Transfer of deuterium from [1,1-²H₂]ethanol to steroids and organic acids in the rat testis

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Rats were given [1,1-²H₂]ethanol in a single dose, and the ²H content was determined in testicular steroids and in organic acids of low molecular mass in the testis, liver and blood. The acids were quantified by capillary gas chromatography/mass spectrometry of *t*-butyldimethylsilyl derivatives with [²H₄]lactate as internal standard. In addition to lactate, pyruvate, 3-hydroxybutyrate and acids of the tricarboxylic acid cycle, the testis was shown to contain 2-hydroxybutyrate, 2-hydroxy-2-methylbutyrate, 2-hydroxyisohexanoate and glycerate. No ²H was found in pregnenolone, 5-androstene-3 β ,17 β -diol or testosterone, whereas the abundance of monodeuterated molecules of 5 α -androstane-3 α ,17 β -diol and its 3 β -isomer were 7.6% and 11.2% respectively. The abundance of monodeuterated lactate was 7.0% in the testis and 5.3% in the blood. The other acids were less labelled but 3-hydroxybutyrate had a higher ²H content in the testis (3.1%) than in the liver. These results support the contention that ethanol is oxidized in an alcohol dehydrogenase-catalysed reaction in testis *in vivo* and that the acute inhibition of the testosterone production is due at least partly to a redox effect. The labelling and increased concentration of 3-hydroxybutyrate in the testis indicate that a change in the mitochondrial redox state might be involved.

INTRODUCTION

The mechanism(s) behind the ethanol-induced inhibition of testosterone production in rats is not fully understood. Different mechanisms seem to be involved depending on the dose and duration of administration of ethanol *in vivo* and on the experimental conditions *in vitro* [1–8]. The relevance of the findings *in vitro* for the conditions *in vivo* is often difficult to evaluate. One possibility is that ethanol inhibits the formation of testosterone by increasing the NADH/NAD⁺ ratio [4,9–14]. This is based mainly on indirect evidence from experiments *in vitro* [9,10,12–14] but the relevance for the mechanism(s) *in vivo* is supported by the increase in the ratios between 3 β -hydroxy-5-ene and 3-oxo-4-ene steroids in the testis of rats after acute administration of ethanol [4]. An analogous increase in the ratio of 5 α -androstane-3 β ,17 β -diol to dihydrotestosterone was also observed which may be related to the increased reduction of dihydrotestosterone to 5 α -androstane-3 β ,17 β -diol induced by ethanol in rat Leydig cells [15].

The concentration of alcohol dehydrogenase in the rat testis is about 10% of that in the liver [16] and the enzyme is localized largely in the Leydig cells [17]. The extent to which local ethanol oxidation may contribute to redox changes affecting steroid biosynthesis *in vivo* is not known. We previously studied redox interaction between the metabolism of ethanol and steroids with the aid of [1,1-²H₂]ethanol [11]. If local ethanol oxidation is sufficiently rapid to change the redox state of the NAD system in the testis, ²H from [1,1-²H₂]ethanol would be incorporated into steroids and hydroxy acids formed in reductions by NADH, as is the case in the liver [18–22]. On the other hand, deuterated hydroxy acids may be transported in blood from the liver and label steroids in the testes. This paper reports the results of analyses of the deuterium incorporation into selected steroids [4], organic acids from the citric acid cycle and other hydroxy acids [23,24] in the testis and into the acids in liver and blood of rats given the labelled ethanol. In addition to providing information on the transfer of deuterium from ethanol to steroids and organic acids, the analyses have allowed the identification of several hydroxy acids in the testis.

