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CONTRIBUTION OF NON-ADH PATHWAYS TO ETHANOL OXIDATION IN HEPATOCYTES FROM FED AND HYPERTHYROID RATS

EFFECT OF FRUCTOSE AND XYLITOL

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Abstract—The metabolism of (1R)[1-^{3}H]ethanol, [2-^{3}H]lactate or [2-^{3}H]xylitol was studied in hepatocytes from fed or T_3-treated rats in the presence or absence of fructose or xylitol. The yields of tritium in ethanol, lactate, water, glycerol and glucose were determined.

A simple model, describing the metabolic fate of tritium from these substrates is presented. The model allows estimation of the ethanol oxidation rate by the non-alcohol dehydrogenase pathways from the relative yield of tritium in water and glucose. The calculations are based on a comparison of the fate of the 1-*pro*R-hydrogen of ethanol and the hydrogen bound to carbon 2 of lactate (or xylitol) under identical condition.

In our calculations we have taken into account that the reactions catalyzed by lactate dehydrogenase and alcohol dehydrogenase are reversible and that lactate or ethanol labelled during the metabolism of the other tritiated substrates will contribute to the tritium found in water.

The contribution of non-ADH pathways to ethanol oxidation varied from 10 to 50% and was correlated to changes in the lactate/pyruvate ratio from 80 to 500. In T_3-treated rats the activity of non-ADH pathways were greater than in fed rats for the same lactate/pyruvate ratio.

The quantitatively most important pathway for the oxidation of ethanol to acetaldehyde is the reaction catalyzed by the NAD^+ dependent alcohol dehydrogenase, which is localized in the cytoplasmic compartment of the liver cells. Besides the alcohol dehydrogenase (ADH) pathway, two other non-ADH pathways have been described [1], the pathway involving hydrogen peroxide and catalase and the microsomal ethanol-oxidizing system [MEOS] involving NADPH, cytochrome P-450 [1] and possibly NADH [2, 3].

The existence of non-ADH pathways for ethanol oxidation, MEOS and catalase reactions, is well established, but the measurements of the contribution to ethanol oxidation have relied largely on experiments with inhibitors. Our approach involves a comparison of the relative amount of labelling of glucose and water from (1R)[1-^{3}H]ethanol or L-[2-^{3}H]lactate, metabolized under identical conditions. In our calculations we have taken into account that the lactate dehydrogenase and the alcohol dehydrogenase reactions are reversible [6] and that lactate and ethanol labelled during the metabolism of other tritiated substrates will contribute to the tritium found in water.

Rognstad [7] and Havre *et al.* [8] used a similar approach, comparing the fate of the (1R)-hydrogen of ethanol with the fate of the hydrogen from a substrate oxidized only via a cytosolic NAD^+-dependent dehydrogenase; however, they have neglected the reversibility of reactions catalyzed by dehydrogenases [5].

We have used cells from fed and triiodothyronine (T_3)-treated rats and the contribution of non-ADH pathways to the ethanol oxidation was measured in hepatocytes in the presence and absence of xylitol or fructose. The T_3-treatment was used in an attempt to increase the utilization of reducing equivalents and because T_3-treatment is reported to cause an induction of the MEOS activity [9].

Xylitol is oxidized in hepatocytes exclusively by a cytosolic NAD^+-linked dehydrogenase (xylitol: NAD^+-2 oxidoreductase E.C. 1.1.1.9) [4]. The product, D-xylulose is converted via the pentose phosphate shunt to fructose-6-phosphate and triosephosphate. It is assumed that lactate oxidation not involving the cytosolic lactate dehydrogenase is negligible and that alcohol, xylitol and lactate dehydrogenase have the same (A) specificity for the coenzyme NAD^+ and use the same pool of cytoplasmic NAD(H) [5].

The results indicate that the activity of non-ADH pathways increases with the lactate/pyruvate ratio.

