1. Montgomery, A. V., Prevedel, A. E., and Swan, H., *Circulation,* 1954, v10, 721.

2. Covino, B. G., Wright, R., and Charleson, D. A., *Am. J. Physiol.,* 1955, v181, 54.

3. Rapport, D., and Beard, H. H., *J. Biol. Chem.,* 1929, v73, 209.

4. Gubner, R., DiPalma, J. R., and Moore, E., *Am. J. Med. Sc.,* 1947, v213, 46.

5. Hegnauer, A. H., Shriber, W. J., and Haterius, H. O., *Am. J. Physiol.,* 1950, v161, 455.

6. Weir, J. B. de V., *J. Physiol.,* 1949, v109, 1.

7. Petersen, J. C., Stripe, M. C., and Himwich, W. A., *Am. J. Physiol.,* 1955, v181, 519.

8. Newburgh, L. H., *Physiology of Heat Regulation and the Science of Clothing.* W. B. Saunders Co., Philadelphia, Pa., 1949, pp128.

Received April 9, 1956. P.S.E.B.M., 1956, v92.

Action of Butyltolylsulfonylurea on Liver Glycogenolysis.* (22465)

JEAN M. TYBERGHEIN, YADVIGA D. HALSEY, AND ROBERT H. WILLIAMS.

Department of Medicine, University of Washington School of Medicine, Seattle.

The hypoglycemic action of a sulfonamide derivative, sulfonamido-isopropylthiadiazole, was first discovered by Janbon and coworkers in 1941(1). Loubatieres(2) suggested that this compound caused hypoglycemia by stimulating insulin secretion. A similar conclusion was reached by Chen and coworkers(3) who investigated the hypoglycemic action of sulfanilamido-cyclopropyl-thiazole. It is possible that one mechanism by which the sulfonamide produces hypoglycemia is by inhibiting the rate of degradation of insulin, as has been reported by Mirsky(4) and Williams(5) to occur with certain other sulfonamides. Holt and coworkers(6) reported that sulfonamido-cyclopropylthiadiazole produces morphologic changes in the alpha cells of the pancreas in rabbits and they suggested that the compound caused hypoglycemia by inhibiting glucagon secretion. Franke and Fuchs(7), Bendfeldt and Otto(8), and Achelis and Hardebeck(9) attributed the hypoglycemic action of butylaminobenzenesulfonylurea to decreased production of glucagon. However, Tyberghein and Williams(10) found that extracts of serum of rabbits treated with butyltolylsulfonylurea (BTSU), which also produces hypoglycemia, had the same glycogenolytic stimulating activity in liver slices as extracts of serum of normal rabbits. Since there was good indication that this glycogenolytic effect was due to glucagon, these observations weaken the hypothesis of a decreased glucagon secretion. Therefore another mechanism was sought to explain the hypoglycemic effect of the sulfonamide preparations. An action on liver glycogen has been discussed by Miller and Dulin(11) and by Loubatieres(12). In the present study we investigated whether or not the hypoglycemic action of BTSU† could be related to an inhibition of glucose release by the liver and whether enzymes involved in glycogenolysis were affected by BTSU.

Methods. Some studies were conducted with rats and others with rabbits.

Rat Experiments. The effect of BTSU on blood sugar and liver glycogen was studied in Sprague-Dawley rats, each weighing approximately 300 g, prepared in one of the 2 following ways: (a) fasted for 24 hours, or (b) fed a standard amount of glucose for 24 hours. One group was fasted since it was believed that in this manner an inhibitory effect of BTSU on glycogenolysis might be more apparent, since normally the glycogen stores are considerably depleted after 24 hours. In all experiments with rats 80 mg of the sodium salt of BTSU, dissolved in 2 ml of water, was given every 12 hours by stomach tube. The first dose coincided with the beginning of the

* This research was supported by funds granted by the Atomic Energy Commission, the U.S.P.H.S. and the Upjohn Co.

† We are indebted to Dr. C. J. O'Donovan of the Upjohn Co. for generous supplies of 1-3-butyl-p-tolylsulfonylurea ("Orinase").

TABLE I. Effect of BTSU on Blood Glucose and Liver Glycogen in Rats.

