Studies on Protection Against the Diabetogenic Effect of Alloxan by Glucose.* (27284)

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Although alloxan is widely used to produce experimental diabetes, the mechanisms of its toxic action on the beta-cells are not known. A promising approach to study this mechanism is the use of protective agents *in vivo*. A biochemically heterogenous group of compounds was found to protect the rat against the diabetogenic effect of alloxan(1,2). However, many of these substances did not prove very valuable since they seem to protect by a nonspecific adrenergic type of mechanism (3), or by reacting nonenzymatically with alloxan, *e.g.*, glutathione(4). Glucose(5) on the other hand seems to be among the few specific protecting agents which do not act by these mechanisms. The following hypotheses were suggested to explain the protection by glucose: (a) the reversal of the inhibitory effect of alloxan on hexokinase(5); (b) substrate and energy production from glucose through the glycolytic and oxidative pathways(6,7); and (c) enhancement of the release of epinephrine by the hexose(3). The present communication deals with testing of some of the metabolic products of glucose and 2 of its analogues for their protective effect against alloxan *in vivo*.

Materials and methods. Sprague-Dawley albino rats of both sexes weighing 100-150 g were used. They were fasted 24-48 hours before injection of alloxan. Unless otherwise stated, the substances to be tested were dissolved in water and injected into the tail vein 5 minutes prior to injection of alloxan through the same route. Routinely a dose of 40 mg of alloxan (Eastman) per kilogram body weight was used. Solutions of pyruvic, fumaric, L-malic and DL-lactic acids were neutralized to pH 7.4 with sodium hydroxide before injection. 3-Methyl-D-glucose† and α-D-methylglucoside (Eastman) were crystallized once from methanol before use. Blood sugar was determined by the Somogyi-Nelson procedure(8,9). Animals were considered diabetic if the non-fasting blood sugar values exceed 150 mg % 48 hours following injection of alloxan. Invariably blood sugar levels of the protected animals were lower than this arbitrary value and those of the unprotected animals were much higher (Tables I and II).

Results. The results summarized in Table I indicate that while I.V. injection of D-glucose at 0.006 Mole/kg of body weight gave full protection none of the citric acid cycle intermediates protected the animals under the same conditions. It should be mentioned, however, that in the case of L-malate and pyruvate it was not possible to use a dose equivalent to the glucose dose (*i.e.*, molar ratio 2:1) because of the toxicity of these compounds at such levels. On the other hand, a relatively large dose of lactate was found to be nonprotective. Two methyl analogues of glucose gave contrasting effects. Thus the introduction of a methyl group on carbon-1 abolished the protective effect of glucose while the introduction of the same group on carbon-3 did not change the efficacy of the sugar.

To test the possibility that glucose and its 3-methyl analogue are acting through enhancing the release of epinephrine, the effect of phentolamine (Regitine) was tested. The data presented in Table II indicate that, unlike adrenaline(10), the protection by glucose and its 3-methyl analogue is not blocked by phentolamine and therefore not mediated through enhancing the release of a vasopressor agent.

Discussion. The failure of phentolamine to reverse the protective effect of glucose and 3-methylglucose argues against an adrenergic type of mechanism. Although the data suggest a more specific mechanism, the protection by 3-methylglucose is hard to explain.

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This synthetic sugar has been reported to be nonmetabolizable(11,12) and was not a substrate for yeast(13), rat brain(14) or intestinal hexokinase(15). If these findings were extrapolated to the beta-cell hexokinase, it would seem unlikely that the hexoses protect by their metabolism through the glycolytic pathway, the citric acid cycle or the hexose-monophosphate shunt. This is in view of the fact that the activation of the sugar by hexokinase is an obligatory initial step for all of these metabolic pathways. However, before such a conclusion can be reached further work is required on the metabolism of sugars by the beta-cells. 3-Methylglucose should be a very useful tool in such studies.

TABLE I. Effect of the Intravenous Injection of Some Glucose Analogues and Some of the Citric Acid Cycle Intermediates on the Diabetogenic Action of Alloxan.

Compounds*	Dose inj., Mole/kg	Range of blood sugar, mg %†
D-Glucose	.006	92-130
α-D-Methylglucoside	.006	434-640
3-Methyl-D-glucose	.006	88-126
L-Malate	.008	325-460
DL-Lactate	.018	382-438
Pyruvate-fumarate mixture	.006 & .003 resp.	333-393

* Compound tested for protection was inj. intrav. 5 min. prior to alloxan inj.
† 4 to 7 rats in each group.

TABLE II. Comparison of Protection against Alloxan by the Hexoses and Epinephrine.

Treatment*	Range of blood sugar, mg %†
Epinephrine & alloxan	106-124
Phentolamine, epinephrine & alloxan	555-698
Phentolamine, glucose & alloxan	122-138
Phentolamine, 3-methylglucose & alloxan	114-128

* When used these substances were inj. in the following dosages and sequence: phentolamine, I.P., 5 mg/kg, 10 min. prior to inj. of epinephrine or sugar; epinephrine, I.P., 400 μg/kg, 5 min. prior to inj. of alloxan; D-glucose or 3-methyl-D-glucose, I.V., 1 g/kg, 5 min. prior to inj. of alloxan; alloxan, I.V., 40 mg/kg.
† 4 rats in each group.

Summary. 3-Methyl-D-glucose protects the rat against the diabetogenic effect of alloxan when injected intravenously at 0.006 Mole/kg body weight, 5 minutes prior to injection of alloxan. This protection was not blocked by phentolamine. α-D-methylglucoside, L-malate, DL-lactate, and a pyruvate-fumarate mixture failed to offer similar protection. The bearing of these findings on the mechanism of protection against alloxan by the hexoses is discussed.

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