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# Monoacetoacetin and protein metabolism during parenteral nutrition in burned rats

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1. The effect of intravenous infusion of monoacetoacetin (glycerol monoacetoacetate) as a non-protein energy source was evaluated in burned rats. During 3 days of parenteral nutrition, in which animals received 14g of amino acids/kg body wt. per day exclusively (group I) or with the addition of isoenergetic amounts (523kJ/kg per day) of dextrose (group II), a 1:1 mixture of dextrose and monoacetoacetin (group III) or monoacetoacetin (group IV), significant decreases in urinary nitrogen excretion and whole-body leucine oxidation were observed in the three groups given additional non-protein energy as compared with group I. 2. Serum ketone bodies (acetoacetate and 3-hydroxybutyrate) were decreased in rats given dextrose, whereas glucose and insulin increased significantly. Monoacetoacetin-infused animals (group IV) had high concentrations of ketone bodies without changes in glucose and insulin, whereas animals infused with both monoacetoacetin and glucose (group III) showed intermediate values. 3. On day 4 of nutritional support, whole-body L-leucine kinetics were measured by using a constant infusion of L-[1-$^{14}$C]leucine. In comparison with group I, the addition of dextrose or monoacetoacetin produced a significant decrease in plasma leucine appearance and release from whole-body protein breakdown. Gastrocnemius-muscle protein-synthesis rates were also higher in the three groups receiving additional non-protein energy. 4. These findings suggest that monoacetoacetin can effectively replace dextrose as an intravenous energy source in stressed rats. Both fuels are similar in decreasing weight loss, nitrogen excretion, leucine release from whole-body protein breakdown and oxidation, in spite of differences in energy substrate and insulin concentrations.

The metabolic response to injury involves both an increased energy expenditure and a mobilization of amino acids, which have been considered as being beneficial adaptations by the host, since they represent an important nitrogen and energy redistribution (Blackburn *et al.*, 1977). Somatic tissues, especially skeletal muscle, provide amino acids not only for hepatic gluconeogenesis but also to support protein synthesis for wound repair, immunocompetence and for visceral and secretory protein synthesis. However, the reutilization of this increased endogenous nitrogen flux for protein synthesis is less than complete, and results in a much higher nitrogen excretion than during prolonged starvation without stress (Kinney, 1977). This adaptative response, which appears appropriate for injuries limited in their duration, leads to a progressive and severe protein depletion in patients with prolonged infections or severe injury.

An increase in branched-chain amino acid oxidation by skeletal muscle has been suggested as a result of a relative energy deficit in that tissue (Birkhahn *et al.*, 1980). This deficit is thought to be secondary to a decrease in the relative availability

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of non-protein substrates as glucose, ketone bodies and long-chain fatty acids. In fact, a decrease in pyruvate dehydrogenase activity secondary to insulin resistance described in sepsis can limit glucose oxidation (O'Donnell *et al.*, 1976). Hepatic ketone-body synthesis is also decreased under similar conditions and therefore cannot be a substantial energy source, as is observed during chronic starvation (Wannemacher *et al.*, 1979).

In a previous review, we have suggested that alternative energy sources to those currently used in intravenous feedings might be useful in critically ill patients in order to spare body nitrogen (Birkhahn & Border, 1981). Medium-chain triacylglycerols, as well as monoacylglycerols of butyric acid and acetoacetic acid, provide carnitine-independent substrates. Monoacetoacetin, the monoacylglycerol ester of acetoacetic acid, is water-soluble. Therefore it does not require the preparation of an emulsion for intravenous administration, as do triacylglycerols. Previous studies in healthy animals receiving continuous intravenous infusion of monoacetoacetin as a supplement to spontaneous oral intake (Birkhahn & Border, 1978) showed excellent tolerance without apparent physiological abnormalities.

The present study was undertaken to evaluate the effect of monoacetoacetin when replacing glucose as a fuel on multiple estimates of protein metabolism in burned rats. In general, the results suggest that monoacetoacetin is as effective as dextrose in decreasing whole-body leucine oxidation, decreasing protein degradation, and increasing muscle protein synthesis.

