EVALUATION OF TESTS FOR LATENT DIABETES
IN THE SAND RAT AND RAT

By

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ABSTRACT

The responses of sand rats and rats to disturbances of their glucose metabolism were tested. Despite their latent diabetes, the sand rats had normal glucose tolerance. They did not show signs of starvation diabetes. Their responses to exogenous and endogenous (tolbutamide-stimulated) insulin were greater than those of laboratory rats. Acute insulin deficiency was induced with mannoheptulose. Both species responded with similar levels of hyperglycaemia, although the response of the sand rat was somewhat slower. The only test used here which indicated a metabolic difference related to latent diabetes, was the induction of transient intolerance to glucose by cortisol. These results suggest that latent diabetes is not associated with any marked metabolic abnormality in the unchallenged sand rat, despite the ease of induction of diabetes by caloric loading.

In this laboratory we have established a colony of a local variety of sand rat (Frenkel et al. 1972). The sand rats have been widely studied because they are latent diabetics (Schmidt-Nielsen et al. 1964). Diabetes can be induced by increasing caloric intake to > 30 Kcal/day (Hackel et al. 1967a).

The purpose of this study was to characterise the regulation of blood glucose concentration in comparison with the laboratory rat. The rat was chosen as a rodent of similar size whose carbohydrate metabolism is well documented and also because it is resistant to the development of diabetes.

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Animals

All the animals were adult males; they were housed in an air-conditioned room, 25 ± 2°C, 50-60% R.H. with 12 hours fluorescent lamp illumination per day. All the rats and 9/25 of the sand rats were colony-bred. The sand rats were fed alfalfa pellets and were given 3% salt solution to drink (Frenkel et al. 1972); the rats received identical care and conditions except that they were fed a diet of standard rat food (Ambar, Hadera) and tap water. All the animals were weighed weekly; only animals which maintained a steady weight were used. The rats weighed between 200 and 240 g and the sand rats 180 to 220 g. Food was withdrawn 14 or 24 hours before experimental treatments, except as noted.

At time zero, the time of administration of the test substances, and at subsequent intervals as noted below, blood was taken from the cut tip of the tail. Sand rats have hairy tails; they were depilated with a cream several days before the experiment and were lubricated with glycerol to avoid tearing the skin. Blood samples, approximately 0.15 ml, were collected in a micro-titration cup containing a small amount of solid sodium heparin and sodium fluoride.

Glucose tolerance test (GTT)

After 0, 14 or 24 h of fasting, and zero-time blood sampling, all the animals received sc injections of 175 mg/100 g body weight of glucose as 17.5% w/v aqueous solution. Blood was taken at 0.5, 1, 2, 3, 4, 5 and 6 h after glucose injection. Changes in the blood glucose concentration were followed using the glucose-oxidase-peroxidase method (Hestrin-Lerner & Ben-Yona 1963).

Cortisol + glucose tolerance test

Each animal was pretreated with 2.5 mg of cortisol acetate sc per day as 0.5% w/v solution in propylene glycol for 7 days. During the cortisol pretreatment they were weighed daily. On the eighth day, after a 14 h fast, the GTT was repeated.

Insulin + glucose tolerance test (IGTT)

The animals were given 0.3 IU of regular insulin and 175 mg/100 g body weight of glucose in 2 separate sc sites after a 24 h fast. The blood was sampled as for the GTT.

Mannoheptulose tolerance test

After a 24 h fast each animal received 20 mg/100 g body weight of mannoheptulose sc as a 20% w/v aqueous solution. Concentrations of mannoheptulose and of glucose were measured 0.5, 1, 2, 3, 4, 5, 6 and 8 h after injection. Mannoheptulose was estimated by Simon & Nelkin's (unpublished) modification of the Dische (1953) reaction; in this modification 1 ml of blood filtrate was heated (60 min, boiling water bath) with 0.2 ml conc. hydrochloric acid; 0.2 ml of 0.48 × 10⁻³ M ferric chloride in 2 N HCl; and 0.1 ml of 0.45% w/v ethanolic orcinol. The cooled mixture was diluted with 3 ml of glacial acetic acid and the O.D. at 610 nm was determined.
**Mannohexulose + tolbutamide tolerance test**

After a 24 h fast, 200 mg/100 g body weight mannohexulose (20% w/v aq.) was injected sc; one hour later 25 mg tolbutamide (Rastinon®, Hoechst, 5% solution) was injected. Blood was taken 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h after mannohexulose injection. The glucose and mannohexulose levels in the blood were measured. After re-feeding the procedure was repeated, substituting an equal volume of saline for the mannoheptulose. The results thus constitute a tolbutamide TT and a control for the mannohexulose + tolbutamide TT.

