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ETHANOL AND ACETALDEHYDE METABOLISM IN RAT STRAINS GENETICALLY SELECTED FOR THEIR ETHANOL PREFERENCE

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Abstract—A study was made of ethanol and acetaldehyde metabolism in both sexes in rat strains genetically selected for their ethanol preference. The strains are denoted by ANA (Alko, Non-Alcohol), which prefers water to a 10% (v/v) ethanol solution, and AA (Alko, Alcohol), which prefers the ethanol solution. Peripheral blood and freeze-stopped livers were used for the *in vivo* studies. A once-through perfusion technique was applied so that in the same liver ethanol and acetaldehyde oxidation, the cytoplasmic redox state and oxygen consumption could be measured. In the female rats of the AA strain there was a higher rate of ethanol oxidation and oxygen consumption, compared with those of the ANA strain. A greater difference was found between the sexes, the female rats of both strains having a more rapid ethanol oxidation and oxygen consumption, compared with the respective males. The AA strain displayed a significantly lower level of acetaldehyde during ethanol oxidation than did the ANA strain. On comparison of the liver acetaldehyde concentrations with the mitochondrial NADH/NAD$^+$ ratio, calculated from the 3-hydroxybutyrate/acetoacetate ratio, strain correlations were observed in both sexes, the ANA strain, with higher acetaldehyde, having a lower 3-hydroxybutyrate/acetoacetate ratio than the AA strain, with lower acetaldehyde. The results are discussed in relation to the regulation of the ethanol and acetaldehyde metabolism. The biochemical basis for ethanol preference is briefly discussed.

THERE IS increasing interest in the genetic factors that determine ethanol preference in animals. The importance of genetic factors has been demonstrated in both mouse[1,2] and rat[3–5] strains. For clarification of the biochemical basis for strain differences in ethanol preference, one method is that of investigating the ethanol metabolism in respective strains. To date, almost all the biochemical investigations of strains with different ethanol preference have been made in mice, with the main concentration being laid upon the characterization of the NAD-dependent alcohol (EC 1.1.1.1) and aldehyde (EC 1.2.1.3) dehydrogenases of inbred-mouse strains. The results obtained in these investigations have indicated that the C57BL/6J mouse strain [which, with no previous exposure to ethanol, prefers 10% (v/v) ethanol to water] has an oxidation capacity in respect of ethanol[2,6] and acetaldehyde[2,7] which is greater than that of the DBA/2J strain (which avoids ethanol). Sheppard *et al.*[7] have also shown that, following intraperitoneal ethanol injection, acetaldehyde accumulates in the blood of DBA/2J mice to a greater extent than is the case in C57BL/6J mice. All of these data support the suggestion of an acetaldehyde-induced avoidance of ethanol.

By outbreeding and selection of animals that differ in regard to alcohol consumption Eriksson has raised two genetically different rat strains.[4,5] Both sexes of these rat strains were used for ethanol and acetaldehyde metabolism investigations, with a view to the discovery of possible differences which could, on a biochemical basis, contribute

to explanation of the ethanol preference. Three experimental techniques were used to study ethanol and acetaldehyde metabolism; ethanol and acetaldehyde were measured in the blood after intraperitoneal ethanol injection, and liver ethanol and acetaldehyde metabolism was studied by the application of freeze–stop and once-through perfusion techniques.

MATERIALS AND METHODS

Animals. Both male and female rats (weighing 200–300 g) of the AA (Alko, Alcohol) and ANA (Alko, Non-Alcohol), of drinking and non-drinking strains respectively, developed by Eriksson,[4,5] were used in these experiments. The rats were given a standard laboratory diet and water *ad lib.* No alcohol had been given to the rats previously.

Blood determinations. In the first part of the investigations, 1·5 g ethanol/kg of body wt was injected intraperitoneally as a 10% (v/v) solution in saline. Blood samples of 0·2 ml were taken at different times from the tip of the tail, pipetted in 1·8 ml of 0·6 M ice-cold perchloric acid, shaken, and the precipitates were centrifuged at 4000 g for 15 min at 4°. The supernatants were used directly for gas chromatography analyses of ethanol and acetaldehyde.

