Control of tissue carnitine contents: Effects of partial hepatectomy and liver regeneration on carnitine concentrations in liver and extrahepatic tissues of the rat

Timothy J. FRENCH1, Anthony W. GOODE1, Paul S. SCHOFIELD2 and Mary C. SUGDEN2*

1The Surgical Unit, The London Hospital, Whitechapel, London E1 1BB, U.K.; and 2Department of Chemical Pathology, The London Hospital Medical College, Turner Street, London E1 2AD, U.K.

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The liver is the sole site of carnitine biosynthesis in the rat. However, the first 24 h after the surgical removal of two-thirds of the liver mass are not associated with depletion of carnitine either in the liver remnant or in a number of extrahepatic tissues with relatively short turnover times of carnitine (<24 h; heart, spleen, kidney). Dietary carnitine was not supplied. The results suggest that the capacity of the remnant liver for carnitine biosynthesis is sufficient to maintain tissue carnitine contents. Liver regeneration influenced the relative proportions of hepatic free and acylated carnitines in a manner compatible with changes in fat disposition in the proliferating tissue.

L-Carnitine is essential for the transfer of activated long-chain fatty acyl groups across the mitochondrial membrane. In addition to this key function, carnitine is involved in the oxidation of branched-chain oxo-acids, the shuttling of acyl moieties chain-shortened by β-oxidation out of the peroxisomes and the modulation of tissue acyl-Coenzyme A:Coenzyme A ratios. The metabolic roles of carnitine have been reviewed recently (Bremer, 1983).

Carnitine is present in dietary components and can also be synthesized de novo. The occurrence of carnitine deficiency syndromes (reviewed by Rebouche & Engel, 1983) indicates that the provision of carnitine by ingestion and/or biosynthesis is often inadequate to satisfy the body's requirement. De novo biosynthesis is obviously of primary importance in conditions associated with restricted food intake, but little is known of the regulation of the biosynthetic pathway, although the sequence has been elucidated (see Bremer, 1983). Carnitine biosynthesis is initiated by post-translational methylation of lysine residues in proteins such as actin, myosin and histones. Trimethyllysine is liberated via protein degradation and is subsequently

*To whom correspondence should be addressed.
converted to butyrobetaine. The final reaction in the biosynthetic pathway is the hydroxylation of butyrobetaine to carnitine, which in the rat occurs only in liver. The newly synthesized carnitine is then released from the liver into the blood and is actively taken up by the other tissues. The inability of extrahepatic tissues to synthesize carnitine from butyrobetaine in situ means that, in the absence of adequate dietary provision of carnitine, they are dependent on hepatic biosynthesis for their carnitine supply. Thus, if liver function is impaired, the provision of carnitine to these tissues might be compromised. Although in man carnitine is synthesized in both kidney and liver, the liver is the major biosynthetic site and the possible deleterious effects of liver damage on extrahepatic carnitine metabolism has not been fully appreciated. A clinical study has indicated decreased post-mortem concentrations of carnitine in liver, muscle, heart, kidney and brain from cachectic, cirrhotic patients (Rudman et al., 1977).

Mature rats survive subtotal hepatectomy (up to 80%) and the remaining tissue regenerates to approximately the original size. Increased DNA synthesis is observed 14 h post-operatively and the first mitosis occurs at about 24 h post-operatively. Cell division continues until the initial cell mass is restored (about 4 days in 200 g rats) (see Gove & Hems, 1978). Because in the rat the liver is the sole site of carnitine biosynthesis the removal of part of the liver is a means by which to investigate the capacity of the remaining liver for carnitine biosynthesis, and specifically to determine whether carnitine biosynthesis in the remaining portion of the liver is adequate to maintain extrahepatic tissue carnitine concentrations. The turnover time of most of the carnitine in liver, heart, kidney and spleen (Brooks & McIntosh, 1975) is such that changes in carnitine content might be expected to be observed during the first 48 h after liver resection if biosynthesis in the regenerating liver were inadequate.

The present study therefore examines effects of partial hepatectomy and liver regeneration on tissue carnitine metabolism in the rat. Because of difficulty in the interpretation of results obtained with fed rats (with variable dietary intakes of carnitine), the experiments were conducted only with rats starved post-operatively. The period of liver regeneration under study in the present work (the first 2 days after resection) is the period of most rapid liver regrowth.

**Materials and Methods**

**Materials**

Sources of materials were as described in Sugden et al. (1982). The NEFA C-test kit for estimation of plasma non-esterified fatty acids was from Alpha Laboratories, Eastleigh, Hants, U.K.

