE OF METHYLENE BLUE.

In all the experiments of this work the lactic acid was neutralised by caustic potash. The reaction therefore follows the equations:

As is seen, part of the carbon dioxide remains combined in the solution. In order to produce it in a free state, 10 drops of 3 % sulphuric acid were added after the termination of the experiment. In that case:

$$\frac{\text{CO}_2}{\text{CH}_3\text{CHO}} = \frac{44}{44} = 1.$$

The experiments were conducted in round-bottomed flasks with long necks, with a capacity of 50 cc. The flasks were closed with rubber corks with three holes. Through one hole a tube passed to the bottom, through

the second, a short tube. Both tubes carried at the top india-rubber tubes which were tightly closed during the experiment by means of stoppers. Into the third opening a funnel with a tap was fitted, through which the sulphuric acid was poured. During the experiment the flasks were shaken up in a special shaking apparatus. On this account even a partial decolorisation of the methylene blue was never observed. After the addition of sulphuric acid a current of air was conducted through the flask and then through a Pettenkofer's tube.

As qualitative reactions for acetaldehyde, the reaction with fuchsin-sulphurous acid and the reaction of Rimini were used. The quantitative determination of acetaldehyde was carried out by distillation into a flask cooled by snow. In the distillate the aldehyde was determined by adding bisulphite and titrating the excess by iodine according to Ripper's method.

Exp. 1. Three flasks. In each 7 g. of hefanol and 25 cc. of 0.5 % methylene blue. In the first and second 25 cc. of 2 % potassium lactate. In the third, 25 cc. of water. The duration of the experiment was 3 h. 20 m. Temperature 17–18°.

Results: No. 1. 75.0 mg. CO₂.
 „ 2. 31.3 „ acetaldehyde.
 „ 3. 40.6 „ CO₂.

After subtracting from 75.0 the carbon dioxide of auto-fermentation, we have 34.4. Therefore,

$$\frac{\text{CO}_2}{\text{CH}_3 \cdot \text{CHO}} = \frac{34.4}{31.3} = 1.1.$$

Exp. 2. Repetition of the preceding. Duration 6 hrs. Temperature 17–18°.

Results: No. 1. 94.6 mg. CO₂.
 „ 2. 46.2 „ acetaldehyde.
 „ 3. 41.2 „ CO₂.
 94.6 – 41.2 = 53.4,

$$\frac{\text{CO}_2}{\text{CH}_3 \cdot \text{CHO}} = \frac{53.4}{46.2} = 1.16.$$

Exp. 3. Repetition of preceding. Duration 7 hrs. Temperature 17–18°.

Results: No. 1. 99.1 mg. CO₂.
 „ 2. 49.0 „ acetaldehyde.
 „ 3. 48.2 „ CO₂.
 99.1 – 48.2 = 50.9,

$$\frac{\text{CO}_2}{\text{CH}_3 \cdot \text{CHO}} = \frac{50.9}{49.0} = 1.04.$$

Thus, in all the three experiments the ratio $\text{CO}_2/\text{CH}_3\text{CHO}$ is, according to the theory, nearly equal to one, if we hold that in the presence of lactic acid auto-fermentation continued with the same energy as in the control experiment.

The question arises, why was not the acetaldehyde further reduced to ethyl alcohol? It is quite possible that the presence of a large quantity of the hydrogen acceptor, which largely takes away the hydrogen, prevents this reaction. It may be expressed in the form of a general rule that *in the presence of a large quantity of the hydrogen acceptor the formation of alcohol is impossible*. If applied in the necessary manner, the hydrogen acceptor can be made to interrupt the alcoholic fermentation at the stage of acetaldehyde.