MATERIALS AND METHODS

Reagents. Enzymes and coenzymes were from Boehringer (Mannheim, FRG). Sodiumboro[^{3}H]-hydride was purchased from Amersham Int. (Amersham, U.K.). Chemicals were of analytical grade. Serum albumin from Armour Pharmaceutical Co. (Eastbourne, U.K.) was treated with charcoal to remove fatty acids [10]. Dowex I was from Fluka A.G. (Buchs, Switzerland), Sephadex G-50 from Pharmacia Fine Chemicals (Uppsala, Sweden) and Instaflour from Packard (Gronningen, The Netherlands).

Animals. Female rats of the Wistar strain weighing

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150–200 g, either untreated or T_3-treated were used. The T_3-treated rats were obtained by intraperitoneal injection with L-3,3′,5-triiodothyronine (sodium salt from Sigma, St. Louis, MO). An injection of 50 μg/100 g body wt. dissolved in 0.5 ml alkali was given every second day for a total of three doses.

Preparation and incubation of hepatocytes. Cells were prepared as described [11]. More than 90% of the cells excluded Trypan blue. Packed cells (about 0.6 ml, approx. 0.66 g wet wt.) were incubated in a 250 ml conical flask at 37° in Krebs–Henseleit bicarbonate buffer containing 1% serum albumin and 7.5 mM glucose in a total volume of 13.5 ml. The gas phase was O_2/CO_2 (19:1).

To the incubation were added 2.5 mM lactate, 0.25 mM pyruvate, 20 mM ethanol, and in some experiments 10 mM fructose or 10 mM xylitol 10 min prior to addition (at time zero) of a trace amount of [2-^{3}H]lactate, [2-^{3}H]xylitol or (1R)[1-^{3}H]ethanol to an initial specific radioactivity of 300 cpm/nmole for lactate, 100 cpm/nmole for xylitol or 50 cpm/nmole for ethanol. 2 ml samples were taken from the incubation at −10, 0, 10, 30, 50 and 70 min and added to 1 ml of 2.2 M perchloric acid. The supernatant was neutralized by KOH containing triethanolamine.

Determination of the distribution of tritium. The neutralized $HClO_4$ supernatant was used for isolation of water, ethanol, glucose, glycerol or xylitol and lactate. The radioactivity in water was obtained as the difference between total radioactivity and the radioactivity in dry matter plus ethanol. Ethanol and water were separated from dry matter by freeze-drying in a Thunberg tube. Tritium in ethanol was determined by incubation of the fraction of ethanol and water with 36 μmol pyruvate, 2 μmole NAD^+, 300 nkat lactate dehydrogenase and 300 nkat alcohol dehydrogenase in 0.2 M Tris buffer, pH = 7.5 for 10 hr at room temperature [12]. After freeze-drying the tritiated lactate formed from (1R)[1-^{3}H]ethanol was counted.

Glucose, lactate and "other anions" were separated by Dowex I (acetate form) ion exchange chromatography [13]. From incubations with fructose, we separated glucose and glycerol and from incubation with xylitol we separated glucose and xylitol by phosphorylation of glucose with ATP and hexokinase [6] and subsequent separation of glucose-6-P and glycerol or xylitol by Dowex I ion exchange chromatography. We did only fine tritium in glycerol, when the labelled substrates were metabolized together with fructose. Samples were counted in a Packard 2425 scintillation spectrometer. Instafluor: Triton X-114 (3:2, v/v) was used as the scintillation liquid with a sample volume of 28%.

The recovery of the tritium removed from the labelled substrates, in the different fractions was 90 to 110%.

The contribution of the non-ADH pathways to ethanol oxidation was calculated from the average yield of tritium in glucose and in water after 50 and 70 min incubation. The tritium content in those fractions was sufficient to allow accurate determination after 50 min of incubation, and the relative yield of tritium after 50 and 70 min differed by less than 10%.

Preparation of labelled substrates. [2-^{3}H]lactate was prepared from [1,1-^{3}H]ethanol by incubation with pyruvate, NAD^+, lactate dehydrogenase and alcohol dehydrogenase in 0.2 M Tris–HCl buffer, pH = 7.5 [6]. After freeze-drying [2-^{3}H]lactate was separated from the dehydrogenases and the coenzymes on a Sephadex G-50 column.

