Ketone bodies as substrates

BY A. J. RICH

Department of Surgery, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH

KETONE BODY (KB) TURNOVER

KB, acetoacetate (AcAc), β-hydroxybutyrate (BHB) and acetone were first recognized as components of human plasma in diabetics in 1865 (Gerhardt, 1865). This unfortunate connection has persisted and the role of KB as substrates has tended to be obscured because of their association as a danger signal in diabetes.

All the KB are small molecules (Fig. 1) with molecular weights less than 104. Although derived from fat they are water-soluble and weakly acidic. BHB and AcAc are interconvertible at a single step by β-hydroxybutyrate dehydrogenase (*EC* 1.1.1.30). This reaction takes place mainly within mitochondria and has NAD^+ and $NADH_2$ as cofactors. In contrast, the decarboxylation of AcAc to acetone is thought to be a non-enzymic reaction and there is evidence that about 60% of this acetone is recovered in glucose under certain conditions (Reichard *et al.* 1974, 1979). Acetone will not be further considered in the present discussion.

Ketone bodies are synthesized almost entirely in the liver, although under certain conditions they can be produced in muscle and kidney in small quantities (Weidemann & Krebs, 1969). Free fatty acids (FFA) released from adipose tissue are transported to the liver. They enter hepatocytes by hydrolysis and are transported into mitochondria as the carnitine ester (McGarry & Foster, 1976). They are then subject to β-oxidation and, after giving up one-third of their energy, undergo several intermediate steps to produce AcAc and BHB. These are able to diffuse freely out of the mitochondrion, and enter the plasma.

The liver itself cannot oxidize ketone bodies, lacking the enzyme 3-oxoacid CoA-transferase (*EC* 2.8.3.5) (Williamson *et al.* 1971). Plasma levels are, thus, the result of the balance between hepatic production and removal, either by oxidation in peripheral tissues, or by renal excretion (Fenselau, 1981). The normal postabsorptive plasma level for the combined KB is less than 1 mM.

KB AS ENDOGENOUS SUBSTRATES

The KB, together with glucose and FFA form a triad of non-nitrogenous substrates which supply energy to most tissues. The energy density of KB falls between the other two substrates with a value of 17·9 kJ (4·2 kcal)/g. Certain tissues, such as brain, are principally glucose-oxidizing (Owen *et al.* 1967); others such as red muscle, mainly FFA-oxidizing (Owen & Reichard, 1971). The scavenger tissues liver, heart, lung and kidney can oxidize glucose or FFA interchangeably depending on the availability of substrate. The KB are alone in providing potential replacements for glucose or FFA in most tissues with the exception of the liver. In general, provided a tissue has the appropriate enzyme systems, its use of KB depends on the concentration of KB presented to it (Williamson, 1973) and hence on the fed state. Cahill (1981) has pointed

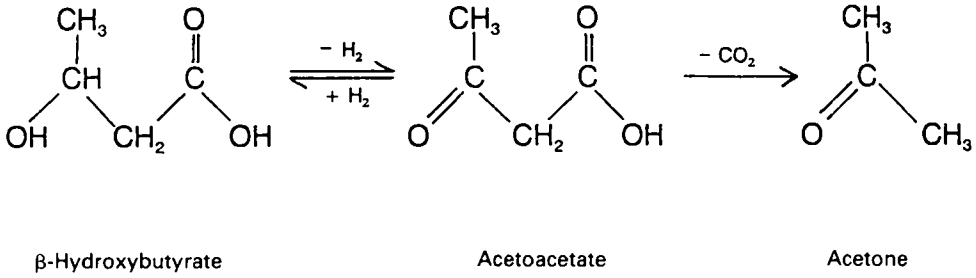


Fig. 1. Ketone molecules and their inter-relations.

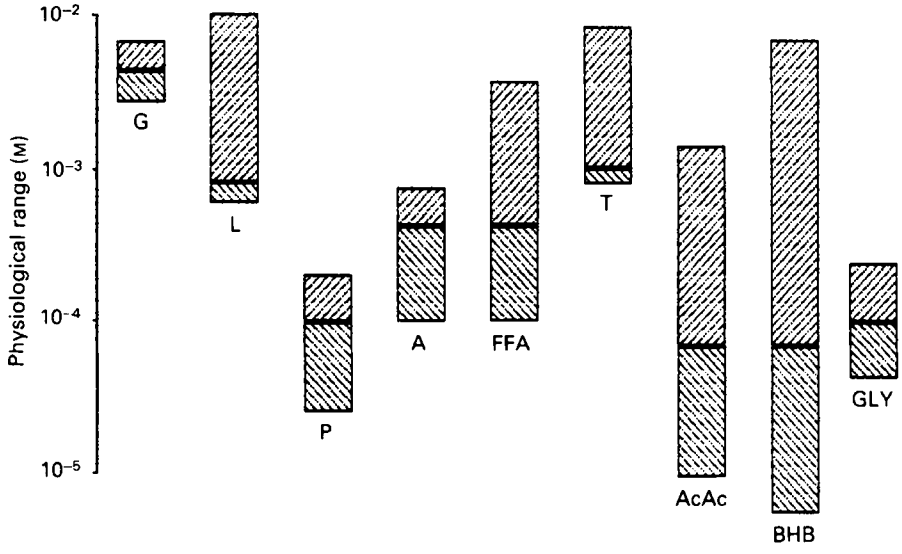


Fig. 2. Physiological ranges of different substrates. —, Normal post-absorptive level. G, glucose; L, lactate; P, pyruvate; A, alanine; FFA, free fatty acids; T, triacylglycerols; AcAc, acetoacetate; BHB, β-hydroxybutyrate; GLY, glycerol (redrawn from Cahill, 1981).

out that the KB are unique in having physiological levels that range over four orders of magnitude. All other substrates are buffered within a much smaller range (Fig. 2) and glucose, in particular, is very tightly controlled. The wide range of plasma concentrations of the KB during different feeding states is responsible for their opportunistic use by many tissues.