Freeze-stop determinations. In the second part of the experiments, ethanol was given to the rats as before. After 50 min, the rats were anaesthetized with 50 mg of pentobarbital (Nembutal R, Abbot S. A., Brussels, Belgium)/kg of body wt intraperitoneally, given as a 1% (w/v) solution in saline. Sixty min after ethanol injection, the livers were frozen *in situ* by means of aluminium clamps precooled in liquid nitrogen. The frozen livers were pulverized in a mortar, and 1·5 g of the liver powder was suspended in 10 ml 0·6 M ice-cold perchloric acid, shaken, and the precipitate was centrifuged at 4000 g for 15 min at 4°. The ketone bodies acetoacetate and 3-hydroxybutyrate from these supernatants were measured by gas chromatography. Ethanol, acetaldehyde, lactate and pyruvate were determined from liver samples, which were precipitated with 0·6 M perchloric acid solution containing 25 mM thiourea ((*p.a.*, E. Merck AG, Darmstadt, Germany).

Perfusion technique. As distinct from the common recirculating perfusion systems a once-through system was employed, without haemoglobin or albumin, which were omitted because of the large volumes of fluid required. The once-through technique applied here has been described earlier by Lindros *et al.*[8] The perfusion medium consisted of Krebs–Ringer-bicarbonate solution[9] equilibrated with $O_2 + CO_2$ (95:5) at 37°, and supplemented with 5·5 mM glucose, 1·25 mM L-lactate and 0·15 mM pyruvate. The flow rate was 40 ml/min. The O_2 concentration in the effluent was measured with a Clark-type O_2 electrode.

The animals were anaesthetized as before with pentobarbital. After the portal vein had been catheterized, oxygenated perfusion medium was immediately pumped through the liver. After about 15 min, when the liver had reached a steady-state, the influent was changed to a perfusion medium to which 4 mM ethanol had been added. Samples were taken from the influent and effluent every 15 min up to 1 hr. No swelling of the liver occurred during this time. Ethanol, acetaldehyde, lactate and pyruvate were measured directly from the perfusion medium.

Analytical techniques. Ethanol, acetaldehyde, acetoacetate and 3-hydroxybutyrate were all measured with a Perkin–Elmer F 40 gas chromatograph and application of

the head-space technique. The samples to be analysed were pipetted into serum bottles, sealed with a rubber stopper, and incubated for 15 min at 65° in a sampling turn-table, after which samples were automatically taken by means of an electropneumatic dosing system. For ethanol determinations, *t*-butyl alcohol was used as an internal standard. Acetoacetate and 3-hydroxybutyrate were measured as acetone, by means of a method described previously.[10] In place of an internal standard, an acetaldehyde standard solution made of redistilled acetaldehyde (from BDH Chemicals, Poole, Dorset, England) was used in the acetaldehyde measurements. The results obtained by Truitt,[11] and in our laboratory,[12] indicate that when ethanol is present acetaldehyde is formed "spontaneously" from supernatants of blood and liver protein precipitations. In a pilot study, it was found that the "spontaneous" formation of acetaldehyde in blood under these conditions could be ignored, but that the effect in liver supernatants was too great to be neglected. To stop this formation of acetaldehyde thiourea was found to be very efficient,[13] and this substrate neither affected determinations of the acetaldehyde itself, nor the measurements of ethanol, lactate or pyruvate. Lactate and pyruvate were assayed enzymatically by a method described by Hohorst *et al.*[14] Enzymes and coenzymes were supplied by C. F. Boehringer (Mannheim, West Germany).