The experiments described above have already appeared in Russian in a paper by Palladin, Sabinin and Lovchinovskaya [1915] published on 1st May, 1915. In September there appeared a paper by Harden and Norris [1915], who, not knowing of our work, had also discovered that lactic acid is decomposed by dried yeast with the help of reductase and carboxylase, and that as a result acetaldehyde is produced. In this work there is mentioned the very interesting fact that only a small part of the oxidised lactic acid is converted into acetaldehyde. As Harden and Norris did not find any pyruvic acid, they suggested that possibly only part of the lactic acid was converted into pyruvic acid, the greater part oxidising otherwise. The following facts speak in favour of such a view: Palladin, Lovchinovskaya and Alexeieff [1915] discovered that methylene blue interrupts the decomposition of pyruvic acid by zymin and wheat germs. Further, according to the equation

it was to be expected that after the end of the experiment the quantity of carbon dioxide in the form of carbonate of potassium would be equal to the quantity of gaseous carbon dioxide evolved.

In reality it was different. In the control portion the combined carbon dioxide was less by 62 %, and in the experimental portion by 82 %, as is seen from the following experiment quoted from their work.

Exp. iv. Three portions of 5 g. of old zymin. 1. 50 cc. of 1 % pyruvic acid neutralised by caustic potash. Air current. 2. 50 cc. of 1 % pyruvic acid neutralised by caustic potash; 1.5 g. methylene blue. Hydrogen current. 3. 50 cc. water, 1.5 g. methylene blue. Hydrogen current. After 24 h. 10 m. the experiment was stopped. There was no decolorisation. Then 10 cc. 5 % H_2SO_4 were added to 1 and the same quantity to 2. 2 cc. of toluene in each flask. Temperature 14.5–15°.

DECOMPOSITION OF LACTIC ACID BY KILLED YEAST 187

Time	1 1 % potassium pyruvate. Air current		2 1 % potassium pyruvate. Methylene blue. Hydrogen current		3 Water. Methylene blue. Hydrogen current	
	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.
2 hrs.	32.2	16.1	22.2	11.1	4.6	2.3
2 „	25.8	12.9	14.2	7.1	4.2	2.1
20 „	41.1	2.0	38.7	1.9	7.0	0.3
24 „	99.1	—	75.1 (–24%)	—	15.8	—
H ₂ SO ₄ added.						
3 hrs. 35 m.	38.6 (–62%)	—	13.2 (–82%)	—	—	—
Total	137.7	—	88.3	—	—	—

It is to be concluded from the evidence given in such experiments that parallel to the decomposition of both the pyruvic and the lactic acids, the formation of some acid or acids decomposing CO₃K₂ with the evolution of carbon dioxide takes place. In presence of methylene blue a greater quantity of these acids is formed, than in the control portions. Still less combined carbon dioxide is produced by decomposing lactic acid by killed yeast in the presence of methylene blue, as was shown in the paper by Palladin, Sabinin and Lovchinovskaya [1915] mentioned above. Lvoff [1913] discovered in our laboratory that in the process of glucose fermentation for each atom of hydrogen removed with the help of methylene blue, the production both of alcohol and of carbon dioxide is diminished by one molecule. This led Lovchinovskaya to try to find some similar laws for lactic acid.

According to the equations:

and taking into account that the molecular weight of methylene blue, C₁₆H₁₈N₃SCl + 3H₂O, is 747.5, it follows that the ratio

$$\frac{\text{CO}_2}{2\text{M}} = \frac{44}{747.5}.$$

It follows from this, that for 1.5 g. of methylene blue before its complete decolorisation in an atmosphere of hydrogen there must be evolved 88.3 mg. of carbon dioxide. Experiments produced greater quantities.

Exp. 4. Three portions of 10 g. of hefanol. 1. 50 cc. 2 % lactic acid neutralised by caustic potash, and 1.5 g. methylene blue. Hydrogen current. 2. 50 cc. 2 % lactic acid neutralised by caustic potash. Air current. 3. 50 cc. of water. Air current. To each flask 2 cc. of toluene were added. After decolorisation of 1, the experiment was stopped. Temperature 16°.

Time	Potassium lactate and methylene blue. Hydrogen current		Potassium lactate. Air current		Water. Air current	
	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.
4 h. 45 m.	78.4	18.4	31.0	7.3	26.0	6.1
23 hrs.	177.6	7.7	63.6	2.7	53.0	2.3
24 h. 45 m.	256.0	—	94.6	—	79.0	—

256 - 94.6 = 161.4 instead of 88.3.