[2-^{3}H]xylitol and (1R)[1-^{3}H]ethanol were prepared from [4A-^{3}H]NADH and D-xylulose plus sorbitol dehydrogenase or acetaldehyde plus alcohol dehydrogenase, respectively [14]. [4A-^{3}H]NADH was prepared from [1-$^3H_2$]ethanol as described [15].

[1-$^3H_2$]ethanol was prepared from freshly destilled acetaldehyde and surplus of sodiumboro[^{3}H]hydride in a Thunberg tube and subsequent freeze-drying.

Metabolite levels. Metabolites were determined in the neutralized $HClO_4$ supernatant. Lactate, pyruvate, glucose, fructose and ethanol were measured by standard enzymatic procedures [16]. The concentration of xylitol was determined with sorbitol dehydrogenase from sheep liver after the method used by Touster and Montesi [17].

For each experiment the lactate/pyruvate ratio was calculated as the average of the values for 0, 10, 30, 50 and 70 min after addition of labelled substrates and thus reflects the average lactate/pyruvate ratio in the time period of tritium incorporation into the metabolites. In cells from T_3-treated animals the lactate/pyruvate ratio was constant with time and in cells from fed animals the ratio varied by less than a factor 2 during the incubation.

RESULTS

Metabolic changes

Rates of xylitol, fructose and ethanol removal and glucose formation were linear with time in the 80 min incubation of time.

The rate of ethanol oxidation, 2.6–2.8 μmole/min ml packed cells (Table 1), was not affected by T_3-treatment, when ethanol was metabolized without xylitol or fructose. We did not find a decrease in the rate of ethanol oxidation after T_3-treatment as reported by Hengsens *et al.* in hepatocytes from starved rats [18].

Fructose metabolism did not change the rate of ethanol oxidation in either hepatocytes from untreated or T_3-treated rats (Table 1). This is in agreement with the results obtained by others that addition of fructose causes the rate of ethanol oxidation to increase in starved [19], but not in fed hepatocytes [20], or in hepatocytes from starved rats when substrates like lactate and pyruvate are added [21].

Xylitol oxidation did not change the oxidation rate of ethanol in hepatocytes from T_3-treated rats, but caused a 20% inhibition of ethanol oxidation in hepatocytes from fed rats (Table 1).

Xylitol is metabolized in hepatocytes from fed rats or T_3-treated rats without ethanol present, at a rate of 1.6 μmole/min g wet wt. [12] or the same rate as in hepatocytes from T_3-treated rats, when xylitol and ethanol were oxidized simultaneously (Table 1). In cells from fed rats ethanol caused a 50% inhibition of xylitol oxidation to 0.75 μmole/min ml packed cells (Table 1).

The lactate/pyruvate ratio (Table 2) was higher when ethanol was metabolized in the presence of

Table 1. Metabolic changes

Treatment	Substrate	Δglucose	Δfructose	Δxylitol	Δethanol
		(μmole/min ml packed cells)			
Fed	Ethanol	0.92 ± 0.06			−2.55 ± 0.15
	Ethanol + fructose	2.36 ± 0.15	−2.49 ± 0.14		−2.32 ± 0.18
	Ethanol + xylitol	1.04 ± 0.17		−0.75 ± 0.06	−2.01 ± 0.17
T_3-treated	Ethanol	0.67 ± 0.03			−2.84 ± 0.11
	Ethanol + fructose	2.04 ± 0.11	−2.60 ± 0.17		−2.50 ± 0.09
	Ethanol + xylitol	1.58 ± 0.08		−1.59 ± 0.09	−2.54 ± 0.09

Hepatocytes were incubated for 80 min and change in metabolite concentrations estimated during the incubation as described in Methods section. Initial concentrations of substrates were 2.5 mM lactate, 0.25 mM pyruvate and 20 mM ethanol in the presence or absence of 10 mM fructose or 10 mM xylitol. Rates are means ± S.E.M. for five different cell preparations.

xylitol, than when ethanol was metabolized in the absence of xylitol in cells from fed rats, but was the same with or without xylitol in cells from T_3-treated rats. These results suggest larger capacity of the cells to oxidize cytosolic NADH in T_3-treated rats and that the capacity to oxidize cytosolic reducing equivalents is rate limiting for xylitol and ethanol oxidation in fed rats only if the two substrates are metabolized together. The average lactate/pyruvate ratio was higher in the presence of fructose, than in its absence, in cells from fed rats during the 80 min incubation time. During the first 20 min of the incubation the lactate/pyruvate ratio in fed rats were lower in the presence of fructose.

The rate of fructose uptake was the same in fed as in T_3-treated rats (Table 1). Glucose was the major end product of both fructose and xylitol metabolism in cells from fed and T_3-treated rats. The rate of glucose formation was correlated to the rate of xylitol and fructose uptake.

Fate of labelling from [2-^{3}H]lactate and (1R)[1-^{3}H]-ethanol

The tritium removed from the labelled substrates during the incubation was recovered in water, glucose, ethanol, lactate and glycerol.

The relative yield of tritium from [2-^{3}H]lactate or (1R)[1-^{3}H]ethanol in water and glucose can be seen in Table 3 and are used to calculate the contribution of non-ADH pathways to the ethanol oxidation (see below).

The tritium not accounted for in water and glucose was found in other neutral compounds, ethanol or lactate and "other anions".

In the neutral fraction after separation of glucose, significant labelling was found only in cells from fed rats incubated with fructose and tritiated ethanol or lactate (yield after 60 min *ca.* 6%, probably representing glycerol) or incubated with xylitol and tritiated ethanol or lactate (yield 3–4%, probably representing xylitol).

Tritium from [2-^{3}H]lactate was found in ethanol in cells from fed rats (yield 16%), because of the reversibility of the reaction catalyzed by alcohol dehydrogenase [6]. In cells from T_3-treated rats and in cells from fed rats in the presence of xylitol we found less (5–7%) tritium from lactate incorporated in ethanol.

The relative yield of tritium incorporated in lactate from (1R)[1-^{3}H]ethanol ranged from 8% in cells from T_3-treated rats, when ethanol was metabolized without fructose or xylitol to 32% when ethanol was

Table 2. The activity of non-ADH pathways and the lactate/pyruvate ratio

Treatment	Substrate	Ethanol via non-ADH pathways (μmole/min ml packed cells)	Lactate/pyruvate
Fed	Ethanol	−0.58 ± 0.15	109 ± 15
	Ethanol + fructose	−0.67 ± 0.27	147 ± 17
	Ethanol + xylitol	−1.30 ± 0.18*	393 ± 57
T_3-treated	Ethanol	−1.02 ± 0.22	38 ± 5
	Ethanol + fructose	−0.54 ± 0.18	33 ± 4
	Ethanol + xylitol	−0.41 ± 0.10**	38 ± 3

Hepatocytes were incubated for 70 min with labelled substrates and the activity of non-ADH pathways estimated as described in the Results section.

Values are presented as means ± S.E.M. for five different cell preparations. *$P < 0.02$ and **$P < 0.05$ compared with incubation in the absence of xylitol (Student *t*-test).

Table 3. The contribution of non-ADH pathways to ethanol oxidation

Treatment	Substrate	The relative yield of tritium from (1R)[1-^{3}H]ethanol in Water	Glucose	[2-^{3}H]lactate in Water	Glucose	% Oxidation via Non-ADH pathways
Fed	Ethanol	75.8 ± 1.8	4.3 ± 0.2	82.0 ± 2.3	6.6 ± 0.5	21.6 ± 4.4
	Ethanol + fructose	52.8 ± 3.5	8.6 ± 1.0	59.0 ± 3.1	17.1 ± 0.8	22.7 ± 7.1
	Ethanol + xylitol	80.3 ± 3.1	3.2 ± 0.6	74.9 ± 2.6	7.6 ± 0.4	56.2 ± 5.6*
T_3-treated	Ethanol	82.6 ± 1.8	6.8 ± 0.7	82.2 ± 2.1	12.3 ± 0.5	35.6 ± 6.6
	Ethanol + fructose	70.0 ± 1.4	9.9 ± 1.5	79.0 ± 1.4	16.7 ± 0.4	21.3 ± 7.9
	Ethanol + xylitol	74.4 ± 1.4	8.9 ± 0.6	81.4 ± 0.7	12.3 ± 0.4	15.6 ± 3.3**

Hepatocytes were incubated for 70 min with labelled substrates, and the distribution of tritium in water and glucose were estimated during the incubation. The recovery of tritium in ethanol, lactate, glucose, water and glycerol or xylitol were 90–110%.
Values are expressed as means ± S.E.M. for five different cell preparations. *$P < 0.005$ and **$P < 0.05$ compared with incubation in the absence of xylitol (Student t-test).

metabolized in cells from fed rats in the presence of fructose.

In the fraction of "other anions" we found less than 2% of the recovered tritium during all the incubation conditions, but when labelled lactate was metabolized in cells from fed rats with ethanol and xylitol (8%).

Calculation of the contribution of non-ADH pathways to ethanol oxidation

Our approach to calculate the contribution of non-ADH pathways to ethanol oxidation is based on the model shown in Fig. 1. If no ethanol is oxidized via non-ADH pathways the fate of the 1R-hydrogen of ethanol and the hydrogen bound to carbon 2 of lactate or xylitol should be identical, since alcohol, lactate and xylitol dehydrogenase use the same pool of cytoplasmic NAD^+ [5]. If, however, some ethanol is oxidized via non-ADH pathways, the yield of tritium from (1R)[1-^{3}H]ethanol in water would be relatively larger, since non-ADH pathways give rise to tritium in water directly without passing through the pool of NADH.

The non-ADH pathways may be quantitated by comparison of the relative yield of labelling of glucose and water from (1R)[1-^{3}H]ethanol and L-[2-^{3}H] lactate, taking into account the reversibility of the reaction catalyzed by lactate and alcohol dehydrogenase [6]. Our approach requires parallel incubations with the same batch of liver cells together with everything identical except for the radioactive labelling.

When [2-^{3}H]lactate is metabolized the relative yield of tritium in glucose (G_L) and water (W_L) can be calculated from the specific radioactivity of NADH (N_L). We consider the model Fig. 1 and set input of tritium into NADH equal to output of tritium from NADH. The relative yield of tritium in glucose (G_L) during the metabolism of L-[2-^{3}H] lactate can then be calculated from the equation

$$G_L = \frac{N_L \alpha f}{\text{the total yield of } ^3\text{H from lactate}}, \quad (1)$$

f is the flux from 1,3-diphosphoglycerate to glyceraldehyde 3-phosphate and α is a constant, including exchange of tritium in the reactions catalyzed by aldolase and triosephosphate isomerase and possible lack of isotopic equilibrium between the 4A and the 4B hydrogen of NADH (6).

The relative yield of tritium in water will be

$$W_L = \frac{N_L \text{ (flux of H from NADH to } O_2)}{\text{The total yield of } ^3\text{H from lactate}} \quad (2)$$

The flux of H from NADH to O_2 will be $(d + b - c + (1 - \alpha) f + y\, c/b)$ or all reducing equivalents, which goes to NAD^+ minus those incorporated in glucose, lactate and ethanol. $N_L y\, c/b$ represents the yield of ^{3}H in water from the ^{3}H incorporated in ethanol via NADH and oxidized via non-ADH pathways. $(d + b - c)N_L$ represents the consumption of NADH in the microsomal and mitochondrial electron transport chain and $N_L(1 - \alpha)f$ represent the yield of ^{3}H in water from the aldolase reaction.

