RELATIVE AND COMBINED EFFECTS OF PROPYLTHIOURACIL, ETHANOL AND PROTEIN DEFICIENCY ON LIVER HISTOLOGY AND HEPATIC IRON, ZINC, MANGANESE AND COPPER CONTENTS


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Abstract — The present study was performed in order to discern the effects of propylthiouracil (PTU) on ethanol and/or protein deficiency-mediated liver histological changes and liver Fe, Zn, Cu and Mn alterations in male adult Wistar rats. The study was performed on 64 animals divided into eight groups, fed with the Lieber-DeCarli control, 36% ethanol-2% protein- and 36% ethanol-2% protein-containing diets, without and with PTU, respectively. PTU was administered at a concentration of 0.05%, an amount which rendered the animals hypothyroid. Two further groups of 5 animals each, with and without PTU respectively, were allowed to consume the control diet ad libitum. Animals treated with PTU showed significantly less fibrosis, but more fat, than animals without PTU. Liver fibrosis was inversely correlated with liver zinc, liver content of this element being higher in the PTU-treated and the ethanol or protein deficiency groups. PTU also reversed ethanol-mediated hepatocyte ballooning and also led to a reduction in nuclear areas.

INTRODUCTION

Ethanol is metabolized mainly in the liver (Lieber, 1994). Early pathological manifestations of alcohol abuse include liver steatosis and hepatocyte ballooning, especially striking in the perivenular cells (Miyakawa et al., 1985), with later deposition of fibrous tissue around the central venule (Nakano et al., 1982). It has been shown that ethanol increases oxygen consumption (Israel et al., 1975) and causes a consequent increase in oxygen tension gradient between periportal and perivenular hepatocytes (Tsukamoto et al., 1990). In addition, the low oxygen tension in the perivenular area exaggerates the redox shift produced by alcohol dehydrogenase-dependent ethanol metabolism (Jaunhonen et al., 1982). Based mainly on this fact and on the ability of propylthiouracil (PTU) to reduce oxygen consumption, this latter drug has been used in the treatment of alcoholic hepatitis (Orrego et al., 1979). However, PTU may also influence other mechanisms involved in ethanol-mediated liver injury. The following is a summary of these mechanisms. Chronic ethanol ingestion and the frequently associated protein-calorie malnutrition result in liver iron overload (Chapman et al., 1982) and iron excess may cause hepatocyte necrosis (Brissot et al., 1983, 1987) enhancing lipid peroxidation (Bacon et al., 1983; Minotti and Aust, 1987) and altering lysosomal membranes (Chapman et al., 1982). Moreover, iron excess increases prolyl hydroxylase activity (Brissot et al., 1987) and activates transcription of genes involved in collagen synthesis (Pietrangelo et al., 1990), increasing hepatic collagen content (Brissot et al., 1983; Chojkier et al., 1989). Scavenging of free radicals is also impaired in alcoholics, partly due to acetaldehyde-mediated liver glutathione (GSH) depletion (Shaw et al., 1983). Interestingly, hypothyroidism decreases iron absorption (Ingbar and Woeger, 1975) and PTU increases hepatic GSH activity (Linscheer et al., 1980). Other trace elements, such as zinc, copper and manganese, whose liver content may become...
affected in chronic alcoholism (Solomons, 1980; Sternlieb, 1980; Scheinberg and Sternlieb, 1994) and/or in the frequently accompanying protein-calorie malnutrition (Mendenhall et al., 1984), are also involved in the enzymatic pathways of collagen synthesis and/or breakdown (Anttinen et al., 1984; Rojkind and Dunn, 1979), and in the case of copper and manganese, also in superoxide dismutase activity (Reicks and Rader, 1990; Zidenberg-Cherr et al., 1990). Urinary zinc levels are increased and tissue and serum zinc levels are decreased in situations of enhanced protein catabolism, a pathway which is also depressed in hypothyroidism (Ingbar and Woeber, 1975).

Thus, the hepatic content of several trace elements becomes affected in alcoholic liver disease, especially with accompanying malnutrition. The alteration in liver trace element content may play a role, by itself, in liver damage. However, tissue levels of these elements may be influenced by PTU, and this drug may also counteract some direct metabolic effects of ethanol. Based on these observations, we have analysed in the present study the relative and combined effects of ethanol and PTU on liver cell size, fibrosis and steatosis, and also on liver iron, zinc, copper and manganese, taking also into account the presence or absence of co-existing protein deficiency.

