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## ORIGINALS

# Diabetes in the Sand-Rat: Diabetogenesis, Responses to Mannoheptulose and Atriplex Ash

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*Summary.* Diabetes was induced in sand rats by increasing the dietary intake of calories. The development of diabetes was followed by progressively greater hyperglycaemic levels in the glucose tolerance test and the appearance of cataract, glucosuria and obesity. Ketonuria was never seen. Diabetic sand rats had a slightly elevated BMR and an RQ of approximately 1. They responded to D-mannoheptulose with hyperglycaemia but not with reduced RQ. *Atriplex halimus* ash did not reduce the hyperglycaemic response to glucose, though it has been reported to do so in alloxanized rats. Alloxanized rats were shown to retain responsiveness to D-mannoheptulose and it is speculated that response to D-mannoheptulose may be dependent on secretion of glucagon.

*Le diabète du rat des sables: Diabétogénèse, réponse au mannoheptulose et à la cendre de l'Atriplex Halimus*

*Résumé.* Le diabète a été provoqué chez le rat des sables par une augmentation de la prise de calories. Le développement du diabète a été marqué par l'élévation progressive des taux d'hyperglycémie dans le test de tolérance au glucose, par l'apparition de cataracte, de glycosurie et d'obésité. La cétonurie n'a jamais été constatée. Les rats des sables diabétiques ont un BMR légèrement élevé et un RQ de 1 approximativement. Ils répondent au D-mannoheptulose par une hyperglycémie, mais non par un RQ réduit. La cendre de l'*Atriplex Halimus* ne réduit pas l'hyperglycémie due au test de tolérance au glucose alors qu'elle la réduit chez les rats alloxanisés. Les rats alloxanisés répondent au D-mannoheptulose, il se peut que la réponse au D-mannoheptulose soit sous la dépendance d'une sécrétion de glucagon.

*Diabetes bei der Sandratte: Pathogenese, Reaktion auf Mannoheptulose und Atriplex-Asche*

*Zusammenfassung.* Bei Sandratten wurde ein Diabetes durch erhöhte Kalorienaufnahme in der Nahrung erzielt. Der Entwicklung des Diabetes folgte eine fortschreitende Erhöhung des Blutzuckerspiegels beim Glucosetoleranztest, sowie Glykosurie, Fettsucht und Katarakt. Eine Ketonurie wurde niemals beobachtet. Die diabetischen Ratten hatten einen leichten erhöhten Grundumsatz und einen respiratorischen Quotienten von etwa 1,0. Auf D-Mannoheptulose reagierten sie mit Hyperglykämie, nicht aber mit einer Erniedrigung des respiratorischen Quotient. Die Gabe von Asche von Atriplex halimus (Staudenmelde) setzte den hyperglykämischen Glucosetoleranztest nicht herab, wie es bei alloxan-diabetischen Ratten beobachtet wurde. Alloxan-diabetische Ratten behalten ihre Reaktionsfähigkeit auf D-Mannoheptulose; es wird vermutet, daß diese Reaktionen auf D-Mannoheptulose von einer Glucagon-Sekretion abhängig ist.

*Key words*: Salt Bush, Atriplex Ash, Mannoheptulose, Sand Rats, Diabetogenesis, Alloxan-diabetic Rats, Glucose Tolerance Test, R. Q.

## Introduction

Sand rats are latent diabetics. When maintained on a low caloric intake (30 kcal/d) they remain normoglycaemic, but caloric loading induces a diabetic syndrome characterized by hyperglycaemia, cataracts and glucosuria (Hackel *et. al.*, 1965, 1966; Miki *et al.*, 1966, 1967).

The aetiology of the diabetes has been described as falling into two stages.

