ON THE GLUCOSE METABOLISM OF TRYPANOSOMES
(TRYPANOSOMA EQUIPERDUM AND TRYPANOSOMA LEWISI)

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Pathogenic as well as saprophytic trypanosomes utilize glucose and probably obtain from it the energy required for life and their especially active motion. It has been shown previously that glucose is decomposed by trypanosomes aerobically as well as anaerobically. Measurements on the oxygen consumption and acid production have been reported (1-4). Little is known, however, about the chemical mechanism of glucose decomposition. The present paper reports experiments by which the main steps in the breakdown of glucose by Trypanosoma equiperdum and Trypanosoma lewisi have been established.

Experiments with Trypanosoma equiperdum

Manometric measurements of the oxygen consumption showed that the amount of oxygen consumed by a certain number of trypanosomes is rather variable. This is probably due to the fact that the oxygen consumption, even in well buffered solutions, is not proportional to the time, but decreases continuously. The rate of the metabolism, therefore, depends to a great extent on the time required for the preparation of the trypanosome suspension. (An example is given in Table I.)

The rate of the oxygen consumption depends also on the medium in which the trypanosomes were kept before and during the experiment. This is strikingly demonstrated in an experiment, the results of which are given in Table II. It shows the effect of cell-

1 A preliminary paper on some of the experiments with Trypanosoma equiperdum was published by Reiner and Smythe (5).
Glucose Metabolism of Trypanosomes

free peritoneal exudate of a guinea pig on the respiration of the trypanosomes. Thus, it was not possible to establish absolute values for the rate of the metabolism. It was necessary to com-

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influence of Number of Trypanosomes (Trypanosoma equiperdum), Time, and Medium on Oxygen Consumption</td>
</tr>
<tr>
<td>Gas, 100 per cent O2; buffer, 0.1 M phosphate; initial pH, 7.6.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medium</th>
<th>Buffer</th>
<th>2 parts buffer + 1 part serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of trypanosomes per cc.</td>
<td>7.2 x 10^7</td>
<td>1.8 x 10^8</td>
</tr>
<tr>
<td>Time</td>
<td>O2 used per 10^8 trypanosomes per hr.</td>
<td></td>
</tr>
<tr>
<td>min.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>0-15</td>
<td>23.5</td>
<td>44.7</td>
</tr>
<tr>
<td>15-45</td>
<td>9.1</td>
<td>41.8</td>
</tr>
<tr>
<td>45-90</td>
<td>5.28*</td>
<td>38.9</td>
</tr>
<tr>
<td>90-210</td>
<td>34.0</td>
<td>31.3</td>
</tr>
<tr>
<td>210-215</td>
<td>33.2†</td>
<td>28.0‡</td>
</tr>
</tbody>
</table>

* Interval 45 to 120 minutes.
† Movement slightly impaired.
‡ Movement strongly impaired.

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of Effect of Glucose and Exudate Plus Glucose on Oxygen Consumption of Trypanosoma equiperdum</td>
</tr>
<tr>
<td>Gas, air; buffer, 0.1 M phosphate; initial pH, 7.6.</td>
</tr>
</tbody>
</table>

| Buffer, cc. | 1.00 | 1.00 | 1.00 |
| 0.85% NaCl, cc. | 0.6 | 0.6 | 0.3 |
| 6% glucose, cc. | 0.2 | 0.2 | 0.2 |
| Exudate, cc. | 0.2 | 0.2 | 0.2 |
| Trypanosomes, cc. | |

<table>
<thead>
<tr>
<th>Time</th>
<th>O2 consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>c.mm.</td>
</tr>
<tr>
<td>20</td>
<td>1.4</td>
</tr>
<tr>
<td>45</td>
<td>1.4</td>
</tr>
<tr>
<td>70</td>
<td>1.4</td>
</tr>
</tbody>
</table>

pare analytical data obtained simultaneously on the same suspension of trypanosomes.

The carbon dioxide liberated from solutions containing bicar-
carbonate was approximately proportional to the oxygen consumption. The ratio of carbon dioxide liberated from media containing bicarbonate (a measure of the total acid produced) to the oxygen consumed averaged $1.8 \pm 0.15$.

In another set of experiments the acids produced (except carbon dioxide) were determined by titration and compared with the glucose decomposed. The ratio of equivalents of acid to moles of glucose was $1.74 \pm 0.20$ in aerobic experiments and $0.92 \pm 0.13$ in anaerobic experiments.