Similar experiments gave the following figures:

161.4
154.2
139.6
148.6
108.8
147.7
Average: 143.4

If we suppose that all the CO₂ had been evolved $88.3 \times 2 = 176.6$ mg. of CO₂ would have been obtained. In reality, the average was found to be 143.4, *i.e.* 81.2 %. Therefore, a small part of the carbon dioxide must have remained in a combined state, and this was confirmed by experiment.

Exp. 5. One portion of 11.5 g. dried yeast (Lebedeff); 50 cc. 1 % lactic acid neutralised by caustic potash; 1.5 g. methylene blue; 2 cc. of toluene. The experiment was stopped after 44 h. 20 m. There was no decolorisation. Afterwards 10 cc. 5 % H₂SO₄ were added. Temperature 14° C.

Duration of the experiment	Potassium lactate and methylene blue. Hydrogen current	
	CO ₂ mg.	CO ₂ in 1 h.
2 h. 30 m.	12.2	4.9
16 h. 10 m.	95.1	5.8
2 h. 40 m.	46.8	17.5
23 hrs.	58.0	2.5
44 h. 20 m.	212.1	—
Addition of H ₂ SO ₄ .		
24 hrs.	19.2	—
Total	231.3	—

Consequently, there appeared to be only 9 % of combined carbon dioxide.

II. THE RATIO OF THE QUANTITY OF CARBON DIOXIDE EVOLVED TO THE QUANTITY OF OXYGEN ABSORBED IN THE PRESENCE OF METHYLENE BLUE.

The experiments in this part of our work were undertaken on the basis of the following equations:

- (1) $2\text{CH}_3 \cdot \text{CHOH} \cdot \text{COOK} + 2\text{M} = 2\text{CH}_3 \cdot \text{CO} \cdot \text{COOK} + 2\text{MH}_2$,
- (2) $2\text{MH}_2 + \text{O}_2 = 2\text{M} + 2\text{H}_2\text{O}$,
- (3) $2\text{CH}_3 \cdot \text{CO} \cdot \text{COOK} + \text{H}_2\text{O} = 2\text{CH}_3 \cdot \text{CHO} + \text{CO}_3\text{K}_2 + \text{CO}_2$,

from which it follows that

$$\frac{\text{CO}_2}{\text{O}_2} = 1.$$

But the experiments of Lovchinovskaya proved that not merely half of the carbon dioxide is evolved, according to the equation, but a considerably greater amount. Therefore, the ratio CO_2/O_2 ought to be much larger than one. Nevertheless, it proved to be equal to one. This shows that, besides oxidising the reduced methylene blue, the oxygen enters into some other oxidation. In the first experiments fermentation took place at the temperature of the room in flat conical flasks similar to those used in cultivating diphtheria. The flasks were sealed by mercury, with two tubes—sealed by mercury—for removing the gas. During the experiment the flasks were shaken once or twice. This proved to be insufficient, the methylene blue having become partly decolorised at the end of the experiment, and high coefficients having been produced.

Exp. 6. Two flasks. In each 2 g. of hefanol and 30 cc. 1 % potassium lactate. In addition, in the second 3 cc. 0.5 % methylene blue, and in the first 30 cc. water. Duration of the experiment 5.5 hours. In all the experiments the gas was analysed in the Polovtzeff-Richter apparatus.

Flask No. 1	Flask No. 2
$\text{CO}_2 = 3.5 \%$	$\text{CO}_2 = 1.41 \%$
$\text{O}_2 = 19.3 \%$	$\text{O}_2 = 19.92 \%$
$\text{N}_2 = 77.2 \%$	$\text{N}_2 = 78.67 \%$
$\text{CO}_2/\text{O}_2 = 4.5 \%$	$\text{CO}_2/\text{O}_2 = 1.65 \%$

Exp. 7. Repetition of the preceding. Duration 5 hrs. 25 cc. of each liquid.

