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The Interconversion and Disposal of Ketone Bodies in Untreated and Injured Post-Absorptive Rats

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[3-^{14}C]Acetoacetate and β-hydroxy[3-^{14}C]butyrate were used to investigate the kinetics of ketone body metabolism in rats 3h after bilateral hind-limb ischaemia and in controls, both groups being in the post-absorptive state and in a 20°C environment. Calculations were carried out as described by Heath & Barton (1973) and the following conclusions were reached. 1. In both injured and control rats, the rates of irreversible disposal (extrahepatic utilization) of β-hydroxybutyrate and acetoacetate were proportional within experimental error to their blood concentrations up to at least 0.4mM (the maximum found in these rats), implying that they were determined, via these concentrations, by the rates of production by the liver. 2. Conversion of blood β-hydroxybutyrate into blood acetoacetate took place mainly in the liver, but the reverse process occurred mainly in extrahepatic tissues. 3. The 'metabolic clearance rate' (the volume of blood which, if completely cleared of substrate in unit time, would give a disposal rate equal to that in the whole animal) was calculated for β-hydroxybutyrate and acetoacetate. Comparison with the cardiac output showed that in control rats the proportion of circulating β-hydroxybutyrate extracted was lower than that of acetoacetate, clearance of which appeared almost complete. After injury both metabolic clearance rates decreased, probably because of the lower cardiac output. 4. After injury, because the average blood concentrations of ketone bodies, especially acetoacetate, were higher, the mean total rate of disposal also increased. Assuming complete oxidation, the mean contribution of ketone bodies to the whole body O_2 consumption rose from 7 to 15%.

One of the most conspicuous early consequences of severe injury in the rat in a 20°C environment is a fall in body temperature and O_2 consumption (Stoner, 1969, 1970). The uptake and oxidation of glucose and the oxidation of free fatty acids are probably decreased (Ashby *et al.*, 1965; Heath & Stoner, 1968; Heath & Corney, 1973), but, despite the importance of ketone bodies (β-hydroxybutyrate and acetoacetate) as a third major metabolic fuel (Williamson & Hems, 1970), nothing is known about the effects of injury upon their rates of metabolism.

Experiments with isotopically labelled ketone bodies were therefore carried out on rats in the 'ebb' phase (Cuthbertson, 1942; Stoner, 1970) after ischaemic limb injury and on controls, both groups being in the post-absorptive state (Heath & Threlfall, 1968). The method of Heath & Barton (1973) was used to determine the rates of irreversible disposal (Baker, 1969; Baker & Rostami, 1969) of ketone bodies, which probably approximate to their rates of oxidation by extrahepatic tissues. Since this method is based upon a completely general model and permits a single blood sample to be taken from each animal at all but the shortest times after injection, it allows for the interconvertibility of blood β-hydroxybutyrate and acetoacetate in all the tissues in which this occurs, and for their low concentrations in the post-absorptive state. These difficulties were not completely overcome by Bates *et al.* (1968) in the only previous study on rats in a comparable nutritional state.

Preliminary accounts of part of this work have appeared (Barton, 1972, 1973).

Experimental

Basic design and procedure

In outline the following procedure, based upon the theory of Heath & Barton (1973), was adopted. The relevant models are 3*a*–3*d* in Heath & Barton (1973), and numbers in square brackets refer to equations in their paper.

1. D_a units of ^{14}C in acetoacetate were injected intravenously into each of a group of rats and the areas to infinity, A_{q1a} and A_{q4a}, under the curves relating the quantities of label in blood acetoacetate, q_{1a}, and blood β-hydroxybutyrate, q_{4a}, to time (t) were estimated.

2. D_h units of ^{14}C in β-hydroxybutyrate were injected and the areas A_{q4h} and A_{q1h} under the q–t curves for blood β-hydroxybutyrate and acetoacetate were estimated.

3. Calculation then gave two generalized rate

coefficients, K_1 and K_4, by the equivalent for single injection of eqn. [3.13], and the overall disposal rate, R, by $K_1Q_1+K_4Q_4$ (eqn. [3.12]) where Q_1 and Q_4 were the total quantities of acetoacetate and β-hydroxybutyrate respectively in blood. The generalized rate coefficients for interconversion of blood acetoacetate and β-hydroxybutyrate, K_{14} and K_{41}, were also given by the single-injection equivalent of eqn. [3.14].

4. After hepatectomy, to confine interconversion of ketone bodies to extrahepatic tissues, rats were infused intravenously at a constant rate with β-hydroxybutyrate and acetoacetate, one of which was labelled. With the assumption of constant rate coefficients, the specific radioactivities in the blood gave values for the generalized rate coefficients K'_{14} and K'_{41}.

5. Eqn. [3.15] then gave the generalized rate coefficients K''_{14} and K''_{41} for interconversion of blood acetoacetate and β-hydroxybutyrate in the liver only, and eqn. [3.17] the coefficients k_1 and k_4 for the net transport through the blood of acetoacetate and β-hydroxybutyrate respectively. The net rates of uptake by the extrahepatic tissues were therefore: for acetoacetate, $R_1=k_1Q_1$; for β-hydroxybutyrate, $R_4=k_4Q_4$.

6. The interchange coefficients also gave values of the rate coefficient for the flow of acetoacetate from blood into liver, k_{12}, and, provided that acetoacetate and β-hydroxybutyrate are in equilibrium in the liver, that for β-hydroxybutyrate, k_{42}, by eqn. [3.19].

For the estimation of q–t integrals values of q were determined at various times after injection, and the areas and their errors were calculated by eqns. [6.2] and [6.10]. Values of q were determined in two ways.

(*a*) For values from 30s after injection, several conscious rats were decapitated for each time-point, a single blood sample being obtained from each rat. The haematocrit value, concentrations of acetoacetate and β-hydroxybutyrate, C_1 and C_4, and quantities of label/ml in these substrates were determined in the blood collected. The haematocrit value, with other published information, gave the blood volume, V, and hence Q_1 and Q_4 by VC_1 and VC_4 and q_1 and q_4 by $V\times$label/ml.

(*b*) For values up to 50s after injection, successive blood samples were taken through a carotid cannula from each rat under anaesthesia. As only the total label/ml was determined, the blood samples could be small, overcoming the main objection to this procedure. To calculate radioactivity/ml in acetoacetate after injection of [^{14}C]acetoacetate and in β-hydroxybutyrate after injection of β-hydroxy[^{14}C]butyrate, small corrections were made for conversion into labelled β-hydroxybutyrate and acetoacetate respectively. The values were multiplied by V, corrected for incomplete mixing of the injected dose in injured rats, to give q.

Experimental detail is now given.

Animal techniques

Rats. Male Wistar rats of the Porton strain, wt. 235.0±5.2g (S.D.) were used, except after carotid cannulation when the wt. was 216.4±12.1g (S.D.). Weights have been corrected to those in the post-absorptive state (Heath & Threlfall, 1968; Heath & Corney, 1973) in which the rats were used. They were housed and experiments were done in a room at 18–22°C, lit from 07:00 to 19:00h. Diet 41B (Bruce & Parkes, 1956) was available until 09:00–11:00h on the day of the experiment.

Cannulation. In single-sampling experiments, rats were cannulated for injection in a lateral tail vein under ether anaesthesia on the morning of the experiment. For serial sampling, the left carotid artery was cannulated 1–3 days before the experiment (Popovic & Popovic, 1960), taking care to avoid damaging the accompanying nerves.

