KETONE-BODY CONCENTRATIONS IN LIVER AND BLOOD AFTER LIMB ISCHAEMIA IN THE RAT

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SUMMARY

1. The effect of a 4 h period of bilateral hind limb ischaemia on the concentrations of ketone bodies in blood and liver of post-absorptive and starved rats has been investigated.

2. The concentration of total ketone bodies in the blood did not change after injury in post-absorptive rats, but fell after injury in starved rats; the blood $\beta$-hydroxybutyrate/acetoacetate ratio fell after injury in both post-absorptive and starved rats.

3. Apart from a transient increase in fed rats, the hepatic $\beta$-hydroxybutyrate/acetoacetate ratio did not change after injury in post-absorptive or starved rats until the terminal stages, indicating adequate hepatic oxygenation during the early response to injury.

4. In control post-absorptive and starved rats the concentration of liver total ketone bodies was correlated with that of plasma non-esterified fatty acids; in post-absorptive rats the liver ketone body concentration rose after injury and was higher than would be predicted from the regression line for these controls, suggesting increased ketogenesis compatible with inhibition of complete oxidation of non-esterified fatty acids after injury. In contrast, in starved rats the liver total ketone-body concentration did not change after injury.

Changes in the oxidation of carbohydrates and fatty acids by the liver shortly after injury, such as that resulting from fatal hind-limb ischaemia in rats in a 20° environment (Ashby, Heath & Stoner, 1965; Heath & Stoner, 1968), were explained by Heath & Threlfall (1968) on the basis of inhibition at the citrate synthase reaction. This might be expected to favour ketone-body synthesis.

There are, however, few reports on the effect of injury on ketone-body metabolism. Engel & Hewson (1953) subjected starved rats anaesthetized with pentobarbital to haemorrhage,

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463
which was judged to be lethal or sublethal according to whether or not there was a rise in plasma amino nitrogen. Haemorrhage was followed either by a further period of starvation, or by infusion of octanoate, both of which increased the blood ketone-body concentration in controls. With the further starvation there was no change in blood ketone bodies after sublethal haemorrhage, and a fall after lethal haemorrhage; in the rats which received the octanoate infusion there was a rise after sublethal haemorrhage similar to that in the controls, but no change after lethal haemorrhage. However, Bloom (1961), working with starved unaesthetized rats which were bled, found a large increase in the blood ketone-body concentration during a further period of starvation, provided the haemorrhage had not increased plasma nitrogen. If plasma nitrogen rose, the blood ketone-body concentration was unchanged.

Little has been done on injured patients but Naftalin (1962) showed that patients who had undergone surgery did not show the usual rise in blood ketone bodies on fasting. In view of the increasing interest in ketone bodies as a metabolic fuel, it was felt that additional animal work was necessary to define the effects of injury on ketone-body metabolism in greater detail and so provide a basis for its further study in injured man.

An important point to be settled when studying the biochemical changes after injury is the extent to which they reflect impairment of the circulation and therefore of the oxygen supply to the tissues. The mitochondrial NADH/NAD$^+$ ratio in a tissue gives an indication of its oxygenation, and in the liver this ratio is thought to be reflected in the $\beta$-hydroxybutyrate/acetoacetate ratio (Williamson, Lund & Krebs, 1967). Measurement of the latter could therefore give valuable support to previous experiments which have suggested that the circulation to essential organs is adequate for several hours after removal of the tourniquets (Stoner, 1958).

We have investigated the effect of ischaemic limb injury on the concentration of ketone bodies in liver and blood, and on the relationship between liver ketone bodies and plasma non-esterified fatty acids (NEFA). A preliminary account of this work has been published (Barton, 1970).

**MATERIALS AND METHODS**

**Rats.** Male albino Wistar rats of the Porton strain weighing 240±8 (SD) g were housed at an environmental temperature of 18–22°C with controlled lighting giving light from 07.00 to 19.00 hours daily. M.R.C. diet 41B (Bruce & Parkes, 1956) was provided. When 'fed' rats were used, diet was available until the start of the experiment (10.00–10.30 hours). When samples were taken (between 14.00 and 19.00 hours) the rats were in the post-absorptive state (Heath & Threlfall, 1968). Such animals will be referred to here as post-absorptive rats. When rats were 'starved', food was removed on the day before the experiment; when rats were left overnight after injury, water but not food was given so that on the second day both injured and control animals were in the starved state.