**Animals**

Female albino Wistar rats (180-220 g) were subjected to a 12 h light/12 h dark cycle (light period started at 0830 h). Rats were fed ad libitum prior to partial hepatectomy or sham-operation and starved post-operatively (grid-bottomed cages). Water was supplied ad libitum.
Partial hepatectomy (removal of both major sublobes of the left lobe, comprising removal of two-thirds of the liver) or sham-operation was performed under ether anaesthesia by the technique of Higgins and Anderson (1931). Rats were starved thereafter and sampled at 1 or 2 days after the operation. Tissues were removed whilst the rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt.). This procedure minimizes changes in tissue carnitine profiles due to ischaemia (see Pearson & Tubbs, 1967).

Tissues were freeze-clamped in liquid N₂. The frozen tissue was ground to a powder at -40°C and processed as described by Brass and Hoppel (1978). In some cases aortic blood samples were obtained and assayed for non-esterified fatty acids (plasma).

Metabolite assays

Tissues were assayed for free and acylated carnitine in HCIO₄-extracts as described by Brass and Hoppel (1978). Free (non-esterified) carnitine was taken to be the carnitine found when HCIO₄-extracts were examined without prior exposure to alkali. HCIO₄-insoluble carnitine content was assumed to be long-chain esters (more than 10 carbon atoms) and the HCIO₄-soluble fraction was assumed to contain short-chain derivatives and free carnitine (see Brass & Hoppel, 1978). Non-esterified fatty acid concentrations were determined on a KEM-O-MAT autoanalyser using a WAKO NEFA kit.

Expression of results

Statistical significance of differences was assessed by Student's unpaired t-test. Results are given as means ± S.E.M. for the numbers of observations given in parentheses.

Results and Discussion

Effects of starvation and partial hepatectomy on liver and body weights

The initial body weights of the rats that were to be subjected to partial hepatectomy or sham operation differed by less than 7% (Table 1). Partial hepatectomy did not accelerate the body weight loss observed in response to either 24 h or 48 h starvation subsequent to surgery.

Partial hepatectomy involved the removal of 55-60% of the liver (Table 1). Liver weight was decreased after 24 h starvation in both sham-operated and partially hepatectomized rats, the decreases accounting for 26% and 19% of the decreases in body weight respectively (Table 1). A decrease in liver weight is a well-documented response to starvation (see e.g. Brass & Hoppel, 1978). The present finding of a decrease in weight of the resected liver is notable since it indicates that the liver remnant after partial hepatectomy may be sensitive to a change in nutritional status. Extending the period of starvation from 24 h to 48 h did not further decrease liver weights of sham-operated rats. Consequently the contribution of the change in liver weight to total body weight loss was decreased (to
Table I. Effects of starvation and partial hepatectomy on liver and body weights

For details see the Materials and Methods section. Rats were subjected to partial hepatectomy or sham operation and starved thereafter for either 24 h or 48 h. Initial body weight refers to the weight of the rats immediately after surgery and consequently does not include the weight of the liver removed in partially hepatectomized rats (4.34 ± 0.16 g in rats to be starved for 24 h (2.38 ± 0.06 g/100 g rat) and 5.0 ± 0.22 g in rats to be starved for 48 h (2.51 ± 0.09 g/100 g rat).

<table>
<thead>
<tr>
<th>Period of starvation</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operation</td>
<td>Partial hepatectomy</td>
</tr>
<tr>
<td>Initial body wt. (g)</td>
<td>195.4 ± 5.6 (4)</td>
<td>182.7 ± 3.6 (7)</td>
</tr>
<tr>
<td>Final body wt. (g)</td>
<td>185.0 ± 6.2 (4)</td>
<td>176.4 ± 4.1 (7)</td>
</tr>
<tr>
<td>Change in body wt. (% initial wt.)</td>
<td>-5.3 ± 0.5 (4)</td>
<td>-3.3 ± 0.9 (7)</td>
</tr>
<tr>
<td>Initial liver wt. (g/100 g rat)</td>
<td>4.28 ± 0.14 (6)</td>
<td>1.90 ± 0.06 (7)</td>
</tr>
<tr>
<td>Final liver wt. (g/100 g rat)</td>
<td>2.92 ± 0.11 (4)</td>
<td>1.28 ± 0.05 (7)</td>
</tr>
<tr>
<td>Change in liver wt. (% initial wt.)</td>
<td>-31.8 ± 2.6 (4)</td>
<td>-32.6 ± 2.6 (7)</td>
</tr>
</tbody>
</table>

Statistical significances of differences between initial and final liver and body wts. are shown by: ***P < 0.001; ** P < 0.01; * P < 0.05.
Statistical significances of differences between rats starved for 24 h or 48 h are indicated by: P < 0.001.

