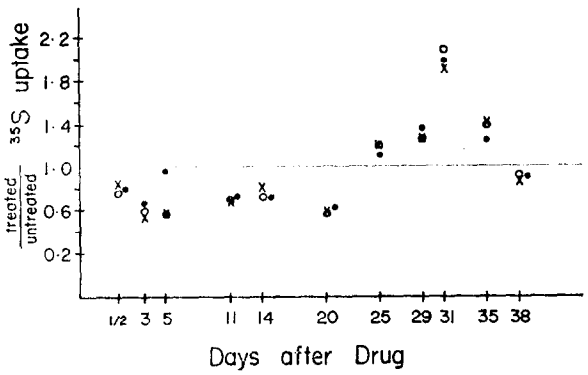

FIG. 2. Aqueous humor and blood. Solid circles, blood; open circles, right eye; ×, left eye.

Also, if one accepts as valid the premise that the ^{35}S levels measured here reflect, mainly, sulfated mucopolysaccharides, then a profound change is occurring in colour MPS. The changes that were measured in blood and nasal tissue would imply that this effect is not localized to the eye.

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Biochemical Pharmacology, Vol. 16, pp. 1117–1120. Pergamon Press Ltd. 1967. Printed in Great Britain

Effect of hypoglycemic agents on liver regeneration

(*Received* 18 *August* 1966; *accepted* 2 *December* 1966)

MANY oral hypoglycemic compounds have been synthesized and investigated in animals, but only a few with activity have survived clinical testing, because of their high toxicity, especially in provoking renal and hepatic reactions. Even with agents under use in man, such as carbutamide and metahexamide, to mention but two, severe liver damage has been reported.[1, 2] The current investigation was instituted with prominent therapeutic sulfonylurea and biguanide derivatives with the view of discerning hepatic changes as reflected in the extent of tissue weight restoration or the liver increment in partially hepatectomized rats, and to study the effect of prolonged systemic hypoglycemia, which must involve the liver directly or indirectly, on the regenerative process. In spite of numerous studies, there is little accord about the site and mechanism of action of the agents except that the hepatic glucose output is diminished. In this conjunction, carbutamide (N-sulfanilyl-N′-butylcarbamide) is claimed to cause a decrease in the rate of liver regeneration in operated rats force-fed one or four dosages, each at 2·0 g/kg and sacrificed 80 hr after surgery.[3, 4] The present communication also describes experiments with insulin NPH, glucagon, and the deproteinated extract Depropanex. The last, which is virtually free of insulin, has been employed clinically as a smooth-muscle antispasmodic and vasodilator.

Tolbutamide [N-(*p*-tolylsulfonyl)-N′-butylcarbamide; Orinase, Upjohn], chlorpropamide (N-propyl-N′-*p*-chlorphenylsulfonylcarbamide; Diabenese, Pfizer), acetohexamide [N-(*p*-acetylphenylsulfonyl)-N′-cyclohexylurea; Dymelor, Lilly], and phenformin·HCl (N′-β-phenylethylbiguanide·HCl; DBI, U.S. Vitamin) were each triturated with ground Rockland rat ration at the specified weight percentages. Insulin NPH and glucagon originated from Eli Lilly and Co., and Depropanex from Merck Sharpe and Dohme. The glucagon sample in amount of 0·07 μg/kg caused a maximal blood sugar rise of 30–40 per cent, 10–15 min after injection into cats.

Adult male Holtzman rats were partially hepatectomized under ether anesthesia by the technique of Higgins and Anderson[5] and the excised portions dried to constant weight at 100°. The animals were maintained in individual cages and given control and supplemented diets and water *ad libitum*. They were sacrificed (ether) 10·5 days after surgery and the entire livers removed and dried; very small sections of fresh tissue were reserved for microscopic study. In one series, insulin, glucagon, and Depropanex were injected subcutaneously daily for the first 7 days, the controls receiving saline; the volume in each case was 1·0 ml. The portion of tissue regenerated or the liver increment was calculated from the dry weights by subtracting the amount removed at surgery multiplied by the factor 0·46 from the liver at necropsy.[6]