## Materials and methods

### *Animal and experimental design*

Male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were used after 1 week of housing in individual cages in a light- and temperature- (26–28°C) controlled room with a stock laboratory diet (Charles River D-3000; Agway Agricultural Products, Minneapolis, MN, U.S.A.) and tap water provided *ad libitum*. Approval to conduct a scald injury in anaesthetized rats had been granted by the Animal Care Committee of the New England Deaconess Hospital. The laboratory is a fully accredited member of the American Association for Laboratory Animal Care and fully adheres to the 'Guiding Principles for Laboratory Animal Care' as promulgated by the American Association for Medical Research.

*Study I.* In order to evaluate changes in urinary nitrogen appearance and serum substrate concentrations induced by the stress used in the second study, 14 rats weighing 205–270g received pentobarbital anaesthesia (30mg/kg body wt.) and a full-thickness scald burn injury by immersing 25% of their body surface area in boiling water for 15s. After the scald injury, the animals were returned to their cages and, along with another 15 non-injured rats, were maintained without food but with tap water *ad libitum*. Urine was collected daily with 3M-HCl. On day 4 of starvation, the animals were decapitated and blood was collected for measurement of serum acetoacetate, 3-hydroxybutyrate and glucose concentrations.

*Study II.* In this, 33 rats weighing 210–260g received pentobarbital anaesthesia and had the external jugular vein cannulated with a 0.50mm × 0.95mm Silastic Catheter (Dow Corning Laboratories, Corning, NY, U.S.A.). At the same time the animals were burned in a similar manner to those in Study I. The catheter was connected to a flow-through swivel (Instech Laboratory, Philadelphia, PA, U.S.A.) permitting continuous intravenous infusion. After 1 day of recovery, in which only 0.9% NaCl was infused, the rats were randomized into four groups to receive 24ml of parenteral nutrition per 100g body wt, via a peristaltic pump (Holter 903; Extracorporeal Co., King of Prussia, PA, U.S.A.) for 3 days. All the solutions contained 5.7% (w/v) amino acids (Aminosyn; Abbott Laboratories, North Chicago, IL, U.S.A.), electrolytes, trace elements and vitamins. Therefore all the animals received comparable amounts of amino acids (13.5–14.3g/kg body wt. per day) (Table 1). Group I did not receive additional non-protein energy. Groups II, III and IV received an additional 531kJ/kg body wt. per day, as dextrose (group II), as a 1:1 mixture of dextrose and monoacetoacetin (group III), or as only monoacetoacetin (group IV). Animals in group III were infused with monoacetoacetin at a rate of 10.25mg/kg per min, and those in group IV at 20.50mg/kg per min. Monoacetoacetin, which was prepared as described previously (Birkhahn & Border, 1978), was dissolved in distilled water and sterilized by passage through a 0.22 $\mu$m-pore filter. The energy concentration of monoacetoacetin solution was estimated from thermodynamic data at 18.41kJ/g.

During the last 3.5h of parenteral nutrition, the rats were housed in metabolic chambers for collection of the expired breath. A tracer amount of L-[1-$^{14}$C]leucine (New England Nuclear Laboratories, Boston, MA, U.S.A.) was added to nutrient solutions so that 1 $\mu$Ci/h was infused. At the end of the radioisotope infusion, the rats were decapitated and blood was collected in chilled empty and heparinized tubes. A section of gastrocnemius muscle was rapidly removed and homogenized in ice-cold 10% (w/v) sulphosalicylic acid.

