Urinary excretion of nitrogenous and non-nitrogenous compounds in the chronic ethanol-fed rat

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SUMMARY

1. The metabolic consequences of chronic ethanol feeding was investigated by assay of urinary metabolites. Male Wistar rats were fed a liquid diet containing 35% of total energy as ethanol or isovolumetric, isocaloric and isonitrogenous amounts of the same diet in which ethanol was substituted by isocaloric glucose (controls).

2. At 6 weeks the entire skeletal muscle mass was reduced by approximately 20%. The urinary excretion of nitrogen, urea and uric acid increased by between 23 and 128%. Urinary creatinine excretion was not significantly altered.

3. Urinary excretion of magnesium was significantly increased by 43%. Urinary excretion of sodium, potassium, calcium and phosphate was increased slightly (i.e. 5-22%), but this change was not statistically significant.

4. Proton n.m.r. spectroscopic analysis showed that ethanol feeding reduced the urinary excretion of citrate and 2-oxoglutarate (by approximately 50%), suggesting decreased citric acid cycle activity. There was an increased excretion of alanine (44%), but excretion of succinate and acetate was not significantly altered. Ethanol in the urine of ethanol-fed rats comprised approximately 2% of total ethanol intake and less than 1% of total energy intake.

5. Lactose was detectable in urine of ethanol-fed rats, but not in control rats, reflecting the reported decreased intestinal lactase activity and increased gut permeability in alcoholics. Urinary galactose excretion decreased by 41%, but relatively large increases in lactate excretion (50%) did not achieve statistical significance.

6. It was concluded that chronic ethanol feeding causes disturbances in whole-body nitrogen homeostasis and alterations in intermediary metabolism.

Key words: alcohol toxicity, nuclear-magnetic-resonance spectroscopy, nitrogen balance, urinalysis.

INTRODUCTION

The ethanol-fed young rat is a suitable experimental model for examining the biochemical effects of chronic ethanol toxicity on skeletal muscle. After 6 weeks of ethanol feeding, the morphological and biochemical changes in rat skeletal muscle [1-5] are similar to those occurring in human skeletal muscle of chronic alcohol abusers [6-9]. These include reductions in skeletal muscle mass, in which type II (anaerobic glycolytic, fast-twitch) fibres or fibre-rich muscle are preferentially affected [3, 4, 6]. In the experimental model strict attention is paid to ensure that both control and treated rats receive liquid diets that are isonitrogenous and isocaloric, albeit with differences in calories derived from ethanol or carbohydrate. The diets are also isovolumetric and isolipidic, with identical amounts of micro- and macro-nutrients, such as trace elements and vitamins [1-5].

As skeletal muscle comprises a major proportion of body weight, there may be evidence of enhanced urinary excretion of nitrogen or changes in whole-body intermediary metabolism as a consequence of ethanol ingestion. Additionally, an explanation for the diminished muscle mass could be a starvation effect caused by excessive urinary excretion of calories in the form of ethanol and/or acetate.

Caution is required in the usage of plasma analytes to assess the nutritional state, as many of these show marked diurnal variations or alter in response to acute nutritional perturbations [10]. The situation is further complicated...
Treatment of animals

by the fact that we have shown that chronically treated, glucose-fed control and ethanol-fed rats have markedly different patterns of feeding[10]. An alternative approach for investigations into whole-body metabolism would be by analysis of urine. Measurement of total urinary urea should be sufficient for assessing nitrogen balance as there is evidence to suggest that, in the rat, urinary nitrogen is predominantly in the form of urea [11]. However, a re-examination is necessary because in the maintenance diet ('R and M Diet No. 1'; SDS, Witham, Essex, U.K.) used in the Animal House of King's College School of Medicine and Dentistry were 8.4%, 21.1% and 70.5%, respectively. The water contents per unit volume of control and ethanol-containing diets were both 0.860 ml of H₂O/ml of diet.