**Method of injury.** Animals were subjected to bilateral hind-limb ischaemia by using the method of Rosenthal (1943); tourniquets were applied under ether anaesthesia and control animals were given an anaesthetic of the same duration (about 3 min) at the same time. Tourniquets were applied at 10.00–10.30 hours and removed 4 h later; this injury gives a mortality of approx. 85% with a mean survival time of 12 h in fed rats (Stoner, 1961). When samples were taken from animals at the end of the injury period this was done without
removing the tourniquets. Colon temperatures were measured with a thermocouple introduced 6–8 cm from the anus.

Methods of sampling. Blood samples were collected from the severed neck after decapitation.

Before investigating the effect of injury on liver ketone-body concentrations, a comparison was made between different methods of removing the liver. Part of the liver was either dropped into liquid N₂ after stunning the animal, or frozen in situ with tongs cooled in liquid N₂ (Wollenberger, Ristau & Schoffa, 1960) in animals stunned or anaesthetized with ether or sodium pentobarbital (Veterinary Nembutal: Abbott Laboratories Ltd, Queenborough, Kent; 45 mg/kg body weight, intraperitoneally).

Table 1. Effect of sampling methods on the concentrations of β-hydroxybutyrate and acetoacetate, and their ratio, in rat liver. Details of sampling methods are given in the Materials and Methods section; rats had access to food and were killed between 10.00 and 12.30 hours. Geometric means and standard error ranges are given; number of rats are in parentheses.

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>β-Hydroxybutyrate (μmol/g wet wt)</th>
<th>β-Hydroxybutyrate ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ether and tongs</td>
<td>0·249 (13) (0·231–0·269)</td>
<td>1·75 (13) (1·45–2·11)</td>
<td>P&lt;0·01</td>
</tr>
<tr>
<td>2. Stunning and lobe removed</td>
<td>0·268 (8) (0·231–0·310)</td>
<td>4·44 (8) (3·71–5·31)</td>
<td>P&lt;0·05</td>
</tr>
<tr>
<td>3. Stunning and tongs</td>
<td>0·211 (9) (0·193–0·230)</td>
<td>2·55 (9) (2·23–2·92)</td>
<td>P&lt;0·01</td>
</tr>
<tr>
<td>4. Pentobarbital and tongs</td>
<td>0·207 (15) (0·193–0·222)</td>
<td>1·56 (15) (1·34–1·81)</td>
<td>P&lt;0·05</td>
</tr>
</tbody>
</table>

The total concentration of ketone bodies was the same irrespective of the method of sampling (Table 1). Lower values for the β-hydroxybutyrate/acetoacetate ratio were found when tongs were used with or without anaesthesia than when lumps were dropped into liquid N₂; using the latter method the greater the size of the lump that was taken, the greater the ratio obtained. This agrees with the results of Brosnan, Krebs & Williamson (1970), who found a 3·4-fold increase in the β-hydroxybutyrate/acetoacetate ratio after hepatic ischaemia of 2 min duration in fed rats. The use of cooled tongs was therefore considered necessary to obtain physiological β-hydroxybutyrate/acetoacetate ratios, but this technique was found unsuitable for routine use in stunned animals and anaesthesia was required. Since pentobarbital lowers the plasma non-esterified fatty acid (NEFA) concentration whereas ether has little effect (Fodor & Grafnetter, 1960), ether was used routinely.

When liver ketone bodies and plasma NEFA were measured in the same animal, this was done at laparotomy under ether anaesthesia by inserting a needle attached to a syringe into the abdominal aorta, then removing a portion of the liver with tongs cooled in liquid N₂, and finally withdrawing blood into the syringe.

Reagents. Sodium DL-β-hydroxybutyrate and NAD⁺ were obtained from Sigma (London) Chemical Co. Ltd, London, S.W.6, NADH and D-β-hydroxybutyrate dehydrogenase (EC
from the Boehringer Corp. (London) Ltd, London, W.5, Florisil (60–100 mesh) from Koch–Light Laboratories Ltd, Colnbrook, Bucks., and Nile Blue A from Matheson, Coleman & Bell, Norwood, Ohio, U.S.A.; other reagents were obtained from BDH Chemicals Ltd, Poole, Dorset.