(a) "Severe" diabetes, in which circulating insulin is high, pancreatic insulin content is normal or reduced, body weight increases due to obesity and there is no ketosis (Hackel *et al.*, 1966); followed by (b) "Fulminating" diabetes, with low circulating insulin, low pancreatic insulin content, weight loss and ketosis with early death. "Fulminating" diabetes may follow the "severe" type, though "severely" diabetic animals sometimes recover. Histologically the B-islet cells are degranulated in both types; in the "fulminating" type the islets are necrotic with glycogen infiltration and vacuolation of the cells (Like and Miki, 1966).

In this study, diabetes was induced in sand rats by caloric loading and insulin secretion was evaluated using three tests. First, the changes in the glucose tolerance test (GTT) during the period of caloric loading were followed. Second, the RQ of diabetic sand rats was measured. This measurement was based on our earlier finding that even during prolonged fasting, the RQ of the non-diabetic sand rat did not fall (Frenkel and Kraicer, 1972a). Blockade of insulin secretion by D-mannoheptulose (M-H) did cause a fall in RQ, indicating a shift to increased fat catabolism (Frenkel and Kraicer, 1972b). Thus, measurement of RQ provides an index of available insulin. Finally, as the ash of the salt bush, *Atriplex halimus*, (the natural

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diet of the wild sand rat) has been reported to potentiate insulin action (Shani *et al.*, 1972) and to reduce hyperglycaemia in diabetic rats (Aharonson *et al.*, 1969), the ability of this ash to reduce the hyperglycaemia produced by a glucose load in diabetic sand rats was tested.

As a result of these studies, the mechanism of action of M-H was called into question. It appeared that M-H may have other endocrine activities besides suppression of insulin release. (Coore and Randle, 1964; Simon and Kraicer, 1957, 1966).

## Materials and Methods

Two of the sand rats used in this study were obtained by the courtesy of Mr. A. Wint of Kibbutz Sdeh Boker, who raised them in the village zoo, where they were fed a diet of bread and rat checkers and were housed in pens. The others were captured in the vicinity of the kibbutz. After quarantine, these sand rats were housed in the conditions described elsewhere (Frenkel *et al.*, 1972), except that they were fed rat checkers with occasional supplements of vitamins; 3% of unpurified salt solution was available *ad lib.* as beverage. Food consumption was measured in metabolism cages. The mean caloric intake was $50.9 \pm 3.3$ kcal/d. Their urine was checked for glucose and acetone once weekly using Clinistix and Acetest (Ames Co.).

Glucose tolerance tests (GTT) were performed 18, 44, 66 and 94 days after loading was begun. Blood glucose levels were followed for 6 h after s.c. injections of 175 mg of glucose/100 g body weight (B.W.). Diagnosis of diabetes was based on glucosuria and diabetic GTT. The hyperglycaemia was quantitated as the area under the individual GTTs. Accompanying signs usually included obesity, due to accumulation of subcutaneous fat, and cataract.

The following tests were performed on diabetic sand rats after 14 h of pretreatment fasting:

a) Mannoheptulose (M-H) tolerance test (Frenkel & Kraicer, 1972c).

b) Measurement of R.Q. after M-H or control (saline injections) (Frenkel & Kraicer, 1972b).

c) Effect of salt-bush (Atriplex halimus) ash on GTT.

The control GTT was performed 2 weeks earlier. For comparison, the effect of an iso-osmotic solution of sodium chloride (9.5% W/V) on GTT was tested 2 weeks later. Salt-bush ash was dissolved in 0.1 *N* HCl by boiling under reflux for 30 min, cooled and neutralized to pH 7 with 0.1 *N* NaOH. The resultant solution was diluted to 14% W/V ash solution. The electrical conductivity was 70 m Mho/cm. Each sand rat received two gavages of 1.8 ml of the above solution 30 min apart, giving a total dose of 0.5 g of ash per sand rat. The GTT was begun 3½ h after the first administration of ash or sodium chloride solution. Plasma $Na^+$ was measured by flame photometry.