Method of injury. Rats were subjected to 4h of bilateral hind-limb ischaemia by using rubber tourniquets (Rosenthal, 1943) applied under ether anaesthesia at 09:00–10:30h. Controls were anaesthetized for a similar period (3–10min). Injured rats were injected 3h after removal of the tourniquets, while in the 'ebb' phase (Cuthbertson, 1942) of the injury, and controls were injected at the same time of day (15:30–17:30h). This injury, which has an 85% mortality (Stoner, 1961), raised the haematocrits from 46.6±2.1 (S.D.; 61 samples) in controls to 59.6±3.1 (S.D.; 64 samples).

Injection and blood sampling in intact rats. Rats were injected with 0.8–1.5μCi of D-β-hydroxy[3-^{14}C]-butyrate, specific radioactivity 10.2 or 13.1μCi/μmol, or 0.5–0.8μCi of [3-^{14}C]acetoacetate, specific radioactivity 4.7 or 10.2μCi/μmol, in 0.2ml of 0.9% (w/v) NaCl. The amounts injected represented 1.4–18% of the blood pool of β-hydroxybutyrate, and 1.2–13% of that of acetoacetate. The quantity of label given was determined by diluting a similar dose from the injection syringe and counting for radioactivity.

Up to 50s after injection rats with carotid cannulae were used. Under ether anaesthesia injection was made directly into a lateral tail vein, and timed serial blood samples (0.05–0.2ml) were collected from the cannula in weighed scintillation vials. A volume of blood equal to at least that of the cannula was allowed to flow just before collection to flush out the previous sample. As sampling was rapid, times were known to about 1s. Finally a sample was collected for haematocrit determination.

From 30s after injection, blood samples were taken by decapitating conscious rats, which were kept in restraining cages from insertion of the tail vein cannula until shortly before decapitation. Label was injected via the cannula and was washed in with about

0.2ml of 0.9% (w/v) NaCl. On decapitation, blood was collected in heparinized beakers for 15s for controls and for 30s for injured animals. The mean durations of sampling were found by collecting and weighing the blood emerging over successive short time-intervals after decapitating other rats, and were 8.8±1.6s (S.D.; six samples) in the injured and 5.8± 0.6s (S.D.; four samples) in controls. Because the circulation was failing, these were overestimates, and the respective sampling times have been taken as 8 and 5s after decapitation.

Infusion and blood sampling in hepatectomized rats. Ether anaesthesia was maintained throughout. Control rats, or rats 2–3h after a 4h period of bilateral hind-limb ischaemia, were laparotomized, and the superior mesenteric artery, hepatic artery and portal vein were ligated and severed. The lobes of the liver were then successively ligated near their base and excised, starting from the anterior lobes; this was necessary since injection of dye into a tail vein demonstrated reverse flow into the liver from the hepatic vein. The temperature of the animal was monitored by a thermocouple inserted 6–8cm from the anus, and was maintained at about 37°C with a lamp.

As soon as hepatectomy was complete, the rats were infused at 0.32ml/min with a solution containing 52mM-glucose (45mM for injured rats), 6.6mM-D-(−)-β-hydroxybutyrate, 5.2mM-acetoacetate and about 1μCi/ml of [3-^{14}C]acetoacetate or β-hydroxy-[3-^{14}C]butyrate in Krebs–Ringer bicarbonate solution (Krebs & Henseleit, 1932), gassed with 5% CO_2 in air until the pH was about 7.4. These infusion rates were mostly within the range of hepatic production rates of glucose (Ashby *et al.*, 1965) and ketone bodies found in post-absorptive rats *in vivo*. The solution also contained L-(+)-β-hydroxybutyrate, since attempts to resolve the DL-form by the method of McCann & Greville (1962) resulted in a product containing only 72.4% of D-β-hydroxybutyrate. At times ranging from 2.8 to 10.9min after the start of the infusion, 2–4ml of blood was removed from the abdominal aorta with a syringe.

Radioactive compounds

Ethyl [3-^{14}C]acetoacetate was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and hydrolysed by the method of Ljunggren (1924) but with a larger excess of NaOH.

For injection, the sodium [3-^{14}C]acetoacetate was purified by a method based on that of La Noue *et al.* (1970). The hydrolysate was chromatographed on a 4ml column, 16cm long, of Dowex 1 resin (AG1X8; formate form; 200–400 mesh; Bio-Rad Laboratories Ltd., St. Albans, Herts., U.K.), with a linear gradient of formic acid (8mmol/ml). Fractions of 2ml were collected and the acetoacetate peak was identified by counting a small portion of each. The fractions comprising the peak were pooled and evaporated to dryness at 25–30°C under reduced pressure to remove formic acid. The residue was dissolved in water, neutralized with 10mM-LiOH and split into portions containing enough label for an experiment (about 10μCi). Each was evaporated to dryness at 25–30°C and stored at −10°C under N_2 (Hall, 1962). No decrease in the recovery from standards was seen over 3 months of storage. The labelled acetoacetate still appeared to contain about 3% involatile impurity, probably due to breakdown on the column. A portion was dissolved in 0.9% (w/v) NaCl immediately before use.

D-(−)-β-Hydroxy[3-^{14}C]butyrate was prepared enzymically from [3-^{14}C]acetoacetate as described by Bates *et al.* (1968). The product was separated from NAD^+ on a 4ml column, 16cm long, of Florisil (60–100 mesh; Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) (Cremer, 1971), and chromatographed and freed from formic acid as described for [^{14}C]acetoacetate. Portions (10μCi) were neutralized with 0.1M-NaOH, and stored at −10°C in 0.9% (w/v) NaCl.

Analytical methods

Blood volumes. These were calculated from the haematocrits as described by Heath & Corney (1973).

In injured, carotid-cannulated rats blood volumes were corrected for incomplete mixing of the injected dose by multiplying by factors (Table 1) determined by carrying out similar experiments on other rats but with ^{131}I-labelled human serum albumin (The Radiochemical Centre) previously passaged through a rat (McFarlane, 1957), instead of labelled ketone bodies. The blood samples were collected in weighed tubes and counted in a Gamma One-Sixty counter (Panax Equipment Ltd., Redhill, Surrey, U.K.). A dose of label similar to that injected was diluted with blood and also counted. The apparent volumes of distribution were calculated after correcting for F_{cells} (ratio of whole body haematocrit value to measured great vessel haematocrit) and specific gravity by using the factors given by Heath (1973) and the haematocrits.

There was a consistent increase in the apparent volume of distribution from 5 to 40s (Table 1) which was not seen in a similar experiment on two control animals (results not shown). The volume of distribution at 40s was always very close to that at 50s, suggesting that by then mixing was virtually complete, and the 50s values have been taken as the actual blood volumes. The large fall in cardiac output after injury (Takács *et al.*, 1962) probably means that the circulation time is considerably increased despite the lower blood volume (Table 3).

Determination of concentrations and radioactivities. Chemicals were bought from BDH Chemicals Ltd., Poole, Dorset, U.K., except for the following:

Table 1. *Volumes of distribution of ^{131}I-labelled albumin at short times after injection into injured rats*

Serial samples were taken from injured, carotid-cannulated rats at the times shown after injection. Volumes of distribution have been expressed as a fraction of that at 50s, assumed equal to the blood volume. Results are given as means±S.E.M. (eight samples).