FEEDING STATES

Glucose predominates as an endogenous substrate during feeding (Table 1). FFA takes over during famine conditions. Between these two extremes ketones are the fuel of choice for many tissues that would otherwise have to oxidize amino acids. The changes

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

Table 1. *Ketone body concentration during different feeding states (from Fenselau, 1981)*

	Arterial concentrations (mM)		
	Ketone bodies	Glucose	Free fatty acids
Fed state			
Resting	0·075	4·33	0·69
During exercise	0·072	4·90	0·83
After exercise	0·183	4·40	0·87
Fasted state			
Overnight	0·29	5·42	0·51
3 d fast	3·06	3·69	0·73
24 d fast	6·80	3·53	0·84

Table 2. *Ketone body kinetics during different fasted states (from Fenselau, 1981)*

	Hepatic ketogenesis (g/d)	Urinary loss (g/d)	Peripheral oxidation (g/d)			
			Brain	Kidney	Heart	Muscle
Short-term fast	115	2·5	20	*	3–5	34
Long-term fast	130	14	40	31	3–5	14

* Not known.

that take place as the post-prandial state is succeeded by fasting and then by prolonged starvation are well-documented (Cahill, 1976). As the meal is digested and glucose disappears from the portal system there is a small fall in systemic plasma glucose and a rise in insulin levels. This is the signal for KB synthesis to begin, and plasma levels of AcAc and BHB become detectable. At the end of an overnight fast KB are the predominant fuel for muscle and only the brain is consuming significant quantities of glucose. Even at this stage one-third of the glucose requirement is derived from gluconeogenesis (Felig, 1975).

If fasting continues, further changes take place (Fig. 3). Plasma concentrations of KB continue to rise. Plasma glucose levels may fall slightly and levels of FFA rise. By the end of the third week plasma KB levels have stabilized, but are almost four times higher than they were at the beginning of the fast. Typically, BHB concentrations are twice as high as AcAc. The liver is now close to its maximum capacity for ketogenesis and is producing about 130 g KB/d (Reichard *et al.* 1974; Table 2), controlled by the interaction of insulin and glucagon (McGarry & Foster, 1976). Brain has doubled its oxidation of KB, utilization by the heart and kidneys is probably unchanged, but muscle oxidation has fallen, its predominant fuel again being FFA. KB handling by the kidney is complex. During a prolonged fast it is able to extract BHB from the plasma in preference to AcAc, and it also plays an important role in resorbing KB from urine. The resulting reduction in urinary losses of KB during late starvation helps to minimize losses of ammonium-nitrogen, thereby further reducing protein degradation (Sapir & Owen, 1975).

This process is known as 'starvation-adaptation' and its importance lies in its effect on N metabolism. KB replace carbohydrates during the onset of starvation and continue to reduce the total carbohydrate requirement even when FFA have become predominant.

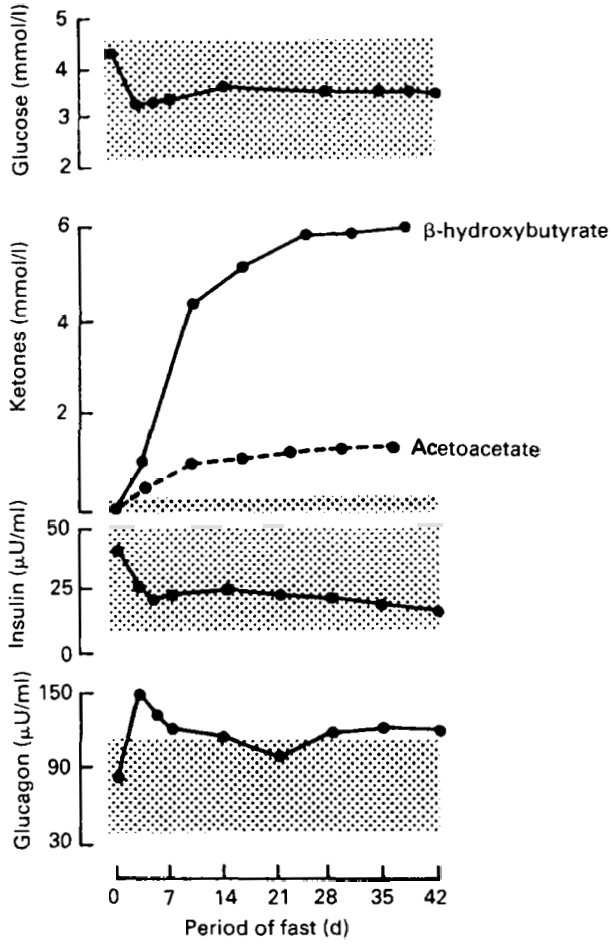


Fig. 3. Changes in plasma levels of glucose, ketone bodies, insulin and glucagon during a prolonged fast. ▨, Normal range.

