LXXXVIII. DISMUTATION OF PYRUVIC ACID IN *GONOCOCCUS* AND *STAPHYLOCOCCUS*

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THE literature on bacterial metabolism contains references which suggest that the anaerobic dismutation of pyruvic acid which we have shown to occur in animal tissues (see the preceding paper) may also take place in certain bacteria. Barron *et al.* [1932; 1933; 1934; 1936], for instance, described experiments concerning the oxidation of pyruvic acid in *Gonococcus* and showed that there is a specific mechanism by which pyruvic acid is oxidized to form acetic acid and carbon dioxide. He assumed that this oxidation is directly effected by molecular oxygen and he named the enzymic system concerned "α-ketonoxidase". His data, however, also allow the assumption that the oxidation of pyruvic acid is brought about by the same type of dismutation which occurs in animal tissues:

$$2 \text{ pyruvic acid} + H_2O = \text{lactic acid} + \text{acetic acid} + CO_2, \qquad \text{.....(1)}$$

and the experiments reported in this paper demonstrate that this view is correct. Pyruvic acid is oxidized in *Gonococcus* by the anaerobic reaction (1), and the oxygen uptake which ensues after addition of pyruvic acid is due to a secondary oxidation of the lactic acid formed by reaction (1). Some other cocci react in the same way, *Staph. aureus* and *albus*, and *Streptococcus faecalis*. For convenience *Staph. aureus* was used for the majority of the experiments and only the salient points were studied in *Gonococcus* and the other organisms.

I. *Methods*

1. *Bacteria.* The organisms used in this work were obtained partly from the National Type Culture Collection, partly from the Department of Bacteriology of this University. Cultures were grown in Roux flasks for 18–24 hours; blood agar was used as medium for *Gonococcus*, broth agar for the other organisms. The bacteria were washed off with 0·9 % NaCl, the washings were filtered through glass wool, and the organism was subsequently washed on the centrifuge. The final sediment was suspended in 0·9 % NaCl and its concentration was determined by measuring the dry weight (drying at 100°). The stock suspension contained usually 5–10 mg. dry bacteria per ml.; it was kept in a refrigerator. Suspensions of *Staphylococcus* or *Streptococcus* showed no significant decrease of activity within a few days.

The stock suspension was diluted with saline for each experiment. Bicarbonate was added to adjust *p*H. The solution was equilibrated with gas mixtures containing N_2 and CO_2, the concentration of the latter usually being 5 %. Yellow phosphorus was employed for the removal of oxygen. The temperature was 40°.

2. *Chemical methods.* The chemical and manometric methods were those described in the preceding paper.

II. *Anaerobic decomposition of pyruvic acid by* Gonococcus *and* Staphylococcus

Barron has shown that *Gonococcus*, in the presence of air, oxidizes pyruvic acid according to the scheme:

$$\text{pyruvic acid} + \tfrac{1}{2}O_2 \rightarrow \text{acetic acid} + CO_2.$$

The following experiments show that CO_2 and acetic acid are also formed in the absence of air, but only 50 % of the theoretical yield of CO_2 and acetic acid is obtained (Exp. 1).

Exp. 1. Decomposition of pyruvic acid by Gonococcus

(*Gonococcus* in saline containing *M*/50 $NaHCO_3$. 3 ml. suspension in each cup. 5 % CO_2 in N_2.

		Cup 1 3 ml. suspension	Cup 2 3 ml. suspension 0·1 ml. pyruvate solution (411 μl.)
μl. CO_2 formed after	60 min.	18·8	68
	120 min.	26·6	117
	180 min.	31·3	152·5
	240 min.	36·0	173
μl. pyruvate initial		0	411
final		0	37
Extra CO_2			+137
Extra pyruvate			−374

Exp. 2 demonstrates the same ratio for $\frac{\text{pyruvic acid used}}{CO_2 \text{ formed}}$ in *Staphylococcus.*

Exp. 2. Anaerobic production of CO_2 from pyruvic acid by Staph. aureus

	Pyruvic acid used	Extra CO_2 formed	Ratio $\frac{\text{pyruvic acid used}}{CO_2 \text{ formed}}$	*p*H
a	503	249	0·496	6·5
b	503	227	0·452	6·5
c	1006	496	0·494	7·5
d	364	165	0·453	7·1
e	270	132	0·488	7·1
f	480	249	0·520	6·5
g	476	233	0·490	6·5
h	466	233	0·500	7·4
i	1808	857	0·474	7·1

