

that aliquot portions of each blood sample had to be used. The data from the remaining 4 experiments are closely similar to those shown in Fig. 2. Four of the 5 diabetic rabbits were sacrificed and submitted to necropsy. Necrosis and disappearance of pancreatic island tissues were evident. It thus becomes clear that our product, like the French, stimulates insulin secretion in rabbits with normal pancreas. The explanation for the occurrence of hyperglycemia in diabetic rabbits following the same compound requires further investigation.

**Summary.** 1. 2-Sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole, like other sulfonamides,

has a chemotherapeutic action against certain pathogenic bacteria. It has a low toxicity. There is no evidence of conjugation as judged by blood examination.

2. When given to normal rabbits, the substance uniformly causes hypoglycemia—with or without a slight initial rise of blood sugar. On the other hand, in diabetic rabbits induced by alloxan, it produces a definite rise of blood sugar.

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## 15647 P

Glycolysis in *Trypanosoma equiperdum*.

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For some time there has been doubt as to whether a phosphorylative mechanism is involved in the breakdown of glucose in *Trypanosoma equiperdum*.<sup>1-3</sup> The purpose of this communication is to report experiments concerned with the metabolic pathway involved in the degradation of glucose by a

blood-free preparation of lysed *Trypanosoma equiperdum*.<sup>\*</sup> Such a preparation was found to transform glucose into fructose 1, 6-diphosphate by phosphorylation, and to catalyze the splitting of fructose 1, 6-diphosphate and the coupled oxidation-reduction of 3-phosphoglyceraldehyde into phosphoglyceric

TABLE I.

The Transformation of Glucose into Fructose and Triose Phosphates by Lysed *T. equiperdum*.

The system contained 0.015 M NaHCO<sub>3</sub>, 0.003 M MgSO<sub>4</sub>, 0.015 M NaF, 0.015 M KCl, 0.012 M glucose, 0.003 M adenosine triphosphate (tipped in from side arm after equilibration), and 0.4 cc of a lysed preparation (equivalent to about  $3 \times 10^8$  parasites) in a total volume of 2.0 cc in Warburg flasks; gas phase 5% CO<sub>2</sub>-95% N<sub>2</sub>; temperature 38°C; time of experiment, 1 hr; 0.2 cc of 100% trichloroacetic acid solution added to terminate the reaction.

| Glucose utilized, | CO <sub>2</sub> evolved, | Fructose formed, | ATP split (7 min. acid P.), | *20 min. alk. P. formed, | †Increase of 2 hour acid P., |
|-------------------|--------------------------|------------------|-----------------------------|--------------------------|------------------------------|
| μg                | μl                       | μg               | μg                          | μg                       | μg                           |
| 1176              | 15                       | 779              | 107                         | 21                       | 50                           |

\* Triose phosphates.

† Glucose-6-phosphate and fructose 1-6-phosphate.

<sup>1</sup> Reiner, L., Smythe, C. V., and Pedlow, J. T., *J. Biol. Chem.*, 1936, **113**, 75.

<sup>2</sup> Barron, E. S. G., *Advances in Enzymology*, Vol. 3, 1943.

<sup>3</sup> Dorfman, A., *Physiological Rev.*, 1943, **23**, 124.

\* Fresh blood of an infected rat was made free of the blood elements by centrifugalization and washing twice with a 0.9% sodium chloride solution. A required amount of the parasites was then lysed with water.

TABLE II.

The Aldolase Activity of Lysed *T. equiperdum*.

The system contained 0.028 M  $\text{NaHCO}_3$ , 0.003 M  $\text{Na}_2\text{CO}_3$ , 0.015 M NaF, 0.015 M KCl, 0.008 M hexose-diphosphate (added from the side arm), 0.4 cc of a lysed preparation (about  $3 \times 10^8$  parasites) in a total volume of 1.8 cc in Warburg flasks; gas phase 5%  $\text{CO}_2$ -95%  $\text{N}_2$ ; temperature  $38.5^\circ\text{C}$ ; reaction time 30 min.; 0.2 cc of 100% trichloroacetic acid added to stop the reaction.

| Fructose split, | 20 min. alk. P. formed, |
|-----------------|-------------------------|
| $\mu\text{g}$   | $\mu\text{g}$           |
| 1140            | 201                     |

acid.<sup>2,4</sup>

The transformation of glucose into triose phosphates was shown by the formation of acid, fructose and 20-minute alkaline hydrolyzable phosphates from glucose and adenosine triphosphate (Table I). The aldolase activity may also be shown by measuring the disappearance of fructose 1, 6-diphosphate and the quantity of triose phosphates formed (Table II). The activity of 3-phosphoglyceraldehyde dehydrogenase is in-

TABLE III.

Effect of Pyruvate and Iodoacetic Acid on 3-Phosphoglyceraldehyde Dehydrogenase in Lysed *T. equiperdum*.

The samples contained 0.026 M  $\text{NaHCO}_3$ , 0.005 M  $\text{Na}_2\text{HAsO}_4$ , 0.03 M NaF, 0.00037 M DPN, 0.005 M fructose 1,6-diphosphate (tipped in from the side arm after equilibration), 0.5 cc of a lysed preparation (about  $2 \times 10^8$  parasites) in a total volume of 2.5 cc. 0.01 M Na-pyruvate or 0.0012 M Na-iodoacetate was added as indicated. Warburg manometers; gas phase 5%  $\text{CO}_2$ -95%  $\text{N}_2$ ; temperature  $37.5^\circ\text{C}$ . The values are the amounts of  $\text{CO}_2$  evolved in microliters.

| Sample                    | Time of reaction | 15'  | 30'  | 45'  | 60'  |
|---------------------------|------------------|------|------|------|------|
| Control system            |                  | 10.1 | 15.7 | 20.6 | 23.9 |
| Arsenate plus pyruvate    |                  | 12.0 | 19.3 | 25.0 | 27.7 |
| Arsenate plus iodoacetate |                  | 3.8  | 4.2  | 4.6  | 5.9  |

TABLE IV.

The Activity of Adenosine Triphosphatase in Lysed *T. equiperdum*.

The samples contained 0.4 cc veronal buffer of pH 7.4, 0.1 cc 0.154 M KCl, 0.1 cc 0.154 M  $\text{NaHCO}_3$ , 0.1 cc 0.0032 M ATP, 0.1 cc of a lysed preparation (about  $2 \times 10^7$  parasites), and 0.1 cc of 0.003 M  $\text{MgCl}_2$ , 0.015 M  $\text{CaCl}_2$ , or 0.015 M NaF; 0.1 cc of 100% trichloroacetic acid was added to terminate the reaction. Temperature,  $39^\circ\text{C}$ ; time, 1 hour.

| Sample               | % of ATP split |
|----------------------|----------------|
| With Mg              | 39.1           |
| With Ca              | 11.2           |
| With F               | 0.0            |
| Without Mg, Ca, or F | 9.8            |

indicated by the production of acid in a system containing fructose 1, 6-diphosphate, diphosphopyridine nucleotide and arsenate. The rate of this reaction is not influenced by the presence of pyruvate. Sodium iodoacetate inhibits the reaction (Table III).

Adenosine-triphosphatase activity is high in the preparation (Table IV).

**Conclusion.** Our experiments indicate that glucose is degraded into pyruvic acid through phosphorylative processes in a blood-free preparation of lysed *Trypanosoma equiperdum*.

<sup>4</sup> Speck, J. F., and Evans, E. A., Jr., *J. Biol. Chem.*, 1945, **159**, 71.