Table 1. *Dietary intake during 3 days on parenteral nutrition*

Each animal was infused with 24ml/100g body wt. per day, which contained the following electrolytes (g/l): NaCl, 1.2; sodium acetate, 1.7; KCl, 1.5; potassium phosphate, 1.1; potassium acetate, 1.0; $MgSO_4$, 1.0; calcium gluconate, 1.3. Trace elements (New England Deaconess Hospital, Boston, MA) (mg/l): $CrCl_2$, 0.2; $ZnCl_2$, 21.2; $CuCl_2$, 9.91; $MnCl_2$, 4.9; NaI, 0.2. Vitamins (MVI-12; USV Laboratories, Tuckahoe, NY, U.S.A.) (per litre): ascorbic acid, 68mg; retinol, 670$\mu$g; ergocalciferol, 30$\mu$g; thiamin, 2mg; riboflavin, 2.4mg; pyridoxine, 2.7mg; niacin, 27mg; dexapanthenol, 10mg; DL-$\alpha$-tocopherol, 6.8mg; biotin, 41$\mu$g; folate, 0.3mg; cyanocobalamin, 3.4$\mu$g; choline chloride, 200mg. Data are presented as means $\pm$ S.E.M. for $n$ experiments: $*P<0.01$ versus group I.

| Group | ($n$) | Total energy (kJ/kg per day) | Total energy (kcal/kg per day) | Amino acids (g/kg per day) | Non-protein Energy: Dextrose (%) | Non-protein Energy: Monoacetoacetin (%) |
|---|---|---|---|---|---|---|
| I | (7) | 226 $\pm$ 4 | 54 $\pm$ 1 | 13.5 $\pm$ 0.3 | 0 | 0 |
| II | (9) | 726 $\pm$ 33 | 174 $\pm$ 8* | 13.8 $\pm$ 0.6 | 100 | 0 |
| III | (9) | 756 $\pm$ 17 | 181 $\pm$ 4* | 14.3 $\pm$ 0.3 | 50 | 50 |
| IV | (8) | 752 $\pm$ 8 | 180 $\pm$ 2* | 14.2 $\pm$ 0.1 | 0 | 100 |

*Analytical methods*

Daily urinary nitrogen excretion for estimation of nitrogen balance was analysed by automated spectrophotometric analysis (Technicon Autoanalyzer) after a micro-Kjeldahl digestion (Technicon Block Digestor) (Fleck & Munro, 1965).

Serum was assayed spectrophotometrically for glucose (Slein, 1963), acetoacetate (Mellanby & Williamson, 1963) and 3-hydroxybutyrate (Williamson & Mellanby, 1963) with $NAD^+$ or $NADP^+$ as a coenzyme. Acetoacetate analyses were conducted on fresh samples (within 24h) that had not been previously frozen. Serum insulin was determined by a double-isotope radioimmunoassay with pig insulin as a standard (Soeldner & Stone, 1965).

During the constant infusion of L-[1-$^{14}$C]leucine, room air was circulated through the chambers at a rate of 1.6l/min, and the appearance of $^{14}CO_2$ as well as the total $CO_2$ production were measured hourly (Sakamoto *et al.*, 1983).

Plasma amino acid concentrations were determined in a D-100 Amino Acid Analyzer (Dionex Corp., Sunnyvale, CA, U.S.A.). Free leucine specific radioactivity in plasma, in the infusate and in the acid-soluble fraction of homogenized tissues, as well as of leucine incorporated into protein in the precipitates of tissues, were measured as previously reported by using a Beckman LS-8000 liquid-scintillation spectrometer (Moldawer *et al.*, 1980).

*Calculations*

Assuming that the plasma compartment achieved a plateau labelling (steady state) when it was reached in the expired breath (between 2 and 3.5h of continuous L-[1-$^{14}$C]leucine infusion) (Laurent *et al.*, 1984), whole-body leucine appearance was estimated from the equation (Waterlow & Stephen, 1967):

$$Q = I/Sp_{max.}$$

where $Q$ (flux) is the amount of leucine leaving and entering the plasma pool ($\mu$mol/h), $I$ is the radioisotopic infusion rate (d.p.m./h) and $Sp_{max.}$ is the specific radioactivity of plasma free L-leucine at the end of the 3.5h of infusion (d.p.m./$\mu$mol).

The percentage of flux oxidized was derived from $^{14}CO_2$ in the expired breath (assuming that 24% did not appear in the breath; Moldawer *et al.*, 1983), the hourly production of $CO_2$ and the radioisotope infusion rate(s). The oxidation rate of leucine was calculated by multiplying the flux ($Q$) by the percentage oxidized.

