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INCREASED KETOGENESIS IN HYPERTHYROID RATS METABOLIZING ETHANOL

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Abstract—The levels of various metabolites were measured in freeze-clamped samples of liver from triiodothyronine-treated and control rats to which either saline or ethanol (2.5 g/kg body weight) had been administered 2 hours earlier. It was found that ethanol led to a sharp increase in the hepatic acetate concentration in both hyperthyroid and euthyroid rats whereas lactate and pyruvate concentrations were lowered in both groups. The lactate/pyruvate ratio rose significantly in euthyroid animals that had received ethanol but the ratio remained relatively low in hyperthyroid rats. The adenine nucleotide phosphorylation potential, already low in hyperthyroid rats, was further lowered by ethanol. However, the most remarkable difference between the responses of euthyroid and hyperthyroid rats to ethanol was in the hepatic concentrations of ketone bodies, particularly 3-hydroxybutyrate. In control animals, administration of ethanol did not affect either the acetoacetate or 3-hydroxybutyrate concentration but, although the level of ketone bodies in the livers of hyperthyroid rats that had not received ethanol was the same as that of controls, there was a greater than fivefold increase in the 3-hydroxybutyrate level when ethanol was given. While this increase in ethanol-dependent ketogenesis is not explicable at this stage, hyperthyroidism did not increase the activity of cytoplasmic acetyl-CoA synthetase, an enzyme that is probably involved in the formation of ketone bodies from ethanol-derived acetate.

Experimental hyperthyroidism in rats lowers markedly the activities of hepatic alcohol dehydrogenase [1–8] and aldehyde dehydrogenase [8]. However, with the exception of two studies [6, 7], treatment with thyroid hormones has not been found to depress either the *in vivo* rate of ethanol elimination or the rate at which ethanol is metabolised through the alcohol dehydrogenase pathway [8]. These observations might indicate that neither enzyme level limits metabolic flux through the pathway in either the euthyroid or hyperthyroid state. Alternatively, they could suggest that hyperthyroidism alters the metabolic status of the liver so as to increase the catalytic efficiency of the residual alcohol dehydrogenase and aldehyde dehydrogenase. In particular, it has been proposed that hyperthyroidism causes NADH to be more rapidly reoxidised, thereby lowering the NADH level and loosening its restraining effect on alcohol dehydrogenase [2, 3].

Reoxidation of NADH could be accelerated either by an increased respiratory rate due to more rapid ATP utilization and a lowered mitochondrial ATP/ADP ratio or by the increased production of reduced metabolites. In this context it is interesting to speculate on the role that ketogenesis could play. Ketogenesis not only requires ATP but 3-hydroxybutyrate, the principal ketone body, is more reduced than acetate, which is commonly considered to be the major end-product of hepatic ethanol metabolism [9–12]. Acetate is an effective ketogenic substrate under some circumstances [13, 14] and, while ketone bodies are not usually produced in anything more than minor quantities during ethanol metabolism in rats [9, 15–17], ketoacidosis is a frequent concomitant of human alcoholism [18–20]. Though alcoholic ketoacidosis is probably a complex phenomenon [18, 20], it has been suggested that the conversion of ethanol to 3-hydroxybutyrate could exist as a device to limit the net production of NADH and ATP, thereby facilitating the metabolic removal of ethanol [21]. As hyperthyroidism is known to increase the formation of ketone bodies from oleate in perfused rat liver [22, 23] and can cause increased ketonaemia in man [24], it appeared conceivable that it might have a similar influence on the metabolic disposal of acetate produced during ethanol metabolism.

To look into this possibility, the pattern of ethanol oxidation was studied by measuring the levels of acetate and the two main ketone bodies, 3-hydroxybutyrate and acetoacetate, in livers of euthyroid and hyperthyroid rats during ethanol metabolism. The levels of lactate and pyruvate were measured in order to assess the cytoplasmic redox state [25] and adenine nucleotides were also determined in order to assess the general energy status of the liver. It was hoped that this approach might provide an insight into why the rate of ethanol metabolism can be maintained in hyperthyroidism even in the face of sharply reduced enzyme levels.

MATERIALS AND METHODS

Chemicals. 3,3′,5′-Triiodo L-thyronine (sodium salt) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethanol was from Ajax Chemi-

cals (Sydney, Australia) and florisil from H. B. Selby and Co. Pty. Ltd. (North Ryde, Australia). Biochemical grade substrates, enzymes and cofactors used in metabolite determinations were obtained either from Sigma Chemical Co. or from Boehringer Mannheim Australia Pty. Ltd. (North Ryde, Australia). General purpose chemicals were A.R. grade.

