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14. About 5×10^5 K-BALB cells were incubated at 37°C with inhibitor in the presence of [^{3}H]leucine (10 μc/ml) (Schwarz/Mann). At sequential time points, cultures were washed twice, and incubated a further 30 minutes with regular medium containing a 100-fold excess of unlabeled leucine. The medium was then removed, and cells were dissolved in 1.0 ml of sodium dodecyl sulfate lysing buffer. Radioactivity in the acid-precipitable fraction was measured in a toluene-based liquid scintillation system. Results are the percent inhibition of incorporation in the absence of drug treatment. RNA and DNA synthesis were measured in the same way except with [^{3}H]uridine and [^{3}H]thymidine (10 μc/ml), respectively.
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Beta Cell Protection to Alloxan Necrosis by Anomers of D-Glucose

Abstract. *Various concentrations of either the α or β anomers of* D-*glucose were injected into fasted rats prior to a diabetogenic dose of alloxan. Plasma concentrations of glucose were measured 24 hours later. There was a significantly greater protection of the pancreatic beta cells by the α anomer of* D-*glucose as compared to the β anomer, which was evidenced by concentrations of glucose in the plasma, and morphology of beta cells.*

Alloxan (mesoxalylurea) produces diabetes in various animal models by necrosis of the beta cell in the islets of Langerhans (*1*). However, D-glucose injected into animals prior to the diabetogenic agent has been shown to diminish or to prevent this effect of alloxan (*2, 3*). Crystalline D-glucose when placed in solution reaches an equilibrium in which 64 percent is in the β and 36 percent in the α configuration (*4*). When the near pure glucose anomers, at various concentrations, are immediately dissolved and injected into rats before significant mutarotation, a difference in the degree of protection between the α and β anomers to alloxan-induced necrosis is observed, the former being more active.

Male rats (Charles River strain) weighing between 190 to 200 g were given free access to Purina Chow and then fasted for 24 hours. A No. 21 butterfly (3-inch tubing Minicath Prn, Deseret Pharmaceutical Co., No. 5084) was placed in a tail vein after dilation induced by hot water. Alpha and beta glucose (Sigma Chemical G-5250, beta lot 052e-0810, analyzed as 99.2 percent β and 0.8 percent α; and Sigma Chemical G-5000 alpha lot 091C-1690 analyzed as 97.6 percent α and 2.4 percent β) were each rapidly dissolved in normal saline by vigorous shaking just before use in each animal. The glucose was administered in a volume of 0.5 ml at doses of 250, 500, or 750 mg per kilogram of body weight. An additional 0.2 ml of saline was injected to flush the tubing and then alloxan (Eastman, 40 mg/kg), freshly dissolved in normal saline in a volume of 0.5 ml, was injected. A final 0.2 ml of saline was then injected. The animals were randomly selected for the experiment, and alloxan-injected animals that were not given glucose were included as controls at the beginning and end of each series. The animals were then allowed free access to their food. Twenty-four hours later a blood sample was obtained by cutting a small section of the tail, and approximately 0.5 ml of blood was captured in a heparinized pipette, and centrifuged; the plasma was analyzed with the use of the Beckman glucose oxidase analyzer. Blood samples, taken 48 or 72 hours later, did not differ from those taken 24 hours after alloxan. Statistical analysis was performed by means of the unpaired *t*-test (Table 1).

Table 1. Either a glucose anomer or saline was injected intravenously 4 to 6 seconds before administration of alloxan (40 mg per kilogram of body weight) to fasted rats, and glucose in the plasma was determined 24 hours later. Similarly treated animals not receiving alloxan showed a mean glucose concentration of 164 ± 2 mg per 100 ml of plasma ($N = 21$). The plasma glucose is given in milligrams per 100 ml of plasma.

Anomer (mg/kg)	Plasma glucose (mean ± S.E.M.)		N	P
	α Anomer	β Anomer		
0	533 ± 21	533 ± 21	38	
250	266 ± 31	409 ± 31	12	< .01
500	240 ± 15	341 ± 20	44	< .001
750	163 ± 4	174 ± 9	23	< .3

Although the exact mechanism in which glucose protects against the diabetogenic effect of alloxan is unknown (*5, 6*), L-glucose, the nonmetabolizable optical isomer of D-glucose has been shown not to protect against alloxan, excluding a purely chemical reaction, while 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose have produced protection as evidenced by permeability in vitro (*6*). The abolition of protection by prior treatment with mannoheptulose has placed credence on the theory that the protective site is moderately stereospecific, probably at the beta cell membrane and not through a common protective metabolic intermediate (*3*). Although the mutarotation of α- or β-glucose into an equilibrium state is rapid in a physiologic setting (≃ 7 minutes) (*7*), the beta cells in the islets of Langerhans are able to distinguish between the injected anomers, as evidenced by the different degree of protection. To further corroborate this observation, the animals protected with α-glucose were morphologically compared with those with β-glucose and those with alloxan alone. Beta cell necrosis was noted in those receiving only alloxan, and, in contrast, "protected islets" demonstrated slight to moderate degranulation of beta cells and only minimal or absent evidence of injury, both proportionate to the protection against diabetes as evidenced by concentrations of glucose in the plasma (*8*).

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