Pair-feeding of control rats was carried out as follows. The exact volume of diet consumed by each ethanol-treated rat over 24 h was measured. This volume was recorded and an identical volume of the glucose-containing control diet was given to its pair-matched control. All diets were freshly prepared each day, and rats were fed their diets between 08.00 hours and 10.30 hours each day. Ethanol-fed rats consumed their diets throughout the day, although there were minor diurnal fluctuations. The attainment of a relative uniform intake of the ethanol-containing diet was essential to maintain the high ethanol concentrations in the blood. This was because treated rats were 'ethanol dependent', and withdrawal of the diet for acute periods caused severe behavioural and physiological responses. When the control rats were presented with their diet, they rapidly engorged themselves because they were semi-starved [10].

Urinary collections

Rats were placed in Tecniplast Metabolic Cages (Labcare Precision Ltd, Aldington, Kent, U.K.) to facilitate collection of urine, and were acclimatized to their new environment for at least a week. On the penultimate day of the experiment, urine was collected over a 24 h period from ethanol-fed rats. The day after, 24 h urine samples were also collected from control rats. Urine was stored at −70°C until analysis. Immediate and complete separation of faeces and urine was obtained by the unique funnel and cone design of the metabolic cages. Urine recovery was also high because of the non-wetting poly-methylpentane surfaces of the apparatus (manufacturer's specifications). Furthermore, it is important to note that the liquid diet was a low-residue preparation, and very little faeces were passed. As subsequent data show, approximately half of the urinary constituents were accounted for by urea, uric acid and creatinine. By implication, the remainder of the urinary nitrogen was probably in the form of ammonia, as well as trace amounts of peptides and imino and amino acids (i.e. hydroxyproline, alanine, 3-methylhistidine, etc.).

Assay procedures

Nitrogen was measured by the Kjeldahl procedure after digestion of samples with sulphuric acid. Sodium and potassium were assayed by flame emission, and calcium and magnesium by atomic absorption spectroscopy, using routine procedures. Urinary phosphorus was measured by the colorimetric procedure described by

METHODS

Source of animals and materials

Male Wistar rats were obtained from Bantin and Kingman (Hull, Humberside, U.K.). All other chemicals were from various suppliers and were of optimum purity.

Treatment of animals

Rats were obtained at 50–60 g initial body weight and were placed in wire-bottomed cages in a temperature-controlled animal house on a 12 h light/12 h dark cycle, commencing at 07.00 hours. At approximately 80 g body weight, rats were pair-matched (n = 6 pairs) and fed a liquid diet containing 35% of total calories as ethanol for 6 weeks ad libitum, as described previously [1–5, 10] and below.

Details of the liquid diets

The control liquid diet was prepared by mixing 121 g of a commercial food drink supplement ('Vita Food', Boots Company Plc, Nottingham, U.K.), 10 g of casein (BDH Chemicals Ltd, Poole, Dorset, U.K.), 85 g of glucose (BDH Chemicals Ltd), a vitamin supplement ('Oravite 7', Beecham Group, Brentford, Middlesex, U.K.) and 800 ml of distilled water. The final volume of the diet was 930 ml and the percentages of total energy provided by fat, protein and carbohydrate were 20.5, 15.7 and 63.8%, respectively. Each litre contained 833 kJ (199 kcal) as fat, 637 kJ (152 kcal) as protein and 2600 kJ (622 kcal) as carbohydrate. For the ethanol-containing diet the 85 g of glucose was replaced with 56.5 ml of ethanol; the percentages of total energy provided by fat, protein, carbohydrate and ethanol were therefore 20.5, 15.7 and 28.7 and 35.1%, respectively. Fat, protein and other nutrients in the two diets were thus identical [1–5, 10]. In both control and ethanol-containing diets the proportions of calories provided by fat, protein and carbohydrate compared favourably with the composition of other commercial laboratory diets. For example, the proportions of calories provided by fat, protein and carbohydrate in the maintenance diet ('R and M Diet No. 1'; SDS, Witham, Essex, U.K.) used in the Animal House of King's College School of Medicine and Dentistry were 8.4%, 21.1% and 70.5%, respectively. The water contents per unit volume of control and ethanol-containing diets were both 0.860 ml of H₂O/ml of diet.

