Effect of Oral Hypoglycemic Drug (Carbutamide) on Glycogen Deposition By Isolated Rat Hemidiaphragm. (22809)

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Recently sulfonylurea derivatives have been introduced in the treatment of diabetes mellitus(1-4). Although these compounds are capable of lowering the blood sugar, their mechanism of action is unknown. Originally it had been postulated that this effect was mediated through destruction of the alpha cells of the pancreas(1-3), but subsequent work has failed to confirm this(5). Mirsky *et al.* have reported an inhibition of insulinase, an enzyme system in the liver capable of degrading insulin, and concluded that such inhibition of insulin destruction was the mode of action of the sulfonylurea compounds(6). Vaughan, using a smaller amount of Carbutamide (N-p-amino-benzene-sulfonyl-N'-n-butyl urea) was unable to detect any inhibition of insulinase(7). However, in other experiments she found that Orinase (N-toluene-sulfonyl-N'-n-butyl urea) significantly reduced the effect of both epinephrine and glucagon on the liberation of free glucose from liver slices(7). The physiological significance of these findings is open to some question in view of the observation that the hyperglycemic response to epinephrine and glucagon is not altered in diabetic patients successfully treated with the sulfonylurea compounds(8).

The present studies were done to ascertain whether Carbutamide* had any insulin-like effect on the metabolism of the isolated rat hemidiaphragm or whether it would augment the effect of a known amount of insulin.

Methods. Insulin *in vitro* is known to increase the glycogen deposition by the isolated rat hemidiaphragm as compared to its control hemidiaphragm not so exposed to insulin, but otherwise handled identically(9). This increment of glycogen deposition as a result of insulin, expressed in micromoles of glucose equivalents/g of tissue, is referred to as the insulin effect. The method of determining the insulin effect was the same as reported previously(10), except for the following modifications. In the experiments designed to determine the effect of Carbutamide alone on the glycogen deposition of the diaphragm no insulin was used. Instead, one hemidiaphragm was exposed to 2 ml of 0.04 M phosphate buffer (pH 6.8) containing 2 mg of Carbutamide for 1 minute while the control hemidiaphragm was exposed to buffer alone, prior to incubation of both for 90 minutes in 0.4% glucose dissolved in buffer. At the end of the incubation, the glycogen content of each hemidiaphragm was determined. In other experiments, one hemidiaphragm was shaken with 0.2 unit of insulin in 2 cc of buffer for 1 minute while the control diaphragm was exposed to only buffer. Subsequently each hemidiaphragm was incubated for 90 minutes in 2 ml of buffer containing 2 mg of Carbutamide and 0.4% glucose. Two other series of experiments were done; in one set one-tenth (0.2 mg) the amount of Carbutamide was used, while in the other series 2 mg of sulfadiazine were substituted for an equal amount of Carbutamide. Since the usual therapeutic blood level of Carbutamide in humans is about 10 to 15 mg %, the lower concentration used represented a physiologic amount while the higher one was in the range of that used by Mirsky *et al.*(6) and Vaughan(7).

Results. Table I summarizes the results. Carbutamide by itself did not exert any insulin-like action on the glycogen deposition of the isolated rat hemidiaphragm. The effect of 0.2 unit of insulin was an increase of 6.29 micromoles of glucose equivalent per gram of tissue. When one hemidiaphragm was exposed to 0.2 unit of insulin and then incubated in glucose and buffer containing 2 mg of Carbutamide, the insulin effect was reduced to 2.90 micromoles per gram of tissue. The

* Carbutamide used was generously supplied by Eli Lilly and Co., Indianapolis, Ind.

TABLE I. Effect of Insulin and Carbutamide on Glycogen Deposition of Rat Hemidiaphragm.

Material used with treated diaphragm	Treated diaphragm	Control diaphragm	Insulin effect
	μmoles (glucose equivalent)/g tissue		
Carbutamide, 2 mg	16.63 ± .74*	16.29 ± .90*	.34 ± .76* (6)§
Insulin, .2 unit	23.54 ± .97	17.24 ± .82	6.29 ± .59 (25)
Idem + Carbutamide, 2 mg	18.50 ± .95	15.60 ± 1.05†	2.90 ± .70 (18)
" + Carbutamide, .2 "	15.65 ± 1.71	10.74 ± 1.26†	4.91 ± .74 (9)
" + sulfadiazine, 2 "	17.37 ± 1.11	15.44 ± 1.69‡	1.93 ± 1.35 (6)

* Mean ± S.E.
† Control hemidiaphragm also treated with Carbutamide.
‡ " " " " " sulfadiazine.
§ No. of determinations in parentheses.