The incorporation of leucine into whole-body protein was then derived from the difference between leucine flux and oxidation, and leucine release from protein breakdown from the difference between flux and intake (Waterlow *et al.*, 1978). The leucine intake as a component of the nutrient solution was constant in all the animals.

The fractional muscle protein-synthesis rate was derived from the equation (Garlick *et al.*, 1973):

$$\frac{S_B}{S_i} = \frac{\lambda_i}{(\lambda_i - k_s)} - \frac{(1 - e^{-k_s t})}{(1 - e^{-\lambda_i t})} - \frac{k_s}{(\lambda_i - k_s)}$$

where $S_B$ and $S_i$ are the specific radioactivities of leucine in the sulphosalicylic acid-precipitated proteins and in the tissue acid-soluble pool respectively (d.p.m./$\mu$mol), $\lambda_i$ is the rate constant for the rise in specific radioactivity of the precursor (33.8 days$^{-1}$; Laurent *et al.*, 1984), $t$ was the length of infusion (days) and $k_s$ was the fraction of protein mass renewed each day (%/day).

*Statistical analysis*

Data are presented as means ± S.E.M. for each group of rats. Student's *t* test was used to compare results in Study I. The four groups of animals in Study II were compared by one-way analysis of variance. Between-group comparisons were conducted by using the least significant difference when the *F* test reached a 95% confidence level. Correlations were obtained by using least squares.

## Results

*Study I*

After 3 days of starvation, the injured and non-injured animals lost 23.9 ± 0.8% and 26.0 ± 1.7% of their body weight respectively (Table 2). However, burned rats lost 18% more urinary nitrogen over the 4-day starvation period ($P<0.05$). Serum acetoacetate and 3-hydroxybutyrate concentrations were significantly lower ($P<0.05$) in injured animals, whereas glucose concentrations were similar.

*Study II*

Cumulative nitrogen balance (Fig. 1) was clearly less negative in the animals receiving additional non-protein energy compared with those receiving only amino acids. No statistical differences were observed in rats infused with dextrose (group II) and those with monoacetoacetin (group IV), although the group with the mixture of both (III) resulted in a slightly more negative cumulative balance ($P<0.05$).

Serum concentrations of substrates and insulin (Fig. 2) were also significantly different among the four groups. Acetoacetate concentrations were decreased by dextrose administration, but were increased with the infusion of monoacetoacetin ($P<0.01$). 3-Hydroxybutyrate concentrations were also lower in burned rats given dextrose and significantly higher in rats given monoacetoacetin. Otherwise, glucose and insulin concentrations followed the pattern of dextrose administration. Although there were not large variations among the groups, those animals which received dextrose (groups II and III) had elevated glucose and insulin concentrations, whereas values from animals receiving only monoacetoacetin as a non-protein energy source were not different from those receiving only amino acids.

Table 3 presents plasma amino acid concentrations. Significantly altered values were found for alanine, threonine, serine, glycine, valine, isoleucine, leucine and tyrosine. Compared with concentrations in group-I animals receiving only amino acids, most concentrations increased, except for the branched-chain amino acids, which were decreased in the groups infused with dextrose as

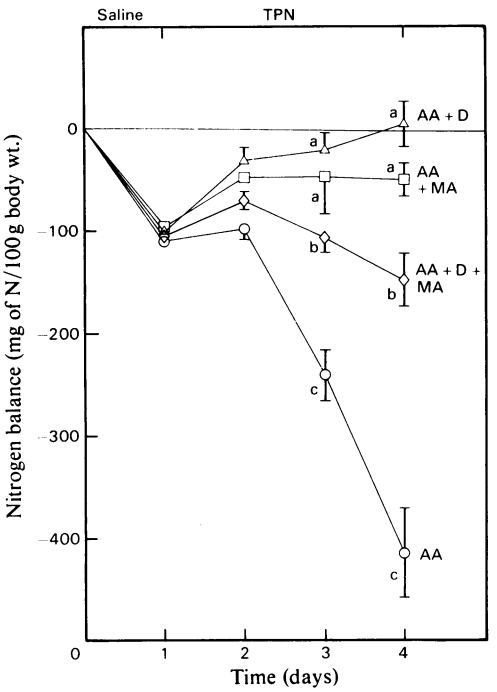


Fig. 1. *Cumulative nitrogen balance in burned rats receiving isonitrogenous parenteral nutrition of different energy composition*

