THE REDUCED SECRETION OF, AND SENSITIVITY TO INSULIN IN ZINC-DEFICIENT RATS

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Insulin has a strong affinity for zinc and is associated with this metal in the Islets of Langerhans. It has been suggested that zinc may be concerned with the binding or storage of the hormone in an inactive form pending its release in response to appropriate stimuli (Grodsky and Forsham, 1966; Logothetopoulos et al, 1964). We have found that in zinc-deficient rats there is a reduced glucose tolerance suggesting a reduced ability to secrete insulin in response to a glucose load. The zinc-deficient rats also show a greatly increased resistance to insulin coma.

Hove, Elvehjem and Hart (1937) found only small differences in oral glucose tolerance curves between zinc-deficient and ad libitum fed control rats and they attributed these differences to a generally poor absorption of nutrients from the gut of the zinc-deficient animal. Results we have obtained in studies of protein digestion and absorption do not support this suggestion (Mills et al, 1967). We have however found a consistent difference in the glucose tolerance curves following intraperitoneal administration of glucose (Fig. 1). Rats were given a diet (Mills and Murray, 1960) containing < 1 p.p.m. Zn ('deficient' diet) or with 6 p.p.m. added Zn (control diet). For the glucose tolerance test they were starved overnight, anaesthetised with pentobarbitone sodium and blood samples of 0.03 ml were taken from the tail for glucose estimation using glucose oxidase (Bergmeyer

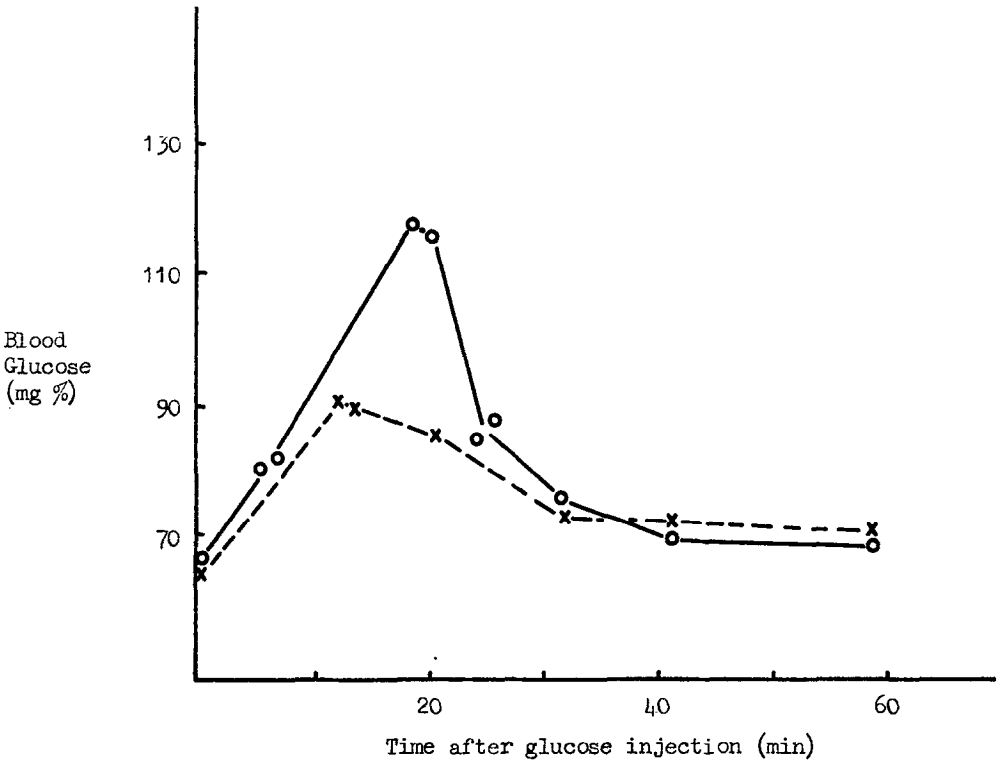

Fig. 1. Glucose tolerance curves of a zinc-deficient (o——o) and a pair-fed (x---x) control rat after an intraperitoneal injection of glucose, 175 mg/100 g body weight.

and Berut, 1963). Maximum glucose values occurred in control rats at 10-12 min and in deficient rats at 16-20 min after intraperitoneal injection of glucose. The increase of blood glucose above the fasting level was always greater in the deficient rats and became three times as much when the dose of glucose was 700 mg/100 g body weight. In 20 experiments there has been no difference between the fasting blood glucose level of deficient (69 $\pm$ 13 mg %, S.D.) or control (75 $\pm$ 12 mg %) rats. When a second dose of glucose was given 1 h after the first the zinc deficient rats showed a serious impairment of blood glucose homeostasis (Fig. 2). These results suggest that the rate of secretion of insulin by zinc deficient rats in response to a glucose stimulus may be less than that of control rats. The poorer response to the first challenge was confirmed by assays of insulin in plasma from jugular