A protocol of the sand rats used is given in Table 1: Nos. 5 and 7 came from the Sdeh Boker zoo.

Alloxan-diabetic rats were prepared as described elsewhere (Aharonson *et al.*, 1969). Their blood glucose level before fasting was $465 \pm 43$ mg% (M + SEM). They were fasted overnight (14 hours), a zero-time blood sample was taken, and 200 mg M-H/100 g B.W. was injected s.c. Blood samples were taken hourly 1 to 6 hours after injection.

Blood was taken from the cut tip of the tail and concentrations of glucose, M-H and ketone bodies were measured (Frenkel and Kraicer, 1972c).

## Results

*Development of diabetes.* The development of diabetic GTT during 3 months is shown in Fig. 1. Glucose, but not ketone bodies, was found in the urine during the period of induction of diabetes. At the time of the tests all sand rats had severe glucosuria. The last GTT of the diabetic sand rats, after 94 days of caloric loading, is shown in Fig. 2: $18.3 \pm 0.9$ mg% of ketone bodies (acetone-equivalent) were found in the zero-time blood samples after glucose injection. Normal values in non-diabetic sand rats of our colony are $3.3 \pm 0.14$ mg% (mean $\pm$ S.E.M. of 7 values). This value is approximately the same as that of rats fed the same checkers.

Table 1. *Protocol of diabetic sand rats used in this study*

| Animal No. | Initial B.W. | Final B.W. | Appearance of cataract | % increase in GTT area after 94 days of caloric induction |
|---|---|---|---|---|
| 1 | 210 | 275 | none | 390 |
| 2 | 174 | 190 | none | 190 |
| 3 | 140 | 205 | 3rd month | 190 |
| 4 | 205 | 225 | none | 170 |
| 5 | 94 | 200 | 1st month | 510 |
| 6 | 206 | 290 | 2nd month | 190 |
| 7 | 104 | 176 | 1st month | 400 |

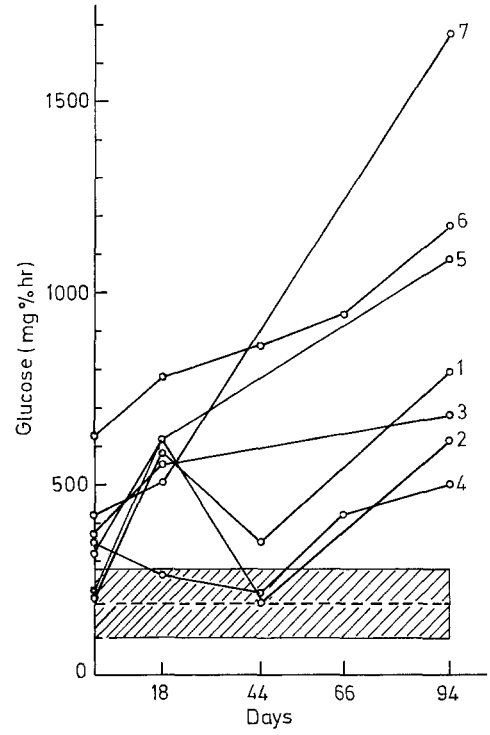


Fig. 1. Changes in glucose tolerance test of individual sand rats (areas under curves), during period of caloric loading. The shaded area is the 95% fiducial limits of the glucose tolerance test of non-diabetic sand rats