These experiments suggest that 1 equivalent of acid is produced from 1 molecule of glucose anaerobically and that 2 equivalents of acid are produced from 1 molecule of glucose aerobically. 1 molecule of oxygen is required for the latter process, that is, for the oxidation of glucose.

To ascertain this further, the rate of glucose decomposition was compared with the rate of oxygen consumption. It was found, as expected from the comparison of acid production and oxygen consumption, that for 1 molecule of glucose, 1 molecule of oxygen was used up during long time intervals. The glucose decomposition took place at a higher rate at the beginning of an experiment and decreased with time more rapidly than the oxygen consumption. Correspondingly, the ratio of oxygen consumed to glucose decomposed was small at the beginning of an experiment and was great at the end of an experiment (when almost all the glucose was used up).

These experiments suggest that glucose, even under aerobic conditions, is first broken down without oxidation by molecular oxygen and that an intermediate product, which is not an acid, is responsible for the oxygen consumption. The resulting product is an acid. (Cf. Table III.)

In further experiments attempts were made to determine the products resulting from the anaerobic and aerobic decomposition of glucose. Carbon dioxide was not produced under anaerobic conditions and only very small amounts of it were found under aerobic conditions. The R.Q. was less than 0.1; i.e., $0.062 \pm 0.004$. The acid produced was not distillable by steam from solutions acidified with a small excess of sulfuric acid. Only a negligible fraction (2 to 12 per cent) of the acid was lactic acid.
A precipitate was obtained when the media in which the trypanosomes had been kept under anaerobic conditions for several hours at 37° were acidified with hydrochloric acid and mixed with a concentrated solution of phenylhydrazine acetate. This precipitate consisted of yellow needles which were centrifuged and recrystallized from alcohol. They had a melting point of 183.2°. The determination of the mixed melting point proved that the precipitate was the phenylhydrazone of pyruvic acid. The hydrazone was redissolved by adding sodium carbonate and recrystallized by acidification, washed, dried, and weighed. It corresponded to 50 to 75 per cent of the acid determined by titration.

Pyruvic acid was also formed under aerobic conditions. It was stated above the total acid produced per mole of glucose was roughly twice as much under aerobic conditions as under anaerobic conditions. The amount of pyruvic acid formed per mole of glucose was also roughly twice as much under aerobic conditions as under anaerobic conditions, the yield being in both cases 50 to 75 per cent of the total acid. This indicated that the acid formed aerobically was also mainly pyruvic acid.

Since it was established by these experiments that molecular oxygen is not involved in the first step of glucose decomposition, even under aerobic conditions, and that a resulting product is oxidized with 1 molecule of oxygen per molecule of glucose (or more exactly the products formed from 1 molecule of glucose),
and since it was established that the resulting acid is in both steps of the reaction pyruvic acid, the path of the glucose decomposition can be expressed by the following two equations.

(1) \(\text{CsH}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{COCOOH} + (\text{C}_3\text{H}_8\text{O}_3)\)
(2) \((\text{C}_3\text{H}_8\text{O}_3) + \text{O}_2 \rightarrow \text{CH}_3\text{COCOOH} + \text{H}_2\text{O}\)

The formula in parentheses is the empirical formula of the anaerobic breakdown product. It is the empirical formula of glycerol.

Kudicke and Evers (6) found that glycerol supports the life of trypanosomes. The substances, gluconic acid, methylglyoxal, glyceric aldehyde, glycic acid, pyruvic acid, lactic acid, glycerol,

| Table IV |
| Glucose Metabolism in Serum-Containing Medium (Trypanosoma equiperdum) |
| Medium, 1 part of rabbit serum + 2 parts of Ringer's solution. |
| Glucose used | Acid formed* | Acid: glucose | Phenylhydrazine of pyruvic acid | Glycerol |
| micro-moles | micro-equivalents | mg. | mole per cent | mg. | mole per cent |
| Anaerobic | | | | |
| 782 | 770 | 0.98 | 68.0 | 50 | 49 | 17.2 | 24 | 24 |
| 428 | 452 | 1.06 | 51.0 | 64 | 67 | 52.4 | 126 | 132 |
| Aerobic | | | | |
| 1050 | 1690 | 1.61 | 185.2 | 62 | 99 | Trace | |
| 630 | 1300 | 2.06 | 145.6 | 63 | 129 | None | |

* Except CO₂.