The liver gained weight between 24 h and 48 h after partial hepatectomy even though food was not available. This indicates that the precursors and energy substrates required for liver growth were provided from endogenous stores.

Effects of starvation and partial hepatectomy on extrahepatic tissue weights

Results are shown in Table 2. Partial hepatectomy did not influence the weights of tissues other than the liver in rats sampled 24 h after surgery, although in rats sampled 48 h after surgery, spleen weight was slightly increased. Spleen enlargement is often observed in conjunction with neoplastic growth, portal hypertension and hepatic cirrhosis (see Harding Rains & Ritchie, 1984). Starvation from 24 h to 48 h post-operatively decreased heart weights in sham-operated rats but not in partially hepatectomized rats. Preedy et al. (1984) have demonstrated a loss of cardiac mass on prolonged starvation of unoperated rats, associated with decreased rates of protein synthesis. It is implied that the physiological changes associated with partial hepatectomy and liver regeneration minimize starvation-induced decreases in heart weights.

Effects of starvation and partial hepatectomy on tissue carnitine contents

As observed by others (Camargo et al., 1966; Glende & Winfield, 1970; Delahunty & Rubenstein, 1970; Fex, 1970; Mangiapane et al., 1973; Gove & Hems, 1977), partial hepatectomy was associated with changes in liver composition, protein content (expressed as a % wet weight) being decreased (results not shown) and triglyceride content being increased (see below). For this reason the results shown in Table 3 are expressed on a whole tissue basis.
Table 2. Tissue weights (g/100 g body wt.) in 24 h- or 48 h-starved partially hepatectomized or sham-operated rats
For details see the Materials and Methods section. Rats were subjected to partial hepatectomy or sham operation and subsequently starved prior to sampling at 24 h or 48 h after surgery.

<table>
<thead>
<tr>
<th>Time after surgery</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operation</td>
<td>Partial hepatectomy</td>
</tr>
<tr>
<td>Liver</td>
<td>2.92 ± 0.11 (4)</td>
<td>1.28 ± 0.05 (7)</td>
</tr>
<tr>
<td>Heart</td>
<td>0.38 ± 0.01 (4)</td>
<td>0.35 ± 0.02 (7)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.05 (4)</td>
<td>0.67 ± 0.02 (7)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.27 ± 0.04 (4)</td>
<td>0.21 ± 0.02 (7)</td>
</tr>
</tbody>
</table>

Statistically significant effects of partial hepatectomy are indicated by: ***P < 0.001; **P < 0.01.
Statistical significances of differences between rats starved for 24 h or 48 h are shown by: †††P < 0.001; ††P < 0.01; †P < 0.05.

As expected from the decrease in tissue mass (Table 2), the total (free and acylated) carnitine content of the liver was significantly decreased, compared with that of the sham-operated rat, in the partially hepatectomized 24 h- or 48 h-starved rat (Table 3). The 33% increase in hepatic carnitine content of sham-operated rats occasioned by extending the period of starvation from 24 h to 48 h was due to an increase in liver carnitine concentrations, liver weight being unchanged (Table 2). An analogous increase in carnitine concentrations, expressed on a g wet wt. basis, was observed in partially hepatectomized rats (an increase of 31%, P < 0.001). Carnitine concentrations are also increased in response to starvation in livers of unoperated rats (Brass & Hoppel, 1978).