Treatment of rats with triiodothyronine (T3). Female Wistar rats weighing between 150 g and 300 g were treated with T3 (20 μg/100 g body weight daily for 6 days) as described previously [8] except that the T3 was dissolved in 0.15 M NaCl containing 10 mM NaOH. Control rats received injections of the alkaline saline vehicle. The animals received food and water *ad libitum* throughout the treatment period.

Administration of ethanol. T3-treated and control rats received a single intraperitoneal injection of either 0.154 M NaCl or 12.5% (w/v) ethanol in 0.154 M NaCl. The volume injected was 2 ml/100 g body weight, thereby delivering, in the case of ethanol solutions, a dose of 2.5 g ethanol/kg body weight. In all experiments the injections were given between 9.00 a.m. and 10.00 a.m. so as to avoid any complications due to diurnal rhythms in ethanol metabolism [26].

Preparation of liver extracts for metabolite determinations. Two hours after the injection of ethanol solution or saline the animals were killed by cervical dislocation. The liver was rapidly excised from each rat, clamped between aluminium tongs precooled in liquid nitrogen and then immersed in liquid nitrogen. The total elapsed time between cervical dislocation and freezing the liver was approximately 12 sec in most cases and was always less than 20 sec. Frozen liver was treated essentially as described by Williamson *et al.* [25]. After pulverizing in a stainless steel mortar with frequent addition of liquid nitrogen, 2–3 g of powdered liver was homogenised with 4 volumes of ice-cold 0.6 M perchloric acid using an Ultra-Turrax homogeniser. In some cases, the perchloric acid contained 45 mM thiourea to lessen the risk of artefactual acetaldehyde formation during processing of the liver [27]. Following homogenisation, the homogenate was centrifuged at 30,000 *g* for 15 min and the resulting supernatant was brought to pH 6–7 by addition of 20% (w/v) KOH. After standing for 10 min on ice the precipitate was removed by centrifugation at 10,000 *g* for 10 min. In most cases, the supernatant from this spin was mixed for approx. 30 sec with florisil (0.1 g/ml) to remove flavins [3, 25] and the florisil was then removed by centrifuging at 10,000 *g* for 10 min. However, because treatment with florisil was found to cause a loss of up to one-quarter of the ATP and ADP, samples used for the determination of these nucleotides and inorganic phosphate were not treated with florisil. A similar precaution was taken by Guynn and Pieklik [16]. Also, as later experiments revealed that pyruvate recovery was also lowered somewhat variably by florisil treatment, only untreated samples were used for the assessment of cytoplasmic redox status. In all experiments acetaldehyde, pyruvate, acetoacetate and adenine nucleotides were determined using only the freshly prepared supernatants but, in some experiments, samples of the supernatant were frozen and kept at −20° for up to 4 days before being used for the analysis of lactate, 3-hydroxybutyrate, ethanol, acetate and phosphate. There was no evidence that such treatment affected the level of these metabolites in the extract.

Analyses. Ethanol, lactate, acetate and ATP were determined by enzymatic methods adapted for the centrifugal analyser [8, 28, 29]. 3-Hydroxybutyrate was measured in the same way as lactate except that 3-hydroxybutyrate dehydrogenase replaced lactate dehydrogenase in the reaction mixture. For the determination of acetaldehyde, pyruvate and acetoacetate it was necessary to employ relatively larger samples than was possible using the centrifugal analyser and so these metabolites were measured using a Varian DMS 90 spectrophotometer. The reaction mixture for acetaldehyde determinations was that used previously [28]. Pyruvate was estimated either alone or serially with ADP using triethanolamine as buffer [30] while acetoacetate was measured by the method of Mellanby and Williamson [31]. Inorganic phosphate was measured as described by Ames [32].

