# Lack of Effect of Carbutamide on Activity of Rat Liver Glucose-6-Phosphatase. (22711)

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Carbutamide (BZ-55, or p-aminophenylsulfonyl butyl carbamide) exhibits hypoglycemic activity in normal animals and humans and in some diabetic individuals(1,2,3). One possible explanation of this physiological activity is that carbutamide interferes with the conversion of glycogen to glucose by inhibiting one of the enzyme systems involved in this conversion. The observation of Miller and Dulin(4) that liver glycogen levels are elevated after the administration of 1-butyl-3-p-tolylsulfonyl urea, an analog of carbutamide, appears to support this explanation.

Glucose-6-phosphatase, one of the enzymes involved in the conversion of glycogen to glucose, is more active in the liver of the diabetic animal(5,6), and is less active in the presence of glycogen storage disease(7). This paper describes a study on the effect of carbutamide on glucose-6-phosphatase activity of rat liver *in vitro*, and correlates these findings with the levels of carbutamide which were found in liver and plasma of animals treated with the drug.

*Materials and methods.* Since it has been shown by deDuve(8) and Langdon *et al.*(6) that most of the glucose-6-phosphatase activity is found in the microsomal fraction of liver homogenates, it was decided to use these sub-cellular particulate fractions as the enzyme source. Therefore active microsomal suspensions were prepared from the livers of normal rats by a method similar to that described by Langdon. These were kept frozen until used. In this condition no decrease in glucose-6-phosphatase activity was observed over a period of several weeks. The reaction mixture consisted of 0.02 *M* substrate, either the sodium salt of glucose-6-phosphate or $\beta$-glycerol phosphate, .02 *M* maleate buffer, pH 6.5, carbutamide or ammonium molybdate solution in concentrations indicated elsewhere, and the microsomal suspension derived from 40 mg of liver and containing about 0.2 mg of nitrogen. The total volume was 0.5 ml and the final pH 6.5. Carbutamide was dissolved in NaOH solution and adjusted to pH 8.0 before addition to the reaction mixture. Phosphate was determined according to Fiske and SubbaRow(9). The enzymatic activity was indicated by percent of total substrate hydrolyzed. The hydrolysis in the presence of inhibitors was compared with that of control experiments for which the activity was expressed as 100. Following the administration of various amounts of carbutamide to normal fasted rats, the concentrations in liver and plasma were estimated by the method of Bratton and Marshall(10). Blood sugar levels were determined by the method of Haslewood and Strookman(11) immediately before treatment and just before sacrifice. To gain some idea of the extent of binding of carbutamide by plasma proteins, a plasma ultrafiltration method similar to that described by Coolidge(12) was used. Fractionation of the livers of treated rats into various subcellular components was made as described by Umbreit, Burris, and Stauffer(13).

*Results.* The enzyme preparation was characterized by determining the effect of substrate and enzyme concentration on the extent of the reaction, by utilizing a known inhibitor of the enzyme, and by using a substrate not readily hydrolyzed by glucose-6-phosphatase. The velocity of the reaction was directly proportional to the enzyme concentration. The reaction was linear for the first 20 minutes, after which time the rate decreased quite rapidly.

For the inhibition studies, an incubation time of 15 minutes was utilized to eliminate variations caused by inactivation of the enzyme(14). The substrate concentration of $2 \times 10^{-2}$ *M* served to minimize changes in substrate concentration during the course of the enzymatic reaction. Thus, in the inhibition studies the reaction followed zero order kin-

TABLE I. Effect of Carbutamide and Molybdate on Glucose-6-Phosphatase Activity of Rat Liver Microsomes.

| Addition, moles/l | % of substrate hydrolyzed 1 | 2 | 3 | 4 | Mean | Relative activity |
|---|---|---|---|---|---|---|
| None | 25.8 | 25.7 | 25.0 | 26.8 | 25.8 | 100 |
| Carbutamide $10^{-5}$ | 31.2 | 28.0 | 31.5 | | 30.5 | 118 |
| $10^{-4}$ | 26.6 | 27.4 | 27.2 | | 27.1 | 105 |
| $10^{-3}$ | 25.5 | 26.7 | 28.1 | | 26.8 | 104 |
| $10^{-2}$ | | 22.6 | 22.9 | | 22.8 | 88 |
| Ammonium molybdate $10^{-4}$ | | | | 16.2 | | 63 |
| $10^{-3}$ | | | | 2.3 | | 9 |
| $3.2 \times 10^{-3}$ | | | | 0 | | 0 |
| $10^{-2}$ | | | | 0 | | 0 |

TABLE II. Distribution of Carbutamide in Plasma and Liver of Rats 4 Hours after Oral Administration.