As a general control of the manipulation technique, the changes in blood glucose concentration were measured following a 24 h fast and sc injection of 1 ml/100 g body weight of isotonic saline.

Thirty minutes later small increases in blood glucose concentration were noted (9.7 ± 2.16 and 7.2 ± 3.45 mg/100 ml in the rats and sand rats respectively). The concentration returned to and remained at the fasting level between 1 and 6 h after the injection.

**Blood analysis**

0.1 ml of blood was diluted with distilled water to 2 or 4 ml; 0.2 ml 10% w/v aqueous ZnSO₄ solution and 0.2 ml NaOH were added to precipitate proteins after which the mixture was centrifuged. The clear supernatant was stored overnight at 4°C.

Sand rats were re-fed to their initial body weight after each experiment, usually this required 1 to 2 months, and they were then re-used.

The means and standard errors of all values were calculated. Significance of differences was evaluated by Student's t-test, using $P < 0.05$ as rejection limit. Significant differences are shown on the graphs as stars.

**RESULTS**

The GTT was studied in unfasted animals and after 14 and 24 h of fasting. With increased time of fasting, both species showed a tendency to greater hyperglycaemia (Fig. 1) and in fact, in rats a striking hyperglycaemia was seen only after 24 h of fasting. In sand rats, the hyperglycaemic peak, during the first 2 h after glucose administration was always higher than that of the rat; the die-away portion of the curve was not affected significantly by fasting. Maximum hyperglycaemic levels for 0, 14 and 24 h of fasting were: for the sand rat 67 ± 19, 96 ± 14 and 147 ± 7 mg/100 ml above the zero-time blood sample level, respectively: for the rats, the values were 35 ± 9, 64 ± 10 and 118 ± 10. Thus the fed sand rat had a somewhat greater hyperglycaemic GTT than the fed rat. The return of the blood glucose concentration to baseline in the rat was prevented by fasting; this was not so in the sand rat.

The sand rat and rat showed similar degrees of hypoglycaemia in the IGTT; the sand rat, however, had a much slower recovery (Fig. 2). Similarly, the hypoglycaemic response of the sand rat to tolbutamide was somewhat greater than that of the rat (Fig. 5). Pre-treatment with cortisol made the
Glucose tolerance tests of rats (black circles) and sand rats (open circles) (all points are m + SEM). "Starred" circles indicate significant ($P < 0.05$) differences between the two species.

(a) Unfasted: Zero-time level (mg/100 ml; m + SEM) = 100.3 ± 6.6 (6 rats) and 58.7 ± 3.3 (6 sand rats).

(b) Fasted for 14 h: Fasting level = 60.5 ± 4.5 (10 rats) and 50.4 ± 1.6 (10 sand rats).

(c) Fasted for 24 h: Fasting levels = 79.4 ± 4.7 (10 rats) and 79.7 ± 8.1 (8 sand rats).

Glucose + insulin tolerance tests of 8 rats and 6 sand rats. Fasting levels were 57.9 ± 3.4 and 54.3 ± 4.4 mg/100 ml respectively.
sand rat, but not the rat, highly intolerant to glucose (Fig. 3). The rat showed a small change in glucose tolerance, the maximum hyperglycaemia being 19.3 ± 6.7 mg/100 ml greater than the maximum of the GTT. The same difference for the sand rat was 109.7 ± 20.2 mg/100 ml. The pre-treatments with cortisol also caused significant increases in the fasting levels to 99.3 ± 3.3 mg/100 ml in the rat and 83.3 ± 6.7 mg/100 ml in the sand rat (cf. Fig. 1). During the week of cortisol treatment all the animals steadily lost weight; during this period the rats lost 16 ± 3.1% and the sand rats 8.1 ± 1.7% of their body weight.