MATERIALS AND METHODS

Materials

The organic solvents were of analytical-reagent grade from E. Merck (Darmstadt, Germany) and were dried and distilled before use. Organic acids and salts were also obtained from E. Merck, except for calcium L-lactate (Kebo, Stockholm, Sweden), citric acid (Riedel-de-Haen, Seelze-Hannover, Germany), fumaric acid and 3-hydroxybutyric acid (Fluka, Buchs, Switzerland). [1,5-¹⁴C]Citric acid, sodium D-3-hydroxy[3-¹⁴C]butyrate and sodium L-[U-¹⁴C]lactate were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Sodium DL-[²H₄]lactate (99.5% ²H) was from MSD Isotopes (Montreal, Quebec, Canada) and [1,1-²H₂]ethanol (99.6% ²H) from Alfred Hempel G.m.b.H. (Düsseldorf, Germany).

Tetrabutylammonium bromide obtained from Eastman Kodak (Rochester, NY, U.S.A.) and hydroxylamine hydrochloride from E. Merck were recrystallized in toluene and methanol respectively. The silylating mixture consisted of 0.5 M *t*-butyldimethylchlorosilane (Janssen Chimica, Beerse, Belgium) and 1.25 M-imidazole (E. Merck, recrystallized from benzene) in pyridine [23].

Radioactivity was measured in duplicate with a liquid-scintillation counter using Quickszint 2000 from Zinsser Analytic (Maidenhead, Berks, U.K.).

Animal experiments

Male Sprague–Dawley rats weighing 370–390 g were used. They were kept in a light- and temperature-controlled room and had free access to food pellets (R3, Ewos, Södertälje, Sweden) and water until the start of the experiments at 09.00–09.30 h. They were injected intraperitoneally with 4.5 ml of 10% (w/v) [1,1-²H₂]ethanol in 0.9% (w/v) NaCl solution or 4.5 ml of 0.9% NaCl solution (controls). After 1 h they were anaesthetized with diethyl ether, blood was collected from the tail vein, and testis and liver samples were quickly removed and frozen in liquid nitrogen. The latter were immediately pulverized in liquid nitrogen when acids were to be analysed, and testes were kept at –70 °C for steroid analysis.

Isolation and derivatization of steroids

One testis from each rat was extracted using a combination of liquid and solid extraction as described previously [4,25]. The steroid extract was then fractionated by straight-phase h.p.l.c. before formation of *t*-butyldimethylsilyl (tBDMS) or enol-tBDMS derivatives, which were then purified by reversed-phase h.p.l.c. [25].

Protein content

The protein content was measured after precipitation with perchloric acid. The pellet was dissolved in NaOH (1 M) at 90–100 °C and the protein content was determined as described by Lowry *et al.* [26]. The concentrations were 20.8 ± 1.7 mg/100 μ l of blood, 80.5 ± 8.7 mg/g of testis and 200.5 ± 8.9 mg/g of liver.

Isolation and derivatization of carboxylic acids

Lactate, pyruvate, 3-hydroxybutyrate, succinate, fumarate, malate, 2-oxoglutarate and citrate were isolated from liver and testis, essentially as reported earlier [23]. The tissues were pulverized in liquid nitrogen together with 5 ml of 0.6 M-HClO₄ containing 10 mg of hydroxylamine hydrochloride. [³H₄]Lactate (24 or 48 μ g) was added to the tissue samples as an internal standard for quantification by gas chromatography (g.c.)–m.s. and [1,5-¹⁴C]citric acid (about 0.04 μ g) was added to monitor separation of the carboxylic acids from phosphate by chromatography on Sephadex G-25, 20–80 μ m (Pharmacia, Uppsala, Sweden). The tBDMS derivatives were prepared as previously described [23].

Lactate and 3-hydroxybutyrate were isolated from blood (100 μ l) by a slightly modified method [24]. [³H₄]Lactate (11 μ g) was added as an internal standard and either [U-¹⁴C]lactate or 3-hydroxy[3-¹⁴C]butyrate was added to monitor the recovery. The carboxylic acids were isolated from the deproteinized blood samples by anion-exchange chromatography on 1 g of DEAE-Sephadex A-25 (Pharmacia) before derivatization with 0.5 ml of the silylating mixture (diluted 1:1 in pyridine).