RESULTS

Ethanol and acetaldehyde in blood after intraperitoneal ethanol injection. Table 1 lists the blood ethanol values of male and female rats of the ANA and the AA strains as

TABLE 1. ETHANOL IN BLOOD AFTER INTRAPERITONEAL ETHANOL INJECTION*

	Blood ethanol at different times after ethanol injection (μmole/ml)				Rate of ethanol elimination (mmole/	Statistical rate comparison (P)		
Animals	0·75 hr	1·5 hr	2·0 hr	2·5 hr	kg/hr)	ANA ♀	ANA ♂	AA ♀
ANA ♀ (9)	30·2 ± 3·9	22·5 ± 2·9	17·0 ± 2·9	10·8 ± 3·2	9·7 ± 0·8			
ANA ♂ (11)	29·9 ± 1·7	26·3 ± 1·4	22·2 ± 1·4	14·7 ± 1·4	8·6 ± 0·4	<0·005		
AA ♀ (11)	30·6 ± 4·3	21·6 ± 2·1	15·4 ± 2·6	8·2 ± 2·9	10·5 ± 0·8	<0·05	<0·001	
AA ♂ (12)	30·3 ± 2·5	24·8 ± 2·1	20·3 ± 1·9	14·9 ± 2·2	8·2 ± 0·7	<0·001	N.S.	<0·001

* Rats were treated and analyses made as described in the Materials and Methods section. The rates of ethanol elimination were calculated from the extrapolated time when ethanol was completely eliminated. The 1·5 g ethanol dose/kg of body wt divided with this time, gives the rate of ethanol elimination. Results are given as means ± S.D. P values were calculated by means of the Student's *t*-distribution.[15]

N.S.—not significant.

a function of time. All the groups of animals exhibited their highest ethanol concentration in the blood within 1 hr. No differences were discernible between the groups 0·75 hr after the administration of ethanol. After 1·5 hr, and subsequently, distinct differences were apparent between male and female rats in both strains. From the extrapolated elimination rates, expressed as mmole ethanol/kg of body wt/hr, it was calculated that the female rats eliminated ethanol 13 and 28 per cent more rapidly than did the males in the ANA and AA strains respectively. Strain differences were also discernible between the female rats. Females of the AA strain eliminated ethanol 8·1 per cent more rapidly than did those of the ANA strain.

The corresponding acetaldehyde values of male and female rats of the ANA and AA strains as a function of time are shown in Table 2, which demonstrates that the

TABLE 2. ACETALDEHYDE IN BLOOD AFTER INTRAPERITONEAL ETHANOL INJECTION*

Animals	Blood acetaldehyde at different times after ethanol injection (nmole/ml)				Statistical group comparisons (P)		
	0·75 hr	1·5 hr	2·0 hr	2·5 hr	ANA ♀	ANA ♂	AA ♀
ANA ♀ (9)	44 ± 12	38 ± 12	41 ± 11	24 ± 8			
ANA ♂ (11)	32 ± 10	28 ± 9	28 ± 9	28 ± 9	N.S.		
AA ♀ (11)	27 ± 16	19 ± 23	15 ± 16	7 ± 10	<0·01	N.S.	
AA ♂ (12)	16 ± 8	20 ± 6	14 ± 11	7 ± 8	<0·01	<0·01	N.S.

* Rats were treated and analyses made as described in the Materials and Methods section. Results are given as means ± S.D. An analysis of variance was applied for the mutual comparison of all groups, and the P values were calculated by means of *F*-distribution.[16]
N.S.—not significant.

blood acetaldehyde, as opposed to the ethanol, maintained a fairly steady level until about 2 hr after ethanol injection. Significant strain differences were apparent in both sexes. The ANA strain displayed a blood acetaldehyde concentration 2–4 times higher than that of the AA strain. This difference increased during the ethanol oxidation. No significant sex differences were found in acetaldehyde concentration taking the whole ethanol oxidation as a basis. If comparison is restricted to the first 2 hr acetaldehyde, significant difference became apparent between the males and females of the ANA strain. During this period the females of this strain displayed a higher level of blood acetaldehyde.