Analytical methods. Blood samples were deproteinized by the method of Williamson, Mellanby & Krebs (1962). In some cases the supernatant from centrifugation of the deproteinized blood was split into two parts, one of which was neutralized with 2 M-KOH and used for β-hydroxybutyrate estimations, and the other was simultaneously neutralized and buffered to pH 6.8–7.0 with 3 M-K₃PO₄ (Bergmeyer & Bernt, 1965), which decreases its dilution for estimation of acetoacetate.

Frozen liver samples (0.6–2.0 g) were rapidly broken up, weighed, and homogenized with 6 ml of ice-cold 5 % (w/v) HClO₄ in a Nelco 10 Homogeniser (Measuring & Scientific Equipment Ltd, London, S.W.1) before thawing could occur. No difference in ketone-body concentrations was found when liver samples were split into two portions, one of which was deproteinized by this method and the other by using frozen HClO₄ (Berry, Williamson & Wilson, 1965) in a percussion mortar. The homogenized samples were centrifuged for 30 min at 30,000 g, and the supernatants were neutralized with 2 M-KOH, allowed to stand in ice for 30 min, centrifuged to remove KClO₄, shaken with Florisil to remove flavines (Berry et al., 1965) and centrifuged again.

Ketone bodies in blood and liver samples were determined by the method of Williamson et al. (1962). To allow for any incompletion of the assay reactions, β-hydroxybutyrate concentrations were calculated by comparison with external standards made up from 97–99 % sodium DL-β-hydroxybutyrate, which were run simultaneously. Despite treatment with Florisil, the extinctions of some incubation mixtures for determination of acetoacetate continued to decrease gradually after completion of the reaction, and were extrapolated back to zero time.

Plasma NEFA concentrations were determined by the method of Dole & Meinertz (1960) using Nile Blue A as indicator.

Distributions of the results given in Tables 1–5 were assumed to be log normal for the reasons given by Heath (1967). The means and standard errors were found for the logarithms of each set of values; comparisons between these means were made by using Student's t test. The antilogarithms of the means ± standard error were then obtained to give the standard error range.

RESULTS

After a 4 h period of bilateral hind-limb ischaemia in the rat, there is a period known as the ‘ebb’ phase (Cuthbertson, 1942) lasting for several hours during which there is a gradual drop in core temperature resulting from decreased heat production (Stoner, 1969). This period is shorter and the decrease in temperature more rapid in starved than in post-absorptive rats (Threlfall & Stoner, 1954). Our rats conformed to this pattern, and most samples were taken during the ‘ebb’ phase. Subsequently a critical period is reached when about 85 % of post-absorptive animals and all starved animals begin the downward progression towards death. The remaining 15 % of the post-absorptive rats start to show the changes associated with recovery. By injuring ‘fed’ rats and leaving them overnight, rats could be obtained in both these states.
Table 2. Concentrations of $\beta$-hydroxybutyrate and acetoacetate in blood of injured and control post-absorptive rats. For details of injury see the Materials and Methods section; rats were guillotined at times shown. Food was available until the start of the experiment. Geometric means and standard error ranges are given; number of rats are in parentheses.

<table>
<thead>
<tr>
<th>Time after removal of tourniquets (h)</th>
<th>Control rats</th>
<th>Injured rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$-Hydroxybutyrate + acetoacetate (mm)</td>
<td>$\beta$-Hydroxybutyrate/Acetoacetate ratio</td>
</tr>
<tr>
<td>10.00</td>
<td>0.134 (14) (0.120-0.151) $P&lt;0.01$</td>
<td>2.63 (14) (2.21-3.13)</td>
</tr>
<tr>
<td>14.30</td>
<td>0.354 (4) (0.340-0.367) $P&lt;0.01$</td>
<td>2.32 (4) (1.58-3.43)</td>
</tr>
<tr>
<td>15.30-16.00</td>
<td>0.317 (9) (0.255-0.395)</td>
<td>1.84 (9) (1.65-2.07)</td>
</tr>
<tr>
<td>16.30-17.00</td>
<td>0.424 (6) (0.346-0.520) $P&lt;0.01$</td>
<td>1.84 (6) (1.68-2.02)</td>
</tr>
<tr>
<td>19.00-19.30</td>
<td>0.821 (6) (0.704-0.956) $P&lt;0.01$</td>
<td>2.91 (6) (2.73-3.11)</td>
</tr>
</tbody>
</table>