The recovery of lactate was $72.1 \pm 6.7\%$ (mean $\pm$ S.D., $n = 11$) and that of 3-hydroxybutyrate $71.4 \pm 9.8\%$ ($n = 10$), after derivatization.

Gas chromatography of tBDMS derivatives of carboxylic acids

A fused-silica capillary column (25 m $\times$ 0.32 mm internal diameter) with a 0.25 μ m layer of chemically bonded poly(dimethylsiloxane) phase (Quadrex Corp., New Haven, CT, U.S.A.) was used with an on-column injection system. The column temperature was 140 °C for 12 min and was then raised to 260 °C at 4 °C/min. The flame ionization detector temperature was 300 °C.

Gas chromatography/mass spectrometry

Steroids were analysed by selected ion-recording g.c.–m.s. by using a VG 7070E double-focusing mass spectrometer (VG Analytical, Manchester, U.K.) with the Quadrex column described above and an all-glass falling-needle injection system. The molecular ions or [$M - 57$]⁺ were monitored using a hardware unit for selected ion recording [25].

The g.c./m.s. analysis of oxime-tBDMS derivatives of carboxylic acids was performed on a Finnigan 4000 instrument equipped with a fused-silica capillary column (30 m $\times$ 0.32 mm internal diameter) having a 0.25 μ m layer of chemically bonded poly(dimethylsiloxane) phase, SPB-1 (Supelco Inc., Bellefonte, PA, U.S.A.). The column temperature was 140 °C for 12 min and was then raised to 260 °C at 4 °C/min. Mass spectra were recorded at an electron energy of 70 eV.

The relative abundances of molecules having 0–1 ³H atoms (steroids), 0–4 ³H atoms (lactate) or 0–2 ³H atoms (other acids) were calculated by comparison with spectra of reference compounds [27]. The intense ions at $M - 57$ contained all original hydrogen atoms in the organic acids [23] and were used in the isotope analyses. Authentic carboxylic acids and steroids in testes of untreated rats were used as reference compounds.

RESULTS

Identification and quantification of carboxylic acids in liver, blood and testis

The method, previously developed for the analysis of carboxylic acids in liver, stomach and pancreas [23], was successfully applied to the analysis of testis (Fig. 1).

The mass spectra of the tBDMS derivatives of hydroxy acids (Table 1) agreed with the fragmentation patterns previously described [28,29]. All tBDMS derivatives were dominated by the [$M - 57$] fragment ion typical for tBDMS derivatives, or m/z 147, derived from the tBDMS function. Other typical ions are m/z 189, 133, 75 and 73 also derived from the tBDMS function [29]. The molecular ions were not detected and [$M - 15$] ions had a low relative intensity (1%). The 2-substituted acids gave ions at $M - 57 - 28$ in the mass spectra of 2-hydroxybutyrate (m/z 247), 2-hydroxy-2-methylbutyrate (m/z 261) and 2-hydroxyisohexanoate (m/z 275) by loss of C(CH₃)₃ and CO [28]. The ion at m/z 231 in the tBDMS derivative of glycerate is probably due to loss of C(CH₃)₃ and tBDMSCOOH as suggested for hydroxylated open-chain carboxylic acids [28]. Fragment ions originating from α -cleavage of hydroxylated open-chain carboxylic acids were given by the tBDMS derivatives of 2-hydroxy-2-methylbutyrate (m/z 187, 4% relative intensity). The identification of the acids was based on retention times and mass spectra as compared with those of reference compounds. The latter were also added to testis samples to confirm recoveries and identity of retention times. In addition to lactate, pyruvate, 3-hydroxybutyrate and citric-acid-cycle acids, significant amounts of 2-hydroxybutyrate, 2-hydroxy-2-methylbutyrate, 2-hydroxyisohexanoate and glycerate were identified in the testis (Fig. 1). These acids have been found in analyses of metabolic profiles in urine or plasma from humans [30].