glycerophosphoric acid, acetaldehyde, ethyl alcohol, glycol, and formaldehyde, were tested concerning their ability to support the life of trypanosomes; glycerol alone did so but only under aerobic conditions. Quantitative experiments with glycerol showed that 1 molecule of oxygen was necessary for the production of 1 molecule of acid (liberation of carbon dioxide from solutions containing an excess of bicarbonate). The average ratio of acid produced per molecule of oxygen consumed was 0.99±0.03. Less than 5 per cent of the acid was carbon dioxide (R.Q. less than 0.05 in three experiments). The acid produced was identified as pyruvic acid. The yields were somewhat higher than those obtained in experiments with glucose.
The amount of glycerol produced from glucose under anaerobic conditions was estimated by the method of Zeisel and Fanto (7). It was found in accordance with Equation 2 that 1 molecule of glycerol is produced from 1 molecule of glucose under anaerobic conditions. 88 moles per cent was the average. No glycerol or only traces were found under aerobic conditions.

The glucose decomposition follows the same course if serum, peritoneal exudate, or other substances are present, which increase the life span of trypanosomes and assist oxygen consumption in vitro (cf. Table IV).

A comparison between normal trypanosomes and trypanosomes resistant to arsenicals indicated no qualitative difference in their carbohydrate metabolism. This part of the study is being continued.

Experiments with Trypanosoma lewisi

The oxygen consumption of these trypanosomes is proportional to the time throughout a fairly long interval. They are apparently less damaged under the conditions prevailing in in vitro experiments than Trypanosoma equiperdum, yet the rate of their metabolism, oxygen consumption, as well as glucose decomposition, is much less than that of Trypanosoma equiperdum. The initial rate of oxygen consumption for Trypanosoma equiperdum was about 50 cc. of oxygen per hour per 10^10 trypanosomes. The same value for Trypanosoma lewisi is about 5 cc. of oxygen (cf. Table V).

The ratio of oxygen consumed to carbon dioxide liberated from solutions containing bicarbonate is 2.38±0.13.

Comparison of the acid (except CO₂) produced with the glucose consumed showed that under anaerobic, as well as under aerobic conditions, about 2.5 equivalents of acid were produced per molecule of glucose. The average of fourteen anaerobic experiments was 2.44±0.36 and the average of twelve aerobic experiments was 2.44±0.34. Thus, the acid production, if carbon dioxide is not included, is the same under aerobic and anaerobic conditions.

Comparison of the oxygen consumption with glucose decompo-
sition showed that less than 1 molecule of oxygen is used for 1 molecule of glucose decomposed. The ratio of oxygen to glucose increases with time and apparently approaches the value 1.0. These facts indicate that *Trypanosoma lewisi*, like *Trypanosoma equiperdum*, first decomposes glucose without oxidation by molecular oxygen even under aerobic conditions. A resulting product

**TABLE V**

*Rate of Oxygen Consumption and Its Variation with Time (Trypanosoma lewisi)*

Gas, air; medium, 0.1 M phosphate buffer; initial pH, 7.6.

<table>
<thead>
<tr>
<th>Time</th>
<th>O₂ per 10⁶ trypanosomes per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>cc.</td>
</tr>
<tr>
<td>0-15</td>
<td>4.84</td>
</tr>
<tr>
<td>15-30</td>
<td>4.68</td>
</tr>
<tr>
<td>30-50</td>
<td>4.83</td>
</tr>
<tr>
<td>50-120</td>
<td>2.23</td>
</tr>
</tbody>
</table>

**TABLE VI**

*Comparison of Rate of Glucose Decomposition with That of Oxygen Consumption and Carbon Dioxide Production (Trypanosoma lewisi)*

Gas, air; medium, 0.1 M phosphate buffer; initial pH, 7.6.

<table>
<thead>
<tr>
<th>Time</th>
<th>Glucose*</th>
<th>Oxygen</th>
<th>O₂:glucose</th>
<th>CO₂</th>
<th>CO₂:O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>c.mm.</td>
<td>c.mm.</td>
<td>c.mm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-45</td>
<td>241</td>
<td>115</td>
<td>0.48</td>
<td>115</td>
<td>1.00</td>
</tr>
<tr>
<td>45-120</td>
<td>141</td>
<td>155</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-120</td>
<td>382</td>
<td>270</td>
<td>0.71</td>
<td>267</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*180 mg. of glucose = 22,412 c.mm.*

is then oxidized. The oxidation of this product yields mainly carbon dioxide. The r.q. approaches the value 1.0 (cf. Table VI).

