Reports and Letters

In vitro Studies on the Action of Sulfonamide Hypoglycemic Agents

It has recently been reported by several German workers (*1, 2*) that a simple sulfonamide compound, N-*p*-amino-benzene-sulfonyl-N′-*n*-butyl urea, referred to as BZ 55, which possesses antibacterial activity, also causes a decrease in blood sugar both in normal animals and in certain diabetic patients. These investigators have used the drug, which can be given orally, for the treatment of selected patients with diabetes mellitus. On the basis of clinical observations and animal experiments, they have suggested that BZ 55 exerts its hypoglycemic action through its effect on the alpha cells of the pancreas, the presumed source of glucagon.

In this country, a related sulfonamide, N-toluene-sulfonyl-N′-*n*-butyl urea, called Orinase, has been found by workers at the Upjohn Company to have similar effects on blood sugar (*3*). They found that the liver-glycogen levels in rabbits that were made hypoglycemic with Orinase were the same as those in a group of untreated controls. The German workers had inferred also that BZ 55 did not cause depletion of liver glycogen (*1*). In view of these observations, it seemed of interest to determine whether Orinase might have some direct effect on the reactions involved in the conversion of liver glycogen to blood sugar. This communication summarizes the results of studies bearing on this problem and the results of experiments on the effect of Orinase (*4*) and BZ 55 (*5*) on the activity of rat-liver insulinase (*6*). It has been suggested that these drugs may inhibit the action of insulinase and thus cause hypoglycemia that is secondary to relative hyper-insulinism.

The insulinase assay system contained I^{131}-labeled insulin (*7*) plus carrier insulin (*8*), so that the final concentration of insulin equaled 0.2 mg/ml. Tris buffer (*9*) *p*H 7.5, containing sufficient versene to give a final concentration of $10^{-3}M$ were used. Orinase and BZ 55 solutions were adjusted to *p*H 7.5 before addition to the reaction mixture. After incubation of the mixture for 30 min at 37°C in air, the reaction was terminated by the addition of TCA, and aliquots of the supernatant were taken for counting in a scintillation counter. Concentrations of Orinase from 5×10^{-4} to $3 \times 10^{-3}M$ were tested, and no effect was observed on the activity of whole rat-liver homogenate or of a partially purified preparation of insulinase from rat liver (*10*). Likewise, no effect was obtained with BZ 55 at a concentration of $3 \times 10^{-3}M$.

In one type of glycogen-storage disease, liver glycogen is maintained at abnormally high levels with the occurrence of hypoglycemia, owing to a deficiency of liver glucose-6-phosphatase (*11*). It was conceivable that Orinase might cause hypoglycemia by interfering with the conversion of liver glycogen to blood sugar at the level of glucose-6-phosphatase. Glucose-6-phosphatase activity was determined by measuring the release of phosphate from glucose-6-phosphate as described by Cori and Cori (*11*). Orinase, at a concentration of $5 \times 10^{-3}M$, had no effect on glucose-6-phophatase activity in homogenates of rat or rabbit liver.

For measurement of glucose release by liver slices *in vitro*, normal, fed animals were killed by decapitation or by bleeding from the neck. The livers were kept in an ice-cold incubation medium of *p*H 7.4. The medium contained, per liter, 148 mmole Na^+, 5.3 mmole K^+, 10.3 mmole PO_4^{---}, and 138 mmole CL^-. For each experiment, a series of five or six flasks (a zero time, a control with medium alone, and three or four flasks with different additions), containing successive slices from a single piece of liver, were used. Each flask contained a single, weighed slice of liver (about 100 mg) in a total volume of 5 ml. Solutions of Orinase in the medium were prepared, and the *p*H of these solutions was adjusted to that of the original medium. When epinephrine was used, it was added immediately after the addition of the slice. Flasks were incubated for 30 min at 37°C in air. Glucose was determined by the method of Somogyi (*12*), using Nelson's color reagent (*13*) on aliquots of the medium after precipitation of proteins with copper sulfate and sodium tungstate.