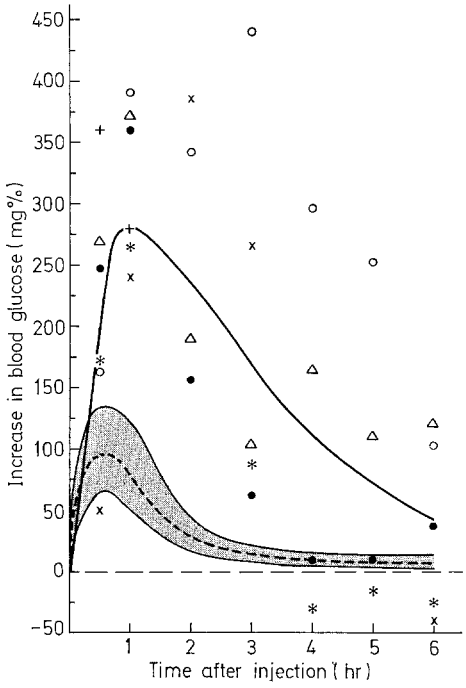


Fig. 2. Glucose tolerance test of diabetic sand rats (Day 94 of Fig. 1). The animals are represented as follows: 1,●; 2,*; 4,×; 5,+; 6,△; and 7,0. The shaded area represents the 95% fiducial limits of the normal glucose tolerance; the upper line is the average of the 6 diabetic responses

responsive to M-H although absorption and elimination may be somewhat slower (Fig. 3).

M-H normally depresses the R.Q. of sand rats to $0.88 \pm 0.032$ from a control fasting value (saline-injected) of $0.98 \pm 0.23$ (Frenkel & Kraicer, 1972b). In the diabetic sand rat no difference between the M-H treated and control groups was found. Variations between times of measurement (Fig. 4) were not significant and the overall means were $0.97 \pm 0.008$ for M-H-treated and $0.97 \pm 0.010$ for the saline-treated. The B.M.R. of diabetic sand rats was $0.65 \pm 0.074$ ml $O_2$/g B.W. h while in normal sand rats it was 0.52 $\pm 0.026$ ml $O_2$/g B.W. h (Frenkel & Kraicer, 1972a).

The administration of salt bush ash had no significant effect on fasting levels of glucose (within the first 3 h. of ash administration) in $^3/_6$ sand rats; one animal, No. 6, showed a rise and 2 others Nos. 3 and 5 had decreases. The GTT was unaffected in Nos. 1, 2 and 5, reduced in Nos. 3 and 4 and elevated in No. 6. Sodium chloride solution had a distinctly hypoglycaemic effect on the GTT but little or no effect on the fasting level (Fig. 5). Sodium levels in the plasma 1 h. after the first administration of ash or NaCl solution rose to $269 \pm 22$ and $318 \pm 33$ mval/l respectively (normal plasma values of $Na^+$ from 8 normal animals were $155 \pm 4.5$ mval/l). The administered salt bush solution contained, 1500 mval/l $Na^+$ and 385 mval/l $K^+$.

The hyperglycaemic response of alloxanized rats to M-H is shown in Fig. 6. Despite the rapid elimination

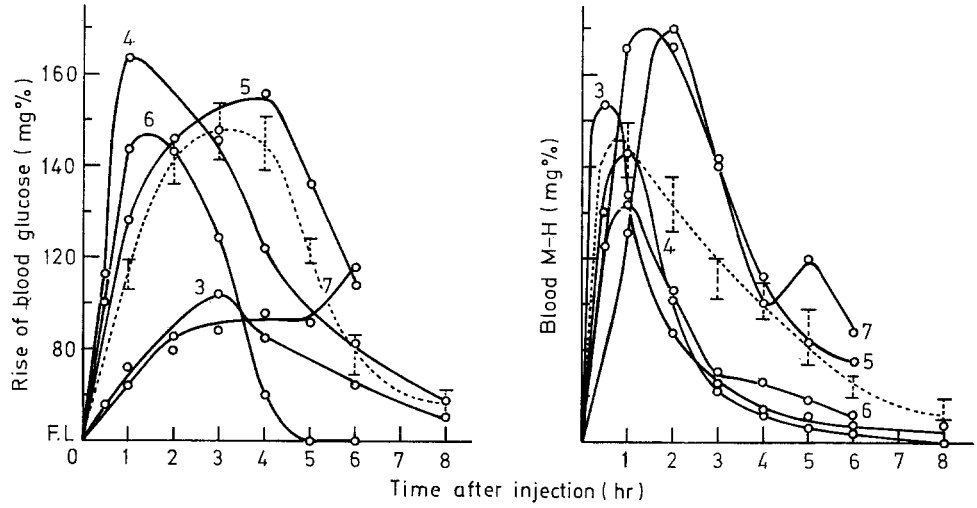


Fig. 3. Mannoheptulose tolerance test of 5 diabetic sand rats. The dotted curves are those of non-diabetic sand rats, I $\pm$ SEM