With ethanol as the labelled substrate the yield in

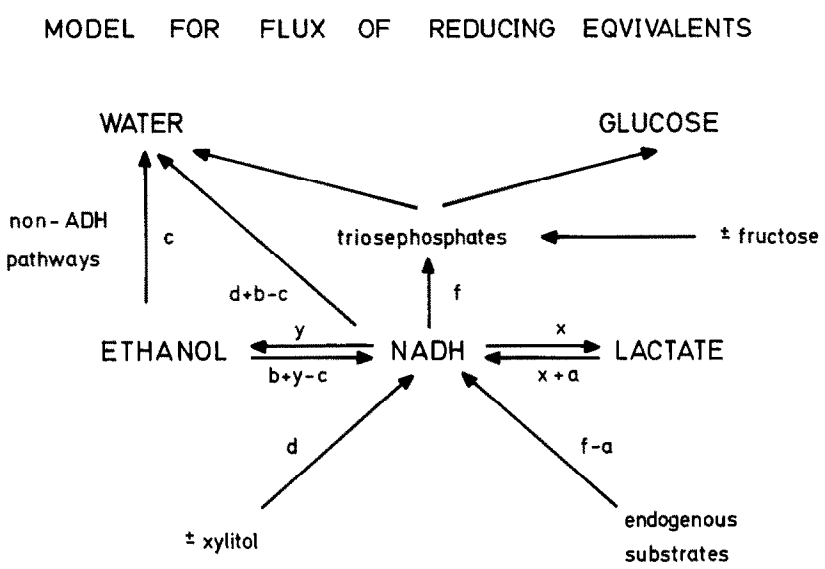

Fig. 1. Model for flux of reducing equivalents from [2-^{3}H]lactate or (1R)[1-^{3}H]ethanol. $b + y - c$, $x + a$, d and $f - a$ represent the formation of NADH from ethanol, lactate, xylitol and endogenous substrates, $d + b - c$ represents consumption of NADH in the microsomal and mitochondrial electron transport chain and f in the triosephosphate dehydrogenase reaction; c represents the amount of ethanol oxidized by non-alcohol dehydrogenase pathways.

glucose and water will be

$$G_E = \frac{N_E \alpha f}{\text{the total yield of } ^3\text{H from ethanol}}, \quad (3)$$

$$W_E = \frac{N_E \text{ (flux of H from NADH to } O_2) + c}{\text{The total yield of } ^3\text{H from ethanol}}. \quad (4)$$

Since the experiments with lactate and ethanol are carried out as parallel incubations, α, f and flux from NADH to water is identical, irrespective of the labelled substrate. Combining equations (1)–(4), the flux of tritium from ethanol to water via non-ADH pathways (c) relative to the total yield of ^{3}H from ethanol (b) is obtained as:

$$\frac{c}{b} = W_E - \frac{G_E}{G_L} W_L. \quad (5)$$

From equation (5) we have calculated the contribution of non-alcohol dehydrogenase pathway (c) to the total ethanol oxidation (b) by using the relative yield of tritium in water and glucose, when labelled ethanol or labelled lactate were metabolized under identical conditions and by hepatocytes from the same batch of cells. The results are shown in Table 3. As can be seen the relative yield of tritium in water from [2-^{3}H]lactate is greater than the relative yield of tritium in water from (1R)[1-^{3}H]ethanol, but at the same time the ratio, between tritium in glucose from ethanol and tritium in glucose from lactate G_E/G_L is less than 1.

In two of the experiments with ethanol and xylitol added to cells from T_3-treated rats we made incubations with [2-^{3}H]xylitol as well as (1R)[1-^{3}H]ethanol and [2-^{3}H]lactate. From the model (Fig. 1) it is seen, that we can compare distribution of tritium from xylitol with the distribution of tritium from ethanol in the same way, as we have done with tritium from lactate and ethanol in the presence of xylitol. If the model is adequate, the same results as regards ethanol oxidation via non-ADH pathways should be obtained. In the experiments with [2-^{3}H] xylitol, we found the average relative yield of tritium in water $W_X = 65.5\%$ and in glucose $G_X = 9.8\%$. From these values and the values from the distribution of tritium from labelled ethanol $W_E = 74.4\%$ and $G_E = 8.9\%$ (Table 3), we can calculate the contribution of non-ADH pathways to the ethanol oxidation to 14.9%, which is the same as the value found with [2-^{3}H]lactate (Table 3).

