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Alloxan Diabetes in Mice.

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Alloxan diabetes has been produced in rabbits, rats, dogs, cats, monkeys, pigeons, and guinea pigs.[1-5] Although Dunn *et al.*,[6] state that alloxan causes islet lesions in mice, no mention was found in the literature of the production of alloxan hyperglycemia in this species. In the course of studies on the effect of hyperglycemia on susceptibility to bacterial infection, it became desirable to find a consistent means of inducing this condition in a small laboratory animal such as the mouse.

Materials and Methods. Male Swiss albino mice of the same age and weight were selected for each experiment. A fresh aqueous solution of alloxan monohydrate (4 mg/ml for intravenous injection and 6 mg/ml for intraperitoneal and subcutaneous injections) was used. Blood sugars were determined by the method of Reinecke.¶,[7] Animals with blood sugar levels over 200 mg% were considered hyperglycemic, since blood sugar determinations on 20 control mice on food and water *ad libitum* were all under 150 mg%.

In view of the wide variation of dosage and route of administration necessary to produce alloxan diabetes in different species of animals,[1-5,8] the following procedures were carried out.

a. Intravenous tail injections of doses varying from 50 mg/kilo to 300 mg/kilo of alloxan were given to 65 mice maintained on Purina Mouse Chow and water *ad libitum* before and after injection. Blood sugars were determined 48 hours after inoculation. Intraperitoneal and subcutaneous injections of similar dosages of alloxan were given to additional groups of mice on the same diet.

b. Since it has been demonstrated[8] that fasting prior to injection increases the susceptibility of rats to alloxan, 131 mice fasted for 8 to 24 hours, were given alloxan intraperitoneally and fed Purina Mouse Chow immediately thereafter.

c. Hard[9] reported that some animals of a given species are resistant to repeated injections of alloxan. A number of mice previously unaffected by an initial dose of alloxan were therefore reinjected with similar dosages and later tested for hyperglycemia.

d. In order to determine the duration of alloxan hyperglycemia blood sugar determinations were made on groups of diabetic mice at various times after the injection of alloxan.

e. Inasmuch as riboflavin, epinephrine, glutathione, cysteine, and niacin have been reported to protect against alloxan[10,8,11,12] an investigation of their protective action in mice was made.

Results. The optimal dose of alloxan for the production of hyperglycemia in mice was found to be 100 mg/kilo injected directly into a tail vein. All of 50 mice given alloxan in this dosage were found to be hyperglycemic at the end of 48 hours, while none of the animals died (Table I) (A).

* Technical assistance of James Samsell.

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¶ Coleman Junior electrophotometer.

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TABLE I.
The Mortality and Blood Sugar Levels of Mice 48 Hours After Inoculation of Alloxan.

No. of animals	Conditions prior to inj. of alloxan	Dose of alloxan mg/kilo	Route of injection	No. of deaths 48 hr	Blood sugar levels after 48 hr mg %			
					50-150	150-200	200-300	Greater than 300
(A)								
5	Food and water *ad lib.*	300	Intravenous	5	0	0	0	0
5		200	"	3				2
50		100	"	0	0	0	0	50
5		50	"	0	2			3
(B)								
18	Same	400	Intraper.	3	4	3	4	4
195		300	"	19	23	52	37	64
8		200	"	0	3	3	2	0
8		100	"	0	4	2	1	1
(C)								
6	Same	750	Subcut.	2	2	0	0	2
6		600	"	4	1	0	0	1
6		500	"	2	2	1	0	1
6		400	"	3	3	0	0	0
6		300	"	0	6	0	0	0
6		200	"	0	1	4	0	1
6		100	"	0	4	1	1	0
(D)								
10	Fasted 24 hr	400	Intraper.	3	0	0	1	6
12	" " "	300	"	0	0	0	0	12*
100	" 8 "	300	"	45	2	6	15	32
9	" 24 "	300	Subcut.	0	4	2	3	0

* Five of these mice died after 60 hours.

