

drug is lowered to 1:8000-1:16000 complete sporulation takes place after a retardation of 3 to 4 days.

Neoprontosil solution (one part) added to agar (4 parts) does not inhibit sporulation of the inoculated bacilli. This result can be compared with the observation of Domagk<sup>3</sup> who found that this drug, while effective against streptococci *in vivo*, does not inhibit their growth *in vitro*.

Since the biochemistry of the process of sporulation in general is not well known, the mechanism by which sulfanilamide suppresses it cannot be understood. The significance of a possible destruction of spore-produced catalase (Ruhle<sup>4</sup>) by the anticatalase properties of the oxidation products of sulfanilamide,<sup>5</sup> is only a matter of conjecture.

*Conclusion.* Sulfanilamide, when added to nutrient media, inhibits spore formation and induces degenerative changes in the bacilli of the *subtilis-mesentericus-mycoides* group.

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### Effect of Carbon Dioxide on Glucose Metabolism of Trypanosomes.

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In the course of the study of the glucose metabolism of trypanosomes it was found that *Trypanosoma lewisi* does not decompose glucose under anaerobic conditions unless the solution contains bicarbonate. This effect has been studied during the last 4 years. Although the mechanism of the carbon dioxide action has not been established definitely, some of the main results are given in this preliminary report.

It is well known that the presence of bicarbonate is necessary for the cultivation of certain bacteria. Werkman and coworkers<sup>1</sup> have

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<sup>3</sup> Domagk, G., *Deut. Med. Wochenschr.*, 1935, **61**, 250.

<sup>4</sup> Ruhle, G. L. A., *J. Bact.*, 1923, **8**, 487.

<sup>5</sup> Main, E. R., Shinn, L. E., and Mellon, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 272.

<sup>1</sup> Wood, H. G., and Werkman, C. H., *Biochem. J.*, 1936, **30**, 48.

found that carbon dioxide is actually used up by the propionic acid bacteria, and Wood and Werkman<sup>2</sup> have suggested that the utilization of carbon dioxide is linked with the succinic acid production. They believe that this 4 C atom compound is formed by the addition of CO<sub>2</sub> to a 3 C atom compound by means of a reaction which can be considered to be the reverse of decarboxylation.

*Trypanosoma lewisi* also forms succinic acid from glucose; this occurs under anaerobic conditions apparently in greater proportions than with any organism or tissue so far studied. At the same time CO<sub>2</sub> is used up. Depending on the concentration of bicarbonate present in the system, the ratio of mols of CO<sub>2</sub> assimilated to mols of glucose used varies from 0.25 to 1.0. The amount of succinic acid produced under these conditions is about 1 mol or sometimes more, per 2 mols of glucose. In addition to succinic acid, lactic acid, pyruvic acid, acetic acid and traces of formic acid are produced by this organism. As stated in a previous paper<sup>3</sup> about 2.5 acid equivalents other than CO<sub>2</sub> are formed per mol of glucose used. Analysis of the acids shows that we have accounted for 90% of the total acids produced. This corresponds to slightly over 6 C atoms per molecule of glucose, suggesting assimilation of CO<sub>2</sub>.

Glycerol does not support the life of *Trypanosoma lewisi* under anaerobic conditions even if NaHCO<sub>3</sub> is present. Under aerobic conditions this organism decomposes glycerol without formation of measurable quantities of titratable acid and with the production of CO<sub>2</sub>. The average respiratory quotient in experiments of long duration (3 hours or more) is 0.85, suggesting complete oxidation of glycerol. Propionic acid bacteria behave differently. Wood and Werkman<sup>2</sup> found that they form some succinic acid even from glycerol.

If glucose is used as a substrate under aerobic conditions, *Trypanosoma lewisi* does not use up, but produces CO<sub>2</sub>. At the same time all the other acids produced under anaerobic conditions can be found but in different proportions. The acetic acid production is increased while the succinic, pyruvic and lactic acid production is decreased. The amount of carbon in the produced titratable acids is less than that present in the glucose used. The shift from anaerobiosis to aerobiosis is thus reflected in the quantitative distribution of the acids produced, the most striking change being that the CO<sub>2</sub> is used under anaerobic conditions and produced under aerobic conditions. This

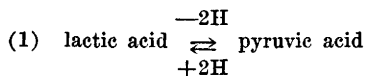
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<sup>2</sup> Wood, H. G., and Werkman, C. H., *Ibid.*, 1938, **32**, 1262.

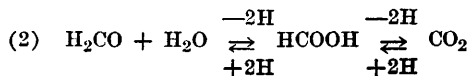
<sup>3</sup> Reiner, L., Smythe, C. V., and Pedlow, J. T., *J. Biol. Chem.*, 1936, **113**, 75.

indicates that the assimilation of CO<sub>2</sub> is linked with oxidation-reduction processes. Furthermore, it was found that under anaerobic conditions pyruvic acid also activates glucose metabolism of *Trypanosoma lewisi* in a similar manner but not to the same extent as CO<sub>2</sub>. In this case pyruvic acid is used up. Carbon dioxide is not produced but the production of lactic acid is increased to approximately the same extent as the utilization of pyruvic acid. This suggests that pyruvic acid functions as a H acceptor. It is, therefore, possible that the CO<sub>2</sub> assimilation occurs through the reduction of CO<sub>2</sub> to formaldehyde and subsequent condensation of formaldehyde.

Since under aerobic and anaerobic conditions, the reactions are essentially identical, the mere difference being a shift in equilibrium of the type



or



one would expect that CO<sub>2</sub> also affects the metabolism under aerobic conditions. This is difficult to demonstrate because CO<sub>2</sub> is quickly accumulated in the medium before quantitative measurements can be carried out. Qualitative experiments suggest, however, that bicarbonate activates glycerol under aerobic conditions.

While our results are in agreement with the assumption that the oxidation-reduction system (2) together with an enzyme acts as a H carrier and thereby activates the metabolism, it is possible that carboxylation of some intermediate is also involved.