Qualitative tests showed that the medium in which the trypanosomes were kept under anaerobic conditions for several hours did not contain lactic acid or any other hydroxy acids, nor did it contain keto acids, formic acid, carbon dioxide, or any substance which, like oxalic acid, would be rapidly oxidized by permanganate at 100° in acid solution. About 10 per cent or less of the
Glucose Metabolism of Trypanosomes

total acid formed was distillable from the aqueous solution by steam. The distillate contained acetic acid.

The non-volatile acid could be extracted with ethyl ether by continuous percolation of the concentrated and acidified solution used as medium. After evaporation of the ether, crystals were obtained in various preparations, which melted between 178–182°. Recrystallization from water yielded a product which melted at 185° and was identified as succinic acid by mixed melting point determination. The crystals obtained from the ether extract corresponded to 64 to 78 per cent (average 73 per cent) of the total acid titrated.

**Table VII**

*Determination of Acetic Acid and Ethyl Alcohol*

<table>
<thead>
<tr>
<th>Medium, Ringer's solution.</th>
<th>Glucose used</th>
<th>Total acid</th>
<th>Acetic acid</th>
<th>Equivalent of total</th>
<th>Uranyl acetate test</th>
<th>Ethyl alcohol</th>
<th>Iodoform test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>micromoles</td>
<td>micromoles</td>
<td>per cent</td>
<td></td>
<td></td>
<td>micromoles</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>1192</td>
<td>3346</td>
<td>250</td>
<td>7.5</td>
<td>+</td>
<td>182</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>1089</td>
<td>3382</td>
<td>247</td>
<td>7.3</td>
<td>+</td>
<td>97</td>
<td>+</td>
</tr>
<tr>
<td>Aerobic</td>
<td>367</td>
<td>1100</td>
<td>175</td>
<td>15.9</td>
<td>+</td>
<td>69</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>728</td>
<td>2000</td>
<td>394*</td>
<td>19.7</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Contains 16 micromoles of formic acid.

Under aerobic conditions 15 to 25 per cent of the total acid was distillable by steam (average 22.3 per cent). Of this 2 to 4 per cent (less than 1 per cent of the total acid) was formic acid, the rest presumably being acetic acid (uranyl acetate test). The remaining (non-volatile) acid was again extracted with ether and identified as succinic acid. The yield in succinic acid thus isolated was 72 to 88 per cent (average 78.3 per cent) of the total non-volatile acid. The purity of these products, as determined by titration with NaOH, varied between 88 and 95 per cent. The melting point was between 177.8–180.6°.

The only non-acidic substance found under anaerobic and also under aerobic conditions was ethyl alcohol (iodoform test). Acetaldehyde and formaldehyde were not present. The yields in ethyl alcohol were small. They corresponded to 1 molecule of ethyl alcohol for 5 molecules of glucose or less. (Cf. Table VII.)
These results indicate that the first step in the glucose metabolism of Trypanosoma lewisi does not involve oxidation by molecular oxygen even under aerobic conditions. It consists in the formation of 1 molecule of succinic acid and 1 molecule of glycol (or acetaldehyde+H₂O) from 1 molecule of glucose.

\[
C_6H_{12}O_6 \rightarrow COOCH(CH_2COOH) + C_2H_5O_2
\]

This is followed under anaerobic conditions by the formation of acetic acid and ethyl alcohol, probably through formation of acetaldehyde and H₂O.

\[
2C_2H_4O_2 \rightarrow 2CH_3COH + 2H_2O \rightarrow CH_3COOH + CH_3CH_2OH + H_2O
\]

The reaction of Equation 4 also takes place under aerobic conditions, and in addition the following oxidations occur.

\[
(5-a) \quad 2CH_2COOH + 4O_2 \rightarrow 2HCOOH + 2CO_2 + 2H_2O
\]

\[
(5-b) \quad 2HCOOH + O_2 \rightarrow 2CO_2 + 2H_2O
\]