Assay of acetyl CoA synthetase. Livers from control and T3-treated rats were homogenised in a Potter Elvehjem Teflon/glass homogeniser with 4 volumes of 0.25 M sucrose containing 10 mM Tris buffer, pH 7.4, and 1 mM EDTA. To obtain the soluble fraction, in which acetyl CoA synthetase (Acetate: Coenzyme A ligase EC 6.2.1.1) is reported mainly to reside [33], homogenates were centrifuged at 40,000 *g* for 45 min. Enzyme activity in the supernatant fluid was assayed using a CentrifiChem centrifugal analyser operating in kinetic mode at 37°. The procedure was based on that used for the determination of acetate [34]. The reaction mixture comprised 60 mM Tris buffer, pH 8.0, 30 mM KCl, 3 mM MgCl, 0.2 mM 2-mercaptoethanol, 4 mM Na acetate, 2 mM ATP, 0.05 mM NADH, 0.7 mM NAD, 0.18 mM coenzyme A, 0.4 mM L-malate, 0.1 mg defatted bovine serum albumin/ml, 15 units of malate dehydrogenase/ml and 0.7 units of citrate synthase/ml. The reaction was started by adding liver extract and readings of absorbance at 340 nm were taken at 30 sec intervals for up to 5 min. Blank runs were performed with systems from which coenzyme A was omitted and the difference between the rates of NADH formation in test and control runs was taken to represent the rate at which malate was oxidised to oxaloacetate for condensation with acetyl CoA generated in the system. Use of blanks from which acetate, rather than coenzyme A, was omitted gave identical results. Enzyme activity was expressed as μmol/min per g liver, assuming that 5 ml of extract were equivalent to 1 g of liver.

RESULTS

Table 1 shows the levels of various metabolites in the livers of control and T3-treated rats 2 hr after the administration of ethanol or saline. In rats receiving saline alone the ethanol and acetaldehyde levels were, predictably, negligible and acetate was also low. In rats dosed with ethanol there was no difference between the ethanol levels in control and T3-treated animals though acetaldehyde was higher

Table 1. Effect of ethanol on metabolite levels in livers of control and hyperthyroid rats

Metabolite	Amount present in liver (μmol/g wet weight) Control saline	Control ethanol	T3-treated saline	T3-treated ethanol
Ethanol	<0.1 (9)	21.5 ± 1.5 (13)*	<0.1 (9)	22.7 ± 1.0 (12)*
Acetaldehyde	0.002 ± 0.002 (9)	0.073 ± 0.003 (13)*	0.004 ± 0.001 (9)	0.095 ± 0.006 (12)*†
Acetate	0.174 ± 0.031 (9)	1.206 ± 0.055 (11)*	0.135 ± 0.027 (9)	1.367 ± 0.055 (10)*
Lactate	1.512 ± 0.163 (9)	0.740 ± 0.077 (13)*	2.188 ± 0.293 (9)	1.108 ± 0.163 (12)*
Pyruvate	0.052 ± 0.006 (9)	0.021 ± 0.006 (10)*	0.062 ± 0.009 (9)	0.014 ± 0.002 (10)*
3-Hydroxybutyrate	0.145 ± 0.034 (9)	0.186 ± 0.024 (13)	0.194 ± 0.043 (9)	1.004 ± 0.071 (12)*†
Acetoacetate	0.044 ± 0.006 (4)	0.045 ± 0.006 (3)	0.052 ± 0.010 (4)	0.070 ± 0.004 (4)*†
ATP	1.867 ± 0.150 (4)	1.757 ± 0.079 (4)	1.318 ± 0.100 (4)†	1.128 ± 0.075 (4)†
ADP	1.306 ± 0.060 (4)	1.648 ± 0.044 (4)*	1.619 ± 0.072 (4)†	1.881 ± 0.061 (4)*†
Pi	4.986 ± 0.200 (4)	5.389 ± 0.150 (4)	6.562 ± 0.260 (4)†	7.722 ± 0.230 (4)*†
ATP/(ADP × Pi)	0.293 ± 0.035 (4)	0.202 ± 0.024 (4)	0.128 ± 0.020 (4)†	0.079 ± 0.007 (4)†

Control and T3-treated rats were injected intraperitoneally with ethanol (2.5 g/kg body weight) in 0.154 M NaCl or with saline alone as described in Materials and Methods. Two hours after being injected the rats were killed, their livers quickly removed, freeze-clamped and immersed in liquid nitrogen. Each frozen liver was then pulverized and a portion of the powdered tissue was extracted by homogenization with 4 volumes of 0.6 M perchloric acid followed by centrifugation. The acid extract was neutralized with 20% (w/v) KOH and samples were used for the determination of ATP, ADP and inorganic phosphate. The remaining neutral supernatant was treated with florisil (0.1 g/ml) and was used for the determination of the other metabolites. Results are presented as mean and S.E.M. values for the number of separate experiments shown in parenthesis.