Ethanol and acetaldehyde metabolism and the extra- and intra-mitochondrial redox levels in liver after intraperitoneal ethanol injection. Table 3 illustrates the ethanol and

TABLE 3. ETHANOL AND ACETALDEHYDE IN LIVER AFTER INTRAPERITONEAL ETHANOL INJECTION*

Animals	Liver ethanol (μmole/g)	Liver acetaldehyde (nmole/g)	Statistical group comparisons (P)			
			ANA ♀	ANA ♂	AA ♀	AA ♂
ANA ♀ (9)	27·4 ± 1·6	197 ± 47		N.S.	<0·01	<0·01
ANA ♂ (9)	29·5 ± 1·7	217 ± 40	<0·025		<0·001	<0·005
AA ♀ (9)	26·8 ± 1·0	135 ± 34	N.S.	<0·001		N.S.
AA ♂ (9)	28·7 ± 1·1	117 ± 67	N.S.	N.S.	<0·005	

* Rats were treated and analyses made as described in the Materials and Methods section. Results are given per wet-wt liver as means ± S.D. P values were calculated by means of the Student's *t*-distribution.[15] In the statistical comparisons left corner below expresses ethanol and the right corner above acetaldehyde comparisons.
N.S.—not significant.

acetaldehyde concentrations in the livers of male and female rats of the ANA and AA strains 1 hr after intraperitoneal ethanol injection. No significant strain differences

were found in the ethanol levels. However, there were significant sex differences which parallel those found in the ethanol elimination-rate determinations. Significant acetaldehyde differences were apparent between the strains, but not between the sexes of the same strain. The liver acetaldehyde concentrations were 85·5 and 45·9 per cent higher in the male and female rats respectively of the ANA strain than in those of the AA strain.

To determine the cytoplasmic redox state in liver during ethanol oxidation lactate and pyruvate were measured. Lactate varied between 1·66–1·83 and pyruvate between 0·038–0·045 μmoles/g of wet wt liver. Corresponding lactate/pyruvate ratios varied between 39 and 48, and no significant differences were observable.

The mitochondrial redox state after ethanol administration, represented by the 3-hydroxybutyrate/acetoacetate ratio, is shown in Table 4. Both strain and sex

TABLE 4. MITOCHONDRIAL REDOX STATE OF THE LIVER AFTER INTRAPERITONEAL ETHANOL INJECTION*

Animals	Liver aceto acetate (nmole/g)	Liver 3-hydroxy-butyrate (nmole/g)	3-Hydroxy-butyrate / aceto acetate	Statistical ratio comparisons (P)		
				ANA ♀	ANA ♂	AA ♀
ANA ♀ (10)	70 ± 32	252 ± 47	3·9 ± 1·1			
ANA ♂ (9)	58 ± 9	322 ± 17	5·7 ± 1·0	<0·005		
AA ♀ (10)	45 ± 11	233 ± 35	5·5 ± 1·5	<0·025	N.S.	
AA ♂ (6)	38 ± 14	349 ± 78	10·0 ± 2·9	<0·005	<0·025	<0·005

* Rats were treated and analyses made as described in the Materials and Methods section. The mitochondrial redox state of the liver was characterized by the 3-hydroxybutyrate/acetoacetate ratio. Results are given per wet wt liver as means ± S.D. P values were calculated by means of the Student's *t*-distribution.[15]

N.S.—not significant.

differences were apparent. In both strains the females had a significantly lower redox ratio than did the respective males. The 3-hydroxybutyrate/acetoacetate ratio was significantly higher for both sexes of the AA strain than for those of the ANA strain (75 and 41 per cent for male and female rats respectively).

Liver perfusion experiments. The rates of ethanol oxidation in the perfused livers of both sexes of both strains are listed in Table 5. The sex differences observed in the blood and freeze–stop determinations were the same as those in the perfusion experiments. No significant strain differences between the same sexes were observed in the perfusion experiments. The ethanol oxidation rates showed a slight increase during the perfusion. The average ethanol oxidation rate values for all four perfusion times of each rat group were compared with the corresponding values obtained from the blood determinations. For transformation of the ethanol oxidation rate values to mmoles ethanol oxidated/hr/kg of body wt, the body-wt/liver-wt ratio must be known. The average ratio for all groups was found to be 29·4 ± 2·6 ($n = 43$), and no significant differences were apparent between the groups. The ethanol oxidation rates of the perfused rat livers, calculated as percentages, were 39, 33, 40 and 37 per cent of the corresponding rate values *in vivo* for ANA ♀, ANA ♂, AA ♀ and AA ♂ respectively.