Exp. 3 shows that the CO_2 is not derived from the bicarbonate of the saline, as the concentration of bicarbonate remains constant while CO_2 is evolved. The CO_2 arises therefore from decarboxylation of pyruvic acid. The constancy of the bicarbonate concentration shows furthermore, as explained in the preceding paper, that the substance remaining after decarboxylation of pyruvic acid is a fixed acid which binds the alkali set free by the decomposition of the pyruvate.

The fixed acid formed from pyruvic acid is steam-volatile and gives a positive lanthanum-iodine test [Krüger & Tschirsch, 1929; 1930]; it is thus acetic acid.

In Exp. 2*c* 498 μl. lactic acid were found in the solution after the evolution of CO_2 was finished. This shows that the second 50 % of pyruvic acid for which we have to account is converted into lactic acid. We have isolated the lactic acid as the zinc salt in a large-scale experiment following the directions of Embden & Kraus [1912] and of Warburg [1925] and identified it as the laevorotatory form which is the same optical isomeride that occurs in animal tissues. It should be mentioned that Kendall *et al.* [1930] and Sevag & Neuenschwander-Lemmer [1936] observed previously, in qualitative experiments, that pyruvic acid may undergo reduction to lactic acid in *Staph. aureus.*

Exp. 3. Anaerobic production of CO_2 and fixed acid from pyruvic acid by Staph. aureus

(Manometric experiment. Cups with 2 side-arms were used. 2 ml. bacterial suspension (8 mg. cocci) per cup. Side-arm 1 of each cup contained 0·2 ml. 5% H_2SO_4.)

	1	2	3	4
Side-arm 2	—	—	0·1 ml. pyruvate (=503 μl.)	0·1 ml. pyruvate (=503 μl.)
Time 0 (after 20 min. equilibration)	H_2SO_4 added	—	H_2SO_4 and pyruvate added	—
Change in pressure (mm.):				
10 min.	+78·5	+1·5	+126·5	+74·5
20 min.	—	+2·5	—	+137·5
30 min.	—	+3·5	—	+140
		H_2SO_4 added		H_2SO_4 added
40 min.	—	+81	—	+114·5
K_{CO_2}	2·036	1·874	1·438	1·622
Calculations:				
Initial bicarbonate (μl.)	160	—	182	—
Final bicarbonate (μl.)	—	152	—	186
Free CO_2 formed (μl.)	—	6·5	—	227
Total CO_2 found (μl.)	160	158·5	182	413
Change in total CO_2 (μl.)	—	−1·5	—	+231

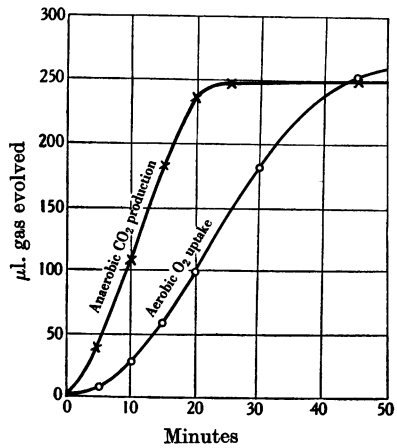

Fig. 1. Anaerobic CO_2 production and aerobic oxygen consumption in the presence of pyruvic acid. (*Staph. aureus.*)

Exp. 4. Aerobic and anaerobic decomposition of pyruvic acid by Staph. aureus

(*p*H 6·8; 3 ml. bacterial suspension containing 11 mg. cocci per cup. 0·1 ml. pyruvate solution (=503 μl.) was added at the time 0 from a side-arm after equilibration. The figures have been corrected for some (small) blanks. Buffer in (1) CO_3-$NaHCO_3$; in (2) phosphate.)