* Significantly different from rats receiving saline; $P < 0.05$.

† Significantly different from control rats; $P < 0.05$.

in the hyperthyroid group. However, when thiourea was used to prevent artefactual formation of acetaldehyde from ethanol during the processing of the liver tissue, the measured acetaldehyde levels were considerably lower and the slight elevation produced by hyperthyroidism (0.050 ± 0.006 μmol/g liver as compared to 0.030 ± 0.008 μmol/g liver; 4 results in each case) was not statistically significant. Acetate was raised markedly and to a similar extent in both groups while the levels of both lactate and pyruvate fell. Acetoacetate varied little between the different groups but hyperthyroid rats dosed with ethanol exhibited slightly higher levels. On the other hand, marked differences were observed in the way ethanol affected the 3-hydroxybutyrate concentration. In control animals, treatment with ethanol had little or no effect on 3-hydroxybutyrate but it caused a marked elevation of this metabolite in the T3-treated animals. If it were to be assumed that the additional 3-hydroxybutyrate was all formed from ethanol-derived acetate, then 3-hydroxybutyrate, rather than acetate, constituted the major end-product of hepatic ethanol metabolism in hyperthyroid rats.

Data in Table 1 also show that hepatic ATP was lower in T3-treated rats while ADP and inorganic phosphate levels were raised. Although ethanol caused no significant change in hepatic ATP in either group, it elicited a rise in ADP in both groups and a significant increase in inorganic phosphate in the hyperthyroid group. These changes in the concentrations of adenine nucleotides and inorganic phosphate meant that the phosphorylation potential

Table 2. Effect of ethanol and hyperthyroidism on hepatic redox status

Cell compartment	Control saline	Control ethanol	T3-treated saline	T3-treated ethanol
Cytoplasm				
Lactate/Pyruvate	11.9 ± 0.4 (4)	38.9 ± 6.2 (4)*	16.8 ± 4.9 (4)	17.5 ± 2.6 (4)†
$NAD^+/NADH$	761 ± 26 (4)	253 ± 44 (4)*	648 ± 131 (4)	564 ± 112 (4)†
Mitochondria				
3-OH butyrate/acetoacetate	2.0 ± 0.5 (4)	3.2 ± 0.9 (3)	2.6 ± 0.5 (4)	12.9 ± 1.2 (4)*†
$NAD^+/NADH$	11.3 ± 1.8 (4)	7.6 ± 2.3 (3)	8.6 ± 1.4 (4)	1.6 ± 0.1 (4)*†

Control and T3-treated rats were given injections of saline or ethanol, and liver extracts were prepared as described in Table 1. Pyruvate and lactate were determined in extracts not treated with florisil whereas 3-hydroxybutyrate and acetoacetate measurements were made using florisil-treated extracts. For the calculation of cytoplasmic and mitochondrial $NAD^+/NADH$ ratios, it was assumed that the equilibrium constants, at pH 7.0 and 38°, were 1.11×10^{-4} for the oxidation of lactate by lactate dehydrogenase and 4.93×10^{-4} for the oxidation of 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase (see ref. 25). Results are presented as mean and S.E.M. values for the number of separate determinations shown in parentheses.

* Within group—significantly different from saline; $P < 0.05$.

† Between groups—significantly different from euthyroid controls; $P < 0.05$.

([ATP]/[ADP][Pi]) in hyperthyroid rats was considerably lower than in the euthyroid animals, especially when ethanol was present.

Intrahepatic redox status was estimated by the conventional method which assumes that the lactate/pyruvate and 3-hydroxybutyrate/acetoacetate couples are at equilibrium with the NAD^+/NADH couple in cytoplasm and mitochondria respectively. The data presented in Table 2 are from experiments in which lactate and pyruvate were determined in freshly prepared liver extracts that were not treated with florisil. In T3-treated rats ethanol caused a pronounced fall in the NAD^+/NADH ratio in mitochondria but had little effect in the cytoplasm, whereas in control animals, ethanol had little or no effect on the mitochondrial NAD^+/NADH ratio but induced a significant fall in the cytoplasmic NAD^+/NADH ratio.