This scheme is in agreement with the results obtained. It accounts for the fact that the total acid, with the exception of CO₂, is approximately the same in aerobic and anaerobic experiments (Equations 3 and 4). It also accounts for the fact that oxygen is consumed only after the glucose decomposition is well started. The R.Q. should, however, be 0.8 according to Equations 5-a and 5-b. As a rule, values close to 1.0 were obtained (0.98±0.02). So far we are unable to account for this discrepancy. It is possible that part of the carboxylic acids is also oxidized or decarboxylated. The possibility that part of the glucose is completely oxidized also has to be considered. Whether or not it goes through the steps of succinic acid and acetaldehyde has not yet been determined. The yields in alcohol and acetic acid were much lower than that expected according to this scheme. 1 molecule of acetic acid and 1 molecule of ethyl alcohol should be formed from 2 molecules of glucose under anaerobic conditions (i.e., 20 per cent of the total acid should be distillable by steam). Less than half of this amount was actually found. This indicates that the reaction of Equation 4 does not go to completion. The fact that the acid distillable by steam was consistently higher under
aerobic conditions than under anaerobic conditions suggests that
the reaction of Equation 6 also takes place.

\[ \text{(6)} \quad 2\text{CH}_2\text{COH} + \text{O}_2 \rightarrow 2\text{CH}_3\text{COOH} \]

It is also conceivable that an intermediate product oxidizes part
of the formic acid formed to CO\(_2\). This would account for the
fact that the r.q. values found were considerably higher than those
calculated on the basis of Equations 5-a and 5-b.

**DISCUSSION**

Both pyruvic and succinic acid are often encountered as break-
down products of the glucose metabolism of bacteria and other
cells. They have been considered mostly as intermediate prod-
ucts or side products. To our knowledge the case of *Trypano-
soma lewisi* reported here is the first in which succinic acid was
found to be the main end-product of uninfluenced anaerobic and
aerobic glucose metabolism. Furthermore, the case of *Trypano-
soma equiperdum* is the first in which the end-product of unin-
fluenced anaerobic glucose metabolism is pyruvic acid and glycerol
and in which mainly pyruvic acid is formed under aerobic
conditions.

It is of interest to discuss what, if any, the intermediate steps
of the anaerobic metabolism might be in these cases, although
little can be said with certainty about them for they are probably
formed and decomposed intracellularly.

The anaerobic formation of glycerol and pyruvic acid may be a
result of a chain of reactions which, except for the participation of
phosphoric acid, which is questionable here, might be similar to
that discussed by Meyerhof (8) as a part of the glucose decom-
position by muscle.

\[ \text{Glucose} \rightarrow 2 \text{triose} \rightarrow \text{glycerol} \rightarrow \text{glyceric acid} \rightarrow \text{pyruvic acid} \]

Phosphorylation was not ruled out here, but there was no evi-
dence suggesting its occurrence. The fact that glyceric aldehyde
did not support the life of trypanosomes does not necessarily
contradict this scheme.

* See this review for additional references.
In the case of succinic acid formation by *Bacillus coli*, Grey (9) suggested that it is formed through the oxidative condensation of 2 acetic acid molecules. De Graaff and Le Fèvre (10) suggested that succinic acid might be formed through condensation, decarboxylation, and oxidation from 2 pyruvic acid molecules. Both of these possibilities are improbable in light of the facts (1) that neither acetic nor pyruvic acid was attacked by *Trypanosoma lewisi* and (2) that oxidation through molecular O₂ was excluded and oxidation of acetic or pyruvic acid by an unknown H acceptor was unlikely. (The environment was strongly reducing as indicated by the negative potentials obtained with blank platinum electrodes and by the complete reduction of methylene blue.)

Our results seem to indicate that the mechanism of glucose decomposition is very different with *Trypanosoma equiperdum* and *Trypanosoma lewisi*. Surprising is the fact that while *Trypanosoma equiperdum* produces two 3-carbon atom compounds, *Trypanosoma lewisi* forms a 2- and a 4-carbon compound.

**SUMMARY**

1. *Trypanosoma equiperdum* decomposes glucose anaerobically by forming 1 molecule of glycerol and 1 molecule of pyruvic acid from 1 molecule of glucose. This reaction is also the first step in the glucose metabolism under aerobic conditions. It is followed by the oxidation of glycerol to pyruvic acid and water so that the oxidative decomposition of glucose yields 2 molecules of pyruvic acid per molecule of glucose. Comparatively small amounts of lactic acid and carbon dioxide were found, possibly due to the metabolism of contaminating cells (leucocytes). The presence of serum or plasma does not alter the course of glucose decomposition. It seems to increase the rate of the anaerobic reaction. The glucose metabolism of normal trypanosomes and trypanosomes resistant to arsenicals showed no qualitative differences.