The biphasic consumption of FFA by muscle is crucial, enabling the limited supply of KB in late starvation to be diverted for brain use. For example, in established starvation it has been calculated that the replacement of glucose by KB as a substrate for brain reduces the overall requirement for endogenous glucose by 60 g/d (Cahill, 1976). Since this carbohydrate requirement would otherwise have to be met by gluconeogenesis there is a net 'sparing' of approximately 55 g muscle/d. In the context of the 'fight or flight' reaction of the starved animal, the preservation of carcass protein is clearly paramount, extending survival time from weeks to months.

EFFECTS OF STRESS, INJURY AND SEPSIS

One of the challenges of clinical nutritional support is the optimal management of N metabolism. The catabolic response to stress and injury in the critically ill is well-recognized. Its principal metabolic features are the result of both a neuroendocrine

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

Table 3. *Effect of injury and sepsis on arterial substrate concentrations*

	Arterial concentrations (mM)			
	Ketone bodies	Glucose	Free fatty acids	Alanine
72 h fast	2·0	3·7	1·2	0·18
72 h post-injury	0·8	5·0	0·84	0·28
Sepsis	0·03	7·1	0·95	0·43

response and a release of inflammatory mediators from damaged tissue (Wilmore, 1986). The pattern of substrate availability is altered (Table 3) as demonstrated by elevated levels of arterial glucose with lower levels of KB and FFA in comparison with fasting (Hartl *et al.* 1984). There is, thus, a shift towards glucose as a principal fuel, away from fat-based substrates. As a result, alanine levels are elevated, implying increased mobilization of amino acids from muscle for gluconeogenesis. This is indicated by increased losses of N in the urine.

Since the period following injury is commonly associated with reduced or absent food intake it might be expected that starvation adaptation would effectively reduce the overall protein loss. However, it is clear that in most patients this response is absent (Birkhahn *et al.* 1981). Experimental studies in man suggest this is the result of reduced ketogenesis by the liver (Watters & Wilmore, 1986), probably mediated by increased concentrations of plasma glucose and insulin. There is no evidence of excessive tissue uptake or urinary losses of KB. The non-availability of KB for extrahepatic oxidation thus increases the overall requirement for glucose and, hence, gluconeogenesis. It has been shown that this is related to increased morbidity and mortality in post-operative patients (Rich & Wright, 1979).

Conventional techniques of parenteral nutritional support in ill patients utilize intravenous glucose or lipid emulsions as exogenous substrates to reduce the requirement for gluconeogenesis. However, there are theoretical and practical objections to the use of either of these substrates. Glucose infusions in stressed patients stimulate additional insulin secretion at a time when plasma levels are already high (Frayn *et al.* 1984). In such circumstances, particularly if sepsis is present, peripheral insulin resistance may prevent complete oxidation of presented glucose (Shaw & Wolfe, 1987). The accumulation of glucose in the plasma may result in excessive hepatic lipogenesis (Sheldon *et al.* 1978) and a fatty liver, or its oxidation may result in excessive carbon dioxide production (Askanazi *et al.* 1980).

Lipid emulsions are difficult to administer in conventional parenteral feeding mixtures because of their tendency to 'cream' if excessive electrolyte additions are made (Davis, 1983). There is also evidence accumulating that these emulsions may have a depressant effect on certain components of the immune response (Hamawy *et al.* 1985), and oxidation of their long-chain fatty acids by peripheral tissues is inefficient (Goodenough & Wolfe, 1984). Thus, conventional intravenous substrates cannot be regarded as ideal.

EXOGENOUS KB

With a view to overcoming these practical problems it has been suggested that the KB bodies might provide an alternative exogenous energy substrate (Sherwin *et al.* 1975).

Their attractions lie not just in their ubiquity as a substrate, water-solubility and insulin independence, but also in their role in reducing the requirement for gluconeogenesis. It will be recalled that hyperketonaemia is not usually observed in stress and sepsis and it has been hypothesized that restoration of this state by the infusion of exogenous ketones or their precursors might result in the normalization of the stress response towards one of adapted starvation (Border *et al.* 1976).

There is ample experimental evidence that KB can be succesfully infused in man and animals with the expected effects on N metabolism. Pawan & Semple (1983) compared the effects of 427 kJ (100 kcal) oral or intravenous glucose or BHB in obese patients undergoing a 2- or 3-week fast. They found that urinary N losses fell significantly during the period when BHB was given, and this reduction in urinary N excretion persisted for several days after the BHB was discontinued. Glucose had no effect on urinary N excretion. A similar reduction was seen in the urinary losses of 3-methylhistidine when BHB was given and the authors (Pawan & Semple, 1983) suggested that this indicated a reduced breakdown of muscle tissue.

Similar results have been reported in long-term fasts (5–10 weeks) in obese individuals (Sherwin *et al.* 1975). In these subjects, who were already hyperketonaemic, BHB infusions reduced urinary N excretion still further and it was again noted that this effect persisted for several days after the BHB infusion ceased.