Time (s) ...	5	10	20	30	40	50
Vol. of distribution	0.401±0.021	0.687±0.031	0.918±0.009	0.954±0.016	0.986±0.004	1.00

sodium DL-β-hydroxybutyrate and NAD^+ from Sigma (London) Chemical Co. Ltd., Kingston-on-Thames, Surrey, U.K.; Tris base and solutes for scintillators from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; and NADH and D-(−)-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30) from the Boehringer Corporation, London W.5, U.K.

Blood samples were analysed in one of three ways.

(*a*) Those obtained after decapitation of intact rats (1.8–5.2ml) were deproteinized and analysed for acetoacetate essentially by the method of Williamson *et al.* (1962). From duplicate assays of blood samples the coefficient of error of a single determination was found to be 1.4% (11 degrees of freedom). β-Hydroxybutyrate was determined by the method described for total ketone bodies by Bates *et al.* (1968), which avoids the use of a trapping agent, except that the quantity of NAD^+ added was increased to 12μmol, and that 0.2ml of 0.5M-diethanolamine–HCl buffer, pH9.0, was used instead of Tris. External standards of DL-β-hydroxybutyrate were run with each set of assays and the extinction found was 98.0±3.5% (S.D.) of the theoretical. In duplicate assays on blood extracts the coefficient of error of a single determination was 1.7% (19 degrees of freedom). Recoveries of the DL-compound added to extracts averaged 104.2± 2.4% (S.E. of slope of standard curve; six pairs of values).

Label in acetoacetate and β-hydroxybutyrate was determined by the method of Mayes & Felts (1967). Label in blood acetone was not determined. The decarboxylations were carried out on 1.0ml portions of the neutralized blood extract (to give label in acetoacetate) and of the cuvette contents after assay of β-hydroxybutyrate (to give label in β-hydroxybutyrate plus acetoacetate and hence in β-hydroxybutyrate). As preliminary experiments showed that up to 10min after β-hydroxy[3-^{14}C]butyrate injection less than 1% of the label in blood β-hydroxybutyrate and less than 2% of that in acetoacetate was in the C-1 position, only the acetone moiety (C-2–C-4) was counted as a routine after decarboxylation, and the CO_2 moiety (C-1) was not trapped or counted for radioactivity.

In each experiment duplicate 1.0ml portions of diluted injection solution, made up to 0.1mM with unlabelled carrier, were decarboxylated by the same procedure. Recoveries of [^{14}C]acetoacetate averaged 87.1±1.3% (S.D.; 17 samples), similar to those found when [^{14}C]acetoacetate was added directly to blood before deproteinization (86.5±2.1%; eight samples). An additional loss of 4.3±3.0% (S.D.; eight samples) took place during deproteinization. Recoveries of β-hydroxy[^{14}C]butyrate from the diluted injection solutions were 90.0±2.4% (S.D.; 14 samples), and only 0.48±0.32% (S.D.; 14 samples) of the label was found in acetoacetate. Results have been corrected for these incomplete recoveries.

The overall coefficients of variation of duplicate radioactivity estimations on blood extracts after injection of β-hydroxy[^{14}C]butyrate were: for β-hydroxybutyrate, 3.3% (six pairs of samples), and for acetoacetate, 2.1% (13); after injection of [^{14}C]acetoacetate they were: for β-hydroxybutyrate, 10.5% (11 pairs of samples), and for acetoacetate, 3.0% (14). The large coefficient of variation for β-hydroxybutyrate after injection of [^{14}C]acetoacetate is due to its dependence upon the difference between two large values.

(*b*) Blood samples from hepatectomized rats (2–4ml) were deproteinized and neutralized as before, but the ketone bodies were separated chromatographically before analysis to permit the estimation of small amounts of label in one in the presence of large amounts in the other. Amino acids were removed from each extract by passing it through a 1ml column, 10cm long, of Dowex 50 resin (AG50WX2; H^+ form; 200–400 mesh) at 4°C. The eluate with washings (5ml) was neutralized with 1M-NaOH and chromatographed, also at 4°C, on Dowex 1 formate resin as described above ('Radioactive compounds') except that the column length was doubled to ensure separation of β-hydroxybutyrate from lactate. (In fact, no label was found in the lactate peak.) Portions of each fraction of the eluate were counted to identify the fractions containing β-hydroxybutyrate and acetoacetate, the remainder of which were then pooled and neutralized with NaOH.

Concentrations of β-hydroxybutyrate and acetoacetate were determined by the method of Williamson *et al.* (1962). For β-hydroxybutyrate, a portion of the neutralized eluate was used, sometimes after concentration at 25–30°C under reduced pressure; the formate present (0.06–0.27mmol) was shown not to affect the results. For acetoacetate, the neutralized eluate was concentrated to about 0.5ml, transferred

with washings to a weighed cuvette, and assayed *in toto* after making the weight of the cuvette contents up to 3.0g. The formate present (1.6–4.0mmol) caused a linear decrease in the extinction of the samples after completion of the reaction, corrected for by extrapolation. Experiments with acetoacetate standards validated this method.

The specific radioactivities of β-hydroxybutyrate and acetoacetate were calculated from the concentrations and from the radioactivity in either the relevant fractions of the column eluate or the cuvette contents counted separately after assay.

(*c*) Label in blood samples from carotid cannulae was determined directly by collecting the blood in weighed counting vials. Concentrations of ketone bodies in these samples were not determined as a routine. Each sample (0.05–0.2ml) was solubilized overnight at 20°C with Soluene 100 (Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.) and decolorized by treating it with 0.4ml of 20% (w/v) benzoyl peroxide, freshly dissolved in benzene, and 0.1ml of 30% (w/v) H_2O_2, and again leaving overnight at 20°C. This procedure is a simpler version, suitable for small blood samples, of that given by Laurencot & Hempstead (1971). Recovery of label added to blood as [^{14}C]acetoacetate was 94.7±3.3% (S.D.; eight pairs of values) and the coefficient of variation of duplicate estimations was 1.2%. Besides correcting for this recovery, the results were corrected in two ways. (i) Analysis of blood specimens from rats decapitated at approximately 30, 60 and 90s after injection of [^{14}C]acetoacetate revealed the presence of label in compounds other than ketone bodies. This was compatible with the presence of a slowly disappearing impurity equivalent to about 3% of the injected dose. Radioactivities have been corrected by assuming linear disappearance of this impurity during the first minute after injection.

(ii) After injection of [^{14}C]acetoacetate, a correction was made for label in β-hydroxybutyrate by assuming a linear increase in the latter to the first time after injection, about 30s, at which both compounds were analysed; similarly for label in acetoacetate after β-hydroxy[^{14}C]butyrate injection.