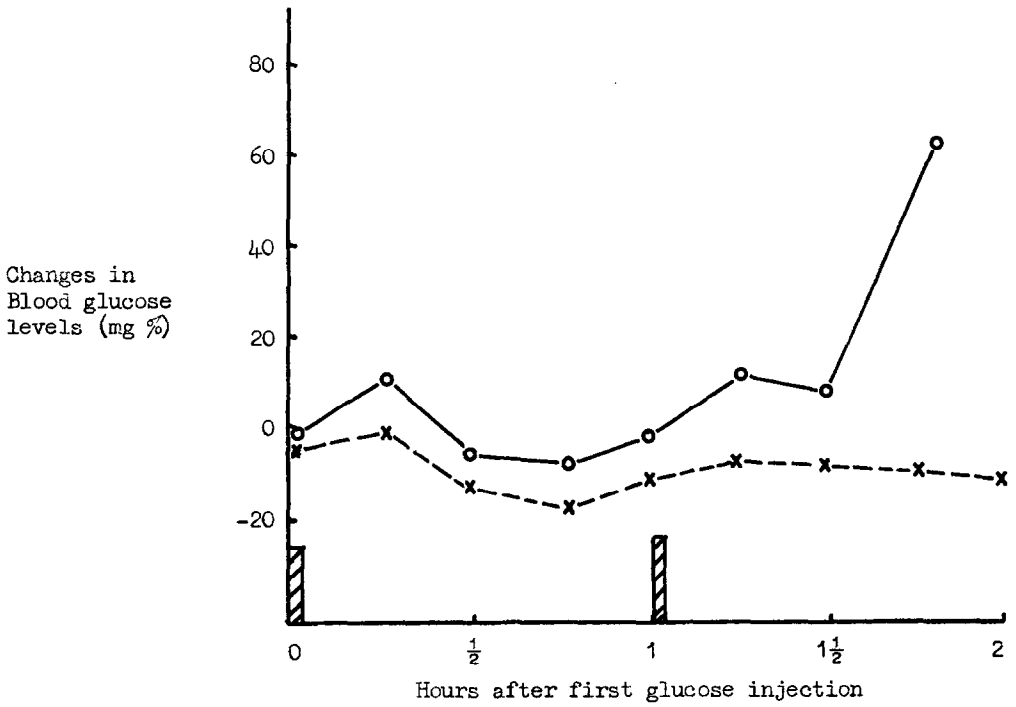

Fig. 2. Changes in blood glucose level in relation to the overnight fasting value of a zinc-deficient (o——o) and a pair-fed control (x---x) rat given intraperitoneal injections of 44 mg glucose per 100 g body weight at 0 and 1 h. The zinc-deficient rat died at 1¾ h.

blood 5 to 15 min after injection of glucose. This showed in four pairs of rats that the zinc deficient rat always had less plasma insulin than its pair-fed control (mean values 24 ± 5 (S.D.) μ units ox insulin equivalent/ml and 30 ± 4 μ units/ml). These assays were made for us by Dr. C. N. Hales, University of Cambridge, by a radio-immunological method (Hales and Randle, 1963) and he points out that the difference is small but probably an under-estimate (Hales and Kennedy, 1964).

In an attempt to show that zinc-deficient do not differ from normal rats in their response to insulin, only in ability to secrete it, deficient rats and their pair-fed controls were given intraperitoneal injections of soluble (zinc-free) insulin, 1 or 2 u./100 g body-weight after being starved overnight. Surprisingly, a very big difference was observed in the time of onset of coma

or convulsions. Most of the control rats became comatose or convulsed within an hour of injection; some took as long as 2 h. None of the zinc-deficient rats was affected before 2 h; about half were affected only to the extent of becoming lethargic and most of the rest entered coma between 3 and 4 h after injection. This effect has been shown in over 30 pairs of deficient and control rats. In 7 pairs of rats glucose concentrations in blood drawn from the tail have been followed during anaesthesia after injections of 1 or 2 u. insulin/100 g and no significant difference between the groups has been found. There is first a rapid drop to a minimum at about 1-1.5 h from injection, then a small rise, a second minimum at about 3-4 h followed by a rise to normal values, the minimum values ranging from 20-30 mg % depending on the amount of insulin given. When blood samples were taken from unanaesthetised rats during insulin coma, the blood glucose values found for deficient and control rats corresponded to those predicted from this pattern according to the time they were taken; they were not necessarily minimum values. Thus the very great difference in insulin sensitivity of zinc-deficient animals cannot be explained by different effects on the level of blood glucose.

Zinc-deficient rats differ from normal rats in many ways which could influence their carbohydrate metabolism and their sensitivity to insulin. They have a much lower content of saponifiable lipid in their tissues and a much higher fasting free-fatty acid level in plasma (a mean of 1056 μ eq./ltr compared with 424 μ eq. in the controls and 368 μ eq. in either group an hour after feeding). They differ in their growth response to pituitary extracts and also in the response of their blood glucose level to tolbutamide, glucagon, adrenalin and guanethidine, an adrenergic blocking agent. Another factor to be taken into account is the difference in feeding pattern. Zinc-deficient rats eat continuously while their pair-fed controls eat their allocation of food in less than 4 h. The metabolic effects of such differences in feeding pattern are being investigated by ourselves and by other groups of workers (Tepperman and Tepperman, 1958; Fabry et al, 1963; Cohn and Joseph, 1960) but it appears unlikely that they can explain our findings in this work. We

have for example shown that normal rats fed continuously become comatose after injection of insulin as readily as the pair-fed controls. Also, it has been shown (Gwinup et al, 1963) that humans eating one meal a day have a greater rise in blood glucose after a glucose load than humans eating continuously. This is, of course, the opposite of our findings in zinc-deficient and control rats.

Our finding in this investigation that glucose produces a reduced insulin response in zinc-deficient animals is not surprising but the observation that zinc-deficient animals are much less sensitive to insulin suggests that there is an, as yet, undefined function of zinc involved.

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