2. *Trypanosoma lewisi* decomposes glucose anaerobically by forming 1 molecule of succinic acid and presumably 1 molecule of glycol. The glycol is decomposed further, presumably to acetaldehyde and water and the acetaldehyde undergoes dismutation so that acetic acid and ethyl alcohol are also formed. The same reactions take place under aerobic conditions but in addition
acetaldehyde is oxidized to formic acid, carbon dioxide, and water. The final products of the aerobic metabolism are therefore succinic, acetic, formic, and carbonic acids and ethyl alcohol.

No indications were found that phosphorylation is involved in the glucose metabolism of either species.

**Methods**

**Trypanosomes**—The trypanosome strains were laboratory strains. The *Trypanosoma equiperdum* strain was kept for 6 years in white rats and guinea pigs. The *Trypanosoma lewisi* strain was kept for 2 years in white rats. The trypanosomes were obtained from the rat blood by fractional centrifugation previously described (2). To separate *Trypanosoma lewisi* from the plasma it was necessary to centrifuge for 15 minutes at 2300 R.P.M.

**Medium**—The trypanosomes were suspended in Ringer's solution heavily buffered with sodium bicarbonate. The Ringer's solution was made up in a 10 times concentrated form, and without buffer. It contained 72 gm. of sodium chloride, 1.92 gm. of anhydrous calcium chloride, and 3.36 gm. of potassium chloride in a liter. This solution was diluted with water, the buffer used, and glucose. The final bicarbonate concentration was usually 0.025 to 0.050. Less bicarbonate was used in some of the manometric experiments. If only the oxygen consumption was measured, phosphate buffer (0.1 m, pH 7.6) was used. The solutions always contained 0.3 per cent glucose. In some instances 2 parts of Ringer's solution were diluted with 1 part of fresh rabbit serum (cf. Table IV). For the preparation of peritoneal exudate a guinea pig weighing about 500 gm. was injected with 20 cc. of phosphate buffer (0.1 m, pH 7.6) containing 0.3 per cent glucose. Exudate was taken through a glass cannula 4 hours later. It was centrifuged free from cells before use. All experiments were carried out at 37° under as nearly as possible sterile conditions.

**Gasometric Measurements**—The oxygen consumption, the carbon dioxide consumption, and the carbon dioxide liberated from bicarbonate were measured in the Warburg-Barcroft apparatus. The carbon dioxide production in a solution buffered with phos-
Phosphate was measured as the difference of the gas expelled by dilute sulfuric acid from the medium containing the trypanosomes, at different time intervals. The carbon dioxide liberated from bicarbonate was determined by Warburg's 2 volume method based on the difference in the solubility of oxygen and carbon dioxide in water.

The acids produced were titrated with 0.02 N sodium hydroxide, with phenolphthalein as indicator. The centrifuged solution was first acidified with an excess of sulfuric acid, then boiled for several minutes.

The "volatile acid" was defined as the acid which could be distilled over by steam from a solution acidified with sulfuric acid. The solutions were aerated for 30 minutes before distillation to drive off carbon dioxide.

**Pyruvic Acid**—The medium was acidified, concentrated in a vacuum, and centrifuged free from insoluble material, then mixed with an excess of phenylhydrazine acetate. The precipitate was redissolved by adding sodium carbonate and recrystallized by acidification, centrifuged, washed, dried, and weighed.

**Glucose**—Hagedorn-Jensen method (11).

**Lactic Acid**—Friedemann, Cotonio, and Shaffer (12).

**Glycerol**—Zeisel and Fanto (7).

**Succinic Acid**—This was extracted with ether from solutions concentrated on the water bath. Continuous extraction (13) was used. The ether, acetic acid, and hydrochloric acid were evaporated. The crystals obtained were weighed and titrated with sodium hydroxide. The melting points taken on this material are uncorrected. The substance was recrystallized from water for identification by mixed melting point.

**Formic Acid**—This was determined by reduction of mercuric chloride (14).

**Acetic Acid**—This was determined by titration of the steam distillate. It was identified as uranyl acetate.

**Acetaldehyde**—The sulfite method and the fuchsin test were used on the distillate.

**Ethyl Alcohol**—The neutralized medium was fractionally distilled and the distillate oxidized with 0.1 N potassium dichromate in acid solution in sealed containers.
Glucose Metabolism of Trypanosomes

The experiments given in Tables I to VII are typical representatives of several experiments. Unless otherwise stated averages were calculated from the results of five or more experiments.

BIBLIOGRAPHY

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