MATERIALS AND METHODS

Thirty-two male Wistar rats were divided into four groups of eight animals each. The control rats received the Lieber–DeCarli control diet (Lieber et al., 1989; Lieber and DeCarli, 1989) (Dyets Inc, Bethlehem, Pennsylvania, USA) containing 18% protein and 1 kcal/ml; a second group was fed an isocaloric 36% ethanol-containing diet; the third group an isocaloric 2% protein-containing diet, and the fourth an isocaloric 2% protein–36% ethanol-containing diet (Lieber and DeCarli, 1989). 6-n Propyl-2 thiouracil (PTU) (Sigma, St Louis, MO, USA) was added to these diets at a concentration of 0.5% (w/w) (i.e. approximately 30 mg/day), based on dosages used in other studies (Kalland et al., 1978; Childs et al., 1991). This amount of PTU leads to the development of hypothyroidism, indeed, serum T3 of the PTU-treated animals was undetectable, and serum T4 ranged from undetectable to 0.53 ng/dl, with a mean value of 0.07 and a median of 0.01 ng/dl (Radiommunoanalysis, Behring, Marburg, Germany).

Another four groups of eight animals each were fed respectively the same diets mentioned above, but without PTU. All these diets (both with and without PTU) were prepared weekly by dissolving the nutrient mixture and the separate vitamin mix in water. Those rats receiving the alcohol, protein-deficient diet consumed the diet ad libitum, and the same amount consumed by these animals was then given to the other groups. This pair-feeding process was repeated every 2 days, always adjusting the amount of liquid diet received by the other groups to that consumed by the animals fed the protein-deficient, ethanol-containing diet. The mean daily amount of diet consumed was (in ml or kcal, mean ± SD): (1) animals without PTU: 61.7 ± 4.2 the controls, 59.6 ± 5.0 the alcoholic animals, 58.5 ± 7.0 the low-protein and ethanol-fed animals, and 60.4 ± 3.5 the low protein-fed animals; (2) animals with PTU: 59.0 ± 3.0 the controls, 60.1 ± 2.0 the alcoholics, 58.2 ± 2.9 the low-protein and ethanol-fed animals, and 55.2 ± 2.0 the low protein-fed animals.

No differences were found between the amount of diet consumed by the animals of the different eight groups ($F = 1.81, P = 0.10$).

Another two groups of five animals each were allowed to consume the control diet ad libitum, with and without PTU, respectively. The amount of diet consumed by these animals was 87.3 ± 5.8 and 92.4 ± 3.1 kcal (ml)/day, respectively, i.e. significantly more than the amount consumed by the study groups.

Two months later the animals were killed by intraperitoneal injection of a lethal dose of pentobarbitol (100 mg/kg). Liver specimens for light microscopic examination were fixed in formalin, embedded in paraffin and cut into serial longitudinal sections. These were stained with haematoxylin–eosin and van Giesson. Samples were mounted for blind histological assessment. The following parameters were histomorphometrically determined using a WIDS II image analyser (Zeiss, Jena, Germany):

(a) Area of the perivenular fibrotic rim of at least 10 randomly chosen pericentral veins. In order to avoid errors due to differences in the
Table 1. Changes in serum albumin and body weight

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Serum albumin (g/dl)</th>
<th>Weight difference (final weight - initial weight, g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>3.25 ± 0.32</td>
<td>+68 ± 14</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>2.84 ± 0.21</td>
<td>-3 ± 23</td>
</tr>
<tr>
<td>3</td>
<td>Low protein</td>
<td>2.70 ± 0.15</td>
<td>-63 ± 15</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol + low protein</td>
<td>2.16 ± 0.44</td>
<td>-106 ± 10</td>
</tr>
<tr>
<td>5</td>
<td>PTU control</td>
<td>3.30 ± 0.32</td>
<td>-55 ± 23</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol + PTU</td>
<td>3.28 ± 0.24</td>
<td>-30 ± 10</td>
</tr>
<tr>
<td>7</td>
<td>Low protein + PTU</td>
<td>2.36 ± 0.19</td>
<td>-121 ± 8</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol + low protein + PTU</td>
<td>2.12 ± 0.25</td>
<td>-123 ± 31</td>
</tr>
</tbody>
</table>