Pair-feeding of control rats was carried out as follows. The exact volume of diet consumed by each ethanol-treated rat over 24 h was measured. This volume was recorded and an identical volume of the glucose-containing control diet was given to its pair-matched control. All diets were freshly prepared each day, and rats were fed their diets between 08.00 hours and 10.30 hours each day. Ethanol-fed rats consumed their diets throughout the day, although there were minor diurnal fluctuations. The attainment of a relative uniform intake of the ethanol-containing diet was essential to maintain the high ethanol concentrations in the blood. This was because treated rats were 'ethanol dependent', and withdrawal of the diet for acute periods caused severe behavioural and physiological responses. When the control rats were presented with their diet, they rapidly engorged themselves because they were semi-starved [10].
Hurst [13], urea by the method of Paulson et al. [14], creatinine by the method of Lustgarten & Wenk [15], and urate on a Coulter Dacos Analyser with urate oxidase (EC 1.7.3.3) [16].

Proton n.m.r. spectroscopy

Portions of urine (0.5 ml) were placed in a 5 mm n.m.r. tube to which 0.05 ml of 2H2O and 0.02 ml of 500 mmol/l 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate were added to lock the magnetic field and to act as a chemical shift reference standard, respectively [17]. The samples were run at room temperature in a Bruker WH400 spectrometer operating at a field frequency of 400 MHz. A single pulse sequence was used, pulse angle 45°, with a pulse recycle time of 2 s. The water signal was suppressed by selective irradiation during the relaxation delay [17].

Statistics

Rats from a single batch of animals were divided into pairs of equal body weight at the commencement of the experimental programme and feeding procedures. A Student's t-test for paired samples was therefore used to assess the differences between means [18]. Differences between means were assumed to be significant when \( P \) was equal to, or less than, 0.05. All data are presented as means ± SEM.

RESULTS

Effect of ethanol consumption on body and carcass weight

Table 1 shows the effect of chronic ethanol feeding on body weight and eviscerated and skinned carcass weight after 42 days of treatment. Significant reductions were seen in body weight (14% decline), carcass weight (16% decline) and skeletal muscle mass (18% decline).

The volumes of diet consumed by the control and ethanol-treated rats were identical, i.e. 58.3 ± 3.1 ml, by virtue of the experimental design. In the control diet this contained 1.29 ± 0.07 g of fat (48.6 ± 2.6 kJ), 9.67 ± 0.51 g of carbohydrate (151.6 ± 8.1 kJ) and 2.22 ± 0.12 g of protein (37.2 ± 2.0 kJ). In the ethanol-containing diet the amounts of fat and protein were identical but the amounts of carbohydrate and ethanol were 4.33 ± 0.23 g (68.0 ± 3.6 kJ) and 2.80 ± 0.15 g (83.1 ± 4.4 kJ), respectively. Table 1 shows that the 24 h urinary volumes were not significantly different between the two groups.

Effect of ethanol consumption on excretion of nitrogenous and non-nitrogenous compounds, as determined by chemical analysis

Ethanol feeding significantly increased the urinary excretion of nitrogen, urea and uric acid by 23%, 62% and 128%, respectively (Table 2). Table 2 also shows that although urinary creatinine excretion was slightly reduced by ethanol feeding (–8%), the reduction did not attain statistical significance \( (P>0.05) \). In control rats the proportions of urea, creatinine and urate nitrogen, as percentages of total urinary nitrogen, were 42.9%, 1.5% and 0.2%, respectively. The corresponding values for ethanol-fed rats were 55.5%, 1.1% and 0.4%, respectively.