	No. of animals		Blood glucose, mg %		Liver glycogen, mg/g tissue	
	Controls	Treated BTSU	Controls	Treated BTSU	Controls	Treated BTSU
Fasted 24 hr	15	15	84 ± 8.4*	49 ± 4.3*	1 ± 1.6*	16.3 ± 4.3*
Fed glucose	8	8	106 ± 8.2	68 ± 5.6	50.5± 6	53.8 ± 3.9

* Stand. error of the mean.

fasting period or of the glucose feeding. The animals were sacrificed between 2 and 3 hours after the third dose. Blood was drawn for glucose determination(13) and the liver excised for glycogen determination(14). In the group of rats fed only glucose, 500 mg was given every 12 hours by stomach tube with or without BTSU.

The effect of BTSU on glucose-6-phosphatase activity was studied in rat liver homogenates. Liver homogenates were prepared from normal fasted rats and from fasted rats given three doses of BTSU, 80 mg each, by stomach tube, according to the method described earlier. Aliquots of the homogenates were incubated at 28.9°C with .08 M glucose-6-phosphate in 0.1 M citrate buffer, pH 6.45 (15). The formation of inorganic phosphate was measured at the end of 30 minutes incubation(16).

Rabbit Experiments. Studies were conducted in rabbits on the effect of BTSU given orally on the glycogenolysis of surviving liver slices. New Zealand white male rabbits, weighing approximately 2 kg were given 500 mg of BTSU every 12 hours for 4 days except for the first and the last dose which were 1 g each. The animals were sacrificed about 3 hours after the last dose was given. Liver slices, weighing approximately 100 mg each, were cut promptly and put in 20 ml beakers containing 2 ml of buffer solution (1 volume 0.1 *N* potassium phosphate buffer and 4 volumes 0.9% sodium chloride solution), pH 7.5; the mixture was incubated at 37° for 45 minutes.

Results. The data presented in Table I indicate that in fasted rats given BTSU the amount of liver glycogen is much greater than in fasted control rats. In rats fed only glucose for 24 hours the amount of glycogen in the liver was the same whether or not BTSU was given. In both the fasted and the glucose-fed rats there was considerable hypoglycemia in the BTSU-treated groups. The conversion of glycogen to glucose is probably impaired since there presumably was relatively little synthesis of glycogen in these animals. The finding that the glycogen content of the glucose-fed rats is the same with or without BTSU treatment, indicates that the conversion of glucose to glycogen is not significantly impaired by BTSU feeding. Data indicating that BTSU decreases liver glycogenolysis *in vitro*, also, are presented in Table II. The glucose output from liver slices of rabbits treated with BTSU was less than that from liver slices of control rabbits. It is of interest to note that this effect was obtained only if the BTSU was given to the animals before sacrifice. The addition of BTSU to the incubation medium *in vitro* did not significantly decrease the glucose output from liver slices of normal rabbits.

TABLE II. Effect of BTSU Treatment in Rabbits on Spontaneous Glucose Output from Liver Slices.*

Spontaneous glucose output, mg/100 mg liver tissue		% decrease in spontaneous glucose output by BTSU treatment
Controls	Treated BTSU	
1.17†	.97†	18
1.54	1.14	26
1.17	.94	19
1.33	1.02	23
1.39	1.18	16
1.51	1.18	22
1.45	1.08	21

* 2 rabbits used in each experiment.
† Mean value for 3 different slice preparations.

If BTSU blocked any step in glycogen synthesis one would expect a decrease in glycogen content in BTSU-treated, glucose-fed rats. This was not found. That there was not an increase in the liver glycogen in these rats as compared to those fed glucose only (despite

TABLE III. Effect of BTSU Treatment on Glucose-6-phosphatase Activity of Rat Liver Homogenates.

	μM inorganic phosphate liberated from glucose-6-phosphate in 30 min./mg protein*	
	Exp. I	Exp. II
Controls	.46 ± .03 (4)†	.48 ± .02 (3)
BTSU treated	.38 ± .01 (4)	.38 ± .02 (4)

* Determined by biuret method; protein values for treated animals averaged ca 95% of those for controls.