Time (min.)	(1) μl. CO_2 formed anaerobically	(2) μl. O_2 absorbed aerobically
5	38	7
10	106	27
15	182	55
20	236	96·5
30	246	180
45	249	251·5
95	249	328

The experiments reported so far show that 50% of the pyruvic acid is decomposed into CO_2 and acetic acid, whilst the second 50% is reduced to lactic acid. The evidence for the reaction (1) is therefore complete. If this reaction is a normal stage in the oxidative breakdown of pyruvic acid it has to be shown that the rate is at least that of the oxidative breakdown in the presence of molecular oxygen. Exp. 4 and Fig. 1 show that the rate of reaction (1) as measured by the anaerobic CO_2 production is in fact higher than that of the oxidation of pyruvic acid by oxygen.

The manometric determination of the CO_2 formed was used to study the effect of *p*H and of oxidation-reduction indicators on the rate of dismutation of pyruvic acid. In Exp. 5 the bicarbonate concentration was varied so that a *p*H range from 5·6 to 8·5 was obtained. The optimum is near *p*H 7·0.

Exp. 5. Effect of p*H on the dismutation of pyruvic acid in* Staph. aureus

(Bicarbonate-CO_2 buffer; 5% CO_2 in N_2; 1 mg. cocci per cup.)

Concentration of bicarbonate (in μl. per ml. solution)	5600	1865	622	207	69	23	8
*p*H	8·48	8·01	7·53	7·05	6·57	6·09	5·61
μl. CO_2 formed in 40 min.	9	81	130	133	146	102	96

The effect of indicators was investigated, as it seemed probable from the work of Green *et al.* [1934] that carriers of the type of oxido-reduction indicators might play a role in the dismutation of pyruvic acid. However, the only effect of indicators was an inhibition (Exp. 6).

Exp. 6. Inhibition of the dismutation of pyruvic acid by indicators in Staph. aureus

Indicator added	Conc. *M*	% inhibition
γ:γ'-Dipyridyl	10^{-2}	80
Benzylviologen	10^{-2}	94
,,	10^{-3}	52
,,	10^{-4}	12
Pyocyanin	$0{\cdot}8.10^{-3}$	90
,,	$0{\cdot}2.10^{-3}$	94
,,	$0{\cdot}5.10^{-4}$	53
,,	$1{\cdot}25.10^{-5}$	22
Lactoflavin	10^{-5}	18

III. *Activator in yeast*

Peters [1936] has shown that the oxidation of pyruvic acid in pigeon brain requires the presence of vitamin B_1. Since it appears to be the same reaction which brings about the oxidation of pyruvic acid in brain tissue and in *Staphylococcus*, and since it is known that vitamin B_1 plays a role in the metabolism of certain micro-organisms [Schopfer, 1935; Tatum *et al.* 1936], we decided to examine the effect of vitamin B_1 on the dismutation of pyruvic acid in *Staphylococcus*. A large acceleration of reaction (1) was found when commercial preparations of vitamin B_1 (Hoffmann-La Roche; Glaxo) were added. Crystalline vitamin, however, had no effect.

Extracts from brewer's or baker's yeast which had been heated to 90–95° for 10 min. also contained a very potent activator. The activation increases with time and may amount to 1000% (Exp. 7, Fig. 2).

Exp. 7. Effect of yeast extract on the dismutation of pyruvic acid

(Bacterial suspension containing 0·6 mg. *Staph. aureus* per 3 ml. in saline, $M/50$ $NaHCO_3$; 5% CO_2 in N_2. $M/50$ pyruvate. Yeast extract: Lebedev extract made from brewer's top yeast heated 10 min. in boiling water-bath, filtered. 1 ml. contained 47 mg. dry matter. The measurement of CO_2 production began 20 min. after the addition of yeast extract. Yeast extract alone had no effect on the CO_2 production. Increase beyond 0·1 ml. original extract had no further effect.)

Quantity of yeast extract added (ml.)	μl. CO_2 formed by 3 ml. suspension: 0	0·1 (100 times dilute)	0·1 (10 times dilute)	0·1 (Original)
Time: 20 min.	6·3	7·0	16·8	33·8
40 min.	12·6	14·7	35·6	87·2
60 min.	20·4	24·5	54·4	170
80 min.	26·8	32·9	73·2	266
100 min.	34·5	43·4	92·2	377
Q_{CO_2} (calculated for the last 20 min.)	39	58	95	555

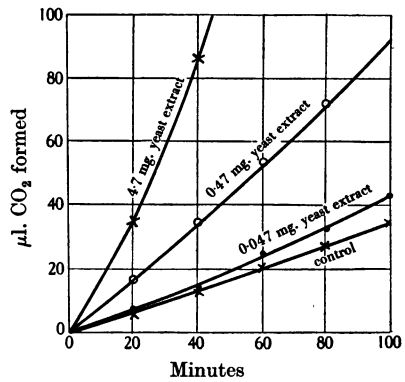

Fig. 2. Effect of yeast extract on the dismutation of pyruvic acid in *Staph. aureus*.