| Tissue | Dosage of carbutamide, mg/kg 1000 | 500 | 200 | 100† | 50 | 25 |
|---|---|---|---|---|---|---|
| Plasma, μg/ml | 1,165 | 676 | 330 | 250 | 95 | 73 |
| Plasma ultrafiltrate, μg/ml | 850 | 460 | 205 | 93 | 31 | 21–31 |
| Liver, μg/g | 665 | 358 | 137 | 94 | 66 | 35 |
| Liver water, μg/ml* | 900 | 485 | 185 | 127 | 89 | 47 |
| Blood sugar change, mg % | −15.5 | −21.0 | −17.5 | −5.2 | −14 | +13.5 |

* Calculated from an assumed moisture content for liver of 74%.
† Avg of 4 rats; all others on single rat experiments.

etics. The enzyme preparation was relatively specific for glucose-6-phosphate as substrate. Under identical conditions the hydrolysis of glucose-6-phosphate was 25 times the rate of hydrolysis of sodium β-glycerol phosphate.

Addition of carbutamide in concentrations from $10^{-5}$ to $10^{-2}$ *M* (0.27 to 270 mg per 100 ml) had no significant effect on the activity of the enzyme (Table I). In contrast, ammonium molybdate, a known inhibitor of glucose-6-phosphatase(15), produced complete inhibition of the enzyme at a concentration of $3.2 \times 10^{-3}$ *M*.

In Tables II and III are presented data on concentrations of carbutamide in plasma and liver after administration of the drug in doses of 25 to 1000 mg per kg orally. Plasma concentrations were higher than the concentrations in liver. When the drug was administered in doses greater than 100 mg per kg there was a fair correlation between the concentration of free carbutamide in plasma and the amounts of the drug dissolved in the water content of the liver. The latter was calculated by dividing the observed concentrations of carbutamide per g wet weight of liver by an assumed moisture content of the tissue of 74%. The concentrations observed in these *in vivo* distribution studies were all within the limits of the concentrations tested with the *in vitro* glucose-6-phosphatase assay, *i.e.* $3.3 \times 10^{-3}$ to $2.4 \times 10^{-4}$ *M*. Very little if any of the carbutamide found in the liver was bound to any particulate fraction.

TABLE III. Distribution of Carbutamide in Rat Liver Intracellular Fractions. Animals received 100 mg/kg orally; sacrificed 4 hr after treatment.

| Fraction | Exp. 1 (μg/g liver) | Exp. 2 (μg/g liver) |
|---|---|---|
| Whole homogenate | 133.0 | 97.0 |
| Nuclear | 5.0 | 2.5 |
| Mitochondrial | 4.3 | 0.7 |
| Microsomal | 2.5 | 2.0 |
| Supernatant | 100.7 | 104.0 |

*Discussion.* Although it seemed reasonable to postulate that carbutamide might exert its hypoglycemic activity by inhibiting glucose-6-phosphatase, the data presented here demonstrate that carbutamide had no significant effect on rat liver microsome preparations of glucose-6-phosphatase in a range of concentrations including those found to be effective in lowering blood sugar. Also the data show that very little of the drug was bound to the liver intracellular particles. Therefore the

hypoglycemic activity of carbutamide must be explained on some other basis than inhibition of glucose-6-phosphatase.

Vaughan(16) has reported a similar lack of effect of 1-butyl-3-p-tolylsulfonyl urea on the glucose-6-phosphatase activity of a rat liver homogenate. However, Hawkins, *et al.*(17) reported that oral administration of carbutamide to rats for a three-week period decreased the glucose-6-phosphatase activity of a liver homogenate prepared from these rats. No information was given regarding an immediate effect of carbutamide on the glucose-6-phosphatase activity of liver. Since the effect of carbutamide on blood sugar is observed within 2 hours, the lowered level of glucose-6-phosphatase reported by Hawkins may have no direct relationship to the hypoglycemic activity of the drug.

*Summary.* The concentrations of carbutamide in the liver and plasma of rats receiving a wide range of doses of the drug have been determined. When carbutamide was added to a rat liver microsome preparation in these concentrations no inhibition of glucose-6-phosphatase activity was observed.

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## Benzyldimethylbufotenin, a Powerful Antimetabolite of Serotonin. (22712)

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Although many antimetabolites of serotonin, capable of antagonizing specifically the actions of this hormone, have been discovered (1,2,3,4) only a few have proven to be active in whole animals. Most of them act only in isolated tissues, or exhibit only low potency in intact mammals(2,4). Even among those capable of affecting the whole animal many are only effective when administered parenterally, and fail by the oral route. Yet it is necessary to develop orally active compounds, as has been pointed out earlier(5), if useful drugs related to serotonin are to be achieved. Consequently we would like to report studies of a new antimetabolite of serotonin which is highly active in combatting the pressor action of this hormone, and one which is very effective even when fed.

The new antimetabolite is 1-benzyl-2, 5-dimethylbufotenin hydrochloride, which may be abbreviated to "benzyl analog of bufotenin" or BAB. The structural formula, as well as that of serotonin, is shown in Fig. 1. Here also is shown the benzyl analog of serotonin (BAS) which recently(4,6) has been found to be a highly active antiserotonin, capable of