Flask No. 1	Flask No. 2
$\text{CO}_2 = 2.8 \%$	$\text{CO}_2 = 1.56 \%$
$\text{O}_2 = 19.29 \%$	$\text{O}_2 = 20.04 \%$
$\text{N}_2 = 77.91 \%$	$\text{N}_2 = 78.4 \%$
$\text{CO}_2/\text{O}_2 = 2.86 \%$	$\text{CO}_2/\text{O}_2 = 2.21 \%$

Exp. 8. Two flasks. In each 5 g. of hefanol and 25 cc. 2 % potassium lactate. In addition, in the first 25 cc. 0.5 % methylene blue, and in the second 25 cc. water. In the thermostat at 34°. Duration of the experiment 3.5 hrs.

Flask No. 1	Flask No. 2
CO ₂ = 7.83 %	CO ₂ = 3.84 %
O ₂ = 18.01 %	O ₂ = 19.53 %
N ₂ = 74.16 %	N ₂ = 76.63 %
CO ₂ /O ₂ = 4.90 %	CO ₂ /O ₂ = 5.48 %

Notwithstanding the very thin layer of liquid, in the last three experiments the aeration was very incomplete, and high coefficients were produced. In the experiments succeeding the flasks used in the experiments 1–3 were taken. During the experiment they were shaken on a shaking apparatus.

Exp. 9. Three flasks. Duration of experiment 3 hrs.

	Flask No. 1	Flask No. 2	Flask No. 3
Hefanol	5 g.	5 g.	5 g.
Methylene blue 0.5 % ...	25 cc.	25 cc.	—
Potassium lactate 2 % ...	25 cc.	—	25 cc.
Water	—	25 cc.	25 cc.

Analysis of gases:

Flask No. 1	Flask No. 2	Flask No. 3
CO ₂ = 7.74 %	CO ₂ = 3.72 %	CO ₂ = 3.84 %
O ₂ = 15.31 %	O ₂ = 19.50 %	O ₂ = 19.04 %
N ₂ = 76.95 %	N ₂ = 76.78 %	N ₂ = 77.12 %
CO ₂ /O ₂ = 1.54 %	CO ₂ /O ₂ = 4.83 %	CO ₂ /O ₂ = 2.90 %

Allowing for the control experiment (No. 2) we have as the final ratio

$$\frac{7.74 - 3.72}{5.00 - 0.77} = 0.95.$$

Exp. 10. Three flasks. Duration of experiment 3.5 hrs.

	Flask No. 1	Flask No. 2	Flask No. 3
Hefanol	7 g.	7 g.	7 g.
Methylene blue 0.5 % ...	25 cc.	25 cc.	—
Potassium lactate 2 % ...	25 cc.	—	25 cc.
Water	—	25 cc.	25 cc.

Analysis of gases:

Flask No. 1	Flask No. 2	Flask No. 3
CO ₂ = 9.84 %	CO ₂ = 4.63 %	CO ₂ = 5.77 %
O ₂ = 13.45 %	O ₂ = 18.44 %	O ₂ = 18.91 %
N ₂ = 76.71 %	N ₂ = 76.93 %	N ₂ = 75.32 %
CO ₂ /O ₂ = 1.44 %	CO ₂ /O ₂ = 2.47 %	CO ₂ /O ₂ = 5.94 %

Allowing for the control as above we have:

$$\frac{\text{CO}_2}{\text{O}_2} = 1.06.$$

DECOMPOSITION OF LACTIC ACID BY KILLED YEAST 191

Exp. 11. Three flasks. Duration of the experiment 5 hrs.

	Flask No. 1	Flask No. 2	Flask No. 3
Hefanol	7 g.	7 g.	7 g.
Methylene blue 0·5 % ...	25 cc.	25 cc.	—
Potassium lactate 2 % ...	25 cc.	—	25 cc.
Water	—	25 cc.	25 cc.

