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The Effect of Alloxan on the *in Vitro* Formation of Glucose by the Liver[1]

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Houssay, Orías, and Sara have recently stated (*3*) that the initial hyperglycemia of alloxan diabetes, which fails to occur in hepatectomized animals, is observed after adrenalectomy or section of the splanchnic nerves, and they expressed the view that the phenomenon must be attributed principally to direct action of alloxan on the liver. Goldner and Gomori (*2*) and Kirschbaum, Wells, and Mollander (*4*) were unable to demonstrate an initial hyperglycemia in dogs and rats, respectively, after adrenalectomy.

The experiments here reported may throw some light on the direct effect of alloxan on the liver. Respiring rat liver slices were suspended in 3 cc. of a glucose-free modified phosphate-buffered Ringer's solution (MPBR) to which, in certain of the flasks, alloxan was added in 0.0014 M concentration, which is roughly equivalent to the concentration that would occur if there is an equal distribution throughout the body after a subcutaneous injection of 200 mg./kilo. The amount of glucose (as total reducing substances by the ferricyanide method of Folin) appearing in the medium after one hour's shaking in oxygen at 37° C. was measured, with the results given in the table.

Each figure represents the average of three observations. Control and alloxan-treated tissues of each experiment were taken from the pooled liver slices of the same animal. The glucose appearing in the medium was the same, within the limits of error, in the alloxan-treated tissues as in the controls. Assuming that the dry weight of rat liver is 28 to 30 per cent of the wet weight, it can be calculated that the glucose in the medium represented about 6 per cent by weight of carbohydrate in the liver slices. Only glycogen could have been the source of most of this material.

In four animals made diabetic by a subcutaneous injection of 200 mg. alloxan/kilo, 48 hours previously, the amount of glucose appearing in the medium is less than one-fifth of the amount seen in the case of the normal liver. Lackey, Bunde, Gill, and Harris (*5*) have shown that in alloxanized rats with moderate to severe diabetes the liver glycogen is low, and we attribute the lessened formation of glucose in the diabetic livers to this fact.

Exp. No.	Blood sugar in mg. %	Liver slices in MPBR: Dry wt. of tissue (mg.)	Liver slices in MPBR: Glucose in medium (mg.)	Liver slices in MPBR: Glucose/mg. dry wt. of tissue (μg.)	Liver slices in MPBR plus alloxan: Dry wt. of tissue (mg.)	Liver slices in MPBR plus alloxan: Glucose in medium (mg.)	Liver slices in MPBR plus alloxan: Glucose/mg. dry wt. of tissue (μg.)
Normal Rats							
1		15.0	4.35	290	16.2	4.20	259
2		17.2	2.55	148	14.0	2.86	204
3		12.9	2.55	197	13.6	2.62	193
4		14.9	2.70	181	15.5	2.92	188
5		16.6	2.85	172	16.1	2.52	156
6		11.6	2.32	200	11.7	2.85	243
		Average		198 ± 20	Average		207 ± 15
Diabetic (Alloxan) Rats							
7	520	13.8	0.345	25	12.9	0.368	28
8	520	13.0	0.555	43	11.8	0.435	37
9	760	16.1	0.270	17	15.7	0.330	21
10	450	12.5	0.600	48	13.2	0.720	55
		Average		33 ± 7	Average		37 ± 8

Our results indicate that alloxan has no direct effect on liver glycogenolysis and are therefore opposed to the concept of Houssay, *et al.* in regard to the origin of the initial hyperglycemia of alloxan diabetes. The possibility remains that alloxan may act upon the liver via nervous pathways, as suggested by the recent observations of Braulio (*1*) in the cat.

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