Main effects

- Low protein: $P < 0.01$, negative
- Ethanol: $P < 0.01$, negative
- PTU: $P < 0.01$, negative

Interactions (two-way)

- Ethanol–PTU: $P = 0.025$, negative
- PTU–2% protein: $P < 0.01$, positive

SNK = Student–Newman–Keuls test.

diameter of the veins, we have calculated the ratio (as %):

area of the fibrotic rim/area of the vessel

(b) Total amount of fat. This was calculated as follows:

mean area of fat droplets \times \text{number of fat droplets in the liver section} \times 100/\text{total area of the section}

(c) Hepatocyte and nuclear areas of at least 20 cells of each of three periportal and three pericentral areas, as far as possible from each other. These measures were made at ×400 magnification.

A part of the liver specimen was dehydrated, weighed, solved in 65% nitric acid (Merck) and 10% hydrogen peroxide, in order to digest organic material. The digestion solutions were quantitatively transferred to volumetric flasks, and diluted to 10 ml with ultrapure water (prepared using a Milli-Q OM-140 deionization system). Flame atomic absorption spectrophotometry (Perkin Elmer 3030 B spectrophotometer) was utilized for trace element determination, using certified patterns of 1000 ppm (Fisher, Fairlawn, NJ, USA). Liver iron, copper, manganese and zinc were determined in such a way.

Blood samples were obtained before killing for serum albumin (Bromocresol green, automated BM/Hitachi 717, Boehringer Mannheim, Germany) and T3 and T4 determination (radioimmunoassay, Behring, Marburg, Germany) (Walker, 1977).

Statistics

The different parameters mentioned above were compared between the first eight groups using analysis of variance and, thereafter, the Student–Newman–Keuls test. Single correlation studies were performed to determine the significance of the relation between two quantitative variables. Moreover, two-way analysis of variance (ANOVA) was used to determine the relative weight of PTU, protein malnutrition and ethanol in
Table 2. Hepatocyte area of animals of the different groups

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Hepatocyte area (µm²; mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pericentral</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>286.2 ± 16.9</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>326.2 ± 21.1</td>
</tr>
<tr>
<td>3</td>
<td>Low protein</td>
<td>245.7 ± 21.5</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol + low protein</td>
<td>249.1 ± 33.4</td>
</tr>
<tr>
<td>5</td>
<td>PTU control</td>
<td>215.3 ± 36.7</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol + PTU</td>
<td>238.5 ± 19.8</td>
</tr>
<tr>
<td>7</td>
<td>Low protein + PTU</td>
<td>208.1 ± 19.4</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol + low protein + PTU</td>
<td>212.7 ± 36.2</td>
</tr>
</tbody>
</table>

Variance analysis: $F = 18, P < 0.001$; $F = 8, P < 0.001$; $F = 14, P < 0.001$

Significance between groups (SNK):
- $2 vs 1, 3-8$
- $1 vs 2-8$
- $4 vs 2-7$
- $3 vs 5, 7, 8$

Main effects:
- Low protein: $P < 0.01$, negative
- Ethanol: $P < 0.01$, positive
- PTU: $P = 0.04$, positive

Interactions (two-way):
- 2% protein–ethanol, $P = 0.046$, negative
- 2% protein–ethanol, $P < 0.01$, negative
- Ethanol–2% protein, $P < 0.01$, negative
- PTU–2% protein, $P = 0.03$, positive

SNK = Student–Newman–Keuls test.

The differences observed, as well as the possible interactions between these three factors.

The ad-libitum-fed groups were not included in the correlation analysis or in the two-way ANOVA; Student's $t$-test was used to compare values of the different parameters of these groups between each other and with those of the control and control–PTU groups respectively.

RESULTS

Results regarding the four groups without PTU have been reported elsewhere (Conde-Martel et al., 1992), but are also summarized in this work.