The ampoules of vitamin B_1 which we obtained from Messrs Hoffmann-La Roche and from Messrs Glaxo had an effect of the same order of magnitude as the extract used above. 0·1 ml. of the tenfold dilute preparation (=5 units vitamin B_1) added to 3 ml. suspension about doubled the rate of reaction. The effect seemed to increase with prolonged washing of the organism.

It is not only the anaerobic dismutation of pyruvic acid which is affected by yeast extracts. The oxygen uptake in the presence of glucose and other substrates is also increased, though less than the pyruvic acid dismutation (Exp. 8).

Exp. 8. Effect of yeast extract on oxidations in Staph. aureus

(Each cup contained 0·87 mg. cocci (dry weight) in 3 ml. phosphate saline; *p*H 7·1. Yeast extract: Lebedev extract heated at 100° (10 min.), filtered, 10 times diluted; 0·1 ml. added to 3 ml. suspension.)

Substrate ($M/300$)	Q_{O_2}: Without extract	With extract
—	2	10
Glucose	107	152
Glycerol	149	214
dl-Lactate	113	143
Pyruvate	81	149

It should be mentioned that the activating effect, or at least the bulk of it, is not due to growth which may occur in the presence of yeast extract. The volume of the cells and the dry weight of the bacteria increase relatively little (not more than 50 %), while the rate of dismutation may increase 1000 %.

The nature of the "activator" in yeast is not clear. It is possible that it is a substance related to vitamin B_1 (phosphorylated vitamin (?), see Lohmann [1937]) or to the "growth factors" described by Hirsch & Müller [1933] and by Knight [1936], but it seems more probable that the effect of yeast extract is due to the combined actions of several substances. We have examined several bodies known to occur in yeast and we have found some compounds which increase the rate of pyruvic acid dismutation, but none of these substances had quantitatively the same effect as the crude yeast extract. Substances which "activate" the pyruvic acid dismutation are fumaric acid, α-ketoglutaric acid, lactic acid, Warburg's yellow enzyme and Warburg's coenzymes (pyridinediphosphonucleotide and pyridinetriphosphonucleotide). Exp. 9 shows the effects of these substances. The yellow enzyme used was impure and contained 4×10^{-7} mol. per g., the pyridinetrinucleotide was pure, the dinucleotide was about 80 % pure.

Exp. 9. Effects of lactate, fumarate, α-ketoglutarate, yellow enzyme and the pyridinenucleotides on the dismutation of pyruvic acid in Staph. aureus

(Each cup contained 3 ml. bacterial suspension (3 mg. cocci); *M*/150 bicarbonate; 5 % CO_2 in N_2. Pyruvate (904 μl.) in 0·2 ml. was added from a side-arm after equilibration in cups 2–11).

	1	2	3	4	5	6
Substances added ...	—	—	0·3 *M*/5 *dl*-lactate	0·1 *M*/5 fumarate	0·1 *M*/10 α-keto-glutarate	37·5 mg. "yellow enzyme"
CO_2 formed after addition of pyruvate (μl.):						
10 min.	2	4·7	6·2	5·6	6·9	4·1
20 min.	3	7·9	10·4	12·7	18·7	9·6
40 min.	4·5	19	25	39·5	49	20·4
80 min.	7·5	41	53	97	115	51
120 min.	9	65	85	166·5	189	88
240 min.	15	150	186	411	425	251
360 min.	19·5	249	295	552	522	448
380 min.	20	263	311	556	523	455

