ness were observed. The time required for these signs to appear was about 15 days. At that time the daily diet was supplemented by oral administration of 25 mg of thiamin hydrochloride in aqueous solution. When the animals became acutely ill with scurvy, usually after 10 days, the thiamin hydrochloride was withdrawn and green food was offered freely. The animals were allowed this diet until definite signs of improvement were seen. At that time the green food was removed, administration of thiamin hydrochloride was resumed and the animals were again brought to the stage of acute scurvy. Repeated treatment in this sequence was employed.

The thiamin effect has been produced in 12 pigs by this procedure, or in roughly one-third of the animals subjected to the treatment. The effect has also been observed in 6 pigs made scorbutic and given thiamin according to variations in the above method.

No explanation for the symptoms or cause of death can be given at this time. Death was apparently not due to starvation, for when food was offered in macerated form enough was ingested to maintain life. Death did not appear to be due to scurvy *per se*, for animals maintained on macerated green food after the development of the symptoms and which had lived for a time after being placed on this diet did not show the usual signs of scurvy at autopsy. Death appears to have been due to some irreversible effect caused by the superimposition of large amounts of thiamin during scurvy. We have not succeeded in producing apparent toxic effects in normal guinea pigs by giving large doses of thiamin.

Pigs have been most susceptible to the thiamin effect during the late winter months.

Histological study of brain sections and peripheral nerves is in progress in an attempt to discover a possible neurological cause of the symptoms and death.

Apparently this effect of large doses of thiamin upon scorbutic animals has not been observed previously.

15646

Hypoglycemic Action of Sulfanilamido-cyclopropylthiazole in Rabbits, and Its Reversal by Alloxan.

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Janbon and his co-workers[1] showed that in man sulfanilamido-isopropylthiadiazole caused a fall of blood sugar, to such an extent that hypoglycemic shock occurred. Loubatières,[2] working with the same compound in dogs, reported that hypoglycemia appeared when the product was given by mouth or by subcutaneous injection. In depancreatized dogs, however, no drop of blood sugar was observed in spite of increased dosage. The author thus concluded that this thiadiazole stimulated insulin secretion, and that this action disappeared if the islets of Langerhans were absent. Bovet and Dubost,[3] and Loubatières[4] studied several analogues of sulfanilamido-isopropylthiadiazole, and found that the butyl, isobutyl, and amyl derivatives were more potent than the isopropyl.

Doctors L. P. Kyrides and F. B. Zienty, of Monsanto Chemical Co., St. Louis, Mo., generously supplied us with a compound closely related to the French product, and suggested an investigation of its effect on blood sugar

