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Blood Glutathione and Alloxan Susceptibility in Inbred Mice.* (24503)

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Male mice of the strain Z(C_3H) have been reported(1) to be more resistant to a given intravenous dose of alloxan than are male mice of strain D_8. Degree of alloxan sensitivity of an experimental animal may possibly be related to the endogenous blood and tissue glutathione (GSH) levels, for GSH and other sulfhydryl compounds protect against the diabetogenic effect of alloxan when given prior to alloxan(2,3). Moreover, sodium deficient diet(4) and repeated acetoacetate injection (5) lower the GSH content of the blood and markedly increase susceptibility to alloxan (4,6). Likewise, such factors as cysteine feeding, thiouracil feeding, and thyroidectomy, which increase the liver and kidney GSH level, also increase resistance to alloxan (7). In view of this evidence that factors which alter the GSH content of the blood or tissues alter alloxan susceptibility, it was thought worthwhile to determine blood GSH levels in these 2 strains of mice. These levels were found to differ in the 2 strains, as did alloxan susceptibility, and therefore it seemed important to determine how these characteristics are transmitted to the F_1 hybrids resulting from the cross between these 2 strains.

Material and methods. Adult mice of strains Z(C_3H) and D_8 were used as well as hybrids resulting from crosses between D_8 female and Z(C_3H) male and between D_8 male and Z(C_3H) female. Male and female mice approximately 6 weeks in age were used for GSH determinations, but only male mice were used for alloxan susceptibility studies. Reduced GSH was determined by the alloxan 305 method as described by Patterson and Lazarow(8). Hematocrit levels were determined by the microhematocrit method of Van Allen (9). Glutathione is reported as mg of GSH/100 cc of red blood cells. In the susceptibility studies, alloxan was injected *via* the tail vein using a No. 26 needle, in doses of 40, 60, or 80 mg/kg body weight. Blood sugars were determined by the method of Folin-Malmros (10) at 48 hours and 7 days after the injection. The average blood sugar of normal mice was 135.6 $\pm$ 13.4 mg % 3 hours after the last meal. A level higher than 200 mg % 3 hours after the last meal was regarded as an indication of diabetes.

Results. Table I shows that mice of strain Z(C_3H) (alloxan resistant) had a significantly higher erythrocyte GSH level (39% higher) than those of strain D_8 (alloxan susceptible).

In determining erythrocyte GSH levels of both types of F_1 hybrids, it was found that, regardless of method of crossing; 1) erythrocyte GSH levels were not significantly different from those of Z(C_3H) (alloxan resistant) strain (see Table I), but 2) were significantly

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TABLE I. Genetic Control of GSH Level in 2 Strains of Mice (± Stand. Dev.).

Strain	No. of mice	Wt (g)	Hematocrit level (%)	Erythrocyte GSH (mg)	p value (for GSH)
Z (alloxan resistant)	18	17.5 ± .9	44.6 ± 3.2	43.0 ± 7.1	.7040
ZD_8 (F_1 hybrids)	12	17.8 ± 1.4	48.8 ± 2.7	43.4 ± 6.9	.6312
D_8Z (F_1 hybrids)	7	17.8 ± 1.4	51.3 ± 3.9	44.9 ± 6.6	<.001
D_8 (alloxan sensitive)	14	16.9 ± 1.0	48.5 ± 5.1	30.9 ± 6.6	

higher than those of the D_8 (alloxan susceptible) strain. This suggests that the factor(s) responsible for control of erythrocyte GSH level are genetically transmited to the offspring, possibly as a dominant characteristic. However additional studies would be needed to establish this point. When levels of erythrocyte GSH were compared for male and female mice of these strains, no significant difference was found (Table II), suggesting that

TABLE II. Erythrocyte GSH Levels of Males and Females of Two Strains of Mice.

Strain	Sex	No. of mice	Wt (g)	Erythrocyte GSH (mg)	p value (for GSH)
Z	♂	18	15.8	41.5 ± 7.7	.60
Z	♀	13	16.7	40.4 ± 3.9	
D_8	♂	13	15.7	31.5 ± 8.1	.22
D_8	♀	24	14.8	34.4 ± 3.2	

sex does not influence the erythrocyte GSH level.