	7	8	9	10	11	12
Substances added ...	0·25 mg. "diphospho-nucleotide"	0·2 mg. "triphospho-nucleotide"	37·5 mg. "yellow enzyme" +0·2 mg. "diphospho-nucleotide"	37·5 mg. "yellow enzyme" +0·2 mg. "triphospho-nucleotide"	37·5 mg. "yellow enzyme" +0·25 mg. "diphospho-nucleotide" +0·20 mg. "triphospho-nucleotide"	0·1 mg. yeast extract
CO_2 formed after addition of pyruvate (μl.):						
10 min.	4·2	4·0	5·1	5·1	5·1	9·2
20 min.	9·8	9·4	10·2	12	12·9	22·1
40 min.	21·3	21·4	27·2	32·6	33	50
80 min.	48	48	71	73	84	148
120 min.	78	79	122	131	144	315
240 min.	178	178	346	357	383	500
360 min.	316	290	498	504	514	504·5
380 min.	—	—	500	505	515	505

M/300 α-ketoglutarate increases the rate of reaction about 300 %, *M*/150 fumarate about 250 %. The effects of the yellow enzyme and of the pyridine-

nucleotides are small if these compounds are added singly, but a very marked effect is obtained if the yellow enzyme is added simultaneously with the nucleotides (100 % increase). Both nucleotides are about equally active and a larger effect is obtained if the two nucleotides are added together. A preparation of "cozymase" kindly supplied by Prof. Myrbäck had the same effect as the pyridinenucleotides.

The results obtained with the yellow enzyme and with the nucleotides suggest that these substances take part as catalysts in the dismutation, but it remains to be shown that the pure substances act in the same way, before the evidence can be considered conclusive. Further work is also necessary before the effects of lactic acid, fumaric acid and α-ketoglutaric acid can be explained. The last two substances increase not only the rate, but also to a small extent the yield of CO_2. They act in quantities which are small compared with the amount of pyruvic acid that they may cause to react; their action appears thus to be catalytic. No appreciable evolution of CO_2 is observed if fumarate or α-ketoglutarate is added to *Staphylococcus* in the absence of pyruvate.

IV. *Oxidations in* Staph. aureus

Since there are only a few data on record concerning the oxidative metabolism of *Staphylococci* [Warburg & Meyerhof, 1912; Meyerhof, 1917; Fujita & Kodama, 1934; Hirsch & Müller, 1933], some experiments are worth mentioning in which the rates of oxidation of several substrates and the total O_2 uptake were measured. In Exp. 10 the oxidations of glucose, *l*(−)lactic acid, pyruvic acid and glycerol were followed quantitatively. These were the only substrates which we found to be readily oxidizable.

Exp. 10. Rates of oxidation of various substrates by Staph. aureus

(Phosphate saline; *p*H 6·8; 3 mg. cocci in 3 ml. Substrates neutralized before addition. Air. Inner cup: 0·2 ml. 2*N* NaOH.)

	μl. O_2 absorbed					
Time (min.)	No substrate	1·040 mg. glucose	1·038 mg. *l*(−)lactic acid	0·973 mg. *d*(+)lactic acid	2·220 mg. pyruvic acid	1·90 mg. glycerol
10	5	34·1	20·4	6·3	8	42
20	7·5	69·5	36·6	12·0	16·8	85
40	12·5	146	70	19·1	35·8	178
60	16·1	216	99	25·4	56·2	—
120	24·7	327	162	34·6	109	—
160	29·7	342	194	40·2	143·5	—
200	32·1	351	228	44·5	176	—
260	38·3	362	253	51·5	215	—
340	42·6	367	278	57·2	254	—
Extra O_2 absorbed (μl.):		324	235	14·6	211	—
Calculated for complete oxidation (μl.):		775	744	725	1393	—
Q_{O_2} (first 40 min.):	6·2	73·0	35·0	9·6	17·4	89

The oxidation of the substrates is not complete. In the presence of glucose about 2·5 mol. O_2 are rapidly taken up per mol. of glucose. Thereafter a very slow O_2 uptake continues. In the case of *l*(−)lactate about one third of the theoretical amount of O_2 is absorbed rapidly and in the case of pyruvate, about one sixth.

Lactic acid is more slowly oxidized than glucose. It is therefore not admissible to assume that the primary step in glucose oxidation is a lactic acid fermentation. Glycerol is the only 3-carbon substance which is oxidized more rapidly than glucose.