The concentrations of acids in testis, liver and blood were determined by g.c.–m.s. with an internal standard ([³H₄]lactate) and values for the concentrations in control and ethanol-treated rats are given in Table 2. The concentrations of 3-hydroxybutyrate both in blood and testis were elevated after ethanol administration ($P < 0.05$). Lactate/pyruvate ratios could not be accurately calculated owing to low and variable recoveries of pyruvate [23].

³H incorporation into carboxylic acids

The isotopic compositions of the carboxylic acids in blood, testis and liver were determined by g.c.–m.s. (Table 3). Lactate, 3-hydroxybutyrate, citrate, succinate, fumarate and malate were all labelled in samples of liver and testis but no ³H atoms were found in pyruvate and 2-oxoglutarate. The content of di-deuterated molecules was low and could not be accurately determined. The isotopic compositions of lactate and 3-hydroxybutyrate in blood were determined and both compounds were labelled.

The percentages of monodeuterated fumarate and malate molecules in the liver were $13.7 \pm 3.0\%$ and $16.0 \pm 3.5\%$ (mean $\pm$ S.D., $n = 6$), respectively (Table 3). If it is assumed that the probabilities are the same for labelling of each of the α -

Fig. 1. Gas-chromatographic analysis of oxime-tBDMS derivatives of carboxylic acids in testis from rat

A fused-silica capillary column with a chemically bonded poly(dimethylsiloxane) phase was used with a falling-needle injection system. The oximes were prepared and the carboxylic acids were isolated and analysed as tBDMS derivatives. The initial column temperature was 140 °C for 12 min and then raised to 260 °C at 4 °C/min. The derivatives of the following compounds were identified: lactate (1), 2-hydroxybutyrate (2), pyruvate (3), 3-hydroxybutyrate (4), 2-hydroxy-2-methylbutyrate (5), 2-hydroxyisohexanoate (6), succinate (7), fumarate (8), phosphate (9), glycerate (10), malate (11), 2-oxoglutarate (12) and citrate (13).

Table 1. Mass spectra of tBDMS derivatives of carboxylic acids

Compound	Principal ions m/z (relative intensity)							Others > m/z 70 more than 10 % relative intensity
	$M-15$	$M-57$	189	147	133	75	73	
2-Hydroxybutyrate	317 (1)	275 (100)	(8)	(99)	(11)	—	(31)	247 (26)
2-Hydroxy-2-methylbutyrate	331 (1)	289 (100)	(5)	(86)	(9)	(6)	(25)	261 (33)
2-Hydroxyisohexanoate	345 (1)	303 (81)	(8)	(100)	(11)	(9)	(34)	275 (40)
Glycerate	433 (1)	391 (31)	(4)	(100)	(26)	(9)	(86)	231 (77)

hydrogens in fumarate, and the hydrogen at C-2 and one of those at C-3 in malate, this corresponds to an ^2H excess of 7.5 % and 8.8 % in each position of fumarate and malate respectively. The corresponding values for the acids in testis were 1.7 % and 2.3 % respectively. Lactate in the liver was significantly more labelled than that in blood and testis ($P < 0.002$ and $P < 0.001$, respectively) which had similar ^2H contents.

Incorporation of ^2H into steroids

When $[1,1-^2\text{H}_2]$ ethanol was given to the rats, ^2H was incorporated into androstane diols in the testis (Table 4). Other steroids with a 17β -hydroxy group, i.e. testosterone and 5-androstene- $3\beta,17\beta$ -diol, and pregnenolone with a 3β -hydroxy-5-ene structure did not become labelled.