Weight changes and serum albumin

Ethanol, protein deficiency and PTU exerted significant, independent effects on body weight (Table 1); a positive interaction (that is, a potentiation of the effect) was observed between protein deficiency and PTU, and a negative one between PTU and ethanol. Regarding serum albumin (Table 1), both ethanol and protein deficiency exerted independent reductions in serum albumin levels; a negative interaction existing between ethanol and PTU. Despite weight loss, serum albumin levels of the animals receiving PTU and the 18% protein-containing diet or PTU and the 36% ethanol–18% protein-containing diet were not altered, in contrast with the reduced levels observed in the ethanol-fed animals without PTU. Serum albumin significantly correlated with final body weight ($r = 0.61; P < 0.0001$).

Hepatocyte area

Animals receiving the diet containing 36% ethanol and 18% protein showed an increased, and animals fed the 2% protein-containing diet showed a decreased, total hepatocyte area, especially when ethanol was present (Table 2). PTU led to a marked decrease in hepatocyte area, especially striking in the pericentral ones (Table 2). All three factors showed independent effects on both pericentral and total hepatocyte areas. A
### Table 3. Nuclear area of animals of the different groups

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Pericentral</th>
<th>Periportal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>39.1 ± 2.8</td>
<td>35.6 ± 2.1</td>
<td>37.4 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>46.1 ± 2.9</td>
<td>40.9 ± 7.8</td>
<td>42.9 ± 3.9</td>
</tr>
<tr>
<td>3</td>
<td>Low protein</td>
<td>33.1 ± 1.5</td>
<td>34.9 ± 2.9</td>
<td>33.9 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol + low protein</td>
<td>37.4 ± 4.3</td>
<td>35.6 ± 4.8</td>
<td>36.5 ± 4.3</td>
</tr>
<tr>
<td>5</td>
<td>PTU control</td>
<td>29.3 ± 4.2</td>
<td>28.4 ± 5.5</td>
<td>28.8 ± 4.8</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol + PTU</td>
<td>30.3 ± 2.1</td>
<td>28.3 ± 1.6</td>
<td>29.3 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>Low protein + PTU</td>
<td>27.7 ± 2.0</td>
<td>28.3 ± 2.7</td>
<td>28.0 ± 1.9</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol + low protein + PTU</td>
<td>27.5 ± 3.2</td>
<td>28.2 ± 5.7</td>
<td>27.8 ± 4.3</td>
</tr>
</tbody>
</table>

Variance analysis

- **Main effects**
  - Low protein: $F = 36, P < 0.01$
  - Ethanol: $F = 8, P < 0.01$
  - PTU: $F = 23, P < 0.001$

- **Interactions (two-way)**
  - Ethanol–PTU, $P = 0.002$, negative
  - PTU–2% protein, $P < 0.001$, positive

SNK = Student–Newman–Keuls test

**Positive interaction** was observed between protein deficiency and PTU, and a negative one between protein deficiency and ethanol, regarding total hepatocyte area. Direct, significant correlations were observed between hepatocyte area and final weight ($r = 0.48; P < 0.001$) and liver manganese ($r = 0.29; P < 0.05$), and inverse correlations with liver zinc and iron ($r = -0.31; P < 0.05$ in both cases) and fat ($r = -0.49; P < 0.001$).

### Nuclear area

Ethanol increased and PTU decreased nuclear areas (Table 3), exerting independent and opposite effects on nuclear areas of pericentral cells. Protein deficiency led to a decrease in pericentral nuclear area, showing a positive interaction with PTU. Total nuclear area showed a positive correlation ($P < 0.01$) with total hepatocyte area ($r = 0.86$), final weight ($r = 0.50$), liver manganese ($r = 0.39$) and liver fibrosis ($r = 0.26; P < 0.05$), and an inverse correlation with liver zinc ($r = -0.37; P < 0.01$) and fat ($r = -0.51; P < 0.001$).

### Fibrosis

Rats fed the 36% ethanol–2% protein-containing diet showed the highest values of pericentral fibrotic rim area (Table 4). PTU-treated animals showed near-normal values; negative interactions existed between PTU and ethanol, and between PTU and protein deficiency (Table 4). A direct, significant correlation was established between fibrosis and liver iron ($r = 0.35; P < 0.01$), and negative correlations between fibrosis and liver zinc ($r = -0.53; P < 0.001$), liver copper ($r = -0.39; P < 0.01$), and weight loss ($r = -0.27; P < 0.05$).