Difference from controls significant at *$P<0.05$; **$P<0.02$. 
TABLE 3. Concentrations of β-hydroxybutyrate and acetoacetate in blood of injured and control starved rats. For details of injury see the Materials and Methods section; rats were guillotined at times shown. Food was withdrawn the day before the experiment. Geometric means and standard error ranges are given; number of rats are in parentheses.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Time after removal of tourniquets (h)</th>
<th>Control rats</th>
<th>Injured rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β-Hydroxybutyrate + acetoacetate (mm)</td>
<td>β-Hydroxybutyrate Acetoacetate ratio</td>
</tr>
<tr>
<td>10.00</td>
<td>0</td>
<td>2.049 (14)*</td>
<td>4.61 (14)*</td>
</tr>
<tr>
<td></td>
<td>(1.903–2.206)</td>
<td>(4.28–4.97)</td>
<td></td>
</tr>
<tr>
<td>14.00–14.30</td>
<td>0</td>
<td>1.575 (9)</td>
<td>3.91 (9)</td>
</tr>
<tr>
<td></td>
<td>(1.420–1.748)</td>
<td>(3.59–4.26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* P &lt; 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.00</td>
<td>1</td>
<td>2.275 (7)</td>
<td>4.39 (7)</td>
</tr>
<tr>
<td></td>
<td>(2.195–2.359)</td>
<td>(4.00–4.83)</td>
<td></td>
</tr>
<tr>
<td>16.00</td>
<td>2</td>
<td>2.013 (9)</td>
<td>4.27 (9)</td>
</tr>
<tr>
<td></td>
<td>(1.837–2.205)</td>
<td>(3.79–4.81)</td>
<td></td>
</tr>
</tbody>
</table>

* Difference from final control values in Table 2 significant at P < 0.02.
Difference from controls significant at: † P < 0.05; ‡ P < 0.01; § P < 0.001.
Ketone-body concentrations after trauma

Concentrations of ketone bodies in rat blood. The sums and ratios of the \(\beta\)-hydroxybutyrate and acetoacetate concentrations in blood taken from post-absorptive and starved rats at the end of, and at various periods after, a 4 h period of bilateral hind-limb ischaemia, and from control rats at the same times, are given in Tables 2 and 3.

In the control post-absorptive rats (Table 2) total ketone-body concentrations gradually rose during the day, but there were no significant differences in the \(\beta\)-hydroxybutyrate/acetoacetate ratio except for an increase between 17.00 and 19.00 hours. After overnight starvation (Table 3) both the total ketone-body concentration and the \(\beta\)-hydroxybutyrate/acetoacetate ratio were increased, and these increases were maintained for the next 6 h. These results fall within the range given in the literature (Berry et al., 1965; Söling, Kattermann, Schmidt & Kneer, 1966; Young & Renold, 1966; Hanson, Ray, Walter & Lardy, 1969; Koundakjian & Snoswell, 1970).

In injured post-absorptive rats (Table 2), the total blood ketone-body concentration was the same as in controls; the \(\beta\)-hydroxybutyrate/acetoacetate ratio tended to fall and the difference was significant 1·5 and 5 h after removal of the tourniquets. In injured starved rats (Table 3) both the total blood ketone-body concentration and the \(\beta\)-hydroxybutyrate/acetoacetate ratio fell and were significantly lower than in the controls at 1 and 2 h after tourniquet removal.

Distribution of ketone bodies within blood. To investigate the effect of injury on the distribution of ketone bodies between red blood cells and plasma, pooled blood samples from injured post-absorptive rats 1·5 h after removal of the tourniquets, and from the corresponding controls, were centrifuged and a sample of plasma was removed. The plasma and the red cells were deproteinized and ketone bodies determined by the method used for whole blood. Concentrations in the red cells were corrected for the small amount of plasma that had not been removed, the volume of which was calculated from the haematocrit measured in triplicate, and for trapped plasma, the volume of which was calculated as described by Chaplin & Mollison (1952). Water was taken to constitute 65% of red cells (Laris, 1958) and 92·7% of plasma (Hatai, 1918). The distribution of \(\beta\)-hydroxybutyrate between plasma water and red cell water was similar to that of acetoacetate and the two compounds were considered together. The ratio of the total ketone-body concentration in plasma water to that in red cell water was 1·87±0·24 (SEM; six samples) in injured rats and this did not differ significantly from the control value of 2·23±0·20 (SEM; six samples).

After removal of the tourniquets there was loss of fluid into the hind limbs which decreased the circulating plasma volume and led to a rise in the haematocrit value. The haematocrit value in these injured rats 1·5 h after removal of the tourniquets was 59·1±0·9 (SEM; six samples), significantly \(P<0·001\) greater than the control value of 45·7±0·7 (SEM; six samples). This rise resulted in a decrease in the proportion of total ketone bodies in the plasma of great vessel blood from 0·798±0·013 (SEM; six samples) in controls to 0·655±0·033 (SEM; six samples) in the injured rats \(P<0·02\).

Concentrations of ketone bodies in rat liver after injury. The effect of a 4 h period of bilateral hind-limb ischaemia on the sums and ratios of concentrations of \(\beta\)-hydroxybutyrate and acetoacetate in the liver of post-absorptive and starved rats is shown in Tables 4 and 5.

Total ketone-body concentrations in the liver of control fed and post-absorptive rats (Tables 1 and 4) gradually rose during the day, with a large rise to maximal values during overnight starvation (Table 5). The \(\beta\)-hydroxybutyrate/acetoacetate ratio also tended to rise
Table 4. Concentrations of β-hydroxybutyrate and acetoacetate in liver of injured and control post-absorptive rats. For details of injury see the Materials and Methods section; livers were removed under ether anaesthesia at times shown. Food was available until the start of the experiment. Geometric means and standard error ranges are given; number of rats are in parentheses.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Time after removal of tourniquets (h)</th>
<th>Control rats</th>
<th>Injured rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β-Hydroxybutyrate + acetoacetate (µmol/g wet wt)</td>
<td>β-Hydroxybutyrate Acetoacetate ratio</td>
</tr>
<tr>
<td>14.00</td>
<td>0</td>
<td>0.212 (12)</td>
<td>1.05 (12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.188–0.238)</td>
<td>(0.88–1.25)</td>
</tr>
<tr>
<td>15.30</td>
<td>1.5</td>
<td>0.290 (14)</td>
<td>1.20 (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.264–0.318)</td>
<td>(1.11–1.31)</td>
</tr>
<tr>
<td>17.00</td>
<td>3.0</td>
<td>0.309 (11)</td>
<td>1.16 (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.285–0.333)</td>
<td>(1.03–1.28)</td>
</tr>
<tr>
<td>19.00</td>
<td>5.0</td>
<td>0.519 (8)</td>
<td>1.40 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.456–0.592)</td>
<td>(1.15–1.69)</td>
</tr>
</tbody>
</table>

Difference from controls significant at: *P < 0.05; †P < 0.01; ‡P < 0.001.
Table 5. Concentrations of β-hydroxybutyrate and acetoacetate in liver of injured and control starved rats. For details of injury see the Materials and Methods section; livers were removed under ether anaesthesia at times shown. Food was withdrawn the day before the experiment. Geometric means and standard error ranges are given; number of rats are in parentheses.

<table>
<thead>
<tr>
<th>Time after removal of tumour</th>
<th>Control rats</th>
<th>Injured rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>(hours)</td>
<td>β-Hydroxybutyrate (μmol/g wet wt)</td>
<td>Acetoacetate</td>
</tr>
<tr>
<td>11.00</td>
<td>1.72 (12)*</td>
<td>1.59 (12)</td>
</tr>
<tr>
<td>14.00</td>
<td>1.42 (1.93)</td>
<td>1.41 (1.93)</td>
</tr>
<tr>
<td>15.30</td>
<td>1.39 (1.57)</td>
<td>1.21 (1.67)</td>
</tr>
<tr>
<td>16.00-16.30</td>
<td>1.37 (1.53)</td>
<td>2.11 (1.53)</td>
</tr>
</tbody>
</table>

Differences between injured and controls were not significant.

* Difference from control value in Table 4 significant at P<0.001.
during starvation, the values at 15.30 and 16.30 hours in starved rats (Table 5) being significantly higher than the corresponding values in fed rats (Table 4). These results fall within the range of those found in the liver of control rats starved for up to 30 h by Berry et al. (1965), Williamson, Herczeg, Coles & Danish (1966), Nordmann, Arnaud, Johnson & Nordmann (1969), Lagunas, McLean & Greenbaum (1970) and Lindros (1970).