*Metabolism of diabetic sand rats.* The absorption of M-H from the s.c. injection site was distinctly retarded in three of five sand rats. The rate of disappearance from the blood was also markedly slower. The time of maximum concentration in the blood was one to two hours after injection. The hyperglycaemic responses to M-H were not different from the normal in $^3/_5$ and were lower in $^2/_5$. Thus, the diabetic sand rat remains of M-H ($T\frac{1}{2} = 1.2$ h.) the blood glucose remains elevated for more than 6 hours.

## Discussion

On the basis of our limited data it is impossible to specify the type of diabetes seen in our sand rats. They

appear to have the "fulminating" type of diabetes, on the basis of their insulin sensitivity. They do not, however, show the striking ketosis and early death described as characteristic of "fulminating" diabetes (Miki *et al.*, 1966). During the development of diabetes, the GTT became progressively more impaired. At the end of the induction period, the sand rats showed the following metabolic characteristics:

1. their GTT was severely diabetic;
2. their BMR was not depressed; and
3. their RQ was close to unity.

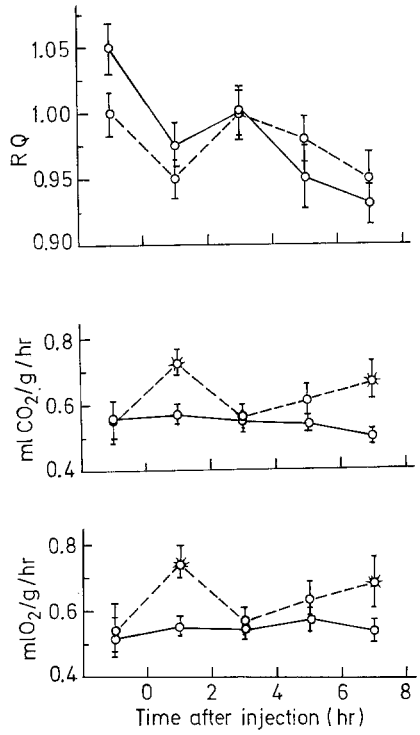


Fig. 4. Oxygen consumption $CO_2$ production and R.Q. after injection of mannoheptulose, (broken line) or isotonic sodium chloride solution (solid line). Starred values are significantly different from those of the control values. Values are ± SEM for 4 sand rats (Nos. 1, 5, 6 and 7)

Thus, although they are intolerant to a glucose load, during fasting they utilize glucose at an essentially normal basal rate.

The utilization of glucose is not affected by the ash of salt bush leaves. Earlier work by Aharonson *et al.*, (1969) indicated the presence of an agent in this plant which reduced the hyperglycaemia of alloxan-diabetic rats. This agent is believed to potentiate the action of insulin (Shani *et al.*, 1972). The same material was inactive in diabetic sand rats, rendered hyperglycaemic by glucose administration. The major cation in this ash is sodium and, in fact, an equivalent amount of sodium chloride alone did have weak hypoglycaemic activity in diabetic sand rats. One of us (J.S.) has tested the effect of sodium chloride (approx. 150 mg) in alloxan-diabetic rats and has seen no reduction of hyperglycaemia.