There is some evidence that KB have a specific effect in limiting protein losses from muscle. When BHB was infused into subjects in the post-absorptive or in the prolonged-fasted state, significant falls in plasma alanine level were seen (Sherwin *et al.* 1975). Plasma levels of other amino acids were stable or varied much less. The infusions had no effect on plasma insulin or glucagon levels, but plasma glucose fell. These changes were thought to be indicative of a direct action of KB on muscle metabolism, and it is interesting to note that the hypoalanineaemic effect was seen at levels of BHB well below those achieved in prolonged fasting. In animals, infusion of very small quantities of BHB has also been shown to reduce alanine efflux from peripheral muscle (Radcliffe *et al.* 1983).

The effect of ketone infusions in stress and sepsis is less clear-cut. In fasted and infected sheep infusion of small amounts of BHB did not result in a reduction of either plasma alanine or glucose levels (Radcliffe *et al.* 1983). Since the contribution of BHB as an oxidative fuel for brain metabolism in sheep is unknown, it is not certain that these results can legitimately be extrapolated to man.

In a group of patients undergoing cholecystectomy, BHB infusion did not reduce post-operative urinary N excretion (Woods *et al.* 1983). The amounts of BHB infused were relatively small (300 kJ) and plasma levels of KB were not measured. This operation is regarded as offering minimal stress (Cuthbertson, 1975) and in this study there was no evidence of the expected increase in post-operative N excretion in either the study group or a control group. There is, thus, at present no unequivocal evidence on the effect of infused KB in stress, nor is the fate known of infused KB during these circumstances.

All these studies have used the racemic mixture of BHB and must be interpreted with some caution because of uncertainty about the pathways available for the metabolism of the L-isomer (Robinson & Williamson, 1980), which has constituted half the infused load. The amounts of KB used in these experiments have been relatively small and almost certainly represent only a fraction of the capability of peripheral tissues to utilize

KB. Animal work suggests that AcAc could be used to provide over 4270 kJ (1000 kcal)/d without saturating oxidative capacity (Bergman *et al.* 1981). Extrapolation of experimental work in diabetics (West & Todd, 1961) suggest similar rates might be achieved in man. However, in practice, other factors are likely to limit the quantities of KB that could be provided as exogenous substrates. The KB are weakly acidic and so have to be administered in solution as sodium or potassium salts. A requirement for 2135 kJ (500 kcal) would impose an electrolyte load of approximately 1200 mM. Such a load would probably be unacceptable in post-operative patients in the Na retention phase.

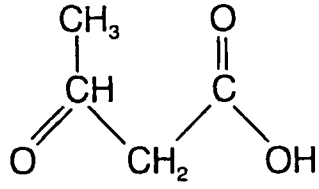
One of the KB, BHB, is optically active and as the chemically-manufactured form is an equal mixture of the D- and L-isomers. Endogenous BHB is the D-isomer and mammalian tissues have no recognized pathways for conventional oxidation of the L-form (Robinson & Williamson, 1980). This would suggest that half of any administered exogenous BHB may be metabolically useless.

There have been two different approaches to this problem, both largely experimental at the present time. Birkhahn & Border (1977) suggested the use of esters of short-chain fatty acids as energy substrates. Their primary rationale for investigating these interesting substances were that they were carnitine-independent and would, therefore, by-pass the putative block to the entry of FFA into the mitochondria resulting from a postulated deficiency of carnitine (Border *et al.* 1970). They also theorized that short-chain fatty acid esters (SCFAE) would be hydrolysed in vivo to their parent fatty acids which would result in hyperketonaemia.

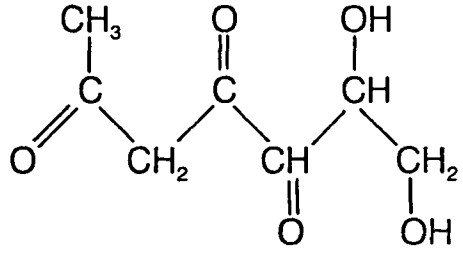
These workers investigated a number of SCFAE and identified two which were water-soluble and non-toxic. Monobutyrin was the ester of butyric acid, and mono-acetoacetin (MA) the ester of acetoacetic acid (Fig. 4). They had energy densities of 24 and 18·5 kJ (5·7 and 4·4 kcal)/g respectively. Clearly these two substances were likely to have ketogenic properties and studies demonstrated that they could be infused at rates up to at least 50 g/kg per d. At these levels a physiological hyperketonaemia without ketonuria was demonstrated (Birkhahn & Border, 1977, 1978).

In subsequent experimental studies on parenteral feeding with SCFAE in stress and injury Birkhahn has only reported work with MA. It is not clear why monobutyrin has been discarded, but in a later review of his work it is suggested that hyperketonaemia was not observed in the initial experiments with monobutyrin infusions (Birkhahn & Border, 1981). In a 7 d study comparing five total parenteral nutrition (TPN) regimens containing equienergetic quantities of non-protein-energy substrates in rats (Birkhahn *et al.* 1986), it was found that body-weight was maintained only in the group given an amino acid–MA mixture. However, this group did not demonstrate the best N balance. This was instead seen in a second group fed on amino acids with MA and glucose. The 7 d cumulative N balance in this group was more than twice that of the amino acid–MA group. In this respect the amino acid–MA–glucose group fared no better than a third group fed on amino acids with glucose and glycerol. The groups which derived their energy requirements from glucose–glycerol tended to be hyperglycaemic in comparison to those receiving MA in the TPN mixtures.