Both rats and sand rats responded to mannoheptulose with marked hyperglycaemia (Fig. 4) but the glucose response was markedly retarded in the sand rats. One factor contributing to the lag in response to mannoheptulose in the

![Graph](image-url)  
**Fig. 3.**
Cortisol + glucose tolerance test. The points are m ± sem for 7 rats and 6 sand rats.
(a) Hyperglycaemic response, and
(b) Circulating mannoheptulose concentrations. Each point represents at least 8 rats or 7 sand rats, after a 24 h fast. Fasting levels were 90.8 ± 8.5 mg/100 ml for rats and 77.4 ± 4.8 for sand rats.
Hypoglycaemic response to 25 mg of tolbutamide of 8 rats and 5 sand rats after a 24 h fast. Fasting levels: 57.9 ± 3.4 mg/100 ml in rats and 59.7 ± 4.2 in sand rats.

sand rats was the retention of significantly higher concentrations in the blood for at least 8 h.

The administration of tolbutamide after mannoheptulose caused rapid fall in hyperglycaemia (compare Figs. 4a and 6). Tolbutamide did not affect the elimination of mannoheptulose from the blood of rats, whereas the elimination was slightly enhanced in the sand rats. The hypoglycaemic response to tolbutamide (i.e. after saline) was significantly greater in the sand rat than in the rat (Fig. 3).

DISCUSSION

In this study we have compared the responses of sand rats and rats to a series of metabolic challenges. The differences between these two species would be expected to reflect the latent diabetes of the sand rat.

The GTT's following 0, 14 and 24 h of fasting indicated that the sand rats of our colony are not sensitive to induction of "starvation diabetes" in contrast to the findings of Brodoff et al. (1967). Although the hyperglycaemic peak in the sand rat was higher throughout, the elimination of excess glucose from the blood was faster in the sand rat.

The sensitivity to endogenous insulin was tested by administration of tolbutamide. Sand rats were found to be more sensitive to tolbutamide than rats.
Fig. 6.
The effect of tolbutamide (25 mg) on response to mannoheptulose in 6 rats and 6 sand rats after a 24 h fast. The fasting levels was 41.7 ± 8.5 mg/100 ml in rats and 40.8 ± 5.8 in sand rats.

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Both were sensitive to exogenous insulin although the sand rats responded with a more prolonged hypoglycaemia. Thus, the sand rat may be more sensitive to endogenous and exogenous insulin; it is certainly not less sensitive than rats. In this respect, our sand rats do not appear to be different from the Egyptian sand rats used by Hackel et al. (1967a) and Brodoff et al. (1967). The sensitivity of the intact sand rat to insulin is in marked contrast to the relative insensitivity of its tissues to insulin in vitro (DeFronzo et al. 1967; Hackel et al. 1967b).

Cortisol depletes pancreatic insulin (BenCosme & Martinez-Palmo 1968; Malaisse et al. 1967) and inhibits the response of target tissue to insulin (De Bodo & Altszuler 1958; Simon et al. 1962). In our sand rats, pre-treatment for 7 days with large doses of cortisol acetate caused a marked but transient hyperglycaemic response to exogenous glucose. This change in the GTT suggests a delay in the secretion of insulin in response to glucose. The GTT of the rat was not affected by cortisol acetate pre-treatment. This suggests that the cortisol-GTT is an appropriate test for the detection of latent diabetes.

The pattern of glucose response of both species to mannoheptulose was similar, but the response developed more slowly in the sand rat. However, the elimination of mannoheptulose from the blood was also slower in the sand rats. Thus, although the responses of rats and sand rats to mannoheptulose were qualitatively similar, the delayed response of the sand rat might be due to a persistance of mannoheptulose in the blood. The secretion of insulin stimulated by tolbutamid was greatly enhanced by mannoheptulose in both species (Kanazawa & Lambert 1970; Renold et al. 1970).

The sand rats of our colony appeared to be different from those used by other investigators. The GTT of non-diabetic sand rats has been reported to be more hyperglycaemic than that of the rat (Hackel et al. 1966, 1967a,b; Brodoff et al. 1967), and also to be very sensitive to exogenous insulin (Brodoff et al. 1967; Hackel et al. 1967a). Our sand rats responded to these tests much less severely. These metabolic differences are probably due to genetic factors and colony conditions. Populations of sand rats are zoo-geographically isolated and hence distinct metabolic differences may have arisen. In our colony the sand rats are fed a basic diet of alfalfa pellets and 3% crude salt solution (Frenkel et al. 1972); other laboratories feed a mixture of ground fresh vegetables. This difference in diet may account for the difference in carbohydrate metabolism. It should be noted that our sand rats become severely diabetic when fed a high carbohydrate diet. Thus, although our sand rats are latently diabetic, their GTT and IGTT are closer to those of the white rat than reported by other investigators.
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