Scintillation counting. All counting was done in a Model 3324 liquid-scintillation spectrometer (Packard Instrument Co. Inc.). Scintillators used were as follows: for aqueous samples, 10ml of standard 2,5-diphenyloxazole–dioxane scintillator; for hydrazine lactate containing acetone from decarboxylation of acetoacetate, the scintillator described by Mayes & Felts (1967); for solubilized decolorized blood, 10ml of the scintillator described by Laurencot & Hempstead (1971) but with the substitution of 2,5-diphenyloxazole for 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen and without ethanol. Counting efficiencies for radioactivity were estimated by using [^{14}C]toluene as an internal standard. For injection experiments, counts were normalized to a dose of 0.382μCi/100g for injection of [^{14}C]acetoacetate and 0.375μCi/100g for injection of β-hydroxy[^{14}C]-butyrate (for calculation of q, values of 0.897μCi/rat and 0.882μCi/rat respectively were used). For infusion experiments, counts were normalized to a dose of 0.15μCi/min per 100g for infusion of both [^{14}C]-acetoacetate and β-hydroxy[^{14}C]butyrate.

Calculations

Areas under q–t curves and their errors. The areas under disappearance curves, i.e. for [^{14}C]acetoacetate in blood after injection of [^{14}C]acetoacetate (A_{q1a}) and for β-hydroxy[^{14}C]butyrate after injection of β-hydroxy[^{14}C]butyrate (A_{q4h}), were determined as described by Corney & Heath (1970) and Heath & Barton (1973). Their methods also gave the errors on the areas from the errors on the values of q at each sampling time, corrected for small differences in the latter. The product curves, i.e. q_{4a} and q_{1h} against time (Figs. 1 and 2), fell into two portions: an initial part, when q was rising and passing through a maximum, and a later part, from the third or fourth sampling time, during which q decreased steadily. The areas under the later parts were determined like those under disappearance curves. Those under the earlier parts were determined by plotting the q–t curves on graph paper and cutting out and weighing the areas under them. Random errors on these areas were determined similarly on 20 sets of normally distributed mean values generated with random numbers. Although systematic errors in drawing the curves were not evaluated, they are unlikely to represent more than 10% of the areas under the early portions or 5% of those under the whole curves.

Errors in rates and rate coefficients. Random errors in rate coefficients (k and K values) were obtained by standard methods (summation of the squares of the products of the error on each component and the partial derivative with respect to that component). Rates of irreversible disposal are the product of k values, which were taken to be normally distributed, and Q values, whose distribution appeared to be log-normal (see the Results section). Since the errors on Q values were much greater than those on k values the latter were converted into log-normal form before combining them with those on Q values to estimate the errors on R_1 and R_4. The error on the total rate of irreversible disposal, R, was calculated from the errors on Q_1 and Q_4/Q_1, which were assumed to be independent, rather than Q_1 and Q_4, which were not.

Results

Intact rats

Experimental data. Table 2 shows values of the concentrations (C), pool sizes (Q, i.e. $C \times V$, the

Table 2. *Quantities of labelled and unlabelled ketone bodies in blood of control rats after injection of* D-β-*hydroxy*[3-^{14}*C*]*butyrate*

Single blood samples were taken after decapitation. For consistency, all distributions are assumed normal, although those of C and Q are probably closer to log-normal. Where possible, S and q have been corrected for errors (not given) in t. Values of q (given per rat) are equal to $2.35 \times (q/100\,g)$, i.e. to $2.35SQ$. Results are given as mean±S.E.M. (coefficient of variation in parentheses).

		β-Hydroxybutyrate				Acetoacetate					
Time after injection (t) (min)	No. of rats	Concentration (C_4) (mM)	Pool size (Q_4) (μmol/100g)	$10^{-4}\times$Specific radioactivity (S_4) (d.p.m./μmol)	$10^{-4}\times$Quantity of label (q_4) (d.p.m.)	Concentration (C_1) (mM)	Pool size (Q_1) (μmol/100g)	$10^{-4}\times$Specific radioactivity (S_1) (d.p.m./μmol)	$10^{-4}\times$Quantity of label (q_1) (d.p.m.)	Concentration ratio C_4/C_1	Specific radioactivity ratio S_1/S_4
0.61	5	0.0653 ±0.0174 (0.594)	0.400 ±0.105 (0.584)	43.03 ±8.77 (0.456)	31.50 ±1.93 (0.137)	0.0726 ±0.0156 (0.481)	0.446 ±0.094 (0.472)	4.553 ±1.366 (0.671)	3.878 ±0.727 (0.419)	0.887 ±0.124 (0.312)	0.115 ±0.029 (0.575)
0.85	4	0.1148 ±0.0326 (0.569)	0.752 ±0.221 (0.586)	15.23 ±3.18 (0.362)	26.59 ±0.83 (0.054)	0.1161 ±0.0300 (0.517)	0.760 ±0.203 (0.535)	4.904 ±0.944 (0.385)	7.715 ±0.894 (0.232)	0.982 ±0.095 (0.193)	0.284 ±0.033 (0.199)
1.18	5	0.1277 ±0.0455 (0.797)	0.771 ±0.260 (0.755)	13.00 ±1.98 (0.340)	18.28 ±0.75 (0.092)	0.1130 ±0.0141 (0.280)	0.690 ±0.079 (0.255)	3.999 ±0.487 (0.272)	6.084 ±0.472 (0.173)	1.050 ±0.226 (0.481)	0.321 ±0.021 (0.148)
2.13	4	0.0862 ±0.0222 (0.515)	0.562 ±0.148 (0.526)	10.03 ±1.48 (0.296)	11.68 ±0.67 (0.115)	0.0795 ±0.0181 (0.455)	0.519 ±0.122 (0.469)	2.384 ±0.174 (0.146)	2.836 ±0.444 (0.313)	1.072 ±0.089 (0.167)	0.254 ±0.037 (0.293)
3.17	6	0.0917 ±0.0124 (0.332)	0.554 ±0.077 (0.339)	7.154 ±1.062 (0.364)	8.295 ±0.454 (0.134)	0.0859 ±0.0097 (0.278)	0.519 ±0.061 (0.287)	1.633 ±0.129 (0.193)	1.920 ±0.126 (0.161)	1.079 ±0.136 (0.308)	0.243 ±0.022 (0.217)
8.12	6	0.1127 ±0.0238 (0.519)	0.708 ±0.148 (0.513)	2.159 ±0.341 (0.387)	2.959 ±0.176 (0.146)	0.0951 ±0.0153 (0.394)	0.596 ±0.092 (0.378)	0.402 ±0.040 (0.241)	0.518 ±0.014 (0.064)	1.189 ±0.189 (0.389)	0.202 ±0.021 (0.258)

Table 3. *Regression of pool size (Q) on concentration (C) for ketone bodies in blood of injured and control rats*

Results obtained at different times after injections of β-hydroxy[^{14}C]butyrate and [^{14}C]acetoacetate are combined. Regressions are given as: $Q = Q_0 \pm$S.E.M.$+(b \pm$S.E.M.$)C$ (numbers of samples in parentheses), the units being: Q and Q_0, μmol/100g; b (equivalent to blood volume, V), ml/100g; C, mM.

β-Hydroxybutyrate, control rats:	$Q_4 = 0.016 \pm 0.010 + (6.08 \pm 0.08)C_4$	(59)
Acetoacetate, control rats:	$Q_1 = 0.001 \pm 0.011 + (6.24 \pm 0.10)C_1$	(59)
β-Hydroxybutyrate, injured rats:	$Q_4 = -0.017 \pm 0.013 + (5.01 \pm 0.07)C_4$	(60)
Acetoacetate, injured rats:	$Q_1 = -0.003 \pm 0.022 + (4.90 \pm 0.08)C_1$	(60)

calculated blood volume), specific radioactivities (S) and quantities of label (q) in the blood of control post-absorptive rats at various times after injection of β-hydroxy[^{14}C]butyrate. The F test showed that q was less variable than S. (F for the overall coefficients of variation at the different times after injection was significant at $P<0.005$ for β-hydroxybutyrate and $P<0.05$ for acetoacetate. The small groups at particular times after injection did not always show the difference.) A similar result was obtained after β-hydroxy[^{14}C]butyrate injection in injured rats and [^{14}C]acetoacetate injection in injured and control rats.

The rate of irreversible disposal, R, of a substrate can be written: $R = kQ$, where k is the composite irreversible disposal rate coefficient [see eqn. (4.5) in Heath & Barton, 1973]. The lower variability of q than S indicates that in both control and injured rats k was more nearly constant than R [see text near eqn. (4.2) in Heath & Barton, 1973]. Moreover, the coefficient of variation of q did not increase with time after injection, as can be seen from semi-logarithmic plots of q against time (Figs. 1 and 2), confirming that the values of k were similar in different rats. Thus R was proportional to Q within experimental error. A mean value of k was therefore calculated which with the range of Q gave the range of R, as described in the Experimental section. Table 3 shows that since the slopes (equivalent to the blood volumes) were nearly constant in each group of rats, Q was closely proportional to C. Consequently the disposal rates of acetoacetate and β-hydroxybutyrate were also proportional to C, their concentrations in the blood.

In these experiments (e.g. in Table 2) the pool sizes (Q_1 and Q_4) and the β-hydroxybutyrate/acetoacetate ratio (C_4/C_1) were substantially time-invariant, suggesting that any such variations in individual rats were small compared with the relatively large differences in Q between rats. (The apparent increase in C_4/C_1 with time in Table 2 was not significant when combined with results from [^{14}C]acetoacetate injections, and was not seen in injured rats.)

For the determination of areas under q–t curves the early parts of the curves were obtained by serial

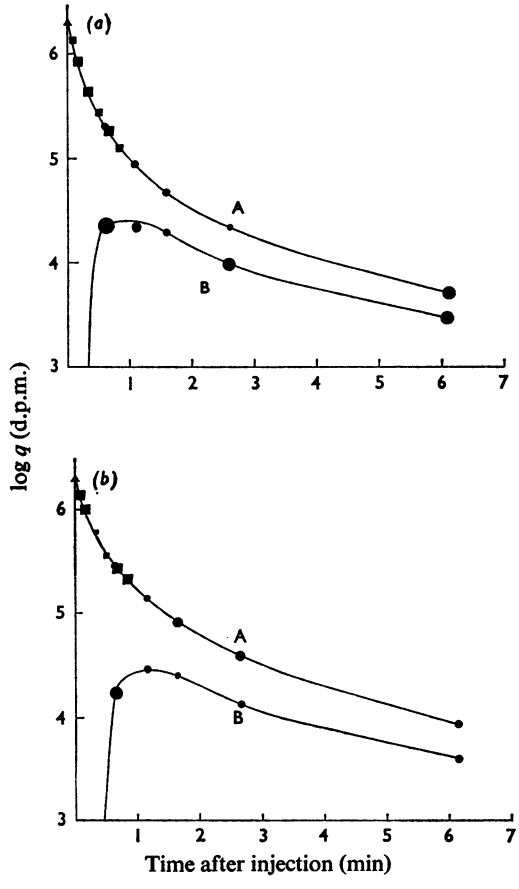


Fig. 1. *Radioactivity–time curves after injection o [3-^{14}C]acetoacetate*

Height of symbol (●, ■) represents the standard error of q (four to seven samples per point). ▲, Dose of label given; ■, serial samples from carotid-cannulated rats under ether anaesthesia; ●, single samples from decapitated conscious rats (from Tables 2 and 3). Curve A, quantity of label in acetoacetate in blood (q_1). Curve B, quantity of label in β-hydroxybutyrate in blood (q_4). (*a*) Control rats. (*b*) Injured rats.

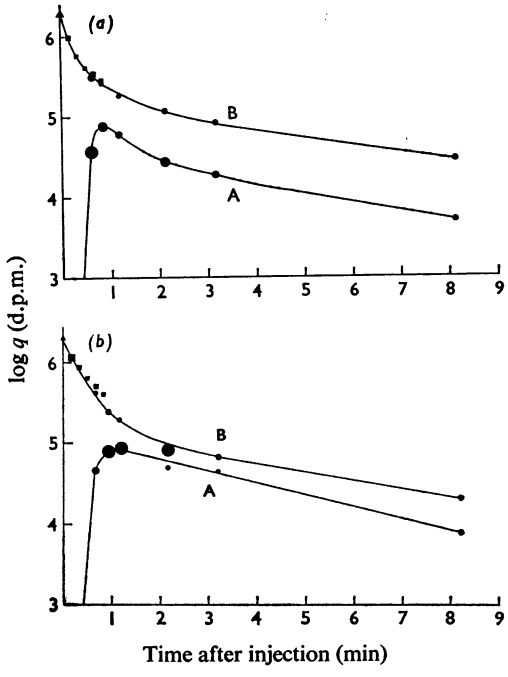


Fig. 2. *Radioactivity–time curves after injection of* D-*β-hydroxy*[3-^{14}*C*]*butyrate*

Details are the same as in Fig. 1.

sampling from carotid-cannulated rats under ether anaesthesia, and are shown with the remainder of the curves in Figs. 1 and 2. (The semi-logarithmic form of these plots, used for convenience, does not indicate the areas.) Unlike the acetoacetate curves (Fig. 1), the β-hydroxybutyrate curves did not run perfectly onto those obtained by single sampling, particularly in injured animals (Fig. 2). This may have been due to the combined effects of blood loss, at up to 1 ml/min, and anaesthesia, resulting in circulatory changes and tissue anoxia. In support of this explanation, other experiments showed that the blood β-hydroxybutyrate/acetoacetate ratio (C_4/C_1) was higher in carotid-cannulated rats decapitated under ether anaesthesia than in decapitated unanaesthetized non-cannulated rats, this difference being greater in injured rats than in controls; when the blood from the carotid-cannulated rats was obtained from the cannula, C_4/C_1 rose even further. Consequently, when simultaneous values were available from both serial- and single-sampling experiments, the latter have been preferred, although the difference between them in fact represents less than 4% of the total area. In neither injured nor uninjured carotid-cannulated rats was the total ketone body concentration (C_4+C_1) higher than in corresponding non-cannulated rats, showing that

Table 4. *Concentrations, pool sizes and rates of disposal of ketone bodies in injured and control rats*

Disposal rates were calculated from pool sizes and from rate coefficients given in Fig. 3. Results are given as geometric means with S.D. ranges (59–60 samples). Difference from controls (Student's *t* test) significant at: * $P<0.001$; † $P<0.05$.

	Control rats			Injured rats		
	Acetoacetate	β-Hydroxybutyrate	Acetoacetate+ β-hydroxybutyrate	Acetoacetate	β-Hydroxybutyrate	Acetoacetate+ β-hydroxybutyrate
Blood concentration (C_1, C_4, C_4+C_1) (mM)	0.095 (0.062–0.147)	0.094 (0.055–0.162)	0.191 (0.119–0.306)	0.234* (0.159–0.343)	0.147* (0.098–0.219)	0.384* (0.266–0.554)
Pool size (Q_1, Q_4, Q_4+Q_1) (μmol/100g)	0.59 (0.38–0.92)	0.59 (0.34–1.01)	1.19 (0.74–1.91)	1.14* (0.77–1.68)	0.72† (0.48–1.08)	1.87* (1.29–2.72)
Disposal rate (R_1, R_4, R) (μmol/min per 100g)	1.99 (1.28–3.09)	0.54 (0.31–0.94)	2.53 (1.63–3.93)	2.84* (1.93–4.19)	0.59 (0.39–0.89)	3.43* (2.32–5.05)

their metabolic state was similar despite considerable weight losses.

Table 4 shows the values of the blood concentrations (C) and the pool sizes (Q) in injured and control non-cannulated rats (combined results from [^{14}C]-acetoacetate and β-hydroxy[^{14}C]butyrate injections). Both quantities were rather variable, and their distributions approximated to log-normal, in accordance with Heath (1967). After injury C and Q for both individual and total ketone bodies increased, whereas the β-hydroxybutyrate/acetoacetate ratio (C_4/C_1, not shown) decreased.

The mean concentration of total ketone bodies (C_4+C_1) in the injured rats was similar to that found by Barton (1971), but that in controls was lower, giving a significant difference between control and injured rats where none was found previously. The β-hydroxybutyrate/acetoacetate ratio in both groups of rats was also lower, although it showed the same tendency to decrease after injury as in the earlier study. A period of about 4 years separated the two sets of experiments, and any changes in the handling and treatment of the rats during this time could have led to differences in the concentrations of ketone bodies in the controls, which are in any case extremely variable. The relative values in the control and injured rats in the present paper are probably the more trustworthy since they were based on much larger numbers and were more closely matched in time, and they are more consistent with the rise in total ketone bodies in the liver after injury (Barton, 1971).

Calculated results. From the above data (Tables 2 and 4; Figs. 1 and 2) two quantities were calculated as described by Heath & Barton (1973) and outlined in the Experimental section of the present paper. First, the generalized rate coefficients (Fig. 3, model *a*) for interconversion (K_{14}, K_{41}) were lower than those for disposal (K_1, K_4), reflecting the disequilibrium between labelled β-hydroxybutyrate and acetoacetate in the blood (e.g. in Table 2). Secondly, the mean rate of disposal of total ketone bodies (R, Table 4) rose after injury because of the increase in pool size (Q), but there was considerable overlap of values with those in control rats because of the variability of the pool sizes in both groups.

Hepatectomized rats

Experimental data. Table 5 shows the specific radioactivities (S') in blood during infusion of labelled β-hydroxybutyrate and acetoacetate into hepatectomized rats. There was no significant change with time, so that an isotopic steady state had been reached by the time of the first sample. In the controls, the ratios of product to precursor specific radioactivities were somewhat lower than those found by McGarry *et al.* (1970) in functionally hepatectomized starved rats, particularly with β-hydroxy[^{14}C]butyrate as the precursor. Infusion experiments on injured rats were only carried out with [^{14}C]acetoacetate; since the results were very similar to those in controls, control values have been used for injured rats during β-hydroxy-[^{14}C]butyrate infusion.

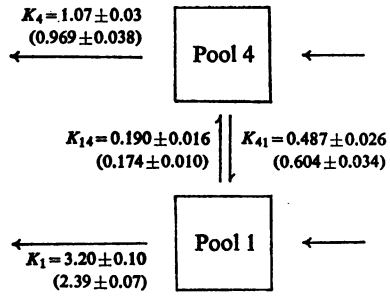
Calculated results. Calculations were carried out as described by Heath & Barton (1973) and outlined in the Experimental section of the present paper. First, the generalized rate coefficients for the system comprising blood plus extrahepatic tissues (Fig. 3, model *b*) were calculated from the specific radioactivities in Table 5. Although K'_{41} was much smaller than K_{41}, K'_{14} was similar to K_{14} (model *a*), and subtraction gave a value of K''_{14} (Fig. 3, model *c*) not significantly different from zero. Therefore, conversion of blood β-hydroxybutyrate into blood acetoacetate takes place mainly in the liver, and the reverse process takes place mainly in extrahepatic tissues. This conclusion does not depend upon the assumption that hepatectomy was without effect on the rate coefficients, provided that they all changed in proportion.

Further calculation gave the individual rate coefficients for transfer from blood to liver (k_{12} and k_{42}; Fig. 3, model *d*). Since these are directly proportional to K''_{14} and K''_{41} respectively, k_{12} was also not significantly different from zero. The values for k_{12} and k_{42} are estimates of the mean and do not give the

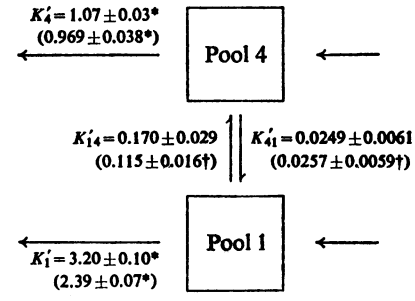
Table 5. *Specific radioactivities in blood during infusion of labelled ketone bodies into hepatectomized rats*

Rats were infused with glucose, unlabelled ketone bodies and [3-^{14}C]acetoacetate or D-β-hydroxy[3-^{14}C]butyrate. Blood samples were taken between 2.8 and 10.9 min after start of infusion. Results are given as mean±S.E.M. (four samples/group).

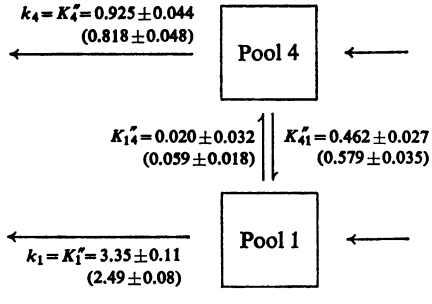
	$10^{-4}\times$Specific radioactivity (d.p.m./μmol)	
	Acetoacetate (S'_1)	β-Hydroxybutyrate (S'_4)
β-Hydroxy[^{14}C]butyrate infusion, control rats	0.948±0.204	33.4±3.0
[^{14}C]Acetoacetate infusion, control rats	41.5±4.3	1.68±0.22
[^{14}C]Acetoacetate infusion, injured rats	36.7±1.5	1.53±0.14



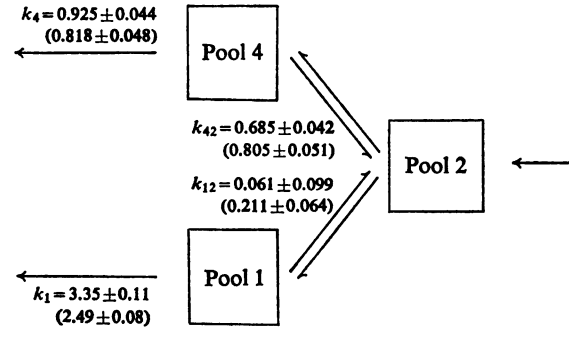
(*a*) Generalized model for whole body



(*b*) Generalized model after hepatectomy
* Assumed equal to K_1 and K_4 (model *a*).
† Calculated by using control values for S_1' and S_4' after β-hydroxy[^{14}C]butyrate infusion (Table 5).



(*c*) Generalized model of liver and blood only



(*d*) Detailed model of liver and blood

Fig. 3. *Rate coefficients for ketone body metabolism in injured and control rats*

The models (*a–d*) shown are those of Model 3 in Heath & Barton (1973). Pool 1 represents blood acetoacetate, pool 4 blood β-hydroxybutyrate, and pool 2 liver acetoacetate plus β-hydroxybutyrate (assumed in equilibrium). All values are generalized or individual rate coefficients and are given in min^{-1} in the form: mean±S.E.M. for controls (mean±S.E.M. for injured rats).

values in individual rats since these depend upon the blood β-hydroxybutyrate/acetoacetate ratio (C_4/C_1) which varied considerably (e.g. in Table 2). If β-hydroxybutyrate and acetoacetate were not in equilibrium in the liver, the calculated value of k_{12} would still be correct but that of k_{42} would be too low.

Finally, the individual rate coefficients for disposal of acetoacetate (k_1) and β-hydroxybutyrate (k_4) were calculated (Fig. 3, model *d*). The maximum difference from the corresponding generalized coefficients (K_1 and K_4; Fig. 3, model *a*) was about 15%, indicating that unlike k_{12} and k_{42} they were not very sensitive to the results from hepatectomized rats, or to the assumptions on which the generalized rate coefficients after hepatectomy were calculated. In both control and injured rats k_1 was greater than k_4, and k_1 fell after injury whereas there was little change in k_4. The rates of irreversible disposal (R_1 and R_4), given by $k_1 Q_1$ and $k_4 Q_4$, are shown in Table 4. The mean disposal rate of acetoacetate (R_1) was greater than that of β-hydroxybutyrate (R_4) in both groups of rats, and increased after injury whereas R_4 did not. In effect, the fall in the β-hydroxybutyrate/acetoacetate ratio (C_4/C_1) more than compensated for the decrease in the fractional turnover rate of acetoacetate relative to β-hydroxybutyrate, i.e. in k_1 relative to k_4.

Discussion

Methods of estimating rates of disposal of ketone bodies

Since the method described by Heath & Barton (1973) and used here is based upon a completely general model with an indefinite number of extrahepatic pools, it is not necessary for blood β-hydroxybutyrate and acetoacetate to be in isotopic equilibrium or for disposal to be slow compared with distribution into extravascular space. These conditions have, sometimes justifiably, been assumed by Bergman *et al.* (1963), Leng & Annison (1964), Bates *et al.* (1968), McGarry *et al.* (1970), Bieberdorf *et al.* (1970) and Balasse & Havel (1971), who have used a one-pool model for ketone body metabolism, but neither condition holds in post-absorptive rats (Table 2, Figs. 1 and 2). Bates (1971) has calculated values for several rates by using a three-pool model and by assuming no transfer from extrahepatic tissues to blood. This assumption is unnecessary for calculation of the disposal rate, R, for total ketone bodies, and can be avoided altogether if additional experiments are done after hepatectomy. In post-absorptive rats it would in fact make little difference to k_1 and k_4, but would cause large errors in k_{12} and k_{42} (Fig. 3).

Most previous work with labelled ketone bodies *in vivo* has involved constant-infusion methods (Bergman *et al.*, 1963; Leng & Annison, 1964; McGarry *et al.*, 1970; Bieberdorf *et al.*, 1970; Balasse & Havel, 1971; Bates, 1971). Besides the general disadvantages of these techniques (Heath & Barton, 1973), their need for serial blood sampling makes them particularly unsuitable for use in either post-absorptive rats, in which the blood concentrations of ketone bodies are low (Table 4), or injured rats, in which the blood volumes are decreased (Table 3). The single-injection method of Heath & Barton (1973) avoids these difficulties by using single samples from several rats at all but the earliest sampling times.

Only Bates *et al.* (1968) have previously studied the disappearance of labelled ketone bodies *in vivo* in the fed or post-absorptive state. They assumed a one-pool model and although they used a single-injection method they still took serial samples. Their results are therefore not directly comparable with those reported here. At the only time for which they give complete results, 2min after injection of β-hydroxy[^{14}C]-butyrate, their values for q_4 and q_1 bracket those given in Table 2 when corrected to the same dose of isotope.

Blood sampled from the great vessels, as in these experiments, is not representative of that in the whole body since its haematocrit is higher. This does not affect calculation of rates of disposal [see the text near eqn. (3.3) in Heath & Barton (1973)]. It will, however, lead to overestimation of rate coefficients and underestimation of concentrations and pool sizes if the concentrations of ketone bodies in erythrocytes are lower than in plasma. The maximum errors, if the concentrations in erythrocytes were zero, would be 16% in injured animals and 9% in controls; since in practice they are about half those in plasma (Barton, 1971), the errors will be correspondingly lower.

Control of ketone body utilization

Dependence of disposal rate on concentration. The results in Table 2 indicate that in control post-absorptive rats the disposal rates of ketone bodies were proportional to their blood concentrations, and similar results show that the same was true after injury. The concentrations in these rats were low (Table 4), and this proportionality between disposal rate and concentration may or may not apply at the higher values found in starvation or diabetes (Bates *et al.*, 1968; Bässler *et al.*, 1970; Balasse & Havel, 1971; Bates, 1971; Bässler *et al.*, 1972). It agrees with the proportionality between the arteriovenous differences across heart and skeletal muscle and the arterial concentrations of ketone bodies under some conditions *in vivo* when the concentrations are low (Rudolph *et al.*, 1965; Hagenfeldt & Wahren, 1968; Whereat & Chan, 1972). These results show that utilization of ketone bodies is a passive process, even after such a drastic change as injury, and is controlled via their blood concentrations by their rates of production by the liver.

Significance of disposal rate coefficients. Values of k cannot be compared directly between states such as the normal and injured where there are differences in blood flow. The significance of the changes in k after injury (Fig. 3, model *d*) is best seen by examining the values of kV (V = blood volume), the metabolic clearance rate (Tait *et al.*, 1962; Tait, 1963). This is the volume of blood which, if completely cleared of substrate in unit time, would give a disposal rate equal to that in the whole animal. Comparison of kV with the cardiac output indicates the whole body extraction coefficient of the substrate. In control rats, the mean values of kV for acetoacetate and β-hydroxybutyrate were 20.9 and 5.8ml/min per 100g respectively, compared with typical cardiac outputs of 28.6ml/min per 100g in unanaesthetized and 20.4ml/min per 100g in anaesthetized rats (Popovic & Kent, 1964). Thus the proportion of circulating β-hydroxybutyrate extracted was lower than that of acetoacetate, clearance of which appeared almost complete. The same was probably true after injury when kV for acetoacetate and β-hydroxybutyrate was respectively 12.1 and 4.0ml/min per 100g, and the cardiac output is also lower (Takács *et al.*, 1962).

The lower metabolic clearance rate for β-hydroxybutyrate than for acetoacetate was probably due to the comparatively low activity of D-β-hydroxybutyrate dehydrogenase in most tissues that utilize ketone bodies (Lehninger *et al.*, 1960; Williamson *et al.*, 1971). Extraction coefficients at low concentrations

have been determined for two of the most important, cardiac and skeletal muscle, from arteriovenous differences in human subjects and dogs, and are as follows: for cardiac muscle, acetoacetate 30–85%, β-hydroxybutyrate 27–45% (Rudolph *et al.*, 1965; Kraupp *et al.*, 1968; Whereat & Chan, 1972); for skeletal muscle, acetoacetate 40–50%, β-hydroxybutyrate 12–50% (Hagenfeldt & Wahren, 1968; Owen & Reichard, 1970). These are broadly in line with the whole body extraction coefficients calculated above. The difference between β-hydroxybutyrate and acetoacetate implies that deductions about the rate of utilization of ketone bodies from their blood concentrations must take into account the β-hydroxybutyrate/acetoacetate ratio as well as the total concentration.

Contribution to body energy requirements

Estimation of the fraction of ketone bodies taken up that is completely oxidized is difficult because of isotopic exchange. Balasse & Havel (1971) found that, at high ketone body concentrations in dogs, about 50% of infused [^{14}C]acetoacetate was rapidly oxidized to $^{14}CO_2$ and that about 20% was recovered in tissue lipids and proteins. If complete oxidation is assumed, the mean value of R in controls (Table 5) is equivalent to an O_2 consumption of about 0.14 litre/h per kg, representing about 7% of the total mean O_2 consumption of the rat in a 20°C environment (2.2 litres/h per kg; Stoner, 1969). After an injury similar to that used in these experiments the mean O_2 consumption falls to 1.23 litres/h per kg, and R increases to the equivalent, at 0.19 litre/h per kg, of 15% of this. This contrasts with the effects of injury on glucose utilization, which decreases roughly in proportion to the fall in O_2 consumption (Heath & Corney, 1973), and on the oxidation of both glucose (Ashby *et al.*, 1965) and free fatty acids (Heath & Stoner, 1968) to CO_2, which also decreases.

The increased rate of disposal of ketone bodies after injury reflects an increased rate of production by the liver. This is compatible with the higher concentrations of ketone bodies found in the liver after injury in the post-absorptive rat (Barton, 1971), apparently due to an increase in the proportion of free fatty acids converted into them.

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References

Ashby, M. M., Heath, D. F. & Stoner, H. B. (1965) *J. Physiol. (London)* **179**, 193–237
Baker, N. (1969) *J. Lipid Res.* **10**, 1–24
Baker, N. & Rostami, H. (1969) *J. Lipid Res.* **10**, 83–90
Balasse, E. O. & Havel, R. J. (1971) *J. Clin. Invest.* **50**, 801–813
Barton, R. N. (1971) *Clin. Sci.* **40**, 463–477
Barton, R. N. (1972) *Abstr. Commun. FEBS Meet.* **8**, Abstr. 1115
Barton, R. N. (1973) *Biochem. Soc. Trans.* **1**, 880
Bässler, K. H., Wagner, G. & Heicke, B. (1970) *Z. Gesamte Exp. Med.* **153**, 131–135
Bässler, K. H., Horbach, L. & Wagner, K. (1972) *Diabetologia* **8**, 211–214
Bates, M. W. (1971) *Amer. J. Physiol.* **221**, 984–991
Bates, M. W., Krebs, H. A. & Williamson, D. H. (1968) *Biochem. J.* **110**, 655–661
Bergman, E. N., Kon, K. & Katz, M. L. (1963) *Amer. J. Physiol.* **205**, 658–662
Bieberdorf, F. A., Chernick, S. S. & Scow, R. O. (1970) *J. Clin. Invest.* **49**, 1685–1693
Bruce, H. M. & Parkes, A. S. (1956) *J. Anim. Tech. Ass.* **7**, 54
Corney, P. L. & Heath, D. F. (1970) *J. Appl. Physiol.* **28**, 672–674
Cremer, J. E. (1971) *Biochem. J.* **122**, 135–138
Cuthbertson, D. P. (1942) *Lancet* **i**, 433–436
Hagenfeldt, L. & Wahren, J. (1968) *Scand. J. Clin. Lab. Invest.* **21**, 314–320
Hall, L. M. (1962) *Anal. Biochem.* **3**, 75–80
Heath, D. F. (1967) *Nature (London)* **213**, 1159–1160
Heath, D. F. (1973) *Brit. J. Exp. Pathol.* **54**, 359–367
Heath, D. F. & Barton, R. N. (1973) *Biochem. J.* **136**, 503–518
Heath, D. F. & Corney, P. L. (1973) *Biochem. J.* **136**, 519–530
Heath, D. F. & Stoner, H. B. (1968) *Brit. J. Exp. Pathol.* **49**, 160–169
Heath, D. F. & Threlfall, C. J. (1968) *Biochem. J.* **110**, 337–362
Kraupp, O., Adler-Kastner, L., Kolassa, N., Nell, G., Plank, B. & Chirikdjian, J. J. (1968) *Eur. J. Biochem.* **6**, 114–125
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
La Noue, K., Nicklas, W. J. & Williamson, J. R. (1970) *J. Biol. Chem.* **245**, 102–111
Laurencot, H. J. & Hempstead, J. L. (1971) in *Organic Scintillators and Liquid Scintillation Counting* (Horrocks, D. L. & Chin-Tzu Peng, eds.), pp. 635–657, Academic Press, New York
Lehninger, A. L., Sudduth, H. C. & Wise, J. B. (1960) *J. Biol. Chem.* **235**, 2450–2455
Leng, R. A. & Annison, E. F. (1964) *Biochem. J.* **90**, 464–469
Ljunggren, G. (1924) *Biochem. Z.* **145**, 422–425
Mayes, P. A. & Felts, J. M. (1967) *Biochem. J.* **102**, 230–235
McCann, W. P. & Greville, G. D. (1962) *Biochem. Prep.* **9**, 63–68
McFarlane, A. S. (1957) *Progr. Biophys. Biophys. Chem.* **7**, 115–163
McGarry, J. D., Guest, M. J. & Foster, D. W. (1970) *J. Biol. Chem.* **245**, 4382–4390
Owen, O. E. & Reichard, G. A., Jr. (1970) *J. Clin. Invest.* **50**, 1536–1545
Popovic, V. P. & Kent, K. M. (1964) *Amer. J. Physiol.* **207**, 767–770
Popovic, V. & Popovic, P. (1960) *J. Appl. Physiol.* **15**, 727–728
Rosenthal, S. M. (1943) *Publ. Health Rep.* **58**, 1429–1436

Rudolph, W., Maas, D., Richter, J., Hasinger, F., Hofmann, H. & Dohrn, P. (1965) *Klin. Wochenschr.* **43**, 445–451
Stoner, H. B. (1961) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **20**, *Suppl.* **9**, 38–48
Stoner, H. B. (1969) *Brit. J. Exp. Pathol.* **50**, 125–138
Stoner, H. B. (1970) *J. Clin. Pathol.* **23**, *Suppl.* **4**, 47–55
Tait, J. F. (1963) *J. Clin. Endocrinol. Metab.* **23**, 1285–1297
Tait, J. F., Little, B., Tait, S. A. S. & Flood, C. (1962) *J. Clin. Invest.* **41**, 2093–2100
Takács, L., Kállay, K. & Skolnik, J. H. (1962) *Circulation Res.* **10**, 753–757
Whereat, A. F. & Chan, A. (1972) *Amer. J. Physiol.* **223**, 1398–1406
Williamson, D. H. & Hems, R. (1970) in *Essays in Cell Metabolism* (Bartley, W., Kornberg, H. L. & Quayle, J. R., eds.), pp. 257–281, Wiley-Interscience, London
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41–47