Table 3 shows that urinary sodium excretion was unaltered by ethanol feeding. Mean increases of between 10 and 22% were obtained for urinary excretion of phosphate, potassium and calcium in ethanol-fed rats, but these changes were not statistically significant. Magnesium excretion significantly increased by 43% as a result of ethanol ingestion (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Effect of ethanol consumption on body composition and urinary volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
</tr>
<tr>
<td>Final body weight (g)</td>
</tr>
<tr>
<td>Carcass weight (g)</td>
</tr>
<tr>
<td>Total muscle mass (g)</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
</tr>
</tbody>
</table>

**P**

< 0.01

< 0.001

< 0.025

> 0.05
Table 2. Effect of ethanol consumption on urinary excretion of nitrogenous compounds, as determined by conventional analysis

Experimental details are described in the legend to Table 1 and in the Methods section. All data are means ± SEM of five to six pairs of observations. In both groups nitrogen intake was 360 ± 19 mg/day. In control rats, the amount of nitrogen excreted in the form of creatinine, urea and urate corresponded to 2.10 ± 0.11, 58.8 ± 6.16 and 0.30 ± 0.05 mg/day, respectively. In ethanol-fed rats the corresponding values were 1.94 ± 0.16, 93.8 ± 6.72 and 0.68 ± 0.08 mg/day, respectively.

Differences between means were assessed by using a Student’s t-test for paired samples.

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Ethanol-fed rats</th>
<th>Difference between means</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine excretion</td>
<td>50 ± 3</td>
<td>46 ± 4</td>
<td>-8</td>
<td>0.05</td>
</tr>
<tr>
<td>(μmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea excretion</td>
<td>2.1 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>+62</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>(mmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urate excretion</td>
<td>5.3 ± 0.9</td>
<td>12.1 ± 1.4</td>
<td>+128</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(μmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen excretion</td>
<td>137 ± 11</td>
<td>169 ± 12</td>
<td>+23</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(mg/day)</td>
<td></td>
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<td></td>
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</tbody>
</table>

Table 3. Effect of ethanol consumption on urinary excretion of electrolytes

Experimental details are described in the legend to Table 1 and in the Methods section. All data are means ± SEM of five to six pairs of observations. Differences between means were assessed by using a Student’s t-test for paired samples.

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Ethanol-fed rats</th>
<th>Difference between means</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium excretion</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>+5</td>
<td>0.05</td>
</tr>
<tr>
<td>(mmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium excretion</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>+17</td>
<td>0.05</td>
</tr>
<tr>
<td>(mmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium excretion</td>
<td>60 ± 4</td>
<td>86 ± 7</td>
<td>+43</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>(μmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium excretion</td>
<td>23 ± 6</td>
<td>28 ± 6</td>
<td>+22</td>
<td>0.05</td>
</tr>
<tr>
<td>(μmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate excretion</td>
<td>532 ± 36</td>
<td>587 ± 60</td>
<td>+10</td>
<td>0.05</td>
</tr>
<tr>
<td>(pmol/day)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Effects of ethanol consumption on excretion of nitrogenous and non-nitrogenous compounds, as determined by proton n.m.r. spectroscopy

Representative proton n.m.r. spectra for urinary constituents in control and ethanol-fed rats are shown in Fig. 1. The vertical scales of the two spectra are different (by a factor of 2) because of the very large signal for the ethanol in the urine of the treated group. For example, although the peak heights of creatinine are apparently higher in Fig. 1(a), the creatinine contents in Fig. 1(a) and Fig. 1(b) were nearly equal, i.e. 1.7 mmol/l. The data from these spectra are summarized in Table 4.

Table 4 shows that, when ethanol-fed rats were compared with pair-fed control rats, there were significant reductions in the urinary excretion of galactose (41%), citrate (53%) and 2-oxoglutarate (44%). Although there was a large mean increase in the urinary excretion of lactate (50%), this did not achieve statistical significance because of the large variance in the control group. There were large increases in the urinary excretion of alanine (44%) as a consequence of ethanol feeding. Excretion of both succinate and acetate was relatively unaltered. Although large amounts of lactose (i.e. 231 mol/day) were excreted in the urine of ethanol-treated rats, the assay procedures were unable to detect any lactose in the urine of control rats. Ethanol was detected in the urine of the treated groups of rats (Table 4). Some ethanol was also detected in the urine of control rats, but this amounted to less than 10% of that detected in urine of ethanol-fed rats.

