Short Communications

Metabolic Changes in Rats during Developing Thiamin Deficiency

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Determinations of rectal temperature, blood glucose, plasma free fatty acids, liver acetyl-CoA and carcass fat of thiamin-deficient rats indicated that during the ensuing anorexia they metabolized their fat reserves more rapidly than did pair-fed normal controls. Their lower metabolic rate indicates that the reserves mobilized are utilized inefficiently.

Thiamin deficiency shows well-defined biochemical disturbances in animals and in man (Peters, 1936; Platt & Lu, 1939; Brin, 1962; Liang, 1962*a,b,c*), and functional derangement of muscular, nervous and cardiovascular systems has been found (Drury *et al.*, 1930; Winthrop *et al.*, 1943).

The present paper reports differences in the rate of starvation as measured by changes in blood glucose, carcass fat content, plasma free fatty acids and liver acetyl-CoA content between animals in the course of developing thiamin deficiency and pair-fed controls.

Male albino rats of the Sprague–Dawley strain, weighing between 230 and 270 g, were used. They were divided into two groups; the first group was fed on a thiamin-deficient diet (Liang, 1962*a*) *ad libitum* and the second group was fed on a restricted basal diet (Liang, 1962*a*), the daily quantity in terms of calories of the diet being the same as that of the thiamin-deficient diet, but with the addition of thiamin hydrochloride (2.0 mg/100 g of diet) and cellulose powder (Liang, 1962*a*). The food intake, body weight and rectal temperature of the rats were recorded every morning. Rats fed on the thiamin-deficient diet survived up to 45–56 days, with the average body weight decreased from 255 ± 15 g (average $\pm$ s.d., $n = 60$) to 128 ± 12 g (about 50%). That of the control rats in group 2 was decreased from 253 ± 18 g (average $\pm$ s.d., $n = 60$) to 181 ± 19 g.

At intervals of 2–3 days, three rats from each group were killed by decapitation after 6 h starvation, and 3–5 ml of blood was collected into heparinized centrifuge tubes. After mixing, the glucose content of 1 ml of the blood was determined by the methods of Nelson (1944) and Somogyi (1945). The rest of the blood was centrifuged for 15 min at 2000 g (r_{av} , 14 cm) to separate the plasma for determination of free fatty acids by the method of Duncombe (1963). The liver was cut out immediately within 30 s after decapitation and immersed in the liquid N_2 ; the acetyl-CoA content was determined by the method of Lumber *et al.* (1969). The dried weight of the carcass was obtained

by drying it overnight at 105°C; then the dried carcass was cut into small pieces and the lipids were extracted with light petroleum (b.p. 60–80°C) in a Soxhlet apparatus. The results are shown in Fig. 1.

The blood glucose concentration of rats fed on the thiamin-deficient diet was 85–88 mg/100 ml of blood initially. It remained at the same value until the 20th day, then it began to decline steadily to 65 mg/100 ml of blood near death. Under the condition of thiamin deficiency, the rats fed on the diet gained weight during the first 10 days and thereafter lost weight owing to anorexia (Liang, 1962*c*); the same trend was obtained in those rats pair-fed on a restricted diet (Fig. 1). The results showed that the carcass fat content of rats fed on the thiamin-deficient diet increased initially from 20.17 to 23.40 g per rat (equivalent to 8.50–9.17% of the body weight) during the first 10 days on the diet, and decreased rather abruptly thereafter, to 2.06 g per rat (1.62% of the body weight) near death, whereas the pair-fed rats also showed an initial increase in carcass fat from 20.17 to 23.75 g per rat (equivalent to 8.5–9.53% of body weight) during the first 10 days, and thereafter declined to 10.07 g per rat (5.5% of the body weight) on the 56th day. The plasma free fatty acid content of the rats fed on the thiamin-deficient diet was 430 μ equiv./litre of plasma at the beginning and remained at the same value for the first 10 days while the food intake was high; then it rose to a peak value of 540 μ equiv./litre from the 20th to 40th day on the diet and then declined abruptly to 50 μ equiv./litre as the fat-depots became exhausted. The acetyl-CoA content of the liver was 26 nmol/g wet wt. of tissue and remained at the same value until the 10th day, then it rose steadily to a peak value of 58 nmol/g between the 34th and 36th day, coinciding with the increase in plasma free fatty acids, and fell abruptly to 8 nmol/g before death. The rectal temperature remained at 38.4°C for the first 20 days on the diet, and then fell steadily to 35.2°C before death. The pair-fed rats under the condition of semi-starvation showed smaller changes in blood

Fig. 1. Changes in (a) rectal temperature, (b) blood glucose concentration, (c) content of plasma free fatty acids, (d) content of liver acetyl-CoA, (e) carcass fat content and (f) body weight of rats fed on a thiamin-deficient diet (●) and of pair-fed rats (○)

Each point represents the average value obtained from three rats.

glucose concentration, carcass fat content, plasma free fatty acid, liver acetyl-CoA content and a steady decline of the rectal temperature.

It has been mentioned previously (Liang, 1962c) that citrate accumulates in the bodies of thiamin-deficient rats and its further metabolism is inhibited by the complex formed with glyoxylate (Ruffo *et al.*, 1962). It seems that during the first few days on a thiamin-deficient diet the rats have a high food intake, and the content of the blood glucose, plasma free fatty acid and liver acetyl-CoA as well as the rectal temperature stay almost at the initial values; however, the food intake decreases afterwards, whereupon the rats use their fat-stores for energy, as shown by the decreased carcass fat content and the mobilization of free fatty acids. There is also an increased excretion of creatine and oxo acids, as shown previously (Liang, 1962a,c). It seems that the thiamin-deficient rats use up their energy reserves faster than do their pair-fed non-deficient controls. Taken together with the finding that under conditions of thiamin deficiency the metabolic rate is decreased (Liang & Ou, 1971), this indicates that the energy reserves that are mobilized are used inefficiently. The formation of metabolic inhibitors (Ruffo *et al.*, 1962) may be responsible.

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