DISCUSSION

Incorporation of ^2H into steroids

We have previously shown that acute administration of ethanol to rats increases the ratio between 5 α -androstane- $3\beta,17\beta$ -diol

and dihydrotestosterone in the testis [4]. In isolated Leydig cells, ethanol increases the reduction of dihydrotestosterone, mainly into 5 α -androstane- $3\beta,17\beta$ -diol [15], whereas in liver homogenates the formation of both the 3 α - and 3 β -epimer is increased [31]. In the present experiments ^2H was incorporated into both epimers, the 3 β -epimer showing the highest degree of labelling (Table 4). This shows that NADH labelled by the metabolism of $[1,1-^2\text{H}_2]$ ethanol participates in the steroid reduction. The low concentrations of steroid did not permit a determination of the position of labelling. The short duration of the experiment and the low labelling of NADPH in the liver exclude a detectable incorporation into the steroid skeleton via cholesterol synthesis [32,33]. Thus C-3 and C-17 are the only potential positions carrying a ^2H . The lack of labelling of the 17α position of 5-androstene- $3\beta,17\beta$ -diol indicates that the ^2H in the analogous androstane diols is attached at C-3. Furthermore, the reduction of 3-oxosteroids in the liver during metabolism of $[1,1-^2\text{H}_2]$ ethanol yields epimeric 3-hydroxysteroids with a deuterium excess of about 10–20 atom % in the 3 α -epimer and 5–10 % in the 3 β -epimer [19,20], and an NADPH-dependent reduction of the 17-oxo group is not observed in male rats [20].

Table 2. Quantification of carboxylic acids in liver, blood and testis

[1,1-²H₂]Ethanol (1.2 g/kg) or NaCl solution (to control rats) was given intraperitoneally. The amount of each carboxylic acid per mg of protein is given as the mean $\pm$ s.d. and the number of rat experiments is in parentheses.

	Liver		Blood		Testis	
	(μ g/mg of protein)	(μ mol/g)	(μ g/mg of protein)	(mm)	(μ g/mg of protein)	(μ mol/g)
Lactate						
Control	0.48 $\pm$ 0.20 (4)	1.07	0.42 $\pm$ 0.05 (3)	0.97	0.54 $\pm$ 0.17 (4)	0.48
Ethanol	0.37 $\pm$ 0.18 (7)	0.82	0.36 $\pm$ 0.11 (4)	0.83	0.63 $\pm$ 0.11 (7)	0.56
Pyruvate						
Control	0.07 $\pm$ 0.04 (4)	0.15	—		0.03 $\pm$ 0.01 (3)	0.03
Ethanol	0.04 $\pm$ 0.03 (3)	0.10	—		0.02 $\pm$ 0.01 (4)	0.02
3-Hydroxybutyrate						
Control	0.12 $\pm$ 0.03 (4)	0.23	0.05 $\pm$ 0.004 (3)	0.09	0.02 $\pm$ 0.01 (4)	0.02
Ethanol	0.22 $\pm$ 0.11 (7)	0.42	0.17 $\pm$ 0.08 (5)	0.34	0.06 $\pm$ 0.02 (7)	0.04
Citrate						
Control	0.18 $\pm$ 0.11 (4)	0.19			0.51 $\pm$ 0.30 (4)	0.21
Ethanol	0.10 $\pm$ 0.05 (6)	0.10			0.65 $\pm$ 0.19 (6)	0.27
2-Oxoglutarate						
Control	0.08 $\pm$ 0.03 (4)	0.10			0.26 $\pm$ 0.23 (3)	0.14
Ethanol	0.03 $\pm$ 0.01 (6)	0.05			0.25 $\pm$ 0.09 (6)	0.14
Succinate						
Control	0.12 $\pm$ 0.05 (3)	0.21			0.07 $\pm$ 0.04 (4)	0.05
Ethanol	0.11 $\pm$ 0.03 (6)	0.19			0.11 $\pm$ 0.03 (7)	0.07
Fumarate						
Control	0.04 $\pm$ 0.02 (3)	0.07			0.02 $\pm$ 0.01 (3)	0.01
Ethanol	0.04 $\pm$ 0.01 (5)	0.07			0.03 $\pm$ 0.02 (6)	0.02
Malate						
Control	0.19 $\pm$ 0.15 (4)	0.28			0.17 $\pm$ 0.15 (4)	0.10
Ethanol	0.21 $\pm$ 0.10 (7)	0.31			0.20 $\pm$ 0.07 (7)	0.12

