Effects of liver damage on ketone-body production and nitrogen balance in starved rats

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The metabolic effects of intraperitoneal administration of carbon tetrachloride (1 ml/kg) were studied in starved rats. The most notable change in circulating substrates was an 80% fall in ketone-body concentrations, which was associated with the doubling of urinary nitrogen losses. The results demonstrate the importance of starvation ketosis in permitting fat mobilization to decrease effectively protein losses during starvation.

Impairment of fat mobilization by injection of antilipolytic agents under conditions of food deprivation elicits massive increases in gluconeogenesis and urea excretion (Talke *et al.*, 1973). Increased oxidation of amino acids in starvation with disease can be attributed in part to lower rates of fat mobilization and/or ketogenesis, owing to the higher insulin concentrations which prevail in states of insulin resistance (Flatt & Blackburn, 1974). Because non-esterified fatty acid and ketone-body concentrations are decreased simultaneously in experimental models of protein-wasting situations (Talke *et al.*, 1973; Blackburn & Flatt, 1974; Neufeld *et al.*, 1976), it has not been possible to assess to what extent decreased rates of release of non-esterified fatty acid from adipose tissue and/or decreased ketogenesis may be responsible for the increased amino acid oxidation. Record *et al.* (1972) found that administration of D(+)-galactosamine to starving rats leads to an increase in liver triacylglycerols and to a decrease in circulating ketone bodies, which suggests that ketogenesis is impaired in this form of liver damage. In the present study, injection of CCl₄ was found to decrease markedly starvation ketosis, while fat mobilization from adipose tissue remained unaffected. This provided an opportunity to investigate how the availability of ketone bodies affects amino acid oxidation and protein losses in starved rats.

Experimental

Male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were housed in individual metabolic cages and maintained on a commercial diet (Agway, Syracuse, NY, U.S.A.). When their weight was approx. 190 g, the

food was removed. Then 24 h later, half of the animals were injected intraperitoneally with CCl₄ (1 ml/kg body wt.) mixed with an equal volume of corn oil (Castro *et al.*, 1972), and the others with the corn oil only. Rats were killed by decapitation, at the time of food removal ('fed animals'), just before the CCl₄ injection (1 day starved), or at 8, 24 or 48 h after the CCl₄ injection. Blood was collected in a heparinized beaker; a portion was centrifuged to obtain plasma for determinations of insulin (Soeldner & Slone, 1965) and non-esterified fatty acids (Dole & Meinertz, 1960); another portion was mixed with an equal volume of ice-cold 10% (w/v) HClO₄. After centrifugation, neutralization with KOH and precipitation of KClO₄ in the cold, the supernatant was used for enzymic assays of substrates (Bergmeyer, 1974). Enzymes and coenzymes were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.). Other samples of the blood were deproteinized with sulphosalicylic acid for determination of amino acid concentrations by using a Durrum amino acid analyser (model D-500). Urine was collected for 24 h periods in vials containing 1.5 ml of 1 M-HCl. After dilution, samples were digested with concentrated H₂SO₄; the ammonia was measured with a Technicon autoanalyser. Urinary urea was also determined with the autoanalyser.

Results and discussion

The weight losses during days 1, 2 and 3 of starvation averaged 23, 10 and 12 g respectively in control and CCl₄-treated rats. The effects of starvation with or without injection of CCl₄ on circulating substrates and insulin are shown in Fig. 1. Similar decreases in glucose and insulin occur in the two groups of animals. Fat mobilization from adipose tissue takes place, as shown by marked

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Fig. 1. Effects of CCl_4 injection on circulating substrate concentrations and on urinary nitrogen excretion in starved rats. Rats starved for 24 h were injected intraperitoneally with CCl_4 (1 ml/kg body wt.) mixed with an equal volume of corn oil (●), or with the corn oil only (○). The points showing substrate or insulin concentrations are the averages of six to ten determinations, except four in the fed state ('0 days of starvation'). Urine was collected in individual metabolic cages; the results for urea and total nitrogen (Kjeldahl) are averages of 8 to 51 determinations. Vertical bars indicate the range of ± 1 S.E.M.; where not shown, this range was less than the diameter of the points. P values were determined by Student's t test; * $P < 0.05$; ** $P < 0.005$.

increases in non-esterified fatty acids. After the administration of CCl_4 , the 3-hydroxybutyrate and acetoacetate concentrations fall abruptly

($P < 0.001$), declining in 8 h to 27% and 45% respectively of the concentrations then prevailing in starving controls. Since the turnover of ketone

bodies in the rat varies approximately in proportion to their circulating concentrations (Bates *et al.*, 1968), a decrease in circulating ketone bodies reflects a lower rate of ketogenesis, rather than increased peripheral extraction. A decrease in the conversion of fatty acids into ketone bodies in the CCl₄-treated rats is consistent with the gradual increase of non-esterified fatty acid concentration, which occurs even though adipose-tissue lipolysis appears to proceed at comparable rates in both situations, as one would judge from the similarity of the glycerol and insulin concentrations (Fig. 1).