Data on the release of glucose from rat- and rabbit-liver slices incubated at 37°C for 30 min with and without $5 \times 10^{-3}M$ Orinase are presented in Table 1. There is no significant effect of Orinase on glucose formation in the absence of epinephrine. The effect of epinephrine on glucose release by liver slices is also shown in this table. The glucose released by the slice incubated in medium alone, expressed as milligrams of glucose per gram of liver, has been subtracted from the glucose released in the presence of epinephrine (8 to 10 μg/ml), expressed in a similar fashion, so that the results are presented as the effect of epinephrine on glucose release in the presence and in the absence of $5 \times 10^{-3}M$ Orinase. It can readily be seen that the effect of epinephrine on glucose release is markedly diminished in the presence of Orinase. A few similar experiments were performed using amorphous insulin as a source of glucagon activity (*14*). In this brief series, it appeared that Orinase also inhibits the

Table 1. Glucose formation by liver slices with and without epinephrine. (Effect of Orinase is given in parentheses in the last three columns.

Material	Orinase $5 \times 10^{-3}M$	No. of animals	No. of experiments	Range	Mean	Standard error of mean
Glucose formation (milligrams of glucose per gram in liver)						
Rat liver	0	4	6	3.7–7.8	6.1	0.60
	+	4	6	5.0–7.6	6.1	0.45
				(−19.3–+43.2%)	(+2.3%)	(8.8)
Rabbit liver	0	3	9	3.6–7.4	4.7	0.48
	+	3	9	3.4–7.0	4.3	0.41
				(−29.8–+30.8%)	(−7.0%)	(5.6)
Epinephrine effect (increment in milligrams of glucose per gram in liver)						
Rat liver	0	3	5	+1.9–+4.5	+3.0	0.44
	+	3	5	+0.2–+1.7	+1.1	0.27
				(−32.0––89.5%)	(−61.9%)	(10.7)
Rabbit liver	0	3	10	+1.8–+4.4	+3.2	0.26
	+	3	10	−0.5–+2.5	+0.6	0.22
				(−43.2––118%)	(−85.7%)	(7.6)

glucagon effect under these conditions.

As Sutherland has pointed out (*15*), when normal, fed animals are used, changes in phosphorylase activity in the liver slices are reflected by changes in glucose output. He and his coworkers have shown that dog-liver phosphorylase is inactivated *in vitro* by liver-phosphorylase inactivating enzyme, which is a phosphatase (*16*), and is reactivated by dephosphophosphorylase phosphokinase, which is referred to as phosphokinase (*17*). He has deduced that epinephrine and glucagon may act by stimulating some portion of the phosphokinase system. If this is the case, it is suggested that Orinase may be effective in the experiments reported here by inhibiting phosphokinase, so that it cannot be activated by epinephrine or glucagon. However, there are several alternative explanations for the observed effect, including the possibility of an action of Orinase on glucose-6-phosphatase or phosphoglucomutase in the intact liver slice. Studies are now in progress to evaluate some of these alternatives and to determine whether the Orinase effect reported here is the mechanism by which it causes hypoglycemia *in vivo*. In view of the failure to demonstrate inhibition of insulinase activity by Orinase in concentrations that might reasonably be expected to occur *in vivo*, it seems unlikely that its hypoglycemic action is due to an effect on this enzyme. In addition, inhibition of insulinase alone would not easily explain the observations mentioned here on liver glycogen levels in animals given Orinase.

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Intravenous Bufotenine Injection in the Human Being

Evarts *et al.* (*1*) found that *n*-dimethyl serotonin, or bufotenine, causes a delay in trans-synaptic transmission at the geniculate ganglion in the optic tract of the cat. When the compound is injected I. V. in the monkey, the hind legs splay out in pseudoparaplegic fashion, and the animal becomes indifferent to noxious stimuli (*2*). A somewhat similar clinical motor response is seen in the rat after intraperitoneal injection, plus a perseverative beating of the forepaws, as if a virtuoso were attacking a piano *fortissimo*. In the dog, I. V. administration of bufotenine in doses of 4 mg/kg causes the same pseudoparaplegic splaying out of the hind legs, salivation, pilomotor response, an unearthly howling, which may persist for the better part of 2 hours, indifference to noxious stimuli, and apparent inability or unwillingness to defend itself when it is attacked by other dogs.