TABLE 5. ETHANOL OXIDATION IN PERFUSED RAT LIVER*

Animals	Ethanol oxidation rate at different times of perfusion (μmole/min/g)				Statistical group comparisons (P)		
	15 min	30 min	45 min	60 min	ANA ♀	ANA ♂	AA ♀
ANA ♀ (4)	1·8 ± 0·40	2·2 ± 0·28	1·9 ± 0·60	2·6 ± 0·86			
ANA ♂ (4)	1·6 ± 0·22	1·4 ± 0·28	1·6 ± 0·31	1·8 ± 0·35	<0·01		
AA ♀ (4)	2·2 ± 0·22	2·4 ± 0·37	2·3 ± 0·30	2·6 ± 0·36	N.S.	<0·01	
AA ♂ (4)	1·7 ± 0·45	1·5 ± 0·45	1·7 ± 0·14	2·0 ± 0·23	N.S.	N.S.	<0·01

* Rat livers were perfused in a once-through system, with cell- and albumin-free Krebs-Ringer bicarbonate medium, as described in the Material and Methods section. The ethanol oxidation rate was calculated from the concentration difference between the in- and out-flowing medium multiplied by the flow rate per wet wt of total liver. Results are given as means ± S.D. An analysis of variance was applied for the mutual comparison of all groups, and the P values were calculated by means of *F*-distribution.[16]

N.S.—not significant.

Table 6 indicates the output of acetaldehyde in the same perfused livers used for determination of the ethanol oxidation rate. Significant differences were observed between both strains and sexes. Here again, both male and female rats of the ANA strain exhibited a higher acetaldehyde output during the ethanol oxidation, compared with those of the AA strain. As opposed to the *in vivo* determinations, the female rats of both strains displayed a significantly higher acetaldehyde output than did the respective males. In all groups of rats, the acetaldehyde output increased during the perfusion. On calculation of the output rate of acetaldehyde as a percentage of the total acetaldehyde formed in ethanol oxidation (the same as the ethanol oxidation rate), the following percentages were obtained: 17 → 28, 12 → 19, 12 → 17 and 5·5 → 12 for ANA ♀, ANA ♂, AA ♀ and AA ♂ respectively (the arrows indicate the direction during the perfusion). It could accordingly be established that the slight increase in ethanol oxidation rate did not provide an explanation for the increase in liver acetaldehyde output during perfusion.

TABLE 6. OUTPUT OF ACETALDEHYDE IN LIVERS PERFUSED WITH ETHANOL*

Animals	Acetaldehyde output rate at different times of perfusion (nmole/min/g)				Statistical group comparisons (P)		
	15 min	30 min	45 min	60 min	ANA ♀	ANA ♂	AA ♀
ANA ♀ (4)	311 ± 200	445 ± 211	527 ± 188	575 ± 177			
ANA ♂ (4)	198 ± 39	272 ± 52	297 ± 49	308 ± 54	<0·01		
AA ♀ (4)	253 ± 56	346 ± 68	395 ± 85	399 ± 87	<0·01	<0·01	
AA ♂ (4)	93 ± 64	161 ± 55	197 ± 61	216 ± 81	<0·01	<0·01	<0·01

* The acetaldehyde was determined from the effluent of the same livers as those used for determination of ethanol oxidation rate (Table 5). The rate of acetaldehyde output was calculated from the effluent acetaldehyde concentration, multiplied by the flow rate per wet wt of total liver. Results are given as means ± S.D. An analysis of variance was applied for the mutual comparison of all groups, and the P values were calculated by means of *F*-distribution.[16]

The oxygen consumption of the same livers as those used for determination of the ethanol oxidation rate is indicated in Table 7. Significant sex differences were observed in both strains. The female rats of the ANA and the AA strains showed respectively 50 and 47 per cent higher oxygen consumption than did the corresponding male rats before ethanol was added to the perfusion medium. When ethanol was added, the oxygen consumption increased in all groups. Calculated as a percentage, the increase was less in the female (7 → 15 per cent) than in the male rats (13 → 21 per cent). No significant strain differences were observed in oxygen consumption.