In order to ascertain whether or not the increased production of ketone bodies during ethanol metabolism in T3-treated rats was due to an induction of hepatic acetyl CoA synthetase, the first enzyme of the metabolic pathway by which acetate is converted to acetoacetate, livers of control and treated rats were assayed for this enzyme. It was found that cytoplasmic acetyl CoA synthetase in T3-treated rats (2.38 ± 0.36 μmol/min per g liver; 3 expts) was the same as that in control rats (2.22 ± 0.19 μmol/min per g liver; 4 expts) and it was therefore concluded that the increased ketogenesis was not attributable to an increased enzymic capacity for acetyl CoA synthesis.

DISCUSSION

Reports that, in rats, experimental hyperthyroidism strongly depresses the activity of both alcohol dehydrogenase [1–8] and aldehyde dehydrogenase [8] without lowering either the rate at which ethanol is eliminated [1–4, 8] or the predominance of the alcohol dehydrogenase pathway [8] cast doubt on claims that the concentration of either alcohol dehydrogenase [35–37] or aldehyde dehydrogenase [28, 38] is the principal control parameter for hepatic ethanol metabolism. The same doubt is raised by the work reported here, for although the hepatic acetaldehyde level in hyperthyroid rats was slightly higher than in controls, probably reflecting a lowered aldehyde dehydrogenase activity, the hepatic ethanol level in T3-treated rats 2 hr after administration of ethanol was not significantly different from that in control rats, suggesting that the rate of ethanol elimination was the same in both groups and confirming earlier results from this laboratory [8].

Interpreted conventionally, the failure of ethanol to cause a significant increase in the lactate/pyruvate ratio in T3-treated rats could be taken to provide tentative support for the idea [2, 3] that, in hyperthyroidism, increased oxidative activity leads to a rise in the cytoplasmic NAD^+/NADH ratio thereby creating a more oxidised environment for the residual alcohol dehydrogenase and leading to an increase in its catalytic activity. By the same token, and somewhat in contradiction, the large increase in the 3-hydroxybutyrate/acetoacetate ratio in T3-treated rats following administration of ethanol would suggest a more reduced environment for the residual aldehyde dehydrogenase, which would not be conducive to increased catalytic efficiency.

However, the assumptions upon which interpretations such as these are based, namely that the lactate/pyruvate couple and the 3-hydroxybutyrate/acetoacetate couple are in equilibrium with, respectively, a single cytoplasmic pool of NAD(H) and a single mitochondrial pool of NAD(H), have been questioned by Berry [39]. In an argument based on observed differential effects of various inhibitors on a number of different redox couples, he proposed that the redox reaction involving the lactate/pyruvate couple takes part in an energy-dependent redox cycle that draws upon different pools of NAD(H) within the cytoplasm. If this proposition is sustainable, then it cannot be assumed that lactate dehydrogenase and alcohol dehydrogenase depend entirely on the same pool of NAD(H) or that the poise of the lactate/pyruvate couple accurately depicts the redox state of the alcohol dehydrogenase environment. A similar argument might also apply to the relationship between the 3-hydroxybutyrate/acetoacetate ratio and the redox state of the aldehyde dehydrogenase environment. It is worth noting that both Guynn and Pieklik [16] and Braggins and Crow [35] have emphasized their observation that faster rates of ethanol oxidation are associated with higher lactate/pyruvate ratios, a relationship which is apparently inconsistent with the frequent claim that alcohol dehydrogenase is tightly controlled by NADH *in vivo*. It therefore seems reasonable to ask whether commonly used indicators of subcellular redox status can be validly applied in all circumstances or whether microcompartmentation of redox enzymes and cofactors renders them less meaningful.

A further point of interest is that, in the present study, ethanol caused the hepatic levels of both pyruvate and lactate to fall in the two groups of animals. Though a lowering of the pyruvate concentration in freeze-clamped liver from rats metabolizing ethanol has been reported previously [3, 15, 40], a fall in lactate has not been observed by other workers who have found either no change [3, 40] or a moderate to large rise [15, 16] in hepatic lactate following the administration of ethanol. In consequence, the rise in the lactate/pyruvate ratio seen in the current work was not as great as that found by others. The explanation for these different observations is uncertain though it could possibly be related to the fact that only well-fed, unanaesthetized female rats were used whereas in the other studies male rats were used and, in some cases, were either fasted [16] or were anaesthetized for the course of the experiment [15]. It is reasonably well established that, in rats, both gender [41, 42] and nutritional status [40, 42, 43] influence ethanol oxidation rates and related metabolic parameters.