control hemidiaphragm was also incubated in glucose and buffer with 2 mg of Carbutamide, but it was not previously exposed to insulin. This represents a significant reduction of the insulin effect by Carbutamide ($P = < .01$). When the same experiment was repeated using a lower concentration of Carbutamide (0.2 mg), the insulin effect was 4.91 micromoles per gram of tissue, not significantly different from the insulin effect in the absence of Carbutamide. It should be noted that at this lower concentration of Carbutamide there was no enhancement of the insulin effect. If Carbutamide acts by inhibiting insulinase, and since insulinase has been reported in muscle (11), one might expect an augmentation of the insulin effect by these sulfonylurea compounds. Even though the rat hemidiaphragm is capable of a greater response to larger amounts of insulin,† the system as used in these experiments might not be sensitive enough to detect a small increment in the insulin effect. Nevertheless, when larger amounts of Carbutamide were used there was an unexpected and highly significant reduction of the insulin effect. Although these experiments do not indicate the mechanism of action of Carbutamide, they do tend to eliminate a direct insulin-like effect.

In an effort to determine the specificity of the inhibition of the insulin effect observed with the larger dose of Carbutamide, similar experiments were done using an equivalent amount of sulfadiazine. With this sulfonamide the insulin effect was also significantly reduced to 1.93 μmole/g of tissue ($P = < .01$). It thus seems that the inhibition of the insulin effect is not limited to the hypoglycemic sulfonylurea derivatives but is also shared by other sulfonamide drugs. The mechanism of this inhibition is not clear at this time. In the higher concentrations, these sulfonamide derivatives may act as non-specific protein poisons affecting several different tissue enzymes. On this basis it might also be possible to explain the inhibition of insulinase reported by Mirsky *et al.*(6) and the reduced epinephrine and glucagon response observed by Vaughan(7). The amounts of the sulfonylurea derivative used by Mirsky were 5 to 25 mg while Vaughan used 6.75 mg/100 mg of liver slices. In the present studies 2 mg were capable of inhibiting the action of insulin on the rat hemidiaphragm while one-tenth this amount had no such effect.

Summary. Carbutamide, a sulfonylurea compound capable of lowering blood sugar, did not have an insulin-like action in increasing glycogen deposition of isolated rat hemidiaphragm. Furthermore, this drug did not produce any enhancement of the insulin effect. In fact, when higher concentrations were used there was a significant inhibition of the insulin effect. This inhibition is somewhat non-specific since it was also observed when sulfadiazine was substituted for Carbutamide. It is suggested that this inhibition, the inhibition of insulinase, and the *in vitro* reduction of the epinephrine and glucagon response which have been attributed to the sulfonylurea compounds might all be due to a non-specific protein poisoning action of

† When 2 units of insulin were used instead of 0.2 unit, the insulin effect was 10.63 ± 0.46.

these drugs in higher concentrations.

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Epinephrine Sensitivity of Blood Vessel Strips from Salt-Fed and Castrated Rats.* (22810)

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The observation that high sodium chloride intake increased blood pressure in rats has been made by several investigators, including Tobian, Fregly, Meneely and associates, Sapirstein, and others(1-4). The salt diet apparently induced hypertension by increasing cardiovascular reactivity, since Raab(5) found that salt and DCA potentiated the pressor effects of epinephrine in rats. That blood vessels were involved was also suggested by findings of Tobian and Binion(6) that the aorta of rats on high salt diet contained more sodium than those on low salt.

In vitro studies of reactivity of blood vessels have been made by Franklin(7) and Furchgott and Bhadrakom(8), who showed that vessel strips constricted a measurable degree, when immersed in solutions of dilute epinephrine. Since high salt diet apparently increased sensitivity of the entire cardiovascular system to epinephrine, it appeared likely that vasoconstrictor functions of blood vessels might be implicated in the response. The object of this study was to determine if blood vessels from salt-fed rats differed from those of control animals with regard to epinephrine sensitivity.

Materials and methods. In the first experiments with 50 albino rats of Sprague-Dawley strain, about same age and size (80-100 g, and 60 days old), were used. The rats were separated at random into 2 groups, one fed a normal laboratory rat diet and tap water, while the other was given the same diet except that 2% sodium chloride by dry weight had been added, and drinking water changed to 0.9% saline. At the end of 10 weeks, control and salt-fed rats were removed in pairs at daily intervals and killed by blow on the head. The aortae were removed immediately from the level of the aortic arch to the origin of renal arteries. A segment 42 mm long was cut out and split longitudinally as shown in Fig. 1A. Incomplete transverse cuts were then made alternately from each side of flattened strip as shown in Fig. 1B. Control and salt-fed preparations were suspended in aerated Evans' Ringer's solution in 37° bath. Strips were attached to long heart levers, counterbalanced so that tension supplied by weight of levers was approximately 4 g. This weight acted to elongate the cut strips in such a way that circular and spiral

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