DISCUSSION

In this study control rats were fed identical amounts of the same diet in which ethanol was replaced by isocaloric carbohydrate [1, 10]. Three other options were also available, namely substitution by fat, by protein or by a combination of carbohydrate, fat and protein. If either fat or protein substitution were used for the control rats then their relative proportions in the control diet would be 55.6% and 50.8% of total calories, respectively. These proportions are clearly unphysiological and are rarely seen in standard solid laboratory diets formulated to provide optimum nutritional intakes. Substitution of ethanol by a combination of carbohydrate, fat and protein was considered to be unacceptable, as the small differences in dietary nitrogen intake would have made the results for the urinary nitrogenous components (i.e. alanine, urea, etc.) difficult to interpret. In contrast, substitution of ethanol with isocaloric glucose resulted in the relative proportions of calories derived from carbohydrate, fat and protein being very similar to the propor-
Urinary analysis in ethanol-fed rats

Fig. 1. Representative proton n.m.r. spectra of urine from control (a) and ethanol-fed (b) rats, using 64 and 128 data accumulations, respectively. Experimental details are described in the legend to Table 1 and in the Methods section. The spectra were recorded at room temperature and at 400 MHz. Chemical shifts are given with respect to 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate. Abbreviations: Ac, acetate; Cit, citrate; Crn, creatinine; Eth, ethanol; Gal, galactose; Lac, lactate; Lat, lactose; Og, 2-oxoglutarate; Suc, succinate.
increases in hepatic uptake of amino acids, hepatic blood flow or peripheral production of ureagenic substrates may also be of importance in increasing urea excretion [26]. Changes in aspartate availability may be due to alterations in the amount of free amino acids derived from either hepatic and non-hepatic tissues, for example changes in amino acid flux, or changes in protein turnover. As protein synthesis declines in many tissues of the ethanol-fed rat, it is possible that increased availability of amino acids may be a causal mechanism [2, 3, 5, 19–21]. Disturbances in hepatic blood flow can be excluded, as we have recently shown that this variable is not altered by chronic ethanol feeding [27].

The results of the proton n.m.r. spectroscopic analysis showed that there were significant reductions in the urinary excretion of galactose and, in contrast, relatively high amounts of urinary lactose, as a consequence of chronic ethanol feeding. Lieber et al. [28, 29] have indicated that ethanol affects the metabolism of both these sugars. It has been shown that acute alcohol administration inhibits utilization of galactose and increases its urinary excretion [28], which is contrary to our observations. These contrasting results are difficult to explain, although Lieber et al. [28] have suggested that enhanced urinary excretion of galactose occurs via redox-state (i.e. NADH/NAD ratio)-induced alterations in the activity of UDP-galactose 4-epimerase (UDP-glucose 4-epimerase, EC 5.1.3.2). Consideration should be given to the possibility that our animal model may be different to those studied by other groups, and that the attenuation of the redox changes due to ethanol consumption over a prolonged period may also be an important adaptive mechanism [28]. The increased urinary excretion of lactose that we observed may reflect ethanol-induced disturbances in intestinal mucosa, for example shortening of villi and reductions in lactase activities and/or changes in intestinal permeability [30].

