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Animal Responses to 2-Deoxy-D-Glucose Administration. (24268)

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Wick(1) has demonstrated that following 2 deoxy-D-glucose (2-DG) administration there is a decreased requirement for glucose to maintain a constant blood glucose concentration in eviscerated-nephrectomized rabbits. 2-DG has been reported to inhibit growth of experimental animal tumors(2) and to inhibit glycolysis in leukemic cells(3), slices of Walker 256 carcinoma(4) and Krebs carcinoma ascites cells(5). These observations are consistent with the hypothesis that 2-DG is a metabolic blocking agent for glucose. A survey of the response of several animal species to 2-DG was undertaken prior to clinical studies to be reported elsewhere(6).

Materials and methods. 2-DG was obtained from the Aldrich Chemical Co., Milwaukee, Wis. Blood 2-DG concentrations were determined by the Quinaldine method(7) and blood glucose concentrations by a modification of the Nelson method(8).

a) In acute toxicity studies 2-DG in doses of 1-3 g/kg body weight as a 10-40% aqueous solution was administered(1) rapidly by tail vein to 20 Swiss mice, each weighing 20 g, and (2) by ear vein over a 2-minute period to white New Zealand rabbits weighing 1 kg. Blood glucose concentrations before and blood glucose and 2-DG concentrations after infusion were determined in the rabbits.

b) In chronic studies 20 male rats of the Sprague-Dawley strain of initial weight 50 g and on a standard purina diet, were divided into 5 groups of 4 rats each. Group I received 0.5 cc of distilled water s.c. twice daily (8 a.m. and 4 p.m.); Group II received 200 mg of 2-DG/kg initial body weight in 0.5 cc of distilled water s.c. twice daily for a total dose of 400 mg/kg; Group III received 400 mg/kg twice daily; Group IV 600 mg/kg twice daily; Group V 800 mg/kg twice daily. Body weight was checked frequently throughout the study. Administration was discontinued after 19 days, and 2 hours after the last injection 2-DG and glucose concentrations were determined on tail vein blood. One week later fasting blood glucose concentrations were determined.

c) On each of 3 white New Zealand rabbits, fasted 24 hours and weighing 3-4 kg, an intravenous glucose tolerance test (500 mg/kg) and an intravenous 2-DG tolerance test (200 mg/kg), and an oral 2-DG tolerance test (400 mg/kg administered by stomach tube) was performed. Also a glucose tolerance test and glucose-insulin tolerance test (0.5 units/kg)* was performed on each rabbit following intravenous administration of 2-DG (200 mg/kg). There was a time interval of several days between tests.

Results. Mice. The LD_{50} for mice receiving 2-DG acutely by tail vein was between 2 and 3 gm/kg *body* weight. Within 5 minutes after administration the mice became ataxic and then were unable to right themselves. Respirations became rapid and shallow and convulsions occurred occasionally. The mice then became unresponsive to painful stimuli. At a dose of 1 g/kg after about 45 minutes, recovery was noted. It was only after several hours that recovery was observed in mice receiving the higher doses.

Rats. The results of administration to rats of 2-DG subcutaneously over a 19-day period

* Glucagon-free-insulin was kindly provided by Dr. W. R. Kirtly, Eli Lilly and Co., Indianapolis, Ind.

TABLE I. Weight, Blood Glucose and Blood 2-DG Levels in Rats Administered 2-DG.

Group	Dose (mg/kg/day)	Initial wt (g)	Wt, day 19 (g)	Blood glucose day 19 (mg %)	Blood 2-DG day 19 (mg %)	Blood glucose day 26 (mg %)
I	0	47 ± 1*	126 ± 9	129 ± 4	0	76 ± 3
II	400	53 ± 1	145 ± 4	135 ± 5	2 ± 1	—
III	800	52 ± 1	111 ± 10	162 ± 12	—	—
IV	1200	49 ± 1	96 ± 13	190 ± 10	6 ± 1	68 ± 4
V	1600	45 ± 1	96 ± 10	212 ± 40	16 ± 3	61 ± 2

* Mean ± stand. error is given.

is presented in Table I. Weight gain was less in those rats receiving the higher doses of 2-DG than in the control rats. Blood glucose concentrations 2 hours after the last injection of 2-DG were elevated above the concentration in the control group, but one week later (last column of the table) fasting blood glucose concentrations were similar in the control group and the 2 groups that received the highest doses of 2-DG.

Rabbits. Within a few minutes after intravenous administration of 1-3 g of 2-DG to a rabbit there was marked incoordination of the extremities. Then the limbs became limp; there was dorsiflexion of the neck; pupils became dilated; occasionally the rabbit cried out. Nystagmus was observed in one case. Breathing was gasping in nature. Death occurred within 6-32 minutes after completion of 2-DG administration. Table II presents the pre- and post-sugar concentrations of these animals and their survival time.

Fig. 1 presents graphically the blood sugar tolerance test responses of 1 of 3 rabbits. All had similar responses. An intravenous glucose tolerance test was performed and several days later an intravenous 2-DG tolerance test (Fig. 1-A). Following administration of 200 mg/kg of 2-DG intravenously, there was a rise in blood glucose to about twice the fasting level. Blood glucose concentrations were high for at least 5 hours with the peak concentration occurring between 1 and 2 hours after administration. In a glucose tolerance test performed 40-50 minutes after 2-DG administration blood glucose decreased much more slowly than on the control tolerance test (Fig. 1-B). A glucose tolerance test with simultaneous administration of insulin (0.5 units/kg) after 2-DG administration resulted in a response similar to the control glucose tolerance test (Fig. 1-C). Blood 2-DG concentration 5-10 minutes after 2-DG administration was near 50 mg%, but decreased rapidly. No effect of insulin or glucose infusion on 2-DG blood levels was noted. 2-DG in doses of 400 mg/kg given by stomach tube (Fig. 1-D) resulted in blood glucose level rises to about 1½ times fasting levels with the peak concentration at about 2 hours and elevated levels for at least 5 hours.

Discussion. Acute administration of 2-DG. The train of symptoms observed in mice and rabbits following intravenous administration of large doses of 2-deoxyglucose is similar to those reported following insulin administration. Woodward and Hudson(9) have demonstrated marked inhibition of glycolysis of brain slices in the presence of 2-DG and 2-DG does enter into the cerebrospinal fluid

TABLE II. Blood Glucose and Blood 2-DG Levels on Intravenous Administration of 2-DG to Rabbits.

Rabbit	Dose (g/kg)	Control blood glucose (mg %)	Post-2-DG blood glucose (mg %)	Post-2-DG blood 2-DG (mg %)	Time of blood sample (min.)*	Time of death (min.)*
1	1	111	177	247	18	Lived
2	1	95	183	146	21	26
3	2	113	171	355	6	6
4	3	—	196	441	12	15
5	3	—	175	568	32	32

* Time is given in min. post-2-DG administration.

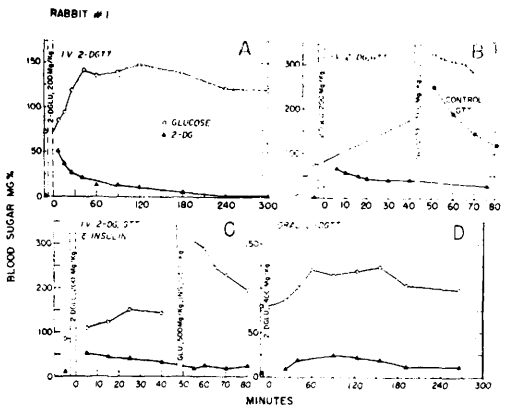

FIG. 1. Rabbit blood glucose and 2-DG tolerance tests: (A) intrav. 2-DG tolerance test; (B) control intrav. GTT and a GTT after 2-DG administration; (C) GTT with insulin following 2-DG administration; (D) oral 2-DGTT.

rapidly after intravenous administration of 2-DG to dogs(10). Sols and Crane(11) have demonstrated that 2-DG is phosphorylated by brain hexokinase but is probably metabolized no further. It therefore appears possible that the symptoms are a result of inhibition of glucose entrance and/or utilization by the brain in the presence of 2-DG, in effect, a "cellular hypoglycemia."

The symptoms of acute administration of 2-DG are very similar to those described by Herring and Hynd(12) on administration of glucosone to mice and rabbits. They noted the similarity of the symptoms to those seen after insulin administration. These authors believed that glucosone might have produced a hypoglycemia for although the reducing power of the blood was high, no methods were available for distinguishing reduction due to glucosone injected from glucose actually present. Glucosone is a hexokinase inhibitor and it seems probable that the symptoms observed were related to an inhibition of glucose phosphorylation by the brain(13).