The addition of non-protein energy as dextrose or monoacetoacetin decreased the negative nitrogen balance in rats receiving amino acids only. [a] versus [b] versus [c]: $P<0.01$. Data are presented as means ± S.E.M. Abbreviations: AA, amino acids; D, dextrose; MA, monoacetoacetin; TPN, total parenteral nutrition.

Table 2. *Nitrogen losses and serum substrates in starved and burned rats*
For details see the text. *$P < 0.05$ versus starved.

| | Cumulative urinary nitrogen loss (mg/4 days) | Serum glucose (mM) | Serum 3-hydroxybutyrate (mM) | Serum acetoacetate (mM) |
|---|---|---|---|---|
| Starved | 720 ± 30 | 6.57 ± 0.22 | 0.990 ± 0.096 | 0.195 ± 0.015 |
| Starved, burned | 850 ± 30* | 7.03 ± 0.39 | 0.503 ± 0.051* | 0.145 ± 0.018* |

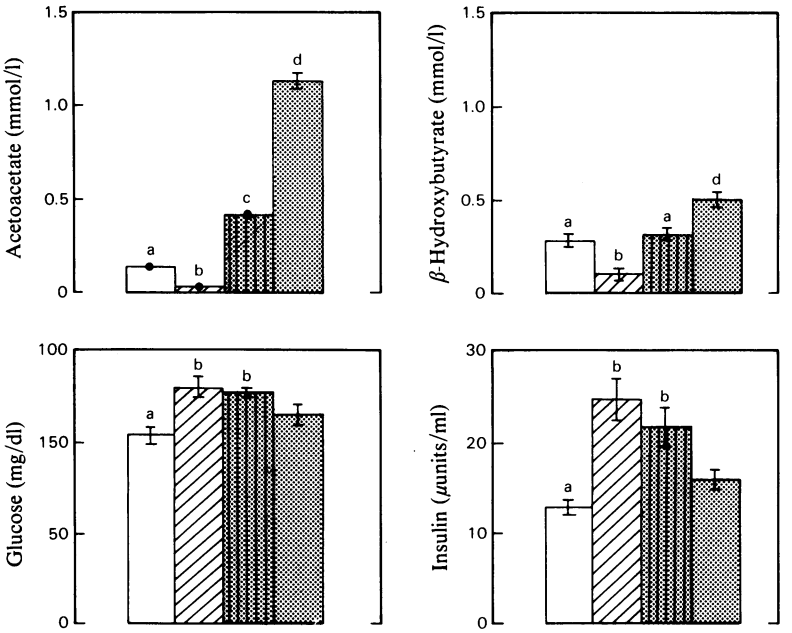


Fig. 2. *Serum substrates and insulin concentrations in burned rats receiving isonitrogenous parenteral nutrition of different energy composition*

Monoacetoacetin increased ketone bodies without changes in glucose and insulin concentrations. Dextrose decreased ketone bodies and increased glucose and insulin concentrations. [a] versus [b] versus [c] versus [d]: $P<0.01$. Data are presented as means ± S.E.M. □, Amino acids; ▨, amino acids + dextrose; ⊡, amino acids + monoacetoacetin; ▥, amino acids + dextrose + monoacetoacetin.