Because of the inter-organ complexities of both intermediary metabolism and the way in which carbon skeletons are shuttled between tissues, the reductions in urinary excretion of citrate and 2-oxoglutarate are difficult to ascribe. They may reflect reduced citric acid cycle activity. Frieden [31] originally suggested that ethanol has an inhibitory effect on the conversion of glutamic acid to 2-oxoglutarate by reducing the activity of glutamic dehydrogenase (EC 1.4.1.2–4), apparently by enhanced NADH formation. Moreover, 2-oxoglutarate is also a common intermediate generated during the metabolism of a number of amino acids, for example arginine, histidine, proline, glutamine and glutamate. Thus, while reductions in excretion may represent a reduction in flux through the citric acid cycle, it may also signify reduced availability of amino acids. This latter possibility is directly contradictory to the fact that alcohol feeding increases the availability of some free amino acids. One might also expect the urinary excretion of other citric acid cycle intermediates to be reduced, yet succinate excretion was unaltered.

Excretion of both citrate and 2-oxoglutarate is known to be affected by changes in acid–base balance. Citrate excretion decreases in metabolic acidosis, and both citrate and 2-oxoglutarate increase in metabolic alkalosis [32]. It is possible that the ethanol-treated rats had a degree of metabolic acidosis, as has been observed previously in chronic alcoholics [33]. Although increased alanine excretion compounded the nitrogen loss, the amount of alanine nitrogen as a proportion of total nitrogen excretion was small, and may have reflected the enhanced alanine release by skeletal muscle observed in vitro (E. Cook, T. N. Palmer, V. R. Preedy & T. J. Peters, unpublished work) or alterations in renal tubular reabsorption of alanine. Similar arguments may be applied to the observation of increased lactate excretion, which may also be an indication of impaired citric acid cycle activity.

When the total urinary ethanol excretion (1.3 ± 0.1 mmol/day per rat) was computed as a fraction of the total ethanol intake, it represented only 2% of total ethanol

### Table 4. N.m.r. spectroscopic analysis of urine

Experimental details are described in the legend to Table 1 and in the Methods section. Urinary analysis was performed by proton n.m.r. spectroscopy. Minimum detection limits were less than 1 μmol/day. All data are means ± SEM of five to six pairs of observations. Abbreviation: ND, not detectable.

<table>
<thead>
<tr>
<th>Metabolite excretion (μmol/day)</th>
<th>Control rats</th>
<th>Ethanol-fed rats</th>
<th>Difference between means (% of control)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>310 ± 44</td>
<td>183 ± 23</td>
<td>-41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lactose</td>
<td>ND</td>
<td>231 ± 21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate</td>
<td>75 ± 12</td>
<td>35 ± 7</td>
<td>-53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>9 ± 2</td>
<td>5 ± 1</td>
<td>-44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Succinate</td>
<td>35 ± 6</td>
<td>31 ± 3</td>
<td>-11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Acetate</td>
<td>210 ± 17</td>
<td>225 ± 27</td>
<td>+7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Alanine</td>
<td>9 ± 1</td>
<td>13 ± 1</td>
<td>+44</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Lactate</td>
<td>14 ± 4</td>
<td>21 ± 2</td>
<td>+50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ethanol</td>
<td>106 ± 23</td>
<td>1320 ± 100</td>
<td>+92</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>
intake (approximately 0.06 mol/day per rat) and therefore less than 1% of total caloric intake. Similarly, the amount of urinary acetate was also a very small proportion of dietary ethanol, i.e. 0.3%, and was similar to the control value. One can therefore conclude that there were no excessive losses of dietary energy intake via urinary losses and the ensuing effects of chronic ethanol ingestion were not a ‘caloric starvation’ effect.

There were slight increases in urinary electrolytes in which statistical significance was only achieved for position in chronic alcohol-fed rats have been reported by Saville & Lieber [34]. Hypomagnesaemia, due to increased urinary excretion of magnesium, is not uncommon in alcoholics [35].

In conclusion, the data reported here provided evidence that the ethanol-induced tissue dysfunctions were of such metabolic significance as to contribute considerably to alterations in whole-body metabolism. Overall, there appeared to be an enhanced excretion of nitrogenous compounds, whereas excretion of non-nitrogenous compounds was reduced.

ACKNOWLEDGMENTS

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REFERENCES