It is also interesting to note that Winter (14), while searching for glucose analogs that would result in recovery of animals in insulin induced convulsions, administered 2-DG to rabbits. He noted a rise in blood sugar and the presence of sugar in the urine, but felt that it was inconceivable that the sugar he was measuring could be glucose since the rabbits remained in convulsions.

Chronic administration of 2-DG. Weight loss on chronic administration of 2-DG has been reported by others(15). Whether this is the result of a diabetic-like state or decreased food intake has not been determined. While the rats receiving 2-DG did have an elevated blood sugar, the concentrations returned to control values following cessation of 2-DG administration.

Tolerance tests. 2-DG enters the blood following oral administration to rabbits and is apparently associated with elevation of blood glucose concentration under our experimental conditions. Sokoloff(15) has reported moderate hypoglycemia in rats receiving 2-DG in their diet. Sols(16) has demonstrated passage of 2-DG across the guinea pig intestinal mucosa and the failure of 2-DG to inhibit glucose transport. The elevation of blood glucose concentration on intravenous administration of 2-DG to the intact rabbit is in agreement with Wick's(1) observations in the eviscerate rabbit. The glucose tolerance test following 2-DG administration is difficult to interpret since the glucose concentrations represent the glucose tolerance curve superimposed on a rising glucose concentration secondary to the 2-DG already administered. However, the tolerance tests have been done when the glucose levels following 2-DG administration are near the peak values. It thus appears that glucose disappearance from the blood is significantly prolonged by 2-DG and that in insulinized animals this prolongation is less. This complements Wick's observation that the amount of glucose oxidized by insulinized eviscerated rabbits receiving 2-DG is about the same as that oxidized by eviscerated animals not receiving insulin or 2-DG.

Summary. The response of mice, rats and rabbits to acute and chronic administration of 2-deoxyglucose (2-DG) has been studied. 1. Mice and rabbits were given large doses of 2-DG intravenously and had symptoms similar to those during insulin hypoglycemia. It is suggested that the symptoms were the result of a cellular glucopenia secondary to inhibition by 2-DG of glucose utilization. 2. Rats receiving 2-DG chronically by subcu-

taneous administration had elevated blood glucose concentrations and weight loss. On discontinuation of 2-DG administration blood glucose concentrations returned to control values. 3. Rabbits following intravenous or oral administration of small doses of 2-DG had a rapid rise in blood glucose concentrations. The glucose concentrations remained elevated for at least 5 hours. In glucose tolerance tests done shortly after 2-DG administration glucose concentrations decreased more slowly than in control tolerance tests, but the animals did respond to insulin administrations.

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Two Local Hemorrhagic Skin Responses to Bacterial Endotoxin in an Inbred Mouse Strain. (24269)

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Although the localized Shwartzman reaction was long undetected in mice and rats, Homma(1), and more recently Kelly *et al.* (2), have demonstrated that this phenomenon, or an analagous one, does indeed occur with adequate "preparation" or in properly selected mouse strains. In addition to the normal Shwartzman reaction, the latter workers have described a "Shwartzman-like reaction" which differs from the classical phenomenon in that it is produced by but a single injection of bacterial polysaccharide. In other respects, however, this reaction is indistinguishable from the normal 2-injection phenomenon.

It was the purpose of the experiments reported here to confirm and extend the abovementioned observations in an effort to elucidate the mechanisms underlying the single-injection reaction, which, because of its morphological characteristics, was thought to be related to the local Shwartzman reaction.

The following experiments were performed with a mouse strain bearing a genetic relationship to one of the "positive" strains (Rockefeller "pen-bred") described by Kelly *et al.,* but differing in that it now reflects some 44 generations of brother-sister inbreeding. Such a strain offers the advantage of genetic homogeneity, thereby defining an important variable of this system.

Materials and methods. The mice used were from a recently established pen-bred subline of the brother-sister, 44 generations inbred BSVS strain, originally extracted by Webster(3) from the Rockefeller Institute Stock. They are maintained under conditions of constant temperature (80° F.) and relative humidity (50%) with a uniform 12 hour artificial (fluorescent lamp) light day.