† Refers to No. of animals.

the hypoglycemia existing) may be explained by the fact that the amount of glucose given was sufficiently large to the make breakdown of liver glycogen of minor importance as compared to synthesis of liver glycogen. In this connection, it may be noted that the amount of glycogen in the glucose-fed rats was larger than in rats fed *ad lib.* (50.5 ± 6 compared to 35.3 ± 3 mg per g). The results described above are consistent with the hypothesis that BTSU-hypoglycemia is the result of a reduced rate of liberation of free glucose from liver glycogen. Since no obvious impairment of glycogen deposition was found, it seems reasonable to suppose that the irreversible step in glucose liberation is involved, *i.e.*, the reaction glucose-6-phosphate → glucose + phosphate.

To determine whether the glucose-6-phosphatase activity of BTSU-treated rats was reduced, compared to controls, the activity of liver homogenates in liberating inorganic phosphate from glucose-6-phosphate was measured. Table III contains data indicating a significant depression of the activity of this enzyme. Similar observations have been made by others(17). One mode of action of BTSU appears, therefore, to be a decrease in the activity of glucose-6-phosphatase, resulting in a decreased release of glucose by the liver. It is possible that this effect of BTSU is the result of a stimulation of insulin secretion or of decreased insulin degradation, caused by the drug. However, this effect of insulin requires 6-12 hours after insulin administration is begun(18), while preliminary experiments indicate that the effect of BTSU on glucose-6-phosphatase activity is more rapidly established.

Summary. 1. In fasted rats butyltolylsulfonylurea (BTSU) produces hypoglycemia and decreased liver glycogenolysis. 2. In rats fed glucose only, BTSU also produces hypoglycemia but the amount of liver glycogen is comparable to that of untreated glucose-fed rats. 3. After treatment of rabbits with BTSU for four days, the spontaneous glucose output from liver slices was less than from liver slices of control animals. 4. The formation of inorganic phosphate by liver homogenate in the presence of glucose-6-phosphate is less in the BTSU-treated rats than in control rats, indicating that the glucose-6-phosphatase activity is less in the BTSU-treated rats than in control rats. 6. These observations tend to indicate that BTSU produces hypoglycemia at least partially by decreasing glucose-6-phosphatase activity in the liver.

1. Janbon, M., Lazerges, P., and Metropolitanski, J. H., *Montpellier Med.*, 1942, v21, 489.

2. Loubatieres, A., *C. R. Soc. Biol.*, 1944, v138, 766.

3. Chen, K. K., Anderson, R. C., and Maze, N., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 483.

4. Mirsky, I. A., Perisutti, G., and Diengott, D., *Metabolism*, 1956, v4, 156.

5. Williams, R. H., *Proc. of Metabolic Conference*, Carmel, Calif., Feb. 3-5, 1956.

6. Holt, C., Holt, L., Kröner, B., and Kühnau, J., *Arch. Exp. Path. Pharmak.*, 1955, v224, 66.

7. Franke, H., and Fuchs, J., *Deutsche Med. Wochnschn.*, 1955, v80, 1449.

8. Bertram, F., Benfeldt, E., and Otto, H., *ibid.*, 1955, v80, 1455.

9. Achelis, J. D., and Hardebeck, K., *ibid.*, 1955, v80, 1455.

10. Tyberghein, J., and Williams, R. H., *Clin. Fed. Proc.*, April, 1956.

11. Miller, W. L., Jr., and Dulin, W. E., *Science*, 1956, v123, 584.

12. Loubatieres, A., *Presse Medicale*, 1955, v63, 1701.

13. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

14. Good, C. A., Kramer, H., and Somogyi, M., *ibid.*, 1933, v100, 485.

15. Swanson, M. A., *ibid.*, 1950, v184, 647.

16. Gomori, G., *J. Lab. and Clin. Med.*, 1942, v27, 955.

17. Cahill, G. F., Jr., and Ashmore, J., personal communication.

18. Ashmore, J., Hastings, A. B., Nesbitt, F. B., and Renold, A. E., *J. Biol. Chem.*, 1956, v218, 77.

Received April 9, 1956. P.S.E.B.M., 1956, v92.