The oxidations of glucose, *l*(−)lactate and glycerol begin at almost full rate as soon as the substrate is added, whilst there is a lag period if pyruvic acid is the substrate. This delay is expected to occur, since the primary dismutation of pyruvic acid shows an induction period. Barron observed a similar lag with gonococci which can be explained in the same way. This phenomenon lends support to the view that pyruvic acid is not oxidized as such but only after reduction to lactic acid.

In Exp. 11 data are given showing the rates of oxidation of a number of other substrates which are oxidized, though slowly, in *Staphylococcus*.

Exp. 11. Oxidation of various substrates by Staph. aureus

Substrate added	Q_{O_2}
—	5
Fumaric acid	6
Succinic acid	9
Formic acid	20
Acetic acid	8
α-Glycerophosphate	12
Glyceraldehyde	11
Glutamic acid	24

Dismutation of pyruvic acid in other micro-organisms

The following organisms were examined with the manometric technique to see if they form CO_2 from pyruvic acid under anaerobic conditions.

Streptococcus faecalis.
Staph. albus.
Sarcina lutea.
Pneumococcus Type I.
Bact. coli commune.
Bact. acidi-propioni 36 (No. 4759, National Collection).
Acetobacter pasteurianum (No. 613, National Collection).
Phycomyces nitens (Department of Botany, Sheffield University).
Phycomyces blakesleanus (National Collection).

The cocci and bacteria were grown on broth agar except *Acetobacter* which was grown on alcohol-malt agar; *Phycomyces* was grown on malt extract.

No evolution of gas was observed in the cases of *Sarcina lutea*, *Pneumococcus*, *Bact. acidi-propioni*, *Acetobacter* and *Phycomyces*. *Streptococcus faecalis* and *Staph. albus* showed the same reaction as *Staph. aureus* (Exps. 12 and 13). Moreover the same activating effect of yeast extract was observed in *Streptococcus faecalis* (Exp. 12).

Exp. 12. Dismutation of pyruvic acid in Staph. albus

(Each cup contained 4 ml. bacterial suspension (27 mg. dry cocci); *M*/300 bicarbonate. 5% CO_2 in N_2. The quantities of pyruvate used, lactate and CO_2 formed agree approximately with reaction (1).)

	1	2
Added to suspension ...	—	0·3 ml. pyruvate (=1400 μl.)
μl. CO_2 formed after addition of pyruvate:		
20 min.	7·1	75·5
40 min.	15·7	148
100 min.	39	379
190 min.	63·5	680
370 min.	86	865
Lactic acid formed (μl.)	106	822
Pyruvic acid used (μl.)	—	−1400
Extra CO_2 formed (μl.)	—	+779
Extra lactic acid formed (μl.)	—	+716

Exp. 13. Dismutation of pyruvic acid in Streptococcus faecalis

(Each manometric cup contained 3 ml. bacterial suspension in saline (7·5 mg. dry cocci; *M*/80 bicarbonate). 5% CO_2 in N_2; yeast extract as in Exp. 8. The experiment shows that the quantities of pyruvic acid used, CO_2 and lactic acid formed agree approximately with reaction (1) and that yeast extract accelerates reaction (1).)

Added to suspension ...	0·1 ml. yeast extract	0·1 ml. yeast extract 0·2 ml. pyruvate (=932 μl.)	0·2 ml. pyruvate (=932 μl.)
μl. CO_2 formed after addition of pyruvate:			
20 min.	11·1	22·6	14
40 min.	16·7	64	28·6
80 min.	23·6	142	46
160 min.	37·6	432	—
	42·0	460	—
Lactic acid found at the end (μl.)	116	512	—
Pyruvate used (μl.)	—	−932	—
Extra CO_2 formed (μl.)	—	+418	—
Extra lactic acid formed (μl.)	—	+396	—

In *Bact. coli*, CO_2 is formed anaerobically as is known from the work of previous investigators [Neuberg, 1914; Mazza & Cimmino, 1934]. The mechanism is, however, different from reaction (1) in that formic acid is first produced:

$$\text{Pyruvic acid} + \text{water} \rightarrow \text{acetic acid} + \text{formic acid}, \qquad \text{.....(2)}$$

and CO_2 and hydrogen are formed secondarily under the influence of "hydrogenlyase":

$$\text{Formic acid} \rightarrow CO_2 + H_2. \qquad \text{.....(3)}$$

In bicarbonate medium the rate of (3) is very slow as compared with the rate of (2) if *Bact. coli* is grown aerobically [Stephenson, 1937], and in the manometric experiment 1 mol. of CO_2 is liberated from the bicarbonate of the medium for each mol. of pyruvic acid fermented (whilst reaction (1) yields 1 mol. of CO_2 from 2 mol. of pyruvic acid).