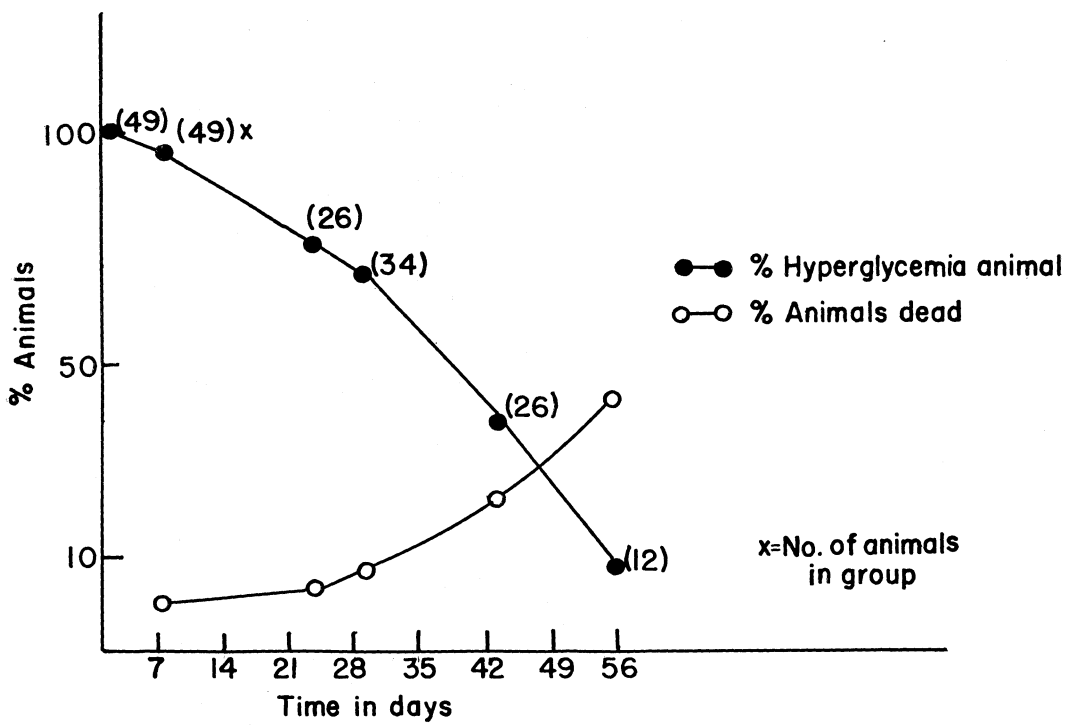

Figure I.— Duration of hyperglycemia and mortality after inoculation with alloxan

TABLE II.
Effect of Riboflavin, Epinephrine, Cysteine, and Niacin on Development of Alloxan Hyperglycemia in Mice.

No. of animals	Compound tested	Route of inj.	Time given before inj. of alloxan*	Dose of compound	Death	Blood sugar levels after 48 hr, mg % 50-150	150-200	200-300	Over 300
20	Riboflavin	Intraper.	6 hr	50 mg	16	1	0	2	1
20	"	"	6 "	10 "	3	5	7	1	4
20	"	Subcut.	6 "	10 "	0	1	3	1	15
60	Epinephrine	"	3 min.	.1 cc, 1:10,000	10	13	8	10	19
10	Glutathione	Intraper.	30 "	40 mg	8	2	0	0	0
20	Cysteine	"	1 hr	5 "	20†	0	0	0	0
20	"	Subcut.	30 min.	3 "	8	6	3	4	2
20	"	"	1 hr	1 "	4	0	4	3	9
20	Niacin	"	6 "	5 "	4	5	1	6	4
195	Control	—	—	—	19	23	52	37	64

* All mice were given 300 mg/kilo of alloxan intraperitoneally.
† All mice died before injection of alloxan.

The optimal dose for the intraperitoneal injection of alloxan was found to be 300 mg/kilo. Of 195 mice given this dose, 101 (52%) showed a hyperglycemia greater than 200 mg% in 48 hours (Table I) (B). Nineteen of the 195 (9%) mice died in 48 hours. Alloxan given in similar dosages by the subcutaneous route was found to be less effective in that only 6 of 42 mice so treated developed hyperglycemia (Table I) (C).

Fasting for 8 to 24 hours prior to injection increases the mortality due to alloxan without increasing the incidence of hyperglycemia in the survivors. Although only 60 of 131 (45%) fasted animals were hyperglycemic 48 hours after injection, 48 mice (37%) died in this interval (Table I) (D).

Five of 22 mice demonstrated to be resistant to an initial dose of alloxan were found to be hyperglycemic after a second injection of the same dose.

The diabetes produced by alloxan was found to be relatively transient in that at the end of 6 weeks 20 or 26 initially diabetic mice had survived and of these only 10 remained hyperglycemic (Fig. 1).

In the dosages used epinephrine, cysteine, niacin and riboflavin gave no significant protection against the action of alloxan (Table II).

Summary. The most effective dose and route of administration of alloxan were found to be 100 mg/kilo injected into the tail vein of the mouse. Fifty mice treated in this way all had blood sugar levels greater than 300 mg% 48 hours later. There were no mortalities.

Fasting before the intraperitoneal injection of alloxan increased the mortality in mice without increasing the incidence of diabetes among the survivors.

Mice resistant to the first injection of alloxan were not necessarily resistant to a second injection of the same dose.

Alloxan hyperglycemia was not permanent in all mice, since the blood sugar levels of one-half of the survivors reverted to normal within 6 weeks.

In the dosages administered epinephrine, cysteine, niacin and riboflavin did not give protection against the action of alloxan.