The effects of MA-containing TPN solutions on the metabolic response to injury were studied in a trauma model (Birkhahn *et al.* 1988). Two groups were studied; equi-energetic amounts of non-protein-energy were provided as either glucose or glucose and MA. These were infused with amino acids as the only source of nutrition for 3 d before and 3 d after experimental injury. Urinary N losses were very similar in the two groups.



Acetoacetate



Monoacetoacetin

Fig. 4. Chemical formula of acetoacetate and monoacetoacetin.

On the third post-injury day, plasma ketone levels were significantly higher in the group receiving MA–glucose. In contrast, glucose levels were significantly higher in the glucose-only group. The authors concluded that in terms of N conservation MA was as good an energy source for parenteral nutrition as was glucose, but had the advantage of normoglycaemia during the post-injury period. Similar observations were made in an experiment designed to test various TPN regimens following burn injury in animals (Maiz *et al.* 1985).

At present, this group of workers have confined their studies to animals. Work in humans is awaited, but it does appear that SCFAE can act as KB precursors and, hence, as energy substrates which are as effective as glucose but without influence on glucose–insulin homeostasis during stress and injury. There is no evidence that they improve protein retention during stress.

As an alternative to SCFAE we have been investigating the ketogenic properties of the polymers of BHB. These are found in nature as a storage product synthesized by certain bacteria (Holmes, 1985). Ketone polymers are 98% D-isomer and have the general formula as shown in Fig. 5. They may be produced in large quantities by fermentation under the appropriate conditions, following which the cells are harvested and broken open chemically to extract the BHB polymer. In native form this has a molecular weight of approximately 600 000 and is solid and insoluble. It has properties similar to a conventional thermoplastic such as PVC, but it is biodegradable by soil bacteria and fungi.

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

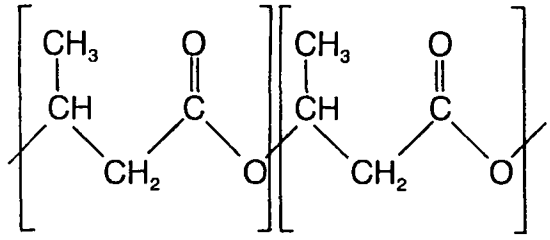


Fig. 5. General formula for β-hydroxybutyrate polymers.

Table 4. *Weight losses (%) in animals fed on various forms of β-hydroxybutyrate*

Treatment group . . .	Control	Group DL	Group D	Polymer group
Mean wt loss	8·1	7·8	12·1	7·7
Range	5–11	7–9	9–18	7–9

DL, β-DL-hydroxybutyrate; D, β-D-hydroxybutyrate; Polymer, β-D-hydroxybutyrate oligomer.

Polymers of BHB might be a suitable alternative to the KB as a substrate for intravenous infusion. They would avoid the problem of excessive loads of cation resulting from infusing BHB monomer because each polymer molecule should only require one molecule of cation to maintain chemical neutrality. Since osmolality is a function of the number of molecules in solution rather than their size, a polymer will have a lower osmolality than the same number of individual BHB molecules in solution. In addition, since they consist almost entirely of the D-isomer, the whole of an administered load will be available for oxidation by conventional pathways. The polymers are readily degraded to BHB by bacterial action so we theorize that in vivo they will be readily hydrolysed to their constituent BHB molecules.

We have been successful in deriving short-chain polymers (oligomers) of BHB. These are water-soluble but at present the quantities yielded are small so we have only been able to carry out some limited work to test for toxicity and for preliminary evidence of protein-sparing during semi-starvation. Our first study in which BHB oligomer was administered orally is described below.

Four groups of adult female Wistar rats were acclimatized to metabolic cages for 1 week. For the experimental period of 5 d all the animals were allowed free access to water and to nutrient-reduced rat chow. This was standard rat feeding mixture to which methyl cellulose had been added to bulk it out so that it provided only approximately one-third of the daily nutrient requirement of the rat.

Daily at 09.00 hours each animal was weighed and 1 ml oral dose of normal saline was given. In the control group, no additive was made. In the other groups 65 mg of either the racemic mixture of BHB (group DL), the pure D-monomer (group D), or the oligomer (polymer group) were added to the orally-administered solution. Stool and urine was collected each day for N analysis by the micro-Kjeldhal method.

All the animals lost weight (Table 4) indicating that their dietary intake was inadequate, as expected. The mean weight loss did not differ significantly between the