Table 3. *Plasma amino acid concentrations (μmol/l)*

For details see the text. Data are presented as means ± S.D.: *$P<0.01$ versus group I; †$P<0.01$ versus group II.

| Group | Tau | Asp | Thr | Ser | Gly | Ala | Val | Ile | Leu | Tyr | Phe |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 298† | 4.6† | 194† | 190† | 367† | 423† | 600† | 229† | 360† | 42 | 100 |
| | ±27 | ±1 | ±13 | ±17 | ±33 | ±33 | ±22 | ±9 | ±12 | ±2 | ±6 |
| II | 219* | 14.9 | 380* | 269* | 658* | 576* | 428* | 156* | 231* | 36 | 102 |
| | ±18 | ±4 | ±12 | ±10 | ±29 | ±22 | ±14 | ±5 | ±6 | ±2 | ±3 |
| III | 282† | 7.9† | 268*† | 214† | 456*† | 473† | 419* | 153* | 228* | 100*† | 91 |
| | ±11 | ±2 | ±21 | ±13 | ±27 | ±33 | ±9 | ±3 | ±5 | ±9 | ±3 |
| IV | 317† | 25.6*† | 316*† | 230*† | 524*† | 556* | 516*† | 190*† | 272*† | 158*† | 97 |
| | ±35 | ±5 | ±22 | ±12 | ±30 | ±23 | ±12 | ±4 | ±6 | ±15 | ±2 |

well as with monoacetoacetin. In contrast, tyrosine concentrations, which were similar in groups I and II, were 2.5 and 4 times higher in groups III and IV respectively. Tyrosine was also determined fluorimetrically by the procedure of Waalkes & Undenfriend (1957). The results were similar to those obtained with the automated amino acid analyser ($r=0.980$). Since phenylalanine remained constant, the phenylalanine/tyrosine ratio showed a remarkable decrease in groups receiving monoacetoacetin: group I, 2.4 ± 0.1; group II, 2.9 ± 0.1; group III, 1.0 ± 0.1; and group IV, 0.6 ± 01 ($P<0.01$).

Whole-body leucine kinetics (Fig. 3) were quite similar among the groups receiving dextrose or monoacetoacetin as additional non-protein energy. A significant decrease (12–17%) in leucine flux, release from protein breakdown (17–43%) and oxidation (29–40%) were observed in rats given additional non-protein energy, but leucine incorporation into protein did not differ, in comparison with group I, which was maintained exclusively on amino acids. Muscle-protein fractional-synthesis rates were also higher in the three groups receiving additional energy (Table 4), without significant differences among them.

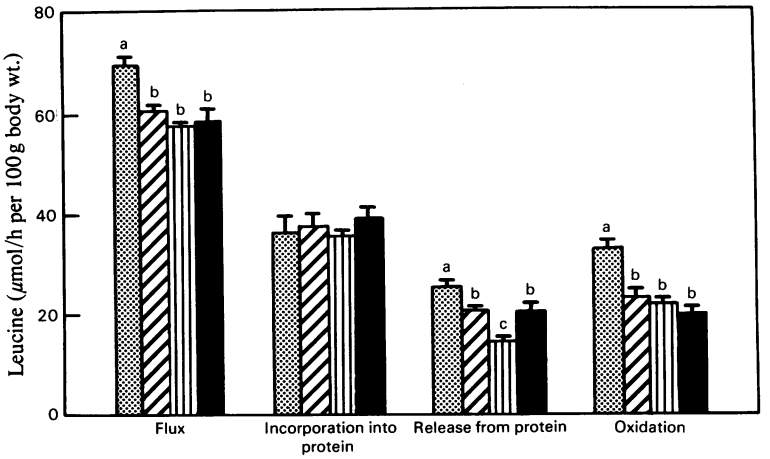


Fig. 3. *Whole-body leucine kinetics measured with* L-[*1*-$^{14}$*C*]*leucine in burned rats receiving isonitrogenous parenteral nutrition of different energy composition*

A decrease in leucine flux, release from protein breakdown and oxidation were observed in rats infused with dextrose or monoacetoacetin compared with those not receiving additional non-protein energy. [a] versus [b] versus [c]: $P<0.01$. Data are presented as means ± S.E.M. ⊡, Amino acids; ▨, amino acids + dextrose; ▥, amino acids + dextrose + monoacetoacetin; ■, amino acids + monoacetoacetin.