TABLE 7. OXYGEN CONSUMPTION OF LIVERS PERFUSED WITH ETHANOL*

	Oxygen consumption at different times of perfusion (μmole/min/g)					Statistical group comparisons (P)		
Animals	0 min†	15 min	30 min	45 min	60 min	ANA ♀ ANA ♂	AA ♀	
ANA ♀ (4)	2·63 ± 0·55	2·80 ± 0·27	2·89 ± 0·23	2·92 ± 0·27	2·98 ± 0·38			
ANA ♂ (4)	1·79 ± 0·18	2·04 ± 0·22	2·09 ± 0·24	2·15 ± 0·24	2·18 ± 0·27	<0·01		
AA ♀ (4)	2·88 ± 0·54	3·08 ± 0·61	3·20 ± 0·73	3·24 ± 0·78	3·31 ± 0·87	N.S.	<0·01	
AA ♂ (4)	1·92 ± 0·14	2·17 ± 0·20	2·21 ± 0·24	2·25 ± 0·21	2·26 ± 0·17	<0·01	N.S.	<0·01

* Oxygen consumption was measured in the same livers as those used for determination of the ethanol oxidation rate (Table 5). The O_2-concentration difference between a control perfusion without liver, and the concentration during the liver perfusion, was multiplied by flow rate, and divided by the total wet wt of the liver. The oxygen consumption results thus obtained are given as means ± S.D. An analysis of variance was applied for the mutual comparison of all groups and the P values were calculated by means of F-distribution.[16]

N.S.—not significant.

† Oxygen consumption before addition of ethanol.

The lactate/pyruvate ratio in the effluent was also determined to assess the cytoplasmic redox state. Before ethanol was added, the lactate/pyruvate ratio in all groups was about 6–8. The corresponding lactate and pyruvate concentrations of the effluent were 1·2–1·4 and 0·17–0·21 mM respectively. When ethanol was added to the perfusion medium, the lactate/pyruvate ratio rose to about 40. This increase in the redox was attributable to a corresponding diminution in the pyruvate concentration. The lactate concentration of the effluent displayed no more than a slight increase after the addition of ethanol. The corresponding lactate and pyruvate concentrations of the effluent were 1·4–1·5 and 0·035–0·045 mM respectively. During the perfusion with ethanol a slight diminution on the lactate/pyruvate ratio occurred in all groups of rats. No significant strain or sex differences were apparent in the lactate/pyruvate ratios before or after the addition of ethanol.

DISCUSSION

Ethanol oxidation. Strain differences similar to those observed here, with the females of the AA rat strain (with high ethanol preference) having a more rapid ethanol oxidation, compared with the females of the ANA strain (with low ethanol preference) have not been reported before. However, Rodgers *et al.*[6] and Sheppard *et al.*[2] have indicated, from differences in alcohol dehydrogenase activity, a greater ethanol oxidation capacity in inbred mouse strains with higher ethanol preference, compared to

mouse strains with a lower ethanol preference. These researchers did not report any sex differences. Such differences have been demonstrated by Büttner,[17] who found a higher alcohol dehydrogenase activity in the livers of female rats than in those of male rats of the Sprague–Dawley strain. Büttner did not observe any differences in activity between the sexes of the Wistar strain. In Wistar rats, Eriksson and Malmström[18] found sex differences in the elimination rate of ethanol that were very similar to those found in this work. They indicated that this difference was attributable to different amounts of oxidizing tissue in proportion to body weight. Since the ratios of body weight to liver weight obtained here were the same for all groups of rats, this could not provide an explanation in this case.

The rate of ethanol oxidation in the liver cytosol can primarily be modified by enzyme activity, acetaