

Alloxan-diabetic rats during acclimation to cold

ROBERT H. POE, JOSEPH W. WHITE,¹ AND
THOMAS R. A. DAVIS

*U. S. Army Research Institute of Environmental Medicine,
Natick, Massachusetts*

POE, ROBERT H., JOSEPH W. WHITE, AND THOMAS R. A. DAVIS. *Alloxan-diabetic rats during acclimation to cold*. *Am. J. Physiol.* 205(1): 184-188. 1963.—The survival rate of alloxan-diabetic rats at 5 C was found to be related to the pre-exposure nonfasting blood sugar level. The majority of deaths occurred within the first 2 weeks of cold. Once cold acclimation had been accomplished, the survival rate was 100%. A significant decrease in skeletal muscle glycogen, and the highest percentage of weight loss were found after 2 weeks of cold exposure. A quantitative relationship between survival in the cold and available insulin is suggested. An augmented ability to utilize carbohydrate, related in part to an increased insulin sensitivity, is hypothesized to account for the postacclimation findings.

THE METABOLIC ALTERATIONS undergone by homeotherms during exposure and acclimation to cold have become the subject of increasing interest and investigation in recent years. The role of the endocrines as regulators of these alterations have been stressed (1). Thyroid hormone, the corticosteroids, epinephrine and norepinephrine, anterior pituitary extract, and insulin have all been investigated to varying extents. Of all, however, insulin has probably been studied the least.

The alloxan-diabetic rat serves as a unique tool with which to study cold acclimation in the presence of an insulin deficiency, because of the selective destruction rendered by alloxan upon the beta cells of the pancreas (2). Such an insulin-deficient, but otherwise normal animal has difficulty in surviving at a temperature of 5 C. This was demonstrated in a previous study where a large percentage of diabetic rats did not live beyond the first few days of exposure (3). Because of this, and the established importance of carbohydrate as an energy source, both initially in the cold and after acclimation (4), this study was conceived to compare carbohydrate stores, as manifested by nonfasting liver and skeletal muscle glycogen, of alloxanized and control rats continuously exposed to a cold environment for periods of time increasing in duration up to and including 6 weeks. Cardiac glycogen levels were included because of the

finding of previous investigators that an elevation in cardiac glycogen existed in alloxan-diabetic rats (5), and a suspicion that such might be altered by exposure to cold.

METHODS

The study utilized female Holtzman rats ranging in weight from 200 to 300 g. All animals remained at 25 C for 3 weeks prior to introduction into the study. The experimental rats, randomly selected, were alloxanized by a single injection of 5% alloxan monohydrate, administered subcutaneously, on a dosage-weight relationship of 120 mg/kg body wt., following a 48-hr fast. In order to insure a stabilization of the diabetic state, and to eliminate any animal with irreversible renal tubule necrosis (2), each experimental rat had to survive at least 7 days postalloxanization at 25 C, maintain its body weight, and have a nonfasting blood sugar level between 300 and 700 mg/100 ml before it was included in the study. Blood sugar determinations were in duplicate using the ultramicro method of Knights, MacDonald, and Ploompou (6). It was believed that for the purposes of providing sufficient separation between normal and diabetic groups, and for classifying the diabetic animals into a moderately severe hyperglycemic class, and therefore having an appreciable degree of insulin deficiency, the method utilized was a satisfactory one. An exact level of insulin deficiency cannot be assumed on the basis of any blood sugar technique, but a qualitative deficiency can be said to exist and is assumed. Control animals did not receive alloxan, and their mean nonfasting blood sugar level was 118 mg/100 ml with an upper range of 140 mg/100 ml.

Alloxanized and control rats, in equal numbers, were placed in continuous cold and were randomly sacrificed following periods of 1, 2, 3, and 6 weeks of cold exposure. Animals which survived 4 weeks or longer were regarded as cold acclimated (7). Sufficient animals were placed in the cold so that at the completion of the study, there were eight animals in each group for sacrifice following each of the four periods of cold exposure. Because of the high mortality observed in the diabetic animals when placed in the cold, considerably more diabetic than control rats were required to yield the

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¹ Present address: Veterans Administration Research Hospital, Chicago, Ill.

satisfactory number for sacrifice. A large proportion of the deaths occurred within the 1st week of cold which rendered the diabetic population surviving 1 week or more and upon which much of the data are based, a somewhat stable entity. Additional randomly selected control and alloxan-diabetic rats were sacrificed without experiencing any cold in order to estimate a base line for their respective groups. The mortality prevented a statistical comparison from being made between cold- and noncold-exposed rats within the group because the populations could no longer be considered uniform.

All cold exposure was accomplished in a lighted, constant-temperature room maintained at 5 ± 1 C with a relative humidity that varied between 90 and 98%. The rats lived in individual metal cages suspended in a manner that allowed the passage of feces and urine through the bottoms of the cages. Food (Purina laboratory chow) and water were available ad libitum to all animals throughout the study. Each rat was weighed using an Ohaus triple-beam animal balance before cold exposure and at the time of sacrifice. No attempt was made to record the amount of food or water consumed, although polyphagia and polydipsia were empirically observed in the diabetic rats.

Sacrificing of the rats followed the specified periods of cold exposure and was performed within the cold room with the animal under pentobarbital anesthesia and in a nonfasting state. Pentobarbital is reported to have no significant effect on tissue glycogen (8). Both gastrocnemius muscles, the median lobe of the liver, and the entire heart were quickly excised, in the given order, prior to the death of the animal. The excised tissue was immediately blotted free of excess blood, divided into two approximately equal portions, and placed in individual preweighed tubes containing 30% potassium hydroxide. The tubes were reweighed and the contents analyzed for glycogen by the alcoholic precipitation method of Good, Kramer, and Somogyi (9). The precipitated glycogen was hydrolyzed to glucose and quantitated in duplicate, by the improved method of Folin and Wu (10) in milligrams of glucose from glyco-

gen per 100 g of wet tissue weight. All final calculations were based upon the average of the duplicate determinations, with the muscle glycogen representing the average of both gastrocnemii. Since the glycogen levels of the cold-exposed rats were less than the controls, in almost all of the parameters measured, any dehydration factor affecting the tissue weight of the diabetics in the cold would serve only to increase the significance of any observed differences, and therefore be of little consequence in the interpretation of most of the results of this study. Caution, however, must be observed in the interpretation of absolute glycogen levels at 5 C when a difference was not apparent. On the basis of body weight, dehydration was not a factor in the diabetics at 25 C.

Venous blood samples obtained at the time of sacrifice were analyzed for sugar by the previously mentioned ultramicro technique.

RESULTS

Because the high mortality experienced by the diabetic group required the introduction of relatively large numbers of animals into the study, it was possible to analyze survival at 5 C with reference to level of hyperglycemia (Fig. 1). There was a significant association ($P < .03$) between survival rate and the pre-exposure level of hyperglycemia following 1 and 2 weeks of exposure (11). The greatest percentage of deaths occurred within the first 2 weeks of exposure. No deaths occurred after 4 weeks of exposure. Relative to hyperglycemia, most deaths occurred in rats with nonfasting blood sugars exceeding 600 mg/100 ml. The survival rate of control animals in the cold was 100%.