Assumptions

All calculations are based on the assumptions, that alcohol dehydrogenase and lactate dehydrogenase share the same pool of NAD(H), and that the apparent isotope effect on the oxidation of the labelled substrates is negligible. We have earlier shown that the first demand is fulfilled, [5, 6]. The tritium isotope effect in the lactate dehydrogenase catalyzed reaction is found to about one [22, 23]. The tritium isotope effect on ethanol oxidation catalyzed by alcohol dehydrogenase is found to be about four, in the absence of acetaldehyde, but when acetaldehyde is present in concentrations near those found *in vivo* and in our hepatocytes, the apparent isotope effect decreases to about one [20]. Because the isotope effect of the cytochrome P-450 mediated oxidation of ethanol also is found to be about one [24] it appears justifiable to neglect isotope effects in the calculations.

DISCUSSION

Effect of fructose and xylitol on the activity of non-ADH pathways in hepatocytes from fed rats

The amount of ethanol, which is metabolized via

non-ADH pathways is about 20% (Table 3) of the total ethanol oxidized by hepatocytes from fed rats, in the absence of fructose or xylitol. When fructose or xylitol is present, the activity of non-ADH pathways increases in cells from fed rats. The rate of ethanol oxidation catalyzed by non-ADH pathways seems correlated to the lactate/pyruvate ratio (Table 2). This may suggest, that NADH activates the hydroxylation of ethanol by the microsomal ethanol oxidizing system (MEOS) [25] by supplying NADH to cytochrome b_5, catalyzed by cytochrome b_5 reductase [26]. Cytochrome b_5 reductase uses NADH preferentially to NADPH [2] and its reduction is dependent of the ratio NADPH/NADH [3]. Another possibility would be that the high NADH concentration caused an increase in the NADPH/$NADP^-$ ratio by the action of transhydrogenase.

The concentration of NADH can be calculated from the lactate/pyruvate ratio (Table 3), the equilibrium constant [pyruvate][NADH]/[NAD^+][lactate] = 1.11×10^{-4} [27] and a NAD^+ concentration of 0.5 mM [28]. Assuming ordinary Michaelis–Menten kinetic for ethanol oxidation catalyzed by MEOS, the apparent K_m can be calculated to 20 μM for NADH and the apparent V_{max} to 2.5 μmole/min ml packed cells in cells from fed rats. The V_{max} found is in agreement with a value found in hepatocytes metabolizing 80 mM ethanol after inhibition of alcohol dehydrogenase with 4 mM 4-methyl pyrazole (2.7 μmole/min ml packed cells [29]). It has been found by others, that xylitol or sorbitol stimulate microsomal hydroxylation [30, 31]. This stimulation was explained by an increased supply of NADPH for mixed function oxidation caused by conversion of xylitol or sorbitol to glucose-6-phosphate or by deinhibition of NADPH generating enzymes [33] in the pentose phosphate pathway due to a loss of adenine nucleotides. The present results, however, suggest that xylitol stimulates microsomal oxidations by an increase in the NADH level and not by deinhibition of NADPH generating enzymes, since fructose, which causes loss of adenine nucleotides as efficient as xylitol [34, 32] and which is converted to glucose to at least the same degree as xylitol (Table 1), has no effect on the metabolism of ethanol via non-ADH pathways. The reported ethanol inhibition of drug oxidation [30] can then be explained by competition between ethanol and other substrates for the microsomal oxidase.

The activity of non-ADH pathways in hepatocytes from hyperthyroid rats

In hepatocytes from T_3-treated rats xylitol did not stimulate the activity of the non-ADH pathways, which is in agreement with the above conclusion, because the lactate/pyruvate ratio did not change by addition of xylitol to cells from T_3-treated rats. On the contrary xylitol seems to inhibit the contributions of non-ADH pathways to the ethanol oxidation (Table 3).

In cells from T_3-treated rats the effect of fructose was the same as that of xylitol, a slight inhibition of the activity of the non-ADH pathways (Table 2).

T_3-treatment is reported to cause an increase in MEOS activity and a decrease in alcohol dehydrogenase activity [7]. This does not agree with our results, unless we take into account the low lactate/pyruvate ratio and the correlation with this ratio and the activity of the non-ADH pathway in fed rats. If we compare the activity in fed rats after extrapolation to the same lactate/pyruvate ratio as in T_3-treated rats, we observe 3–4 times activation of the microsomal oxidizing system in T_3-treated rats.

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