Table 3. Abundance of monodeuterated fragment ions (*M*–57) in mass spectra of the oxime-tBDMS derivatives of carboxylic acids isolated from rats given [1,1-²H₂]ethanol

Values, which are percentages of monodeuterated molecules, are given as means $\pm$ s.d., and the number of rat experiments in each case is in parentheses.

	Liver	Blood	Testis
Lactate	14.2 $\pm$ 2.7 (5)	5.3 $\pm$ 0.8 (3)	7.0 $\pm$ 1.7 (7)*
Pyruvate	0		0
3-Hydroxybutyrate	2.2 $\pm$ 0.5 (7)	4.2 $\pm$ 0.6 (4)	3.1 $\pm$ 0.6 (7)†
Citrate	5.2 $\pm$ 1.2 (4)		3.2 $\pm$ 0.2 (6)
2-Oxoglutarate	0		0
Succinate	3.2 $\pm$ 1.4 (4)		0.6 $\pm$ 0.2 (7)
Fumarate	13.7 $\pm$ 3.0 (6)		3.4 $\pm$ 0.4 (6)†
Malate	16.0 $\pm$ 3.5 (6)		4.5 $\pm$ 0.4 (6)†

* $P < 0.05$, compared with values for liver in individual rats.

† $P < 0.01$, compared with values for liver in individual rats.

Theoretically, the deuterated androstanediols in testis may be formed locally, represent plasma steroids or accumulate by exchange with the latter. The mean concentrations of the 3 α - and 3 β -epimers are 0.28 and 0.22 ng/ml respectively in plasma [34], and 5.7 and 1.7 ng/g respectively in the testis [4]. These values and the low blood content of the testis (11.5 μ l/g of tissue [35]) exclude significant contamination by plasma steroids. The maximum contribution by exchange can be calculated from the concentrations [4,34] and the results of steady-state infusion experiments [36]. Infusion for 3 h of 17 β -hydroxy-5 α -[1,2-³H]-

Table 4. Incorporation of ²H into testicular steroids in rats given [1,1-²H₂]ethanol (1.2 g/kg body weight), 1 h before being killed

Results are presented as means $\pm$ s.d. ($n = 5$).

Steroid	Abundance of ² H ₁ molecules (%)
Pregnenolone	0
5-Androstene-3 β ,17 β -diol	0
Testosterone	0
5 α -Androstane-3 α ,17 β -diol	7.6 $\pm$ 0.4
5 α -Androstane-3 β ,17 β -diol	11.2 $\pm$ 1.3

androstan-3-one resulted in testis/plasma ratios of 0.43 for radioactive 5 α -androstane-3 α ,17 β -diol and 2.7 for radioactive 5 α -androstane-3 β ,17 β -diol. The corresponding values obtained after infusion of 5 α -[1,2-³H]androstane-3 α ,17 β -diol were 0.28 and 3.1 respectively. The values indicate that less than 2% of the pool of the 3 α -epimer in testis could have been exchanged for steroids in plasma. The apparent uptake of the 3 β -epimer from the blood was most likely due to formation in testis from the labelled 3-oxo- or 3 α -hydroxy-steroid, and it may be assumed that the uptake of the 3 β -epimer was approximately the same as that of the 3 α -epimer. If the circulating androstanediols were deuterated in the liver [20], less than 5% of the deuterated molecules in the testis could then have been supplied by exchange with plasma steroids. Thus the results indicate that the androstanediols became labelled in the testis.