While the results of the present work fail to resolve the argument about whether hepatic ethanol metabolism is governed primarily by alcohol dehydrogenase concentration or by the NAD^+/NADH ratio, an argument which might, in any case, be thought sterile in the light of more integrated theories of metabolic control [44, 45], some observations, particularly the marked increase in hepatic 3-hydroxy-

butyrate in hyperthyroid rats dosed with ethanol, merit consideration within the wider context of the total metabolic activity of the liver and especially in relation to energy balance.

It has been argued with conviction for over four decades [46, 47] that metabolic flux through the various anabolic and catabolic pathways of an organism is regulated so as to preserve an energy balance. If this is accepted as a reasonable general proposition, then it may be useful to consider how the energy yield from ethanol oxidation could vary under conditions in which ethanol is the major oxidisable substrate presented to the liver, as is common in both experimental and natural situations in which well over half the oxygen used by the liver is employed in ethanol oxidation [10]. Halperin *et al.* [21] calculated that if ethanol were to be completely oxidised to CO_2 and H_2O, the theoretical yield of ATP (16 mol/mol ethanol) would be over six times greater than if it were to be oxidised to 3-hydroxybutyrate (2.5 mol/mol ethanol) while its oxidation to acetate or acetoacetate would give intermediate yields of 6 and 4 mol ATP/mol ethanol respectively. Such differences could clearly have important implications for the energy status of the liver and, therefore, for the amount of ethanol that could be metabolised to the different end-products.

There is ample evidence to show that in normal, well-fed, euthyroid rats very little ethanol is completely oxidised in the liver and that the principal end-product of hepatic ethanol metabolism is acetate [9–12]. However, the present study shows that in hyperthyroidism there is a strong shift towards the production of 3-hydroxybutyrate as the principal end-product. What causes this shift is not known at this stage though the increased ketonaemia sometimes associated with clinical hyperthyroidism has been attributed to a beta-adrenergic mechanism [24] while the more rapid conversion of oleate to ketone bodies seen in isolated perfused livers from T3-treated rats has been attributed to an increase in the rate at which oleoyl-CoA is transported into the mitochondria [23]. Whether this latter proposed mechanism is relevant to ketogenesis from ethanol is, however, questionable. Although rat liver acetyl-CoA synthetase is primarily a cytoplasmic enzyme [33, 48], approximately one-fifth of the activity is mitochondrial [48] so it is possible that ethanol-derived acetate might be activated within the mitochondria where it is first formed. Certainly, the present study showed no increase in cytoplasmic acetyl-CoA synthetase in T3-treated rats.

Whatever the mechanism involved in the switch towards 3-hydroxy butyrate as the chief end-product of hepatic ethanol metabolism in hyperthyroid rats, the fact that such a switch occurred might help to explain why the hepatic ATP level in T3-treated rats following ethanol administration was so much lower than in euthyroid controls in which acetate was the principal end-product. At the same time, it should be noted that ATP levels were depressed in hyperthyroid rats even when ethanol was not administered, so the effect appears to be due primarily to the hyperthyroid state *per se* and not to differences in the way ethanol is metabolised. As well as affecting ATP levels, treatment with T3 led to rises in hepatic ADP and inorganic phosphate, thereby causing the adenine nucleotide phosphorylation potential to fall to a low level in hyperthyroid rats. This fall was exacerbated by the administration of ethanol which caused the ADP and inorganic phosphate levels to rise still further. Although it is reasonably well established that hyperthyroidism lowers the phosphorylation potential [49, 50], not through an uncoupling action as was first proposed [51] but more likely through stimulation of ATP hydrolysis via the Na^+-K^+ ATPase [2, 52] or, perhaps, through an increase in the rate of energy-dependent redox cycle activity [39], the further effect of ethanol has not been noted before. A fall in the phosphorylation potential has been found in rats following chronic ethanol treatment [53] but a single dose of ethanol such as that used in the present study was previously reported to be ineffective in this respect [16]. The results of the present study suggest that, in hyperthyroid rats, even a single dose of ethanol augments the effect of thyroid hormones though whether the two act through the same agency is not known.

This study raises a number of questions concerning the relationships between thyroid status, ethanol metabolism, ketogenesis and general energy metabolism. The work might have implications for still unresolved discrepancies between reports concerning the effects of thyroid hormones on ethanol elimination in humans [54–56] as well as for clinical conditions such as alcoholic ketoacidosis. Investigations are therefore continuing in an attempt to gain a greater understanding of the nature of some of these relationships.

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