Liver increment and body-weight data for animals fed the supplemented and control diets or injected with insulin NPH, glucagon, or Depropanex at daily dosages per kg of 3·3 units, 0·33 μg, and 0·33 ml, respectively, together with the Fisher *t* values in the requisite comparisons with the controls, appear in Table 1. Experiments with the higher concentrations of the agents were instituted toward evaluation of gross and microscopic alterations, but the findings for the various hematoxylin and eosin-stained liver sections were comparable to those of the given controls. The data from three or four animals of each of the series fed chlorpropamide (0·40%), tolbutamide (0·75%), or acetohexamide (0·75%), and from six animals on 1·50% tolbutamide, were discarded because of body-weight losses in excess of 12–15 per cent. Aside from the fact that starvation markedly depresses the liver increment, another consideration is that the response of animals to sulfonylureas is decreased under this condition.[7]

The sulfonylureas, acetohexamide, and chlorpropamide depressed liver regeneration at levels of 0·75 and 0·15 per cent respectively. The decrease with acetohexamide at 0·50 per cent was not statistically significant, and chlorpropamide at a more clinically realistic concentration (0·015%) was without effect, but 0·40 per cent caused marked inhibition, with body weight losses becoming greater. In direct contrast, tolbutamide at an enormous level was without action on the rate of regeneration but, as indicated, about 40 per cent of the rats were excluded because of weight losses. Phenformin·HCl was ineffective at 0·003 per cent but proved an extreme inhibitor at 0·075 and 0·15 per cent (in either case, $P < 0.01$).

Insulin NPH given up to concentrations of 3·3 units/kg daily for 7 days, like glucagon and Depropanex at the specified dosages, was without effect on the extent of regeneration. At the high dosage, nine of the insulin-injected rats succumbed and no attempt was made to force-feed the animals. Also, consistent with the design of these experiments, pair feeding was not employed. However, as based on only a few rats, the inhibitory action of several oral hypoglycemic compounds, described above, likewise extended to pair-fed series. Uberto[8] has demonstrated that the growth curve of the rat is not influenced by carbutamide or tolbutamide.

That hypoglycemia occurs with the sulfonylureas in partially hepatectomized animals can be inferred from the report of Dulin and Johnston,[9] who carried out perfusion experiments with both eviscerated and completely hepatectomized rats and dogs. The amides maintained their activity in the absence of the liver but were ineffective when the pancreas was removed. Accordingly, some insulin is required for the action of the ureas, according to this and a number of other reports. On the other hand, lowering of blood sugar occurs in the eviscerated guinea pig given phenformin,[10] and the action is thought to involve an indirect stimulation of anaerobic metabolism of glucose.[11, 12]

Although the mechanisms for the hypoglycemia are distinct, the inhibition of liver regeneration occurs with both the sulfonylureas (acetohexamide and chlorpropamide) as well as the biguanide. It might be construed that certain effects on the liver are common to both mechanisms or that the findings merely reflect a direct action of the agents on the regenerating liver at the higher or more pharmacologically significant levels. Furthermore, there is also a difference in inhibitory activity among the three sulfonylureas, tolbutamide being the least active, acetohexamide (0·75%) intermediate, and chlorpropamide (0·15%) most extreme. These differences might stem from the fact that chlor-

propamide is little metabolized and, therefore, the biological half-life depends on the rate of its excretion, which averages 35 hr,[13] whereas the period is about 5 hr for both tolbutamide and acetohexamide.[14–17] The clinical potencies based on biological half-life and acute potency have been advanced by McMahon and co-workers and follow a similar sequence.[18] The possibility that these compounds might elicit amino acid antagonism or inhibition of proteinogenesis in regenerating liver should not be overlooked. Determination of the hepatic nucleic acid turnover at various postoperative periods and of the rate of radiophosphorous uptake by the RNA fraction would be invaluable in this approach. It is unlikely that the observed inhibitory activity stems mainly from the

TABLE 1. LIVER REGENERATION IN THE PRESENCE OF HYPOGLYCEMIC AGENTS, GLUCAGON, AND DEPROPANEX

Duration: 10·5 days.

