Effect of Insulin on Glycosuria, Polyuria and Food Intake in Alloxanized Rats

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INTRODUCTION

In recent studies of the quantitative relationships among glycosuria, food intake and body weight in alloxan-diabetic rats, we utilized an index of disturbance in glucose metabolism that was independent of variations in food intake and body weight.[1] This index was expressed as the ratio of glycosuria to food intake, which is readily converted to the percentage dietary carbohydrate that is utilized. Utilization thus calculated was found to be significantly increased by adrenalectomy and reduced by lethal doses of cortisone or thyroxin.[2] Sublethal doses of cortisone or thyroxin, adrenocorticotrophic hormone, diethylstilbestrol, cobaltous chloride, and partial thyro-parathyroidectomy-thiouracil treatment had no significant effect.[2] Starvation[1] and treatment with an anorexigenic drug[3] (nitrogen mustard) were also ineffective. The change in carbohydrate utilization, averaged over five days of treatment with insulin, was linearly related to the log dose of insulin.[3] The present report details the temporal progression of glycosuria, polyuria, food intake, body weight, and the calculated carbohydrate utilization before and during the time of insulin administration.

MATERIALS AND METHODS

Routine Alloxanization Procedure. Male Sprague-Dawley rats, 120 to 160 gm. body weight and fed granulated Rockland Rat diet and tap water ad libitum, are injected intraperitoneally with 160 mg./kg. body weight of a freshly prepared, 1 per cent aqueous solution of alloxan monohydrate (Eastman) on Monday and again on Wednesday. On Friday, urine is collected for twenty-four hours. Those rats with a urine volume less than 30 ml. receive a third injection of alloxan the following Monday; those failing to respond with polyuria to this injection are discarded. All of the remaining alloxanized, supposedly-diabetic rats are then kept for three weeks before collecting another twenty-four-hour urine sample for glucose analysis and confirmation of diabetes. During this three-week interval, some rats show remissions of glycosuria, while the remainder develop a fairly stable diabetic state as indicated by a plateau in glucose excretion.[4] At this time, variations in food intake and body weight account for nearly 88 per cent of the variability in glycosuria.[1] The rats are never starved before alloxan injection[5] because we have found this procedure to increase mortality disproportionately to the increase in the number of diabetics produced. Substitution of 1 per cent glucose solution for tap water, or glucose injection,[6] in order to prevent fatal post-alloxan hypoglycemia,[7] has been of such slight benefit to us that it is not believed to be worth while.

A summary of the results of alloxanization of twenty representative series of approximately fifty rats each follows: 1,024 rats received the first and second injections of alloxan, after which 61 (6.0 per cent) died. Of the survivors, 484 (47.3 per cent) developed a permanent diabetes and the 479 (46.8 per cent) alloxan-resistant rats received the third injection. Of these 479, 160 became permanently diabetic. Therefore, from 1,024 rats, a total of 644 (62.9 per cent) diabetics was obtained. These data do not support previous observations that the ED_{50}[8] and the LD_{50}[9] for alloxan are essentially the same.[10]

Experimental. Thirty-five diabetic rats, prepared as above, were placed in individual, paraffin-coated, metabolism cages and offered tap water and a medium carbohydrate diet[1] ad libitum. Spilled drinking water was trapped to prevent dilution of the urine; the diet was given in the form of a thick paste to minimize spillage. The maximal available glucose and energy contents per gram of dry diet were calculated from all nutrients as 0.64 gm. and 4.8 kcal. respectively. Urine was collected daily under toluene and analyzed for glucose by a modification of the Hanes-Hagedorn-Jensen method. Glycosuria (G) was recorded daily for each rat in grams glucose, urinary glucose concentration in gm./L., urine volume in ml., food intake (I) in grams dry food, and body weight in grams.

After ten days of control observations, each rat re-

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ceived seven daily subcutaneous injections of protamine zinc insulin (Lilly), 10 U/kg. body weight. For purposes of calculation, the data obtained the first three control days were omitted in order to delete any variations attributable to adaptation of the rats to the experimental conditions.

On each day of observation, the glucose excretion of each rat was divided by the intake of available glucose (0.64I) to obtain the percentage excreted (100G/0.64I); subtraction from 100 yielded percentage utilization. These percentages, as well as the glycosuria, urinary glucose concentration, urine volume, food intake, and body weight, were then averaged for all of the rats on each day.

RESULTS AND DISCUSSION

During the pretreatment week, glycosuria (G) was significantly related to food intake (I) and body weight (W). The respective regression equations, calculated from the thirty-five means of the last five pretreatment days, were as follows: $G = -0.73 + 0.359\ I$ and $G = 4.79 + 0.016\ W$. These relationships compared favorably to those obtained in previous experiments.[1, 2] Also during this week, the daily mean urine volumes and urinary glucose concentrations remained fairly constant, averaging approximately 115 ml./day and 75 gm./L., respectively (figure 1); however, during the week of insulin administration, these values progressively decreased to approximately 20 ml. and 20 gm./L. on the seventh day. Cohen[11] has shown that the urinary glucose concentration of alloxan-diabetic rats increases progressively with urine volume until a limiting value of 60-80 gm./L. is attained, whereafter the concentration plateaus. This ceiling of renal concentrating ability is reached at a blood sugar level of approximately 300 mg./100 ml. Our data confirm Cohen's observations.

As a result of the decrease in glucose concentration and urine volume (figure 1), glycosuria fell from a pretreatment average of 8.4 gm./day to 0.4 gm. on the final day (figure 2). The regression of glycosuria on urine volume was linear and without a plateau, again confirming Cohen's results. The percentage excretion of available dietary glucose tended to parallel glycosuria because of the relative constancy of food intake: while the percentage excretion dropped from a pretreatment average of 50.9 per cent to a final value of 2.8 per cent (table 1), the food intake ranged between 24 and 30 gm./day both before and during insulin therapy (means: 27.1 and 26.8 gm./day, respectively). In other words, the untreated diabetic rats utilized almost 50 per cent of their consumption of 68 gm. available glucose/

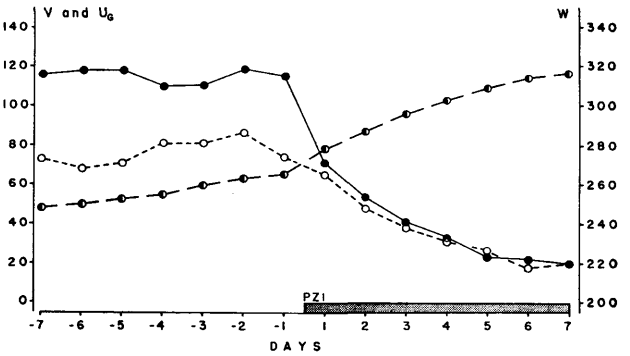

FIG. 1. Temporal responses to 10 U/kg./day of protamine zinc insulin (PZI) of alloxan-diabetic rats. Urine volume in ml. (V: solid circles), urinary glucose concentration in gm./L. (UG: open circles), and body weight in gm. (W: halved circles).

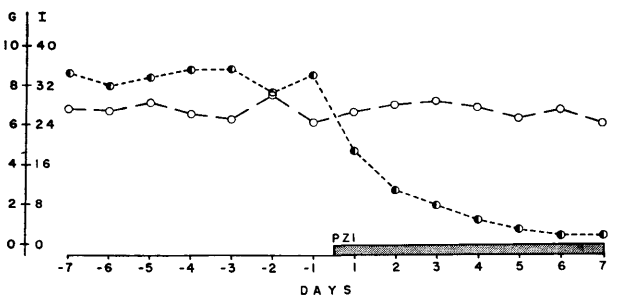

FIG. 2. Temporal responses to 10 U/kg./day of protamine zinc insulin (PZI) of alloxan-diabetic rats. Glycosuria in gm. (G: halved circles) and dry food intake in gm. (I: open circles).

kg. body weight/day, while seven days of insulin-treatment increased utilization to 97 per cent of the intake of 49 gm. glucose/kg./day. Notwithstanding the constant food intake, gain in body weight rose from a pretreatment average of 2.4 gm./day to a treatment average of 5.6 gm./day (table 1); the nature of this acceleration was not determined.

It is of interest to compare our results to those obtained by Dohan, Fish, and Lukens[12] on anterior pituitary-diabetic dogs fed raw beef heart ad libitum. Insulin treatment reduced to practically nil the urinary excretion of 80 to 90 per cent of the available glucose consumed without markedly altering the food intake. It is also desirable to compare our figures to those derived by Stetten, Welt, Ingle, and Morley[13] from experiments on several fasted, anesthetized, alloxan-diabetic and normal rats, previously force-fed. Under intravenous glucose infusions, their diabetic rats utilized 39 per cent as much glucose as the normals. This figure seems in fair agreement with ours of 50 per cent considering the differences in experimental approach.[14] Comparable

TABLE 1
Effect of insulin in alloxan-diabetic rats

Daily Values	Pretreatment Mean	Days of Insulin Therapy						
		1	2	3	4	5	6	7
Weight gain, gm./day	2.4	12.8	9.4	9.0	7.0	5.4	5.6	2.6
Glucose excretion, per cent of intake	50.9	27.0	14.7	11.4	6.7	5.0	2.9	2.8
Glucose retention, per cent of intake	49.1	73.0	85.3	88.6	93.3	95.0	97.1	97.2

results were obtained by Chernick and Chaikoff,[15] who found the oxidation of added glucose by diabetic liver slices to be 10 to 60 per cent of normal.

SUMMARY

A detailed description is given of the procedure used to induce alloxan diabetes in 1,024 rats with a 63 per cent success.

In alloxan-diabetic rats fed ad libitum, daily insulin therapy caused progressive falls in urine volume, glucose, and glucose concentration, together with a transient increase in weight-gain. Since no change in food intake was observed, the improvement in calculated glucose utilization (49 to 97 per cent) paralleled the improvement in glycosuria.

SUMMARIO IN INTERLINGUA

Effectos de Insulina Super Glycosuria, Polyuria, e Ingestion de Alimentos in Rattos Alloxanisate

Es presentate un description detaliate del technica usate pro inducer diabete a alloxano in 1.024 rattos. Le methodo succedeva in 63 pro cento del animales.

In rattos con diabete alloxanic, mantenite sin restriction de dieta, un diurne therapia a insulina causava progressive reductiones del volumine de urina, del glucosa, e del concentration de glucosa, insimul con un transiente acceleration del augmento de peso. Nulle alteration del ingestion de alimentos esseva observate, e le melioration del calculate utilisation de glucosa (ab 49 usque a 97 pro cento) esseva parallel al melioration del glycosuria.

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