### Liver lipids

Ethanol and 18% protein-fed animals showed a modest, although significant, increase in fat content in the liver; a more marked increase was observed in the animals treated with ethanol and a 2% protein-containing diet (Table 4). Addition of PTU led to a marked increase in the fat content in animals treated with 2% protein and/or with ethanol. All the three factors showed independent,
Table 4. Liver fat and fibrosis and liver iron

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Fibrosis (%)</th>
<th>Fat (%)</th>
<th>Iron (µg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.10 ± 0.06</td>
<td>0.20 ± 0.01</td>
<td>382 ± 34</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>0.24 ± 0.05</td>
<td>1.41 ± 0.73</td>
<td>585 ± 122</td>
</tr>
<tr>
<td>3</td>
<td>Low protein</td>
<td>0.22 ± 0.06</td>
<td>1.46 ± 1.07</td>
<td>637 ± 159</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol + low protein</td>
<td>0.70 ± 0.04</td>
<td>3.28 ± 1.93</td>
<td>1019 ± 210</td>
</tr>
<tr>
<td>5</td>
<td>PTU control</td>
<td>0.11 ± 0.01</td>
<td>3.00 ± 1.66</td>
<td>696 ± 134</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol + PTU</td>
<td>0.15 ± 0.05</td>
<td>7.88 ± 1.34</td>
<td>544 ± 238</td>
</tr>
<tr>
<td>7</td>
<td>Low protein + PTU</td>
<td>0.12 ± 0.03</td>
<td>6.94 ± 1.29</td>
<td>1037 ± 196</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol + low protein + PTU</td>
<td>0.15 ± 0.03</td>
<td>6.45 ± 2.92</td>
<td>767 ± 246</td>
</tr>
</tbody>
</table>

Variance analysis

- Significance between groups (SNK)
  - F = 12, P < 0.01
  - F = 26, P < 0.01
  - F = 15, P < 0.01

Main effects

- Low protein: P < 0.01, positive
- Ethanol: P < 0.01, positive
- PTU: P < 0.01, positive

Interactions

- Two-way
  - Ethanol–PTU, P = 0.001, negative
  - PTU–2% protein, P < 0.001, negative

- Three-way
  - Ethanol–PTU, P = 0.001, positive
  - Ethanol–2% protein, P = 0.014, positive
  - Ethanol–PTU, P < 0.01, negative
  - Ethanol–2% protein, P < 0.001, negative

SNK = Student–Newman–Keuls test.

additive effects on fat amount (Table 4).

Lipid concentration inversely correlated with serum albumin (r = -0.36; P < 0.01) and final weight (r = -0.53; P < 0.001) and directly with liver iron (r = 0.42; P < 0.001).

Liver trace elements

Animals treated with ethanol and/or the 2% protein-containing diet showed a decrease in liver zinc, especially marked in the group treated with both factors (Table 5). PTU tended to increase the liver zinc content: the control rats with PTU showed a significant increase in liver zinc, when compared with the controls without PTU, and rats treated with ethanol and/or the 2% protein-containing diet plus PTU showed significantly higher values than those without it. All the three factors exerted independent significant effects on liver zinc content; PTU increasing, and ethanol and protein deficiency decreasing it. Liver zinc content directly correlated with serum albumin (r = 0.46; P < 0.001).

Both ethanol and protein deficiency led to an increase in liver iron, especially marked in the group treated with both factors. PTU also increased liver iron content. In animals treated with PTU, protein deficiency, but not ethanol, led to a further increase in liver iron content; however, rats treated with PTU, ethanol and 2% protein showed significantly higher values of liver iron than those without PTU. PTU and protein deficiency exerted independent, significant effects on liver iron, a negative interaction between ethanol and PTU having also been observed (Table 4). Liver iron inversely correlated with final weight (r = -0.71) and albumin (r = -0.46; P < 0.001 in both cases).