A few preliminary tests (Frenkel, unpublished) suggest that the response of the sand rat to exogenous insulin is not reduced during diabetes, i.e. that the diabetes is not of the insulin-resistant type. This is consonant with the hyperglycaemic response to M-H if it is assumed that the hyperglycaemia reflects insulin lack. It must be stressed, however, that the Israeli sand rat of our colony is somewhat different from the Egyptian animals used in other laboratories. Hence, the analysis of the stages of sand rat diabetes made by others may not be valid for our subspecies.

The high RQ of the diabetic sand rat cannot be accepted without reservation. It may, in fact, be somewhat elevated by fat anabolism, since the diabetic sand rat accumulates abnormally large quantities of subcutaneous fat. Whereas the fat anabolism of diabetic sand rats is elevated, fat catabolism appears to be decreased. In contradistinction to results reported by Miki *et al.*, (1966) there is no ketosis. M-H also did not cause any fall in the RQ.

In short, it may be speculated that, in addition to the decrease in available insulin during diabetes, there is also an impaired utilization of fat.

The paradoxical increase of hyperglycaemic response of diabetic sand rats to M-H is not explicable on the basis of insulin suppression alone. In this study it has been shown that M-H also induces very strong hyperglycaemia in alloxan-diabetic rats. Since it is inactive in pancreatectomized rats (Simon *et al.*, 1961) the response appears to be mediated by a glucagon secretion from the $\beta$-cells. Although glucagon secretion does not appear to be stimulated *in vitro* by M-H (Vance *et al.*, 1968), Müller *et al.*, (1971) have recently reported a rise in circulating glucagon in M-H — infused dogs. The glucagon response was independent of insulin levels.

The rise in glucagon, induced by M-H, may, therefore, explain the hyperglycaemic response of diabetic sand rats and alloxan-diabetic rats. The source of the glucose, in this case, could be fat. Glucagon has been shown to enhance the conversion of fat to glucose by the rat liver (Williamson, 1966). This utilization of acetyl-CoA for glucose synthesis may also explain the lack of ketosis.

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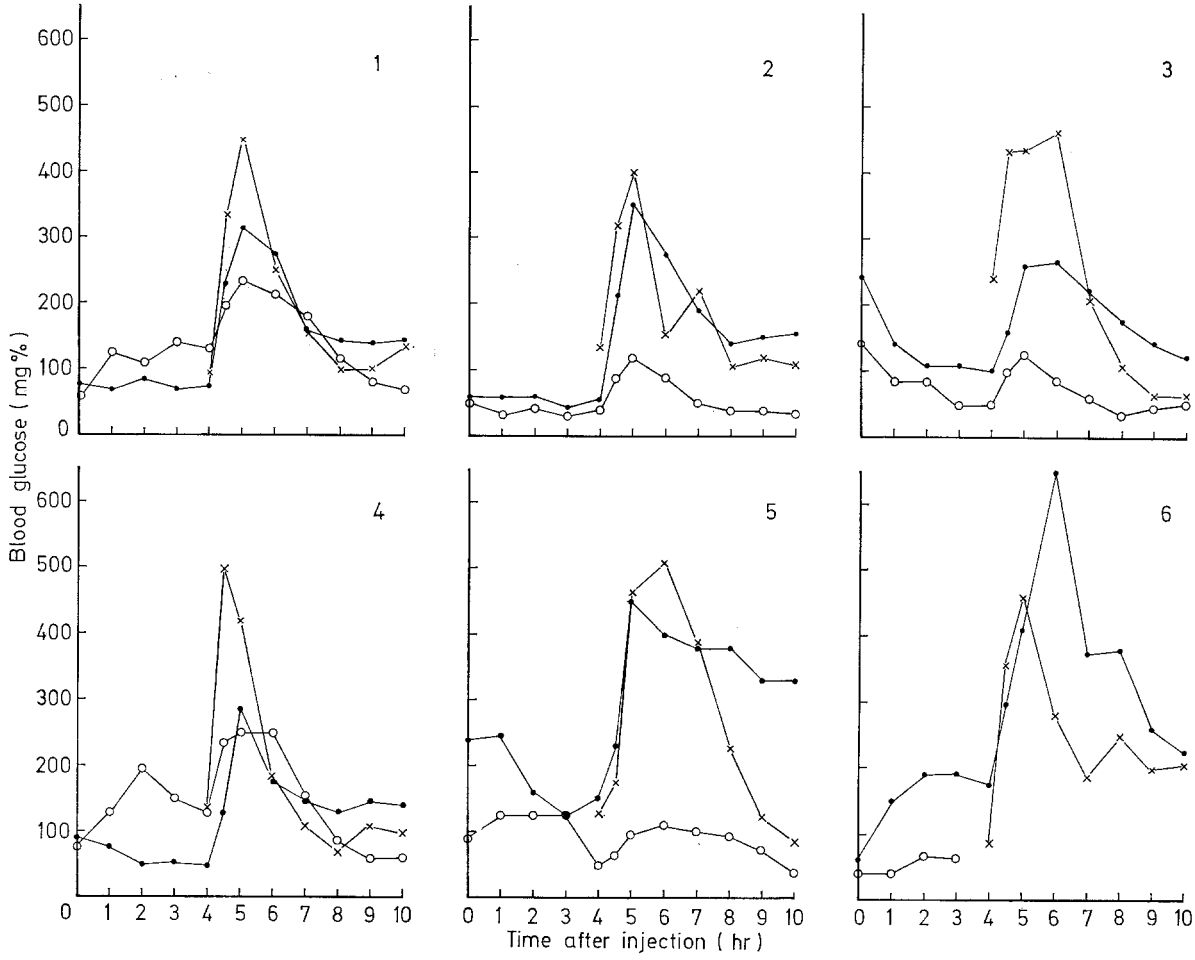