After injury in post-absorptive rats (Table 4), the total ketone-body concentration of liver rose, the maximum difference from the controls being at 3 h after removal of the tourniquets. This rise cannot be explained by changes in liver weight or water content (Threlfall, 1970b).

**Fig. 1.** Relationship between liver ketone bodies and colon temperature in rats surviving overnight after injury; (a) total ketone-body concentration and (b) β-hydroxybutyrate/acetoacetate ratio. For details of the injury see the Materials and Methods section; food was withdrawn at 10.00 hours on the day of injury, and livers were removed from surviving rats under ether anaesthesia at 10.30–11.00 hours on the following day.
The $\beta$-hydroxybutyrate/acetoacetate ratio was only significantly different from the controls 1.5 h after tourniquet removal, when it was higher.

In further experiments, rats were injured in the ‘fed’ state and then left without food until the following morning, when colon temperature and liver ketone-body concentrations were measured in the few rats which survived. The total ketone-body concentration and $\beta$-hydroxybutyrate/acetoacetate ratio were both negatively correlated with temperature (Fig. 1). At this late stage it was possible to select two groups of rats, those with a colon temperature below 30° which would die (Stoner, 1958) and those with a colon temperature above 35° which would recover. In recovering animals the total ketone-body concentrations and $\beta$-hydroxybutyrate/acetoacetate ratios were lower than in control rats starved for 24 h (Table 5), whereas in those that were going to die, the total concentrations were as high as the control values and the $\beta$-hydroxybutyrate/acetoacetate ratios very much higher.

In rats injured in the starved state, there was no difference from the controls at any of the times investigated in either the total ketone-body concentration or the $\beta$-hydroxybutyrate/acetoacetate ratio (Table 5).

**Relationship between liver ketone-body and plasma NEFA concentrations.** In another series of experiments, the concentrations of liver ketone bodies and plasma NEFA were determined

![Graph](image.png)

**Fig. 2.** Relationship between liver total ketone-body and plasma NEFA concentrations in control post-absorptive and starved rats. Samples were taken at 10.00-17.30 hours from post-absorptive rats and 10.00-12.00 hours from starved rats; for details of the procedure used see the Materials and Methods section.

in simultaneous samples (see the Materials and Methods section) from control post-absorptive and starved rats and injured post-absorptive rats. There was a positive correlation between the logarithm of the liver total ketone-body concentration and the plasma NEFA concentration in the control post-absorptive and starved rats taken together (Fig. 2). In Fig. 3 the same control regression line is given but with the points for the injured post-absorptive rats super-
imposed. At the end of the injury period there was a correlation between ketone bodies and fatty acids with a regression line parallel to that in controls, but 1·5 and 3·0 h after removal of the tourniquets there was no correlation and with one exception all the points lay above the regression line for the controls so that the liver total ketone-body concentrations were higher than would be predicted from the corresponding plasma NEFA.

![Graph showing correlation between liver total ketone-body and plasma NEFA concentrations](image)

**Fig. 3.** Relationship between liver total ketone-body and plasma NEFA concentrations in injured rats. For details of the injury and of the procedure used for obtaining the samples, see the Materials and Methods section; food was withdrawn at the start of the experiment. The regression line for controls (---) is taken from Fig. 2. Regression line for injured rats at end of ischaemia. Time after tourniquet removal: ▲, 0 h; ○, 1·5 h; ●, 3 h.

The NEFA concentrations in these experiments were lower than those obtained by Stoner (1962), particularly in injured rats at the end of the ischaemic period. A direct comparison was therefore made between plasma NEFA concentrations in blood samples taken by the procedure described in this paper and by decapitation of unanaesthetized rats, as in the earlier experiments. Injured rats at the end of a 4 h period of bilateral hind-limb ischaemia were used. In the animals which were decapitated, care was taken to minimize excitement immediately beforehand, and blood was collected for the shortest period of time necessary. In the laparotomized rats, the mean plasma NEFA concentration was 0·428 mEq/l (eleven samples; standard error range 0·390–0·470); in the guillotined rats it was 0·605 mEq/l (twelve samples; standard error range 0·566–0·648), approximating to that found by Stoner (1962). The difference between these values was significant at $P<0·02$.

**DISCUSSION**

*Redox state of tissues after injury.* It would be interesting to have an estimate of the mitochondrial NADH/NAD$^+$ ratio in the extra-hepatic tissues after injury, but this is probably not given by the $\beta$-hydroxybutyrate/acetoacetate ratio in the blood (Barton, 1970). It is difficult
to draw conclusions from the fall in this ratio after injury (Tables 2 and 3) until more is known about some of the factors influencing it.

However, these strictures do not apply to the liver, in which the mitochondrial NADH/NAD$^+$ ratio is thought to be reflected in the β-hydroxybutyrate/acetoacetate ratio. In the experiments reported here there was no difference in this ratio from the controls except for an increase 1.5 h after removal of the tourniquets in post-absorptive rats. The reason for this change is not known, but it was not sustained and at later times, 3 and 5 h after tourniquet removal, when any impairment of the circulation should have become more apparent, the hepatic β-hydroxybutyrate/acetoacetate ratio had returned to control values. There was also no difference from control values in injured starved rats, whose condition, as indicated by the fall in core temperature and by the survival time (Threlfall & Stoner, 1954), deteriorates much more quickly than that of injured post-absorptive rats.

There is therefore no evidence of any serious impairment of the hepatic circulation after removal of the limb tourniquets for at least 5 h in the post-absorptive rat and 2 h in the starved rat. This agrees not only with physiological data but also with the NADH/NAD$^+$ ratio in the cytosol as indicated by the lactate/pyruvate ratio (Threlfall, 1970a).

This interpretation of the hepatic β-hydroxybutyrate/acetoacetate ratio is supported by the results for moribund rats, which showed an increase (Fig. 1), with a similar increase in the lactate/pyruvate ratio (Threlfall, 1970a). It is possible that hepatic hypoxia in these animals may have been exaggerated by the ether anaesthesia, which tended to depress their already weak respiration.

The reason for the low hepatic β-hydroxybutyrate/acetoacetate ratio in recovering rats (Fig. 1) is not known, but it should be emphasized that these animals are entering a different phase after the injury, the 'flow' phase (Cuthbertson, 1942).

Liver ketone-body metabolism after injury. Since the fraction of the cardiac output through the liver is increased during the period shortly after removal of the tourniquets (Takács, Kállay & Skolnik, 1962; Ashby et al., 1965), it is unlikely that the increased concentration of total ketone bodies in the liver of post-absorptive rats from 1.5 to 5 h after injury (Table 4) is due to a decreased rate of removal. This may not be the case in moribund rats, whose high hepatic concentration of total ketone bodies (Fig. 1) is probably due to a deficient circulation since the liver is then definitely hypoxic.

In the present experiments where there was a direct comparison between plasma NEFA and liver ketone bodies, the relationship in the controls was consistent with the hypothesis that under 'physiological' conditions mobilization of NEFA, leading to an increase in their oxidation by the liver, is mainly responsible for ketogenesis (Krebs, 1966). Previous correlation studies between ketone bodies and NEFA in non-ruminants have been confined to the blood in human subjects (Werk & Knowles, 1961; Hanson, Johnson & Zaharko, 1965; Hagenfeldt, 1968; Jenkins, Welborn & Goff, 1970).

After injury fat mobilization occurs (Stoner & Matthews, 1967) but owing to variable circulation through the fat depots this is reflected to a variable extent in the plasma (Stoner & Matthews, 1966; Kováč, Rosell, Sándor, Koltay, Kováč & Tomka, 1970). The relationship between plasma NEFA and liver ketone bodies seems to have been disturbed by the injury in a manner which shows a clear distinction between the periods before and after removal of the tourniquets. Although the cause of the disturbance while the tourniquets were in place is not known, the regression line was parallel to that for the controls, showing that liver ketone
bodies were still responsive to NEFA. The uptake of NEFA by the liver is proportional to
the concentration in the plasma (Fine & Williams, 1960), and the increase in ketone-body
concentrations over those expected from the plasma NEFA after tourniquet removal suggests
an increase in the proportion of NEFA taken up that is converted into ketone bodies. This
would be expected from the decrease in oxidation of fatty acids to carbon dioxide (Heath &
Stoner, 1968).

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