ventral wound may cause the early death of the embryo or later on lead to the formation of a permanent fistula.

In one series of 16 anuran combinations, 12 reached late larval life and of these 8 metamorphosed into frogs. Each showed by the skin coloration the regions of the 2 species in combination. In another series of 15 combinations 7 are now in late larval life and one has metamorphosed. Four of these consist of 2 perfect reciprocal pairs.

In one series of 9 pairs of urodele combinations 3 have metamorphosed and one is approaching metamorphosis. In another series of 9 pairs, 3 are in mid-larval life. All of these are perfect specimens. Several were imperfectly united and lived to mid and late larval life when they were discarded.

The head, thorax and forelimbs of the anterior half of the combination attained the normal size and appearance of the species it represented. It, however, exerted a marked influence over the posterior half of the combination. The normally smaller anterior half inhibited the growth of the normally larger posterior half. The normally larger anterior half reversed the conditions by increasing the size of the normally smaller posterior half. In other words, the anterior half was always species dominant in its effect. This was very striking in the late larval and early adult life.

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Glucose Metabolism of the Trypanosoma Equiperdum in Vitro.

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It has previously been found that trypanosomes can live either aerobically or anaerobically. The oxygen consumption has been measured and in some instances also compared with the glucose utilization.[1, 2, 3, 4] In the present paper a report is given of measurements of the glucose and oxygen consumed and the products formed aerobically and anaerobically.

1 Yorke, W., and Nauss, R. W., *Ann. Trop. Med.*, 1912, **5**, 199.

2 Fenyvessy, B. v., and Reiner, L., *Z. Hyg.*, 1924, **102**, 109; *Biochem. Z.*, 1928, **202**, 75.

3 Issekutz, B. v., *Arch. Path. u. Pharmakol.*, 1933, **173**, 479.

4 Brand, T. H. v., *Z. vergleich. Physiol.*, 1933, **19**, 587.

The first set of experiments in which the oxygen consumed was compared with the total acid produced was carried out using the Barcroft-Warburg apparatus. The medium consisted as a rule of glucose dissolved either in Ringer solutions equilibrated with CO_2 or in isotonic phosphate buffer solutions, pH 7.4. It was found in experiments with phosphate buffer that the CO_2 produced is comparatively small. Hence, the CO_2 found liberated from bicarbonate solutions was chiefly due to the production of a non-volatile acid. The ratio of the mols acid produced to the oxygen consumed was fairly constant in all experiments and averaged 1.74 ± 0.07.

In another set of experiments the fixed acid produced was determined by titration with NaOH and compared with the glucose utilized, as determined by the Hagedorn-Jensen method.[5] The ratio of the mols of acid produced to the mols of glucose consumed was again near to 2, *viz.*, 1.80 ± 0.22 in aerobic experiments and near to 1, *viz.*, 0.87 ± 0.11, in the anaerobic experiments.

Lactic acid determinations by the Friedemann, Cotonio and Shaffer method[6] showed that only a negligible fraction of the non-volatile acid is lactic acid.* The fact that in aerobic experiments the ratio of mols acid produced to mols glucose consumed was near to 2 and that nearly 2 equivalents of acid were produced per mol oxygen consumed, suggested that 2 molecules of pyruvic acid or an isomer thereof, were produced by oxidation of one molecule of glucose. Indeed, the presence of pyruvic acid in the solutions was shown by the isolation of its phenylhydrazone. In more quantitative experiments 53-75% of the titratable acid was isolated and identified by mixed melting point as the phenylhydrazone of pyruvic acid. The acid produced under anaerobic conditions was again chiefly pyruvic acid. Fifty-two to 72% of the titratable acid was again recovered and identified as the phenylhydrazone of pyruvic acid. If one molecule of glucose is split to form one molecule of pyruvic acid, the empirical formula of the substance which remains is that of glycerol. This same empirical formula requires one molecule of oxygen for conversion to pyruvic acid.

It has been reported that glycerol is able to "revive" trypano-

[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

[6] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

* Traces of lactic acid may be produced by glycolysis by red cells. The trypanosomes were obtained by fractional centrifugation from an infected rat's blood, and as a rule a small number of red cells were present.

somes which have lost their motility because of lack of food.[7] We found that this "revival" takes place only under aerobic conditions. Trypanosomes suspended in glycerol containing buffered salt solution lose their motility in a very short time if kept anaerobically. Studies of the glycerol metabolism under aerobic conditions were made by the same methods as described above for glucose. The amount of total acid produced was, here, 1 mol per 1 mol of oxygen. No appreciable amounts of CO_2 were found. The presence of pyruvic acid was demonstrated by the action of yeast carboxylase and most of the titratable acid was recovered as the phenylhydrazone of pyruvic acid.

It remained to be seen whether or not the non-acid substance produced by trypanosomes anaerobically from glucose actually is glycerol. Determinations of glycerol according to the method of Zeisel and Fanto[8] showed that, in emulsions of trypanosomes containing glucose and kept anaerobically, this substance was present in amounts approximately equal to the amount of acid found. The average result of 3 experiments was 0.87 ± 0.11 mols acid and 0.97 ± 0.24 mols of glycerol per mol of glucose.

Preliminary experiments indicate that the presence of serum does not alter the described course of glucose metabolism. The evidence presented here for the production of large amounts of non-volatile acid agree with the fact that a decrease of the alkaline reserve is one of the symptoms of the experimental *Trypanosoma equiperdum* infection of rats.[9]

Summarizing, the following scheme is suggested for the main path of glucose utilization by trypanosomes *in vitro:*

$$\text{Glucose} \longrightarrow \text{Glycerol} + \text{Pyruvic Acid}$$
$$\text{Glycerol} + O_2 \longrightarrow \text{Pyruvic Acid} + 2\ H_2O$$

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[7] Kudicke, R., and Evers, R., *Z. Hyg.*, 1924, **101**, 317.

[8] Described in Allen's Commercial Organic Analysis, 5th ed., Vol. II, 666.

[9] Kligler, I. J., and Geiger, A., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 229; Kligler, I. J., Geiger, A., and Comaroff, R., *Ann. Trop. Med.*, 1929, **23**, 325; Scheff, G., *Biochem. Z.*, 1928, **200**, 307; Linton, R. W., *J. Exp. Med.*, 1930, **52**, 103, 695.