Fig. 5. Individual responses of diabetic sand rats to glucose administration (× — ×); effect of pretreatment *per os* with salt-bush ash (● — ●) or isoosmotic NaCl solutions (○ — ○)

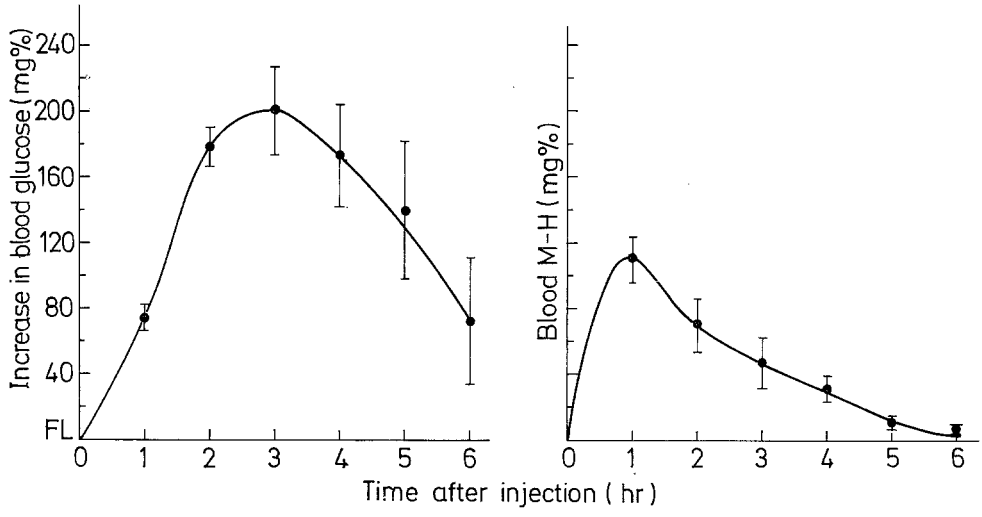


Fig. 6. Mannoheptulose tolerance test of 7 alloxan-diabetic rats. Values are ± SEM. Blood glucose fasting level (FL) was 93.4 ± 8.7

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