Treatment	No. of rats	Body weight (g ± S.E.) Initial	Body weight (g ± S.E.) At necropsy	Liver increment (g ± S.E.)	t
Dietary series (g/100 g ration)					
Group 1–8M					
Control	15	313 ± 4·8	321 ± 5·5	1·964 ± 0·060	
Chlorpropamide (0·015)	14	311 ± 3·3	304 ± 2·0	1·904 ± 0·105	0·50
Phenformin·HCl (0·0030)	16	305 ± 3·1	292 ± 5·5	1·902 ± 0·114	0·56
Group 2–12F					
Control	17	252 ± 4·0	275 ± 6·3	2·117 ± 0·075	
Chlorpropamide (0·15)	10	264 ± 7·1	263 ± 6·5	1·618 ± 0·098	4·05*
Phenformin·HCl (0·075)	9	266 ± 5·5	265 ± 4·4	1·721 ± 0·144	3·17*
Group 3–1F					
Control	15	239 ± 4·8	254 ± 6·0	2·041 ± 0·093	
Acetohexamide (0·50)	12	248 ± 6·1	259 ± 6·0	1·875 ± 0·081	1·30
Group 4–15P					
Control	13	243 ± 3·4	268 ± 7·5	2·013 ± 0·111	
Tolbutamide (0·75)	9	237 ± 3·6	241 ± 7·0	1·836 ± 0·066	1·21
Acetohexamide (0·75)	7	242 ± 3·7	238 ± 6·0	1·629 ± 0·077	2·36†
Group 5-13F					
Control	13	248 ± 5·9	283 ± 7·6	2·249 ± 0·115	
Chlorpropamide (0·40)	9	257 ± 7·9	229 ± 7·8	1·606 ± 0·067	4·47*
Phenformin·HCl (0·15)	11	250 ± 5·7	239 ± 8·3	1·818 ± 0·075	3·00*
Group 6–5G					
Control	14	271 ± 7·3	292 ± 8·5	2·090 ± 0·114	
Tolbutamide (1·50)	8	260 ± 6·8	263 ± 7·8	1·851 ± 0·077	1·46
Subcutaneous injection‡					
Group 8–9G					
Control	13	288 ± 5·7	302 ± 8·6	2·175 ± 0·112	
Insulin NPH (3·3 units/kg)	6	294 ± 4·3	316 ± 4·3	2·123 ± 0·175	0·26
Glucagon (0·33 μg/kg)	12	288 ± 8·1	308 ± 8·0	2·374 ± 0·151	1·06
Depropanex (0·33 ml/kg)	12	295 ± 7·8	302 ± 10·1	2·130 ± 0·096	0·30

* $P < 0·01$.
† $P < 0·05$.
‡ Dosages per kg body weight injected daily for the first 7 days after surgery.

extent of the hypoglycemia engendered by the agents, considering the innocuous behavior of the high level of insulin (group 8-9G; in Table 1).

The inhibition of liver regeneration by carbutamide as described in the published accounts might not be too surprising. The agent is actually a sulfanilamide derivative, and the unsubstituted or parent compound has been shown to depress liver regeneration when added to the diet at 0·40%.[19] Sulfanilamide, which does not induce hypoglycemia, and the sulfonylureas have been shown to decrease

glucose 6-phosphatase activity of liver slices and, because of the higher dosages needed, presumably the involvement of this enzyme is inconsequential to the mechanism of the action.[20] In regard to other enzymes, the insulinase levels of rat liver are depressed in the presence of sulfonylureas. However, this effect requires relatively large amounts of the agents, and also, tolbutamide does not influence the half-life of the injected ^{131}I-insulin.[21] A greater effect of the enzyme might be engendered in relation to regenerating liver, a point not investigated in the present study.

The structural configuration of phenformin may predispose to the observed inhibition of liver regeneration since the parent compound, guanidine, depresses liver regeneration in the rat, an effect which extends even to arginine.[22] Guanidine itself is hypoglycemic[23, 24] and, as it displays high toxicity, it has served as a model for the screening of a large number of allied derivatives.

Acknowledgements—The author is indebted to Dr. E. Goldman, pathologist, for aid in the examination of the liver sections. This study was aided by the Ben M. Hurwitz Metabolic Fund of Northwest Institute for Medical Researdh.

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Biochemical Pharmacology, Vol. 16, pp. 1120–1126. Pergamon Press Ltd. 1967. Printed in Great Britain

Studies of the chemical nature of the α-adrenergic receptor—II. Investigation of the labeling procedure

(*Received* 26 *September* 1966; *accepted* 2 *December* 1966)

TWO PROCEDURES have been described for labeling the α-adrenergic receptors of rabbit aorta with ^{3}H- or ^{14}C-dibenamine hydrochloride.[1–3] In procedure I the receptors were partially protected with