Regarding liver copper content, no differences were observed between animals fed the ethanol–18% protein-containing diet with and without PTU. However, both ethanol and protein deficiency led to a decrease in liver copper, especially
Table 5 Liver zinc, copper and manganese

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Zinc (µg/g dw)</th>
<th>Copper (µg/g dw)</th>
<th>Manganese (µg/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>57.2 ± 12.6</td>
<td>14.1 ± 1.22</td>
<td>5.45 ± 1.29</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>35.3 ± 5.6</td>
<td>11.7 ± 1.26</td>
<td>4.57 ± 0.89</td>
</tr>
<tr>
<td>3</td>
<td>Low protein</td>
<td>21.3 ± 8.9</td>
<td>8.5 ± 2.35</td>
<td>2.39 ± 0.82</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol + low protein</td>
<td>15.9 ± 4.5</td>
<td>7.9 ± 2.15</td>
<td>3.10 ± 0.94</td>
</tr>
<tr>
<td>5</td>
<td>PTU control</td>
<td>72.1 ± 6.1</td>
<td>18.6 ± 2.24</td>
<td>3.55 ± 0.47</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol + PTU</td>
<td>49.8 ± 10.5</td>
<td>11.5 ± 2.69</td>
<td>4.13 ± 1.10</td>
</tr>
<tr>
<td>7</td>
<td>Low protein + PTU</td>
<td>61.4 ± 10.4</td>
<td>15.7 ± 2.96</td>
<td>3.25 ± 0.58</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol + low protein + PTU</td>
<td>46.4 ± 10.2</td>
<td>10.2 ± 2.10</td>
<td>2.80 ± 0.82</td>
</tr>
</tbody>
</table>

Variance analysis

Significance between groups (SNK)

Main Low protein effects Ethanol PTU

Interactions Two-way

SNK = Student–Newman–Keuls test.

marked in the group treated with both factors (Table 5). Liver copper directly correlated with serum albumin ($r = 0.33$) and liver zinc content ($r = 0.85; P < 0.01$ in both cases).

Ethanol, PTU and the 2% protein-containing diet tended to decrease liver manganese content, although only protein deficiency exerted a significant, independent effect. Liver manganese directly correlated with serum albumin ($r = 0.28$) and final weight ($r = 0.60; P < 0.05$ in both cases).

Ad-libitum-fed animals

Results regarding these groups are shown in Table 6. Differences of serum albumin levels between the ad-libitum-fed animals and the respective pair-fed controls without or with PTU were not significant.

Animals fed the control diet ad libitum showed significantly larger periportal hepatocytes and total hepatocyte area than the control animals ($P < 0.01$ and $< 0.03$ respectively). Ad-libitum-fed animals with PTU showed larger pericentral hepatocytes and total hepatocyte area than the PTU control group ($P < 0.02$ and $P < 0.05$, respectively). Differences of periportal hepatocyte area between ad-libitum-fed animals with and without PTU reached a borderline statistical significance. Periportal nuclear area of the ad-libitum-fed animals with PTU, although larger than that of the PTU control animals ($P < 0.01$), was significantly smaller than that of the ad-libitum control animals ($P < 0.02$). Both pericentral and periportal nuclear areas were significantly larger in these two groups, when compared with the pair-fed control and control + PTU animals, respectively.

Ad-libitum-fed animals both with and without PTU showed values of the pericentral fibrotic rim area and liver fat similar to those of the controls.

Liver zinc content of the ad-libitum-fed animals with and without PTU was similar to that of the
controls; however, control-PTU rats showed higher values of liver zinc content than these two groups (P < 0.001). Control rats showed higher liver iron content than ad-libitum-fed rats (P < 0.01) and control rats with PTU, and a slightly, but significantly, higher liver iron content than the ad-libitum-fed animals with PTU (P < 0.05). No differences existed between the ad-libitum-fed animals with and without PTU, nor between the respective controls regarding liver copper content, but ad-libitum-fed animals with PTU showed significantly higher liver manganese levels than the control animals with PTU (P < 0.001).

DISCUSSION

Our results show that PTU decreases hepatocyte ballooning and fibrous tissue deposition, and increases liver fat. This last feature seems to be dependent on the calorific restriction suffered by the pair-fed animals, since liver fat of the ad-libitum-fed control animals with PTU is similar to that of the ad-libitum-fed animals without PTU.

Hepatocyte ballooning depends on the accumulation of fat, protein and water. An excess of reduced equivalents derived from ethanol metabolism may lead to increased fatty acid synthesis and decreased oxidation, both resulting in increased liver lipids. Hypothyroidism decreases fatty acid oxidation, and also synthesis and catabolism of proteins, more intensely the former than the latter. This could explain the changes observed in our study. The few reports on liver changes in hypothyroidism show pericentral congestion and fibrosis (Baker et al., 1972), fatty liver in chicks treated with PTU (Akiba et al., 1985), slight steatosis and reduced hepatocyte area in developing mice treated with PTU (González-Reimers et al., 1988) and reduced pericentral fibrosis (Israel et al., 1975). Our results are thus in accord with these reports.

Besides reducing oxygen consumption, PTU increases portal flow (Kawasaki et al., 1989) and, perhaps it also prevents liver damage by altering liver content of certain trace elements involved in free radical scavenger mechanisms and/or in metabolic pathways of collagen metabolism, such as iron and zinc (Underwood, 1977; Rojkind and Dunn, 1979; Anttinen et al., 1984). In the present study, liver iron correlated both with liver fat and liver fibrosis; these results are in accordance with the mechanisms mentioned in the Introduction. Ethanol, especially when associated with protein deficiency, strongly increases liver iron content (Conde-Martel et al., 1992).
PTU leads to an increase in liver iron both in the control group and in the low protein-fed animals, but tends to reduce iron overload in the alcoholic animals. Only a few studies examined trace elements in hypothyroidism. Al Khayat et al. (1982) found a slight elevation in liver iron in thyroidectomized rats, but a significant decrease in PTU-treated animals; we have observed, in another experimental model, that PTU- and PTU + ethanol-treated mice showed a slight increase in liver iron at an age of 85 days, when compared with controls. However, liver iron of the ethanol-treated animals without PTU was higher than that of the animals given PTU, as in the present work (González-Reimers et al., 1989).

Zinc depletion is a classical marker of protein wastage (Solomons, 1980). The low liver zinc levels described in alcoholics (Mills et al., 1983; Valberg et al., 1985; Milman et al., 1986) may be responsible for progressive liver fibrosis; Anttinen et al., (1984) have shown that zinc supplementation hampers carbon tetrachloride-induced liver fibrosis. In our study, PTU counteracted the lowering effect of ethanol on liver zinc, a finding which has not been described previously. Interestingly, we have also observed an inverse correlation between liver zinc and fibrosis.

Manganese depletion is commonly observed in malnutrition (García-Aranda et al., 1990). In our study, animals fed the low protein diet, either with or without PTU and/or ethanol, showed the lowest liver manganese values. Theoretically low liver manganese may cause liver damage, as superoxide dismutase is a manganese metalloenzyme, and manganese deficiency could thus impair liver free radical scavenger mechanisms (Keen et al., 1985). Liver manganese overload has been reported in alcoholic liver disease, probably due to impaired biliary excretion (Milman et al., 1986); it may affect liver fibrogenesis, since manganese acts as cofactor of enzymes involved in collagen synthesis (Rojkind and Dunn, 1979), although ethanol-fed miniature pigs showed enhanced activity of manganese superoxide dismutase when compared with controls (Ziedenberg-Cherr et al., 1990), thus being theoretically protected against hepatocyte necrosis. In our study, no correlation was found between liver manganese and fibrosis.

PTU-treated animals showed higher liver copper levels than those without PTU, a finding in accordance with other studies. Liver copper was related to the amount of fibrosis. Liver copper excess, by promoting necrosis (Sternlieb, 1980) and enhancing lysyl-oxidase activity (Rojkind and Dunn, 1979), may theoretically affect liver fibrogenesis. However, liver copper depletion may also promote fibrogenesis, since it is associated with low hepatic cytosolic Cu-Zn superoxide dismutase (Reicks and Rader, 1990) and selenium-dependent glutathione peroxidase activities (Allen et al., 1988).

Thus, our study shows that propylthiouracil may profoundly influence ethanol-mediated liver changes, leading to a decrease in fibrous tissue deposition, which may be related to an increase in liver zinc.

REFERENCES


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