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

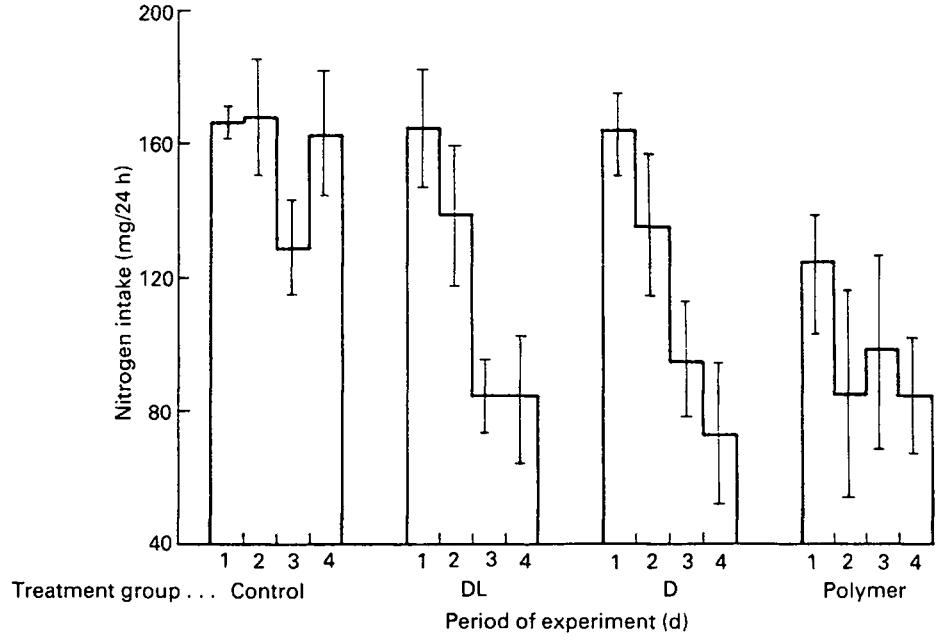


Fig. 6. Mean nitrogen intakes for days 1–4 in animals fed on saline (9 g sodium chloride/l; controls), β-DL-hydroxybutyrate (DL), β-D-hydroxybutyrate (D) or β-D-hydroxybutyrate oligomer (Polymer). Values are means and standard deviations represented by vertical bars.

groups. No animal died and throughout the experiment all appeared healthy and active. Voluntary N intake, however, varied considerably, those intakes in all the experimental groups showing a decline with time (Fig. 6), so that on the last day of the experiment, for example, group DL was taking only half the N of the first day. In contrast, the N intake of the control group was unchanged.

Both the control and all the experimental groups were in negative N balance on the first day, but in the control group N balance had become positive by day 4 (Fig. 7). Groups DL and D became progressively more negative, but the polymer group showed a similar improvement in N balance to the control group, although because of its considerably reduced N intake it did not reach a positive N balance by the fourth day. The change in N balance between days 1 and 4 was significantly better in the polymer group than in groups D or DL.

All the animals remained well during the experiment and had no abnormal gastro-intestinal symptoms. Post-mortem examination demonstrated no abnormalities.

This experiment suggests that oligomers of BHB are non-toxic by the oral route in the doses given, and demonstrate a protein-sparing effect during semi-starvation greater than that of the monomer. A larger protein-sparing effect may have been obscured because of the apparent anorectic effect of KB. This has been described elsewhere in man (Pawan & Semple, 1983) and animals, (Langhans *et al.* 1985). Work is proceeding to produce larger quantities of BHB oligomers to enable studies of the effect of intravenous infusion to be carried out.

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

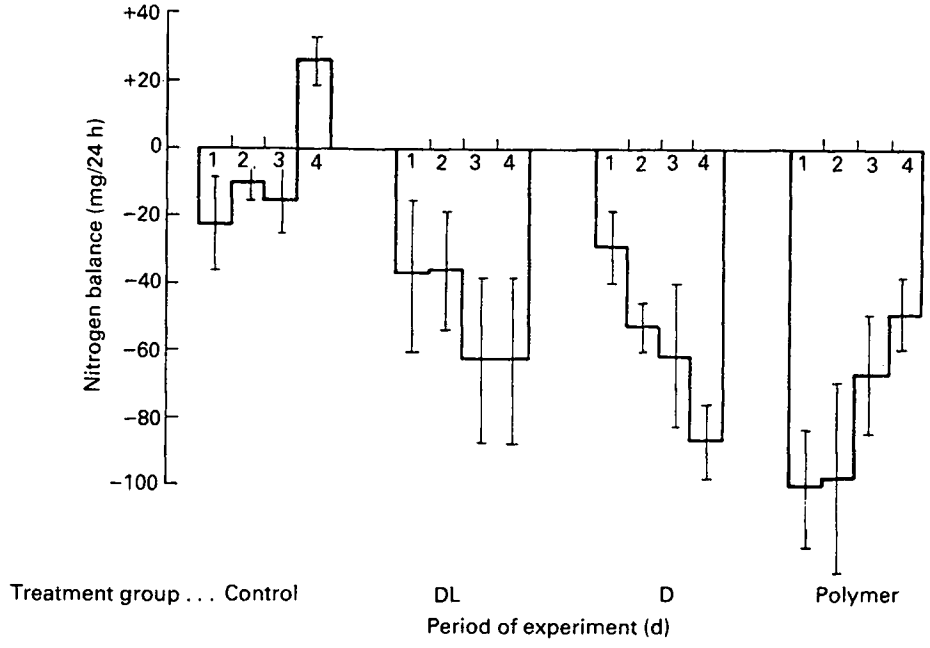


Fig. 7. Mean nitrogen balances for days 1–4 in animals fed on saline (9 g sodium chloride/l; controls), β-DL-hydroxybutyrate (DL), β-D-hydroxybutyrate (D) or β-D-hydroxybutyrate oligomer (Polymer). Values are means and standard deviations represented by vertical bars.

CONCLUSIONS

There seems to be little doubt that KB can act as an endogenous substrate but a convincing protein-sparing effect has only been demonstrated in non-stressed fasting individuals. Investigation in man of the effects in injury and sepsis are limited. However, there is no firm evidence of a protein-sparing effect from KB or ketogenic substrates in the doses reported over and above those seen with equienergetic amounts of glucose. In animals, plasma levels of KB similar to those seen in adapted starvation have been obtained by KB infusion. In man, only small amounts of KB have been infused and elevations of plasma KB levels have been correspondingly minimal. It must, therefore, remain questionable whether an adequate stimulus to protein sparing has been attained. Indeed, since KB are not a normal constituent of plasma during stress the fate of exogenous KB administered during this state deserves investigation.

However, one particular attraction of KB as substrates is their independence of insulin. It has been clearly demonstrated that they will act as an energy source following injury without potentiation of an existing hyperglycaemia. This aspect of KB may be more useful than their N-sparing potential. Although the KB themselves are chemically unsuitable for intravenous administration as an energy substrate, investigation of SCFAE and ketone polymers suggests that these are promising substitutes.

It is possible to speculate that a future use for ketogenic substrates may be as part of a 'trauma support solution'. Recent reviews (Askanazi *et al.* 1988) have highlighted the

https://doi.org/10.1079/PNS19900042 Published online by Cambridge University Press

post-injury stress response as an unwanted and unnecessary phenomenon, given the support available by modern medicine, and have suggested its abolition by pharmacological blockade. If this is indeed a therapeutic possibility, then it will obviously be necessary to provide a substrate mix which will optimize healing of damaged tissues and maintain organ function during the period when oral intake is impossible. Since the KB are a normal component of plasma in the post-digestive, resting phase, its seems reasonable to provide them as part of such a mixture. This area, together with the targeting of the amino acid content of substrate mixtures to specific disease states, offers an exciting prospect for nutritional support techniques in the future.

REFERENCES

Askanazi, J., Kvetan, V. & Goldiner, P. (1988). Nutrition in the acutely ill. *Anaesthesiology Clinics of North America* **6**, 49–71.

Askanazi, J., Rosenbaum. S. H., Hyman, A. I., Silverberg, J. M.-E. & Kinney, J. M. (1980). Respiratory changes induced by the large glucose loads of total parenteral nutrition. *Journal of the American Medical Association* **243**, 1444–1447.

Bergman, E. N., Kon, K. & Katz, M. L. (1981). Quantitative measurements of acetoacetate metabolism and oxidation in sheep. *American Journal of Physiology* **205**, 658–662.

Birkhahn, R. H., Askari, A. & Thomford, N. R. (1986). Total parenteral feeding of rats with an acetoacetate monoglyceride and glucose mixture. *Journal of Nutrition* **116**, 851–864.

Birkhahn, R. H., Askari, A. & Thomford, N. R. (1988). The influence of ketosis on the metabolic response to skeletal trauma. *Journal of Surgical Research* **44**, 160–165.

Birkhahn, R. H. & Border, J. R. (1977). Intravenous feeding of the rat with short chain fatty acid esters. I. Glycerol monobutyrate. *American Journal of Clinical Nutrition* **30**, 2078–2082.

Birkhahn, R. H. & Border, J. R. (1978). Intravenous feeding of the rat with short chain fatty acid esters. II. Monoacetoacetin. *American Journal of Clinical Nutrition* **31**, 436–441.

Birkhahn, R. H. & Border, J. R. (1981). Alternate or supplemental energy sources. *Journal of Enteral and Parenteral Nutrition* **5**, 24–31.

Birkhahn, R. H., Long, C. L., Fitkin, D. L., Busnardo, A. C., Geiger, J. W. & Blakemore, W. S. (1981). A comparison of the effects of skeletal trauma and surgery on the ketosis of starvation in man. *Journal of Trauma* **21**, 513–519.

Border, J. R., Burns, G. P., Rumph, C. & Schenk, W. G. (1970). Carnitine levels in severe infection and starvation: a possible key to the prolonged catabolic state. *Surgery* **68**, 175–179.

Border, J. R., Chenier, R., McMenamy, R. H., La Duca, J., Seibel, R., Birkhahn, R. H. & Yu, L. (1976). Multiple systems organ failure: muscle fuel deficit with visceral protein malnutrition. *Surgical Clinics of North America* **56**, 1147–1167.

Cahill, G. F. (1976). Starvation in man. *Clinics in Endocrinology and Metabolism* **5**, 397–415.

Cahill, G. F. (1981). Ketosis. *Journal of Enteral and Parenteral Nutrition* **5**, 281–286.

Cuthbertson, D. (1975). Nutrition in relation to trauma and surgery. *Progress in Food and Nutrition Science* **1**, 263–287.

Davis, S. S. (1983). The stability of fat emulsions for intravenous administration. In *Advances in Clinical Nutrition*, pp. 213–239 [I. D. A. Johnston, editor]. Lancaster: MTP Press Ltd.

Felig, P. (1975). Amino acid metabolism in man. *Annual Review of Biochemistry* **44**, 933–941.

Fenselau, A. (1981). Ketone body metabolism in normal and diabetic man. In *Handbook of Diabetes Mellitus*, vol. 3, pp. 143–208 [M. Brownlee, editor]. New York: Garland STPM Press.

Frayn, K. N., Little, R. A., Stoner, H. B. & Galasko, C. B. (1984). Metabolic control in non-septic patients with musculo-skeletal injuries. *Injury* **16**, 73–79.

Gerhardt, J. (1865). Diabetes mellitus und Aceton. *Wiener Medizinische Presse* **6**, 672.

Goodenough, R. D. & Wolfe, R. R. (1984). Effects of total parenteral nutrition on free fatty acid metabolism in burned patients. *Journal of Parenteral and Enteral Nutrition* **8**, 357–360.

Hamawy, K. J., Moldawer, L. L., Georgieff, M., Valicenti, A. J., Babayan, V. K., Bistrian, B. R., & Blackburn, G. L. (1985). The effect of lipid emulsions on reticuloendothelial function in the injured animal. *Journal of Parenteral and Enteral Nutrition* **9**, 559–565.

Hartl, W., Gunther, B., Wicklmayr, M., Tiechmann, R. & Dietze, G. (1984). Substrate balances across skeletal muscle tissue in severe sepsis. *Clinical Nutrition* **3,** 221–226.

Holmes, P. A. (1985). Applications of PHB – a microbially produced biodegradable thermoplastic. *Physics and Technology* **16,** 32–36.

Langhans, W., Pantel, K. & Scharrer, E. (1985). Ketone kinetics and D-(−)-3-hydroxybutyrate-induced inhibition of feeding in rats. *Physiology and Behaviour* **34,** 579-582.

McGarry, J. D. & Foster, D. W. (1976). Ketogenesis and its regulation. *American Journal of Medicine* **61,** 9–12.

Maiz, A., Moldawer, L. L., Bistrian, B. R., Birkhahn, R. H., Long, C. L. & Blackburn, G. L. (1985). Monoacetoacetin and protein metabolism during parenteral nutrition in burned rats. *Biochemical Journal* **226,** 43–50.

Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G. & Cahill, G. F. (1967). Brain metabolism during fasting. *Journal of Clinical Investigation* **46,** 1589–1595.

Owen, O. E. & Reichard, G. A. (1971). Human forearm metabolism during progressive starvation. *Journal of Clinical Investigation* **50,** 1536–1545.

Pawan, G. L. S. & Semple, S. J. G. (1983). Effect of 3-hydroxybutyrate in obese subjects on very-low-energy diets and during therapeutic starvation. *Lancet* **i,** 15–18.

Radcliffe, A. G., Wolfe, R. R., Colpoys, M. F., Muhlbacher, F. & Wilmore, D. W. (1983). Ketone–glucose interaction in fed, fasted and fasted-infected sheep. *American Journal of Physiology* **244,** R667–R675.

Reichard, G. A., Haff, A. C., Skutches, C. L., Paul, P. & Holroyde, C. P. (1979). Plasma acetone metabolism in the fasting human. *Journal of Clinical Investigation* **63,** 619–626.

Reichard, G. A., Owen, O. E., Haff, A. C., Paul, P. & Bortz, W. M. (1974). Ketone body production and oxidation in fasting obese humans. *Journal of Clinical Investigation* **53,** 508–515.

Rich, A. J. & Wright, P. D. (1979). Ketosis and nitrogen excretion in undernourished surgical patients. *Journal of Parenteral and Enteral Nutrition* **3,** 350–354.

Robinson, A. M. & Williamson, D. H. (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiological Reviews* **60,** 143–187.

Sapir, D. G. & Owen, O. E. (1975). Renal conservation of ketone bodies during starvation. *Metabolism* **24,** 23–33.

Shaw, J. F. H. & Wolfe, R. R. (1987). Energy and protein metabolism in sepsis and trauma. *Australian and New Zealand Journal of Surgery* **57,** 41–47.

Sheldon, G. F., Petersen, S. R. & Sanders, R. (1978). Hepatic dysfunction during hyperalimentation. *Archives of Surgery* **113,** 504–508.

Sherwin, R. S. Hendler, R. G. & Felig, P. (1975). Effects of ketone infusions on amino acid and nitrogen metabolism in man. *Journal of Clinical Investigation* **55,** 1382–1390.

Watters, J. M. & Wilmore, D. W. (1986). Role of catabolic hormones in the hypoketonaemia of injury. *British Journal of Surgery* **73,** 108–110.

Weidemann, M. J. & Krebs, H. A. (1969). The fuel of respiration of rat kidney cortex. *Biochemical Journal* **112,** 149–166.

West, E. S. & Todd, W. R. (1961). *Textbook of Biochemistry*, p. 1003. New York: Macmillan.

Williamson, D. H. (1973). Tissue-specific direction of blood metabolites. In *Symposia of the Society for Experimental Biology* no. XXVII: *Rate Control of Biological Processes*, pp. 283–298 [D. D. Davies, editor]. Cambridge: Cambridge University Press.

Williamson, D. H., Bates, M. W. & Page, M. A. (1971). Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. *Biochemical Journal* **121,** 1133–1140.

Wilmore, D. W. (1986). Are the metabolic alterations associated with critical illness related to the hormonal environment? *Clinical Nutrition* **5,** 9–20.

Woods, W. G. A., Pawan, G. L. S., Semple, S. J. G. & Ralphs, D. N. L. (1983). Effect of intravenous 3-hydroxybutyrate on post-operative nitrogen balance. *Human Nutrition: Clinical Nutrition* **37**C, 391–394.

Printed in Great Britain