The similar ²H contents of the two androstanediols in testis may seem unexpected in view of the preferential reduction to the

3 β -epimer during ethanol metabolism in isolated Leydig cells [15]. However, the degree of incorporation of ^2H does not necessarily reflect the rates of the reactions, since incorporation may take place even when the enzyme is saturated with reduced coenzyme when the rate is independent of the coenzyme concentration. Thus differences in the K_m for NADH of the 3 α - and 3 β -hydroxysteroid oxidoreductases might explain the differences in 3-hydroxy/3-oxo ratios [4] and degrees of labelling of the two androstenediol epimers.

The oxidoreductase catalysing the oxidation of pregnenolone is the same as that catalysing the reduction of dihydrotestosterone to the 5 α -androstane-3 β ,17 β -diol [37]. The absence of ^2H from pregnenolone can be explained by the slow reduction of 3-oxo-4-ene to 3 β -hydroxy-5-ene steroids [38,39]. Under physiological conditions the oxidation of pregnenolone is essentially irreversible but is inhibited by NADH (see ref. [39]), i.e. under conditions induced by ethanol oxidation [40]. Neither 5-androstene-3 β ,17 β -diol nor testosterone was labelled. This can be explained by the fact that the 17 β -hydroxysteroid oxidoreductase in the testis utilizes NADP rather than NAD as coenzyme [41]. NADPH is expected to have a lower isotope excess than NADH because of dilution in the transfer of ^2H from NADH to NADPH [21,33,42].

Comparison of the labelling of organic steroids and acids

Ethanol increases the lactate/pyruvate ratio in testis, reflecting an effect on the cytosolic redox state [40]. The marked increase in the 3-hydroxybutyrate concentration observed in the present study indicates an effect on the mitochondrial redox state, as recently demonstrated with isolated Leydig cells [13]. 3-Hydroxybutyrate had a higher isotope excess in the testis than in the liver (Table 3). This indicates that the increased concentration of 3-hydroxybutyrate is due to a redox effect of local ethanol metabolism. The intramitochondrial redox effect might be due to transfer of reducing equivalents from the cytosol or to oxidation of acetaldehyde in the mitochondria [43]. It has been proposed that such a change can be important for the inhibition of testosterone synthesis [13].

Lactate in testis was significantly labelled, but the difference between the labelling of lactate in blood and testis was small and the concentration of lactate was about the same in blood and testis. Thus it could not be determined whether the labelling of lactate in the testis was due to uptake of lactate from the blood or to labelling during metabolism of [1,1- $^2\text{H}_2$]ethanol in the testis. The labelling of the other acids in the testis was lower than that of lactate, and could therefore be due to transfer from lactate in the testis and to uptake from the blood. The labelling of 5 α -androstane-3 β ,17 β -diol was higher than that of lactate in the testis ($P < 0.001$). Thus it cannot be explained solely by uptake of lactate from the blood. Rather, it supports the contention that ethanol oxidation in the testis provides NADH which is used by the 3 β -hydroxysteroid oxidoreductase.

In conclusion, the results indicate that ethanol is oxidized by alcohol dehydrogenase in the testis *in vivo*. This can explain the increased ratio between 5 α -androstane-3 β ,17 β -diol and 17 β -hydroxy-5 α -androstane-3-one in testis after an acute dose of ethanol [4]. Since the same 3 β -hydroxysteroid dehydrogenase catalyses the oxidation of 3 β -hydroxy-5-ene to 3-oxo-4-ene steroids [37], which is inhibited by NADH [39], the oxidation of ethanol can also explain the increased ratio between such steroid couples in the testis *in vivo* [4,5]. This is most probably one of the mechanisms for acute inhibition of testosterone synthesis by ethanol. Whether it can explain the inhibition of testosterone production in human-choriogonadotropin-stimulated rats [3] remains to be established. Ethanol also inhibits the formation of

pregnenolone, most markedly during chronic ethanol feeding [5,14,44]. It is not known whether this is an indirect effect or also related to the oxidation of ethanol in the testis.

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