1 Janbon, M., Lazerges, P., and Métropolitanski, J. H., *Montpelier Méd.*, 1942, **21-22**, 489.

2 Loubatières, A., *C. R. Soc. de biol.*, 1944, **138**, 766.

3 Bovet, D., and Dubost, P., *C. R. Soc. de biol.*, 1944, **138**, 764.

4 Loubatières, A., *C. R. Soc. de biol.*, 1944, **138**, 830.

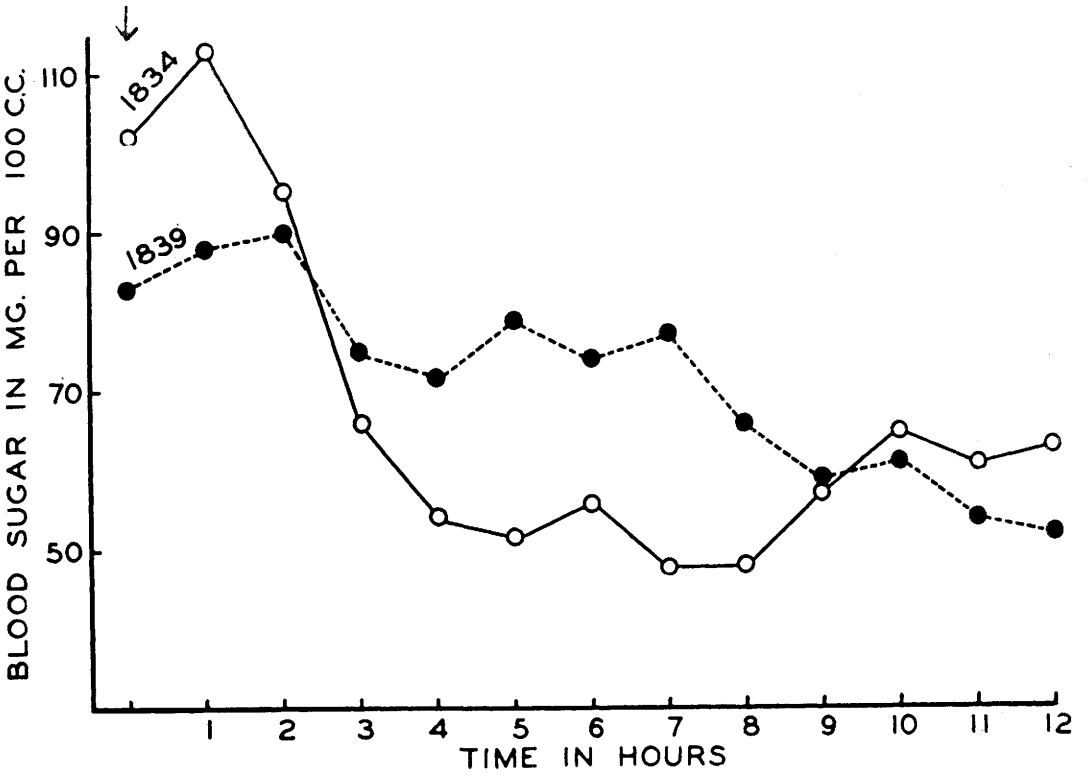
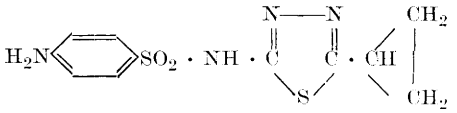


FIG. 1.

Hypoglycemic Action in Normal Rabbits.

The blood sugar readings of 2 rabbits are plotted in the chart. Both rabbits were male. At arrow, 2-sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole suspended in water was given by stomach tube—the dose being 1 g per kg. No convulsions took place at the height of hypoglycemia.

in addition to its other properties. Its chemical name is 2-sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole, and has the following formula:



The material melts at 221-222°C, and is very slightly soluble in water.

The present compound, like many sulfonamides, has a chemotherapeutic action against bacteria. According to the unpublished results of Dr. H. M. Powell of our own laboratories, it inhibits the growth of streptococcus and pneumococcus, type I, *in vitro,* and is capable of saving lives of mice infected with pneumococcus, type I, dysentery bacillus, and hemolytic streptococcus.

When given by mouth to rabbits in the dose of 296 mg per kg, it was appreciably absorbed but not to the same extent as sulfadiazine. The peak of blood concentration of the free form with that dose varied from 3 to 6.5 mg per 100 cc in 6 hours. No conjugation could be demonstrated in the blood stream. The toxicity of 2-sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole is low—failing to kill mice in a single dose of 25 g per kg given orally.

Six groups of 5 rats each were fed diets containing 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5% of this substance, respectively, for 22 days. All animals survived. Upon sacrifice, no renal lesions were discovered in spite of the high blood concentration, which varied from 20 to 23.5 mg per 100 cc. Lack of con-