Table 3 also shows the distribution of total carnitine between free and acylated carnitines in livers of starved rats at 24 h or 48 h after partial hepatectomy or sham operation. In 24 h-starved sham-operated rats, most of the carnitine was present as free carnitine and short-chain acylcarnitine (41% and 52% respectively). In contrast only 22% of total hepatic carnitine was present as free carnitine in 24 h-starved partially hepatectomized rats, 65% of total carnitine being short-chain acylcarnitine and 15% being long-chain acylcarnitine. The plasma non-esterified fatty acids (NEFA) concentrations in the partially hepatectomized and sham-operated rats at 24 h after surgery were 0.67 ± 0.15 (6) mEq/l and 0.63 ± 0.08 (6) mEq/l respectively. This indicates that the change in the relative proportions of free and acylated carnitines in the regenerating liver is a consequence not of a change in availability of NEFA in the blood, but of a perturbation of hepatic fat metabolism. The increased percentage of acylated carnitine in regenerating livers (78% of total carnitine vs. 59% in sham-operated rats) occurred concomitantly with an increase in liver lipid content (142 ± 7 (6) mg/g compared with 36 ± 3 (6) mg/g in
Table 3. Effects of partial hepatectomy on tissue free and acylated carnitine in 24 h- or 48 h-starved rats
For details see the Materials and Methods section and Table 2.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Conditions</th>
<th>No. of rats</th>
<th>Total tissue carnitine</th>
<th>Free carnitine</th>
<th>Short-chain acylcarnitine</th>
<th>Long-chain acylcarnitine</th>
<th>F/(S+L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>24h-starved: SO 4</td>
<td>1329.9 ± 100.9</td>
<td>540.0 ± 59.0</td>
<td>688.9 ± 87.0</td>
<td>101.1 ± 11.2</td>
<td>0.72 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 7</td>
<td>530.7 ± 69.5***</td>
<td>120.6 ± 20.1***</td>
<td>337.4 ± 47.9*</td>
<td>72.7 ± 9.2</td>
<td>0.29 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h-starved: SO 4</td>
<td>1768.2 ± 103.3†</td>
<td>636.3 ± 12.5</td>
<td>922.3 ± 46.1</td>
<td>209.4 ± 49.9</td>
<td>0.57 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 6</td>
<td>1278.6 ± 108.2+++**</td>
<td>519.0 ± 68.1+++††</td>
<td>586.1 ± 71.4+++***</td>
<td>125.5 ± 14.7††</td>
<td>0.77 ± 0.13+++††</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>24h-starved: SO 3</td>
<td>319.9 ± 44.1</td>
<td>154.7 ± 36.4</td>
<td>136.4 ± 21.3</td>
<td>28.7 ± 1.8</td>
<td>0.96 ± 0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 6</td>
<td>294.1 ± 28.6</td>
<td>121.4 ± 12.0</td>
<td>122.4 ± 18.0</td>
<td>38.1 ± 6.0</td>
<td>0.79 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h-starved: SO 4</td>
<td>352.0 ± 12.1</td>
<td>172.7 ± 8.6</td>
<td>154.4 ± 14.4</td>
<td>24.9 ± 2.7</td>
<td>0.99 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 7</td>
<td>310.7 ± 12.0*</td>
<td>172.7 ± 17.8†</td>
<td>105.5 ± 8.8*</td>
<td>32.6 ± 2.3</td>
<td>1.36 ± 0.14†</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>24h-starved: SO 4</td>
<td>220.6 ± 19.9</td>
<td>100.7 ± 14.0</td>
<td>109.7 ± 13.0</td>
<td>10.2 ± 2.1</td>
<td>0.88 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 6</td>
<td>303.1 ± 25.9*</td>
<td>120.6 ± 12.9</td>
<td>169.2 ± 17.8*</td>
<td>22.9 ± 2.7*</td>
<td>0.64 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h-starved: SO 3</td>
<td>351.9 ± 25.8</td>
<td>137.9 ± 19.2</td>
<td>194.6 ± 11.3+++</td>
<td>19.5 ± 4.2</td>
<td>0.65 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 7</td>
<td>360.2 ± 21.9</td>
<td>169.9 ± 9.8††</td>
<td>165.5 ± 17.6</td>
<td>24.8 ± 1.1</td>
<td>0.95 ± 0.11†</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>24h-starved: SO 4</td>
<td>50.3 ± 10.3</td>
<td>32.0 ± 5.2</td>
<td>15.9 ± 4.7</td>
<td>2.3 ± 0.9</td>
<td>1.76 ± 0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 6</td>
<td>47.0 ± 4.7</td>
<td>24.0 ± 3.2</td>
<td>22.7 ± 1.7</td>
<td>3.8 ± 0.3</td>
<td>0.93 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h-starved: SO 3</td>
<td>71.0 ± 5.7</td>
<td>38.4 ± 3.6</td>
<td>29.1 ± 4.3</td>
<td>3.0 ± 0.7</td>
<td>1.21 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PH 7</td>
<td>94.3 ± 7.1</td>
<td>50.6 ± 3.2+++**</td>
<td>38.2 ± 3.8+++</td>
<td>5.8 ± 0.8*</td>
<td>1.18 ± 0.08+++*</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant effects of partial hepatectomy (PH) compared with sham-operation (SO) are shown by:
***p < 0.001; **p < 0.01; *p < 0.05. Statistically significant differences between rats starved for 24 h or 48 h are shown by: +++ P < 0.001; ++ P < 0.01; † P < 0.05.
sham-operated rats, P < 0.001) suggesting that acylcarnitine accumulates in liver when the supply of NEFA exceeds the hepatic capacity for its metabolism.

At 48 h after surgery, by which time the weight and total carnitine content of the regenerating liver was 70-80% that of the control, disturbances in hepatic carnitine metabolism were less apparent. However it is of particular interest that at 48 h after partial hepatectomy, despite a decrease in total carnitine content, the amount of free carnitine in the regenerating liver was substantially increased (Table 3). The finding of increased amounts of free carnitine in the regenerating liver in the period from 24 h to 48 h after partial hepatectomy indicates either that the rate of acylcarnitine utilization exceeds that of its formation or that there is some limitation on the formation of acylcarnitine. Others have suggested that fatty acids are important as a fuel for the regenerating liver (Nakatani et al., 1982), fat oxidation enhancing hepatic regeneration by increasing energy charge (Nakatani et al., 1981a,b). Fat oxidation is also required for the DNA synthesis associated with cell division (Nakatani et al., 1982) and it is notable that the increase in free carnitine occurred during the period of liver weight gain (24 h-48 h).

However, plasma NEFA concentrations and liver lipid contents remained high in 48 h-starved partially hepatectomized rats (0.99 ± 0.13 (6) mEq/l and 162 ± 14 (6) mg/g respectively) indicating that fat oxidation and acylcarnitine formation in the regenerating liver may be restricted by factors other than fat availability. Extension of the period of starvation from 24 h to 48 h did not significantly affect the liver contents of free and acylated carnitines in sham-operated rats, probably because plasma NEFA concentrations were not further increased (concentrations of 0.71 ± 0.10 (6) mEq/l in 48 h-starved sham-operated rats).

As observed with liver, the free carnitine contents of hearts, kidneys and spleens of partially hepatectomized rats were increased from 24 h to 48 h after partial hepatectomy but the increases were less marked than those observed in liver (Table 3). There were also changes in the long and short-chain acylcarnitine contents (Table 3) reflected in the values for total tissue carnitine contents (Table 3). The interpretation of the changes in total carnitine and relative amounts of free and acylated carnitines between 24 h and 48 h after partial hepatectomy is complicated because effects of starvation per se may occur concomitantly with effects of liver resection and regeneration. For example, starvation from 24 h to 48 h after surgery increased renal short-chain acylcarnitine contents in sham-operated rats (Table 3). It should however be noted that extending the period of starvation from 24 h to 48 h did not significantly increase plasma NEFA concentration in sham-operated rats (see above) and did not generally cause marked changes in carnitine profiles in extrahepatic tissues (see Table 3). Despite these reservations, the present results unambiguously demonstrate that only in heart after 48 h-starvation is partial hepatectomy associated with decreased total carnitine content, and indeed under some circumstances, increases in total tissue carnitine are observed in kidneys and spleens of partially hepatectomized rats. The biochemical basis for the decrease in heart total carnitine content observed at 48 h after partial hepatectomy is not
known but is related to a decrease in cardiac short-chain acylcarnitine content (Table 3).

Although most of the carnitine in the body occurs in skeletal muscle (Brass & Hoppel, 1978), its turnover time in this tissue (105 h, Brooks & McIntosh, 1975) is such that marked depletion of carnitine would not be expected as a consequence of partial hepatectomy, most of the liver mass being restored during this period. In confirmation of this, concentrations of carnitine in gastrocnemius muscles of sham-operated or partially hepatectomized rats were found in the present study to be $736.4 \pm 96.5$ (3) and $761.3 \pm 64.4$ (5) nmol/g wet wt. respectively at 24 h after surgery and $918.8 \pm 106.4$ (3) and $808.6 \pm 50.3$ (6) nmol/g wet wt. respectively at 48 h after surgery.

Conclusions

Liver resection is not associated with marked depletion of carnitine in heart, spleen or kidney in the first day after partial hepatectomy in the rat. The turnover time of carnitine in these tissues is less than 24 h (Brooks & McIntosh, 1975). It is therefore suggested that the capacity of the remaining liver for carnitine biosynthesis is sufficient to accommodate the requirements of these extrahepatic tissues. Either the control of total body carnitine content is exerted through changes in rates of excretion or degradation rather than synthesis (see Brass & Hoppel, 1978) or hepatic biosynthesis is stringently regulated. If control is exerted at the level of biosynthesis, it is implied that the hepatic supply of butyrobetaine is adequate, even (as in the present study) when the rats are starved. A further finding is that liver cell proliferation is associated with disturbances in hepatic carnitine profiles which are suggestive of changes in intrahepatic fat disposition.

Acknowledgements

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