Plant preparations containing bufotenine have been used by primitive man to produce temporary ecstatic states of depersonalization and hallucination. Cohoba, the narcotic snuff of the Indians of Hispaniola and South America, was found by Stromberg (*3*) to contain this substance. The *mouch-more* (*4*) of the Koryaks and other Siberian tribes of the Kamchatka peninsula and the *flugsvamp* (*5*) of the Vikings, which also produced temporary psychoses, were the *Amanita muscaria* mushroom, which contains bufotenine (*6*). This indole is a constituent of the skin of poisonous toads and seems to be present in normal human urine in minute amounts (*7*).

On 12 October 1955, experiments on the I. V. injection of bufotenine were conducted at the Ohio State Penitentiary (*8*). Four healthy, young male convicts were used as subjects. All were above the normal intelligence level, all had been college students, none were recidivist criminals, and all were considered to be relatively stable emotionally. They were denied breakfast on the morning of the experiment.

Bufotenine (*9*) was dissolved in sterile, distilled water and drawn into a syringe. After venepuncture, blood was drawn into the syringe and admixed with the aqueous solution to a volume of 10 ml. This was injected slowly and steadily over a 3-minute period. The first subject received 1 mg of bufotenine in this fashion. Within 1 minute, after one-third of the injection had been completed, he complained of a tight feeling in the chest and a prickling sensation in the face as if he had been jabbed by nettles. Before the injection was completed, he experienced a fleeting sensation of pain in both thighs and a mild nausea. The prickly sensation in the face persisted for 6 minutes. There was no significant change in blood pressure or pulse.

The second subject received 2 mg of bufotenine over the same 3-minute period. During the first minute, he felt a tightness in his throat and a racing pulse, but objectively the pulse remained at the basic rate of 84 beats per minute. There followed a complaint of "tightness in the stomach," then a tingling in both pretibial areas. His face developed a purplish hue, and he had questionable nystagmus. Within 7 minutes after the end of the injection, all subjective complaints were gone, the facial color was normal, and no nystagmus was seen. No significant changes in blood pressure or pulse occurred.

The third subject received 4 mg of bufotenine in the afore-described fashion. Within a minute, he complained of a tingling and burning sensation in the face. In the second minute of injection, he complained of chest oppression, which quickly changed to "a load is pressing down from above and my body feels heavy." Before the injection was completed, he experienced a hollow feeling in the stomach, a numbness of the entire body, "a pleasant Martini feeling —my body is taking charge of my mind." At the time of completion of the injection, his pupils were dilated and he had bilateral nystagmus. Within a minute he reported, "I see red and black spots—a vivid orange-red—moving around." The spots changed in size and shape and persisted for 2 minutes. His face perspired and became purplish. Pupillary dilation and nystagmus were absent by the tenth minute of the experiment, but the facial color did not return to normal for 15 minutes. In retrospect, he stated that it was difficult to concentrate but that he had a feeling of great placidity during the experiment. No significant change in blood pressure or pulse occurred.

The fourth subject received 8 mg of bufotenine in the same fashion. He developed an almost immediate sensation of light-headedness as the injection began, then complained of a burning sensation in the face, which turned purple. Nausea and air hunger followed. He developed a transient hyperpnea for 30 seconds. The pupils were grossly dilated and there was moderate nystagmus. As the needle was withdrawn he blurted, "I see white straight lines with a black background. I can't trace a pattern. Now there are red, green, and yellow dots, very bright, like they were made out of fluorescent cloth, moving like blood cells through capillaries, weaving in and out of the white lines." This visual experience was present with eyes both open and closed, facial sweating and purpling was intense, nausea had abated, and the