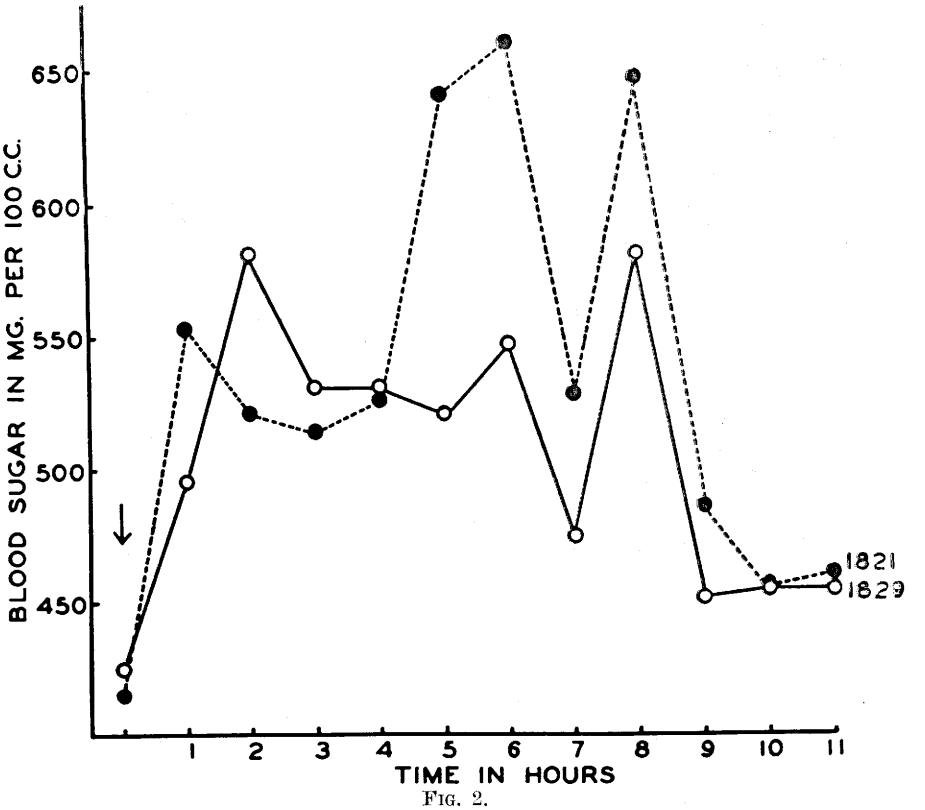


FIG. 2.

Hyperglycemic Action in Diabetic Rabbits.

Diabetes of rabbits was induced by intravenous injection of alloxan. Rabbit No. 1829 received a dose of 175 mg per kg; and the other, No. 1821, 200 mg per kg. Glucose in the amount of 2 g per kg was administered following alloxan at 2-hour intervals until each rabbit recovered from the crisis. At arrow, 2-sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole was given *per os* to rabbit No. 1829 in the dose of 1 g per kg, and to rabbit No. 1821 in the dose of 2 g per kg. The rise of blood sugar in each case is clearly shown by the curves.

jugation probably accounts for the absence of kidney damage. The only change uniformly observed was hypertrophy of the thyroid gland, which also occurs with other sulfonamides.[5]

The most unique feature of the compound is its hypoglycemic action following oral administration. Our experiments were carried out in rabbits. Fig. 1 shows the blood sugar curves of 2 starved animals, the method of Hagedorn and Jensen[6] being employed for the sugar determination. It should be noted that a dose of 1 g per kg reduced the blood sugar to as low as 48 mg per 100 cc in one, and 52 mg per 100 cc in the other, with a slight initial rise of blood sugar. Four additional rabbits received a smaller dose, 0.6 g per kg each, and all exhibited a fall of blood sugar without primary hyperglycemia.

A second group of rabbits was made chronically diabetic with alloxan according to Duffy's technic.[7] In our hands, about one-half of the animals died before diabetes was established. Six experiments were performed on 5 diabetic rabbits with oral administration of 2-sulfanilamido-5-cyclopropyl-1,3,4-thiadiazole. The dose varied from 1 to 2 g per kg. The results of 2 of them are charted in Fig. 2. It is obvious that an opposite effect occurred, namely, a rise of blood sugar. The figures of the latter were so high

[5] Mackenzie, C. G., and Mackenzie, J. B., *Endocrinology*, 1943, **32**, 185.

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

[7] Duffy, E., *J. Path. and Bact.*, 1945, **57**, 199.

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.

The authors are indebted to Dr. Paul N. Harris for his post-mortem examination of our diabetic rabbits, and to Miss Marian H. Ellaby and Mr. Harold M. Worth for their assistance in making the charts.

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.*[1-3] 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.** 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 $NaHCO_3$, 0.003 M $MgSO_4$, 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 3×10^8 parasites) in a total volume of 2.0 cc in Warburg flasks; gas phase 5% CO_2-95% N_2; temperature 38°C; time of experiment, 1 hr; 0.2 cc of 100% trichloracetic acid solution added to terminate the reaction.

Glucose utilized,	CO_2 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.

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

2 Barron, E. S. G., *Advances in Enzymology*, Vol. 3, 1943.

3 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.