It should be borne in mind that although in reaction (2) the elements of water are taken up it is not a hydrolysis, any more than reaction (1) is a hydrolysis. Reaction (2) is an intramolecular oxido-reduction and is physiologically equivalent to reaction (1) which is an intermolecular oxido-reduction. Both (1) and (2) are "fermentations" of pyruvic acid, i.e. energy-yielding reactions.

V. *Side reactions*

Reaction (1) is the main, but not the only reaction which occurs in *Staphylococcus* if pyruvic acid is available. This is indicated by the fact that the yields of CO_2 and lactic acid are not always exactly theoretical, and furthermore by the formation of other products such as succinic acid and acetoin. Succinic acid is regularly formed if large quantities of pyruvic acid are fermented anaerobically by *Staphylococcus*. For instance 76 μl. of succinic acid (118 mg. = 22400 μl.) were formed in an experiment in which 5600 μl. of pyruvic acid were fermented. The formation of traces of acetoin was qualitatively indicated by the positive Voges-Proskauer test (carried out according to the very sensitive modification of Barritt [1936].

VI. *Barron's "α-ketonoxidase"*

Barron [1936] showed that the oxidation of pyruvate by molecular oxygen in gonococci is inhibited by narcotics and fluoride, while the oxidation of lactate is not or is less affected. From this and similar work he concluded that two

different enzymes are concerned with the oxidations of the two substrates. The conclusion is certainly correct, but it does not follow from the evidence that the two enzymes are an "α-hydroxyoxidase" and an "α-ketonoxidase". The present paper demonstrates that the second enzyme is a "dismutase" and the specific inhibitions of pyruvic acid oxidation are due to the inhibition of the dismutation. If the view is correct, and if the O_2 uptake in the presence of pyruvate is due to an oxidation of lactate, it should be impossible to inhibit the O_2 uptake in the presence of pyruvate without inhibiting the oxidation of lactate. Barron was of opinion that there was evidence against this conclusion; he believed that HCN, H_2S and CO inhibit lactate oxidation more than pyruvate oxidation. In our view, however, his evidence is not valid, since (1) in the case of H_2S the CO_2 production instead of the O_2 uptake was taken as measure for the oxidation; (2) the concentration of HCN acid is decreased in the presence of pyruvate owing to reactions between pyruvic and hydrocyanic acids. The inhibition of cell respiration by HCN is generally diminished in the presence of pyruvate (see e.g. van Heyningen [1935]); (3) the differences in the effects of CO described by Barron are not sufficiently significant to allow any conclusion. A slightly smaller inhibition of pyruvate oxidation may be explained by Warburg's "saturation" hypothesis [1927], according to which the inhibition by CO must be less at low concentrations of the substrate. The actual concentration of the substrate for the oxidation (lactate) is necessarily low, if pyruvate is the added metabolite.

Summary

1. Pyruvic acid reacts anaerobically in gonococcus, *Staph. aureus*, *Staph. albus* and *Streptococcus faecalis* according to the equation: 2 pyruvic acid + water = lactic acid + acetic acid + CO_2. The data indicate that this "dismutation" is the preferential reaction by which pyruvate is broken down in these organisms. There is no direct oxidation of pyruvic acid by molecular oxygen. The oxygen consumption occurring in the presence of pyruvic acid is due to the oxidation of lactic acid formed by the dismutation.

2. The rate of the dismutation in *Staph. aureus* and *Streptococcus faecalis* is increased (up to 1000%) if boiled yeast extract is added. The nature of the "activator" in yeast is discussed.

3. As by-product of the anaerobic metabolism of pyruvic acid small quantities of succinic acid, equivalent to 1–2% of the pyruvic acid metabolized, are formed.

4. Data concerning the rate and degree of the oxidation of various substrates by *Staph. aureus* are given. The oxidations are also activated by yeast extracts.

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