The total urinary nitrogen excretion over 24 h and the amounts of nitrogen excreted in the form of urea are shown in Fig. 1. In the fed state, the mean urinary nitrogen excretion ($\pm$ S.D.) was 316 ± 40 mg of N/day, of which 83% was in the form of urea. During the 3 days of starvation, the daily urinary nitrogen decreased rapidly, to 186 ± 18 , 114 ± 19 and 117 ± 11 mg of N/day in the control animals. A significantly greater urinary nitrogen loss occurs in the CCl₄-treated rats: 157 ± 29 mg of N on day 2 (+38%; $P < 0.001$), and 235 ± 42 mg of N on day 3 (+101%; $P < 0.001$). Concomitantly urea-N excretion increased from 71 ± 36 mg in controls to 109 ± 34 mg on day 2 (+54%; $P < 0.005$) and from 95 ± 12 to 173 ± 32 mg on day 3 (+82%; $P < 0.001$). Blood amino acid concentrations, shown in Fig. 1 for alanine and the branched-chain amino acids leucine, isoleucine, and valine, were generally elevated to 2–3 times control values 24 h after the CCl₄ injection. These changes were transient, having essentially vanished by the next day.

Concentrations of non-esterified fatty acids must be high and those of insulin must be low for ketogenesis to proceed at rapid rates (Bieberdorf *et al.*, 1970). Since circulating insulin concentrations were unchanged and those of non-esterified fatty acids were equal to or higher than those in starved control animals, the failure to maintain starvation ketosis after exposure to CCl₄ must be attributed to the liver damage caused by this substance. By contrast, the livers of CCl₄-treated rats retained the capacity of synthesizing urea, at nearly twice the control rate, and to generate the ATP required for this process. Gluconeogenesis was adequate to maintain blood glucose concentrations and probably proceeded at a faster rate than in the control animals, given the increased urinary nitrogen and urea excretions. In the starved state hepatic ATP production can be presumed to occur primarily by oxidation of fatty acids to acetyl-CoA and to CO₂. Thus, among the various metabolic processes mentioned, the pathway that converts acetyl-CoA into ketone bodies appears to be most affected by the lesions induced by CCl₄. The reason for this is not known, but it may be recalled that the capacity

for production of ketone bodies by rat liver slices was observed to be lost more readily than that for the formation of urea (Krebs, 1970).

Since the demonstration by Owen *et al.* (1967) that ketone bodies can replace glucose as a fuel for energy production in the brain, the importance of fat mobilization and of ketone-body metabolism in decreasing protein losses during starvation (Cahill, 1970) and during protein-sparing therapy (Flatt & Blackburn, 1974) has come to be increasingly appreciated. Infusion of ketone bodies decreases alanine release from muscle, and urinary nitrogen excretion decreases (Sherwin *et al.*, 1975), suggesting that the supply of alanine may be a rate-limiting factor for gluconeogenesis *in vivo* (Snell, 1979). The striking increases in alanine concentration and in urinary nitrogen excretion which follow the CCl₄ injection in our experiments thus appear to be due to the decreased availability of ketone bodies. The increase in alanine may have been accentuated by less effective hepatic extraction of alanine, as observed in perfused livers from rats in which liver damage was caused by injection of D(+)-galactosamine (Record *et al.*, 1972) and in hepatocytes isolated from CCl₄-treated rats, though the overall capacity for gluconeogenesis remained high in these cells (Kamp & Hornbrook, 1977).

The high nitrogen losses suffered by the rats in which non-esterified fatty acid concentrations were high, but fatty acid conversion into ketone bodies was impaired by CCl₄ injection, demonstrate the importance of starvation ketosis if fat mobilization is to be effective in preserving body proteins during periods of severely restricted food intake. This finding provides an explanation for the elevated nitrogen losses incurred by surgical patients in whom the development of starvation ketosis is curtailed (Wedge *et al.*, 1976).

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