The results of the glycogen analyses are shown in Fig. 2. The mean liver glycogen of the alloxan-diabetic rats was significantly less ($P < .05$) than the controls when the group means for 1, 2, 3, and 6 weeks of cold were compared. This appeared to be a reflection of the pre-exposure difference which was significant upon *t* test ($P < .01$). An interesting observation was that the level of liver glycogen in the control rats became de-

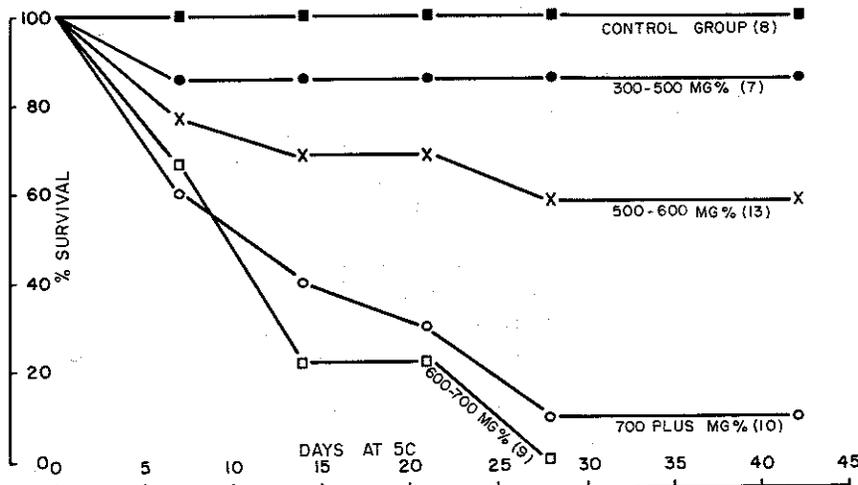


FIG. 1. Survival rate of alloxan-diabetic rats at 5 C vs. level of hyperglycemia. The association of survival rates with respect to hyperglycemic level is significant ($P < .03$) at both 7 and 14 days. The number of animals that initially constituted each group are in parentheses following the notation of hyperglycemic level.

pressed during the cold experience while that of the alloxan-diabetics did not differ from its pre-exposure level. The possible existence of a dehydration factor could have masked a depression in the latter group.

The skeletal muscle glycogen of the alloxan-diabetic rats did not differ significantly from the controls at 25 C. This supports the findings of Cullimore et al. (12) who found similar results in their postprandial animals. However, there was significance using the Mann Whitney test ($P < .003$) after 2 weeks in the cold. The diabetics at this point had much less muscle glycogen than the controls. Since this difference disappeared upon additional cold exposure, it appeared that the 2-week exposed control animal either had less of a need of muscle glycogen for calorigenesis, or possessed the ability to replenish its stores more readily than the diabetic under similar circumstances.

The heart of the alloxan-diabetic rat has been shown to have an elevated glycogen content (5). Our data confirmed this finding. However, once the variable of cold was introduced, the relationship was reversed. A comparison of group means following the introduction of cold revealed that the alloxan-diabetics had significantly less cardiac glycogen than the controls ($P < .05$).

A study of body weight change revealed that the diabetic rats were less able to maintain their weight in the cold than the controls (Fig. 3). The highest percentage of weight loss occurred at 2 weeks for the diabetics but at 1 week for the controls. Using the Mann Whitney test, a comparison of the change in weight for all rats

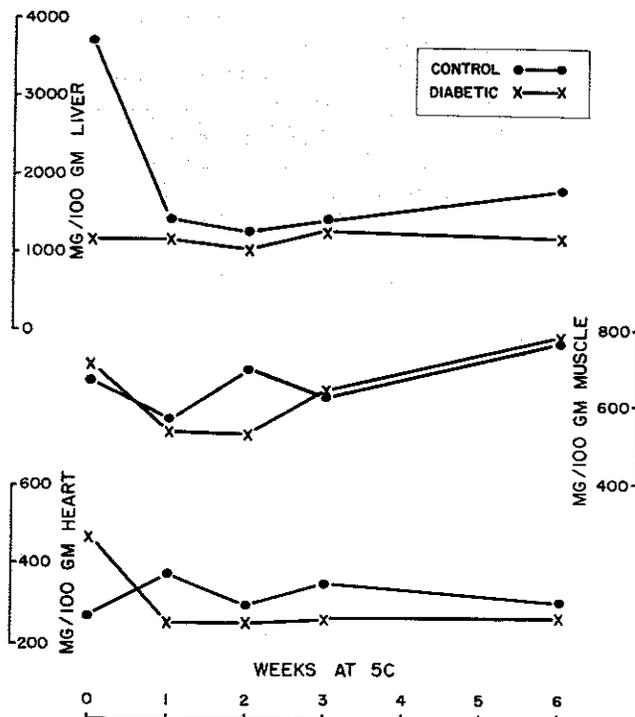


FIG. 2. Glycogen content of liver, skeletal muscle, and heart of alloxan-diabetic and control rats living at 5 C. Each point represents the mean value for eight animals.

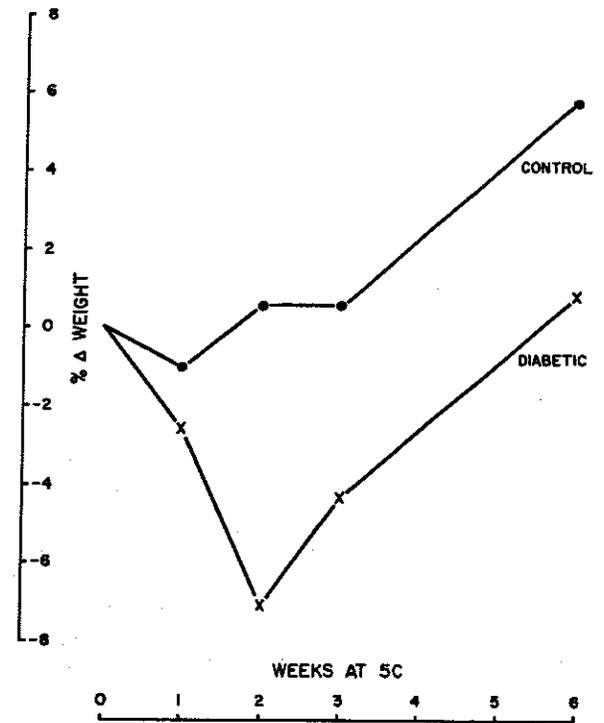


FIG. 3. Per cent weight change of alloxan-diabetic and control rats at 5 C. Each point represents the mean of eight animals.

in both groups over the first 2 weeks of cold exposure revealed a significant difference between diabetic and control ($P < .03$) with the diabetics losing the most weight.

Mean nonfasting sugar levels of the rats at time of sacrifice were compared (Table 1). The cold did not exert any significant effect upon blood sugar in either group. A similar comparison of nonfasting blood sugar was made using two groups of alloxan-diabetic rats with similar levels of hyperglycemia because a difference in populations could have affected the former data. One group lived at 5 C and the other at 25 C for 42 days (Table 2). The cold did not significantly alter the level of hyperglycemia supporting the former data.

DISCUSSION

The precise role of insulin in the survival and cold acclimation of small mammals such as the rat is, as of yet, not fully understood. It is known that the survival rate of the alloxan-diabetic rat is markedly reduced by a continuous exposure to 5 C. The present data and previous work (3) attest to this fact. Such an animal is qualitatively insulin deficient (2), but the extent of the deficiency must obviously vary from animal to animal. However, some of the diabetics do survive, and exhibit certain criteria of cold acclimation (3), indicating that at least a "normal" amount of insulin must not be an absolute necessity for acclimation, and suggesting that some compensatory mechanism might be involved.

Certain studies have indicated that normal cold-

TABLE 1. *Nonfasting blood sugar in alloxan-diabetic and control rats at 5 C*

GROUP	MG% NON - FASTING BLOOD SUGAR				
	NO EXPOSURE	ONE WEEK	TWO WEEKS	THREE WEEKS	SIX WEEKS
MEAN	578	468	489	483	560
DIABETIC RANGE	377-667	303-600	203-675	312-579	358-727
MEAN	118	112	102	93	103
CONTROL RANGE	83-140	85-132	67-121	60-121	85-128

acclimated rats have an augmented capacity for the absorption, formation, and utilization of glucose. Pagé and Babineau (13) have shown that the intact acclimated rat forms liver glycogen from ingested glucose at a more rapid rate than control animals and Hannon (14) has demonstrated the increased activity of glucokinase and glucose 6-phosphatase in the liver of cold-acclimated rats. Baker and Ashworth (15) point out an increased insulin sensitivity in the cold-acclimated rat with a decrease in pancreatic-extractable insulin. It is, therefore, not improbable that such mechanisms as these make possible cold acclimation in the presence of an insulin deficiency.

The large number of deaths of diabetics within the first 2 weeks of cold indicates that the presence of an adequate supply of insulin has a function relative to survival. Ordinarily, the food intake of the normal rat in the cold does not match utilization until after the 10th day of exposure (16). The addition of a metabolic derangement such as that produced by the lack of adequate insulin undoubtedly places an additional stress upon the animal already stressed by the cold. The relationship of survival and hyperglycemic level indicates that a quantitative factor, albeit an inadequately measured one, regarding insulin probably is present and affecting the survival of the diabetic animals.

It is during the initial 2-week period that the greatest metabolic demand is placed upon the rat living in the cold. The inadequacy of food intake has been mentioned. Furthermore, the 2-week period marks the time when both shivering and nonshivering thermogenesis are relatively low, with the former decreasing and the latter increasing (17). Add the factors of a decrease in muscle mass in the diabetic rat (18), an increased rate of weight loss and a low muscle glycogen content as noted in the present study, the importance of insulin to the activity of certain enzymes, namely, glucokinase (19) and the importance of this enzyme in muscle glycogen synthesis, particularly in the cold (14), and it becomes quite

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TABLE 2. *Nonfasting blood sugar of 11 cold-exposed and 9 noncold-exposed alloxan-diabetic rats over a 6-week period*

GROUP	MG% NON-FAST BLOOD SUGAR			SIGNIFICANCE	
	BEFORE	AFTER	△		
DIABETIC 25 C	529	498	-31	n.d.	n.d.
DIABETIC 5 C	507	421	-86	n.d.	

Significance was tested using the standard *t* test.

apparent why a diabetic animal would conceivably have a problem in surviving the first 2 weeks of cold. Once the diabetic rat has passed this critical period, survival is more assured. Once full acclimation has been achieved, i.e., 4 weeks, death is unusual. In the present study, the survival of diabetics after 4 weeks of exposure was 100%. Muscle glycogen no longer differed from normal and the rats were gaining weight. The increased ability to metabolize glucose after acclimation would account for these findings. The absence of a decrease in hyperglycemia does not necessarily detract from the hypothesis, because it could reflect only the insensitivity of blood sugar as a means of estimating the amount of insulin present.

The decrease in cardiac glycogen in cold-exposed diabetic rats could be attributed to an increased utilization by the heart itself. Cardiac rate is increased in the cold (20). Glycogen stored in the heart is considered to be a source of energy for the heart itself, and an increase in heart rate would require an increased rate of utilization. Augmentation of glucose metabolism by the cold could make this possible. The original relationship of the increased glycogen level in the diabetic could be attributable to a mass action effect (21).

In summary, much data indicate that an increased ability to utilize glucose, i.e., an increased insulin sensitivity, occurs in the cold. This would make possible the survival of the alloxan-diabetic rat once it has survived an initial but critical exposure period. The muscle and liver glycogen levels of the diabetic rats as found in this study support such a hypothesis. The explanation of an increased tissue sensitivity with cold acclimation would also agree with findings for other endocrine substances, including norepinephrine which is reported to be the mediator of nonshivering thermogenesis by some investigators (22).

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