Table 4. *Muscle and liver protein fractional synthesis rates (FSR)*

For details see the text. Data are presented as means ± S.D.: *$P<0.01$ versus group I.

| Group | Muscle (gastrocnemius) protein FSR (%/day) |
|---|---|
| I | 3.3 ± 0.2 |
| II | 4.5 ± 0.4* |
| III | 4.4 ± 0.2* |
| IV | 4.1 ± 0.3* |

## Discussion

As has been repeatedly described in animals as well as in humans (Blackburn & Flatt, 1974; Clowes *et al.*, 1976; Wannemacher *et al.*, 1979; Neufeld *et al.*, 1982), injury and infection decrease the usual elevation of ketone-body concentrations observed as an adaptation to starvation. By using a burned rat as a model for generalized metabolic stress, a significant decrease in acetoacetate and 3-hydroxybutyrate and an increase in nitrogen loss compared with starved unstressed rats were also noted.

Studies have been conducted to evaluate the capacity of ketone-body infusions to affect amino acid and protein metabolism in humans and animals (Sherwin *et al.*, 1975; Haymond *et al.*, 1983; Pawan & Semple, 1983). These studies have been complicated by the use of a sodium salt of 3-hydroxybutyrate, resulting, in some cases, in $Na^+$ overload and alkalemia that can itself modify the concentration of some metabolic substrates such as alanine (Birkhahn, 1983). Furthermore, it is impossible to administer sufficient energy as ketone bodies in the form of free acids without inducing metabolic acidosis.

These problems can be overcome by using a glycerol ester of short-chain fatty acids, such as monoacetoacetin, which is neutral, stable and water-soluble. In the bloodstream, monoacetoacetin is hydrolysed to acetoacetate and glycerol (Birkhahn, 1983) by enzymes other than lipoprotein lipase. This explains the increased concentrations of serum acetoacetate found in groups III and IV, even higher than 3-hydroxybutyrate concentrations, which were already elevated (Fig. 2). Despite a direct stimulation of insulin secretion by ketone bodies, which has been described for the rat (Hawkins *et al.*, 1971), we did not observe elevated insulin concentrations in animals receiving monoacetoacetin infusion. Insulin increased, depending on dextrose administration and serum glucose concentration. This is noteworthy, since, although insulin did not increase with monoacetoacetin and was significantly lower than in animals given dextrose, the cumulative nitrogen balance (Fig. 1) as well as the decrease in whole-body leucine oxidation (Fig. 3) and the increase in muscle protein fractional-synthesis rate (Table 4), were comparable in the two groups of animals. These observations agree with the concept that either glucose or fatty acids (as well as their

metabolites, ketone bodies) are able to spare nitrogen, as has been postulated (Sherwin, 1982; Jeejeebhoy *et al.*, 1976).

The elevations in plasma concentrations of some amino acids in rats infused with monoacetoacetin were unexpected. The plasma tyrosine concentration was 439% higher in rats given all of their non-protein energy as monoacetoacetin as compared with animals receiving dextrose infusions of equal energy content (Table 3). The unexpected results of the present study allow only speculation as to the potential mechanism, which may be due to an increase in production and/or a decrease in catabolism of tyrosine. In clinical situations associated with high concentrations of ketone bodies, such as diabetic ketoacidosis (Aoki *et al.*, 1975) or prolonged starvation (Felig *et al.*, 1969), similar changes in tyrosine have not been reported. Certainly, this metabolic finding is a cause for some concern about the safety of monoacetoacetin for intravenous nutrition and therefore requires further investigation.

Nevertheless, we can conclude from this study that monoacetoacetin can effectively replace dextrose as an intravenous energy source in stressed rats. Monoacetoacetin, like dextrose, decreased body-weight loss and negative nitrogen balance. Both substrates increased muscle protein fractional-synthesis rate and altered whole-body leucine kinetics by decreasing appearance, release from protein breakdown and oxidation of leucine. The effects on protein metabolism were observed despite substantial differences in serum concentrations of ketone bodies, glucose and insulin.

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