In alloxan susceptibility studies, initially reported by Martinez *et al.*(1), the Z(C_3H) strain was shown to be more resistant to alloxan than the D_8 strain. In the present study it was observed that F_1 hybrids resulting from crosses between Z(C_3H) and D_8 strains were as resistant to alloxan as was the Z(C_3H) parental strain (Table III). Furthermore, at a given dose of alloxan, blood sugar levels of the diabetic group of both the Z(C_3H) strain and of hybrids were similar (Table III). These studies suggest that the factor(s) which controls alloxan resistance appears to be genetically transmitted to the F_1 hybrids.

Discussion. These findings imply a relationship between erythrocyte GSH level and resistance to intravenous alloxan in these inbred strains of mice, supporting the hypothesis that the endogenous GSH level is related to the alloxan susceptibility.

Since the erythrocyte GSH level differed in these inbred strains of mice, it alone might determine their differing susceptibility to alloxan. In this case, since GSH is not found in the blood plasma(11,12), alloxan would have to enter the red blood cells and be detoxified there. It seems probable however, that GSH levels within the B-cell of the islets of Langerhans may also determine the resistance to diabetogenic agents. Therefore, it is important to measure the sulfhydryl level in the B-cells since it is these that are selectively destroyed by administered alloxan(13), possibly because of their deficiencies in GSH(14).

In considering all factors determining alloxan resistance, the level of erythrocyte GSH should be taken into account as possibly determining the extent of detoxification of the diabetogenic agent before it reaches the B-cell. The susceptibility to diabetogenic agents, therefore, is probably due to a number of factors, one of which is erythrocyte GSH.

Summary and conclusions. Studies on

TABLE III. Alloxan Susceptibility in 2 Strains of Mice and Their F_1 Hybrid Crosses.

Strain of mouse*	% of mice developing diabetes when inj. with specified dose of alloxan (mg/kg)			Blood sugar of diabetic mice when inj. with specified dose of alloxan (mg/kg)			Erythrocyte GSH level of mouse strain used (mg)	
	40	60	80	40	60	80	GSH	Stand. dev.
D_8	25	100	100	291	382	417	30.9	6.6
Z (C_3H)	0	58	91		292	358	43.0	7.1
Hybrid crosses	0	75	93		305	357	43.9	7.1

* 10-14 mice were used in each group for alloxan inj.

erythrocyte GSH were carried out using 2 inbred strains of mice and their F_1 hybrids. 1. The erythrocyte GSH level in the $Z(C_3H)$ (alloxan resistant) strain and the F_1 hybrid was significantly higher than it was in the D_8 (alloxan susceptible) strain. 2. The $Z(C_3H)$ strain and the F_1 hybrids were equally resistant to alloxan, whereas the D_8 strain was significantly more susceptible to alloxan. 3. Thus, both the blood GSH level and the degree of alloxan susceptibility also appears to be genetically controlled.

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Effect of Volatile Fatty Acids, Sodium and Potassium Bicarbonate in Purified Diets for Ruminants.*† (24504)

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A previous report(1) showed that growth of lambs fed a purified diet containing a mixture of glucose and salts of acetic, propionic and butyric acid was superior to that of lambs fed diets containing a mixture of either glucose and starch or glucose, starch and cellulose. After a period of 2 years, all 3 animals on glucose-starch diet and 2 of 3 animals on cellulose diet died, whereas 5 of 6 animals on volatile acids diet survived. Further evidence for the adequacy of "acids" diet was furnished by the fact that the 5 survivors were normal in weight and in appearance. The present study was undertaken to investigate the factor(s) responsible for the superiority of volatile-fatty-acids diet. Data obtained from 2 experiments are presented.

Procedures.‡ Details of biological procedures were the same as those described previously(1) except that in this study all lambs were restricted to amounts they would consume in 3 feedings daily. Prior to placing the lambs on experiment, they were treated with *Clostridium perfringens* type D bacterin vaccine to prevent enterotoxemia. Volatile fatty acids were determined by Keeney's method (2) on samples of rumen fluid taken by stomach tube 6 hours after feeding, during latter part of first experiment. In Exp. I, diets 2, 4, 5 and 6 shown in Table I were tested. Diet 2, used as positive control, has the same formulation as the "complete" volatile acids diet used previously(1); diet 4 is similar to 2 except that Na butyrate is omitted, and diets 5 and 6 correspond to 2 and 4, respectively, except that triacetin is substituted for acetate salts. Ten lambs averaging 31 lb initially were assigned to the 4 diets; 2 lambs to each of diets 2 and 4, and 3

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