Analysis of gases:

Flask No. 1	Flask No. 2	Flask No. 3
CO ₂ = 12·96 %	CO ₂ = 4·92 %	CO ₂ = 6·09 %
O ₂ = 11·24 %	O ₂ = 18·07 %	O ₂ = 18·40 %
N ₂ = 75·80 %	N ₂ = 77·01 %	N ₂ = 75·51 %
CO ₂ /O ₂ = 1·48 %	CO ₂ /O ₂ = 2·18 %	CO ₂ /O ₂ = 3·98 %

Allowing for the control, we have:

$$\frac{\text{CO}_2}{\text{O}_2} = 0·80.$$

The results of the last three experiments show that with good aeration the ratio CO₂/O₂ during the decomposition of potassium lactate by yeast in the presence of methylene blue is only slightly less than one.

Just as in respiration, according to the theory of Palladin, the oxygen is absorbed in order to remove the hydrogen; in the last three experiments the oxygen absorbed oxidised the hydrogen which was removed from the lactic acid by means of methylene blue. Thus, the methylene blue has taken the place of the respiratory pigment.

III. THE DECOMPOSITION OF LACTIC ACID IN THE PRESENCE OF PYRUVIC ACID.

The problem for our further experiments was to find the conditions in which the lactic acid would produce, instead of acetaldehyde, alcohol. The question then arose, what was the substance that in natural conditions substituted methylene blue during alcoholic fermentation in the process of decomposition of lactic acid? Of course, only acetaldehyde can be this substance. From our point of view the scheme of decomposition of lactic acid by yeast with the production of alcohol is represented in the following manner:

- (1) CH₃·CH(OH)·COOH + CH₃·CHO = CH₃·CO·COOH + CH₃·CH₂·OH,
- (2) CH₃·CO·COOH = CO₂ + CH₃·CHO.

The acetaldehyde produced oxidises new quantities of lactic acid, and so on. Consequently, in the presence of an insignificant quantity of acetaldehyde it is possible to convert an indefinitely large quantity of lactic acid into

alcohol. In order to test the correctness of the view expressed, we took, instead of acetaldehyde, pyruvic acid, which when decomposed by carboxylase, produces acetaldehyde:

We had two reasons for taking pyruvic acid. Firstly, because it produces acetaldehyde *in statu nascendi*; secondly because Oppenheimer [1914] demonstrated that pyruvic acid evokes the decomposition of lactic acid by yeast juice.

Exp. 12. Nine flasks with 5 g. of zymin in each. In addition, in three flasks 25 cc. 0.2 % pyruvic acid neutralised by caustic potash, and 25 cc. of water; in three flasks 25 cc. 2 % lactic acid neutralised by caustic potash, and 25 cc. of water; in the remaining three flasks 25 cc. 2 % lactic acid, and 25 cc. 0.2 % pyruvic acid neutralised by caustic potash. The following quantities of carbon dioxide in mg. were evolved:

		18 hours	24 hours	60 hours
1.	Pyruvic acid	116	174	248
2.	Lactic acid	117	164	219
3.	Lactic and pyruvic acids	147	223	325

The average quantities of carbon dioxide evolved in one hour were as follows:

Duration of the experiment. Hours	Pyruvic acid		Lactic acid		Lactic and pyruvic acids	
	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.	CO ₂ mg.	CO ₂ in 1 h.
18	116	6.5	117	6.5	147	8.2
6	58	9.5	47	7.8	76	12.6
36	74	2.0	55	1.5	102	2.8
60	248	—	219	—	325	—

Thus, the addition of a small quantity of pyruvic acid increased the evolution of carbon dioxide at the expense of lactic acid in the following manner:

18 hours.	147 - 117 = 30 (+ 25.6 %)
24 hours.	223 - 164 = 59 (+ 36.0 %)
60 hours.	325 - 219 = 106 (+ 48.4 %)

Taking into account, that from the decomposition of the pyruvic acid only 25 mg. of carbon dioxide could be evolved, it follows that the remaining 81 mg. (106 - 25 = 81) of carbon dioxide were evolved at the expense of the lactic acid.

DECOMPOSITION OF LACTIC ACID BY KILLED YEAST 193

Therefore, it is to be concluded that *pyruvic acid evokes the decomposition of lactic acid by killed yeast, though in a weaker degree than methylene blue.*

This surplus of carbon dioxide cannot be attributed to the stimulation of auto-fermentation by the pyruvic acid, as, in comparison with the quantity of carbon dioxide evolved in presence of lactic acid, in the experiments in which auto-fermentation proceeded in the presence of pyruvic acid only a slight surplus was produced, hardly exceeding the 25 mg. produced from the decomposition of pyruvic acid:

18 hours.	116 - 117 = 0
24 hours.	174 - 164 = 10
60 hours.	248 - 219 = 29

It must also be noted that the pyruvic acid stimulated the decomposition of lactic acid at first in a weak degree (25.6 %), but at the end of the experiment this stimulation gradually increased (48.4 %).

Exp. 13. Three flasks with 5 g. zymin in each. In addition, in the first flask 25 cc. 0.2 % pyruvic acid neutralised by caustic potash and 25 cc. of water, in the second flask 25 cc. 2 % lactic acid neutralised by caustic potash and 25 cc. of water, in the third flask 25 cc. of 2 % lactic acid and 25 cc. 0.2 % pyruvic acid neutralised by caustic potash. During fermentation the flasks were firmly closed with india-rubber stoppers. After 25 hours the quantity of acetaldehyde was estimated:

1. Pyruvic acid...	...	...	13.5 mg.
2. Lactic acid ...	...	...	0 „
3. Lactic and pyruvic acids	...	...	11.5 „

In the third portion, according to the quantity of carbon dioxide evolved in the first experiment, it was to be expected that 59 mg. of acetaldehyde would be produced. But in reality only 11.5 mg. were produced.

It is to be concluded, therefore, that *the decomposition of lactic acid by killed yeast in the presence of pyruvic acid takes place without acetaldehyde being produced, i.e. in a different manner from that which takes place in the presence of methylene blue.*

Exp. 14. Two flasks with 5 g. zymin in each. In addition, in the first flask 25 cc. 2 % lactic acid neutralised by caustic potash and 25 cc. of water; in the second flask 25 cc. 2 % lactic acid and 25 cc. 0.2 % pyruvic acid neutralised by caustic potash. The experiment was conducted for 24 hours. In order to liberate the combined carbon dioxide, at the end of the experiment 10 cc. 10 % sulphuric acid were added to each of the two flasks. The quantity of alcohol was then estimated by the method of Nicloux.

	Carbon dioxide	Alcohol
1. Lactic and pyruvic acids	205	167
2. Lactic acid	138	143
	67	24

$$\frac{C_2H_5O}{CO_2} = \frac{24}{67} = 0.35.$$

Exp. 15. Three flasks with 7 g. zymin in each. In addition, in the first flask 25 cc. 0.2 % pyruvic acid neutralised by caustic potash and 25 cc. of water, in the second flask 25 cc. 2 % lactic acid neutralised by caustic potash and 25 cc. of water, in the third flask 25 cc. 2 % lactic acid and 25 cc. 0.2 % pyruvic acid neutralised by caustic potash. The experiment was conducted for 62 hours. The estimations of carbon dioxide and alcohol were made as in the preceding experiment.

	Carbon dioxide	Alcohol
1. Pyruvic acid	490	476
2. Lactic acid	447	445
3. Lactic and pyruvic acids	695	514

Therefore, the third portion gave the following surplus of carbon dioxide and alcohol, as compared with the second:

Carbon dioxide	695 - 447 = 248 mg.
Alcohol ...	514 - 445 = 69 „

If we suppose that in the third portion the pyruvic acid not only evoked the decomposition of lactic acid, but also stimulated auto-fermentation, we must subtract from the figures of the third portion those of the first portion, not of the second:

Carbon dioxide	695 - 490 = 205 mg.
Alcohol ...	514 - 476 = 38 „

Therefore, even if we allow for the stimulation of the process of auto-fermentation by pyruvic acid in the presence of lactic acid, we must conclude that *pyruvic acid evokes the decomposition of lactic acid by killed yeast with the production of alcohol and carbon dioxide*. But a sharp deviation from the normal alcoholic fermentation is observed, for to a great quantity of carbon dioxide evolved, only a small quantity of alcohol is produced:

$$(1) \quad \frac{C_2H_5O}{CO_2} = \frac{38}{248} = 0.15,$$

$$(2) \quad \frac{C_2H_5O}{CO_2} = \frac{38}{205} = 0.18.$$

What has caused such a great predominance of carbon dioxide over alcohol? It is well known from organic chemistry that reactions very rarely correspond to the theoretical equations: usually the main reaction is accompanied by a greater or smaller number of accessory reactions. Moreover, all the

chemical reactions studied by us in killed organisms differ more or less widely from the same reactions which take place in living organisms, in that they are accompanied by different accessory reactions, absent from living organisms.

In all probability, in the case investigated by us, the accessory reactions have also partly obscured, and partly altered, the main reaction of decomposition of lactic acid into alcohol and carbon dioxide. It is possible, that one of these accessory reactions was the reaction of Cannizzaro, very common in killed plants and animals. It is unknown whether it takes place in living organisms. This reaction alone could have reduced the production of alcohol by half. It may be also that part of the lactic acid was decomposed with the evolution of carbon dioxide, but without the formation of the aldehyde. Lastly, it is possible that the surplus of carbon dioxide was produced by further oxidation of the acetaldehyde. Further investigations are needed to solve these questions.

A surplus of carbon dioxide over alcohol is a usual phenomenon during anaerobic respiration of seminiferous plants. It is to be hoped that some of these cases will be explained after further studies on the decomposition of lactic acid by yeast in the presence of pyruvic acid.

The alcohol which was found in portions with lactic acid alone, was, of course, not produced from the latter, but appeared as the result of auto-fermentation, as the control experiment proved that the addition of neutralised lactic acid to zymin scarcely increases the quantity of carbon dioxide evolved and alcohol produced. Thus, 7 g. of zymin after 62 hours of auto-fermentation evolved 400 mg. of carbon dioxide and produced 417 mg. of alcohol.

Just as the negative results of Buchner's experiments do not prove that lactic acid cannot be the intermediate product of alcoholic fermentation; our own positive results—on account of the discovery of the wide capacity of killed yeast for decomposing the most dissimilar compounds, which are known to have no relation whatever to alcoholic fermentation¹—do not yet conclusively prove that lactic acid is the intermediate product of alcoholic fermentation. It remains to stop the alcoholic fermentation in the stage of lactic acid, and this is what we are now engaged upon. At any rate, our experiments give, firstly, a scheme which shows in which direction the work must be conducted, and, secondly, prove that *alcohol does not present the result of decomposition, but the product of reduction of acetaldehyde by removing the hydrogen from one of the intermediate products of decomposition of glucose.*

¹ For example, it is impossible, on account of the formation of aniline from nitrobenzene by killed yeast, to say that these substances are the normal products of metabolism in yeast.

The investigations on the significance of hydrogen during alcoholic fermentation and respiration have made a complete revolution in our ideas on these processes. They have proved that the reactions of reduction are of main importance. Firstly, on them depend the anaerobic oxidations. Secondly they have made it clear that, *during the formation of the intermediate products, in the molecule of glucose the hydrogen is displaced, and not the oxygen*, as Hoppe-Seyler supposed [1876]. Thirdly, the chemical processes of alcoholic fermentation and respiration cannot be explained by reactions of decomposition alone, as the hydrogen is displaced not only within the confines of one molecule, but also passes from one molecule into another. This means that during alcoholic fermentation and respiration the molecules act on each other reciprocally. Grey's experiments [1913] on the anaerobic fermentation of glucose by normal *Bacillus coli communis* (Escherich) and a strain derived from the normal organism by selection with sodium chloroacetate also speak in favour of the theory that lactic acid presents an intermediate product in alcoholic fermentation. The normal bacteria produce besides lactic acid,—acetaldehyde, alcohol and carbon dioxide. But artificially selected bacteria decompose glucose only as far as lactic acid, without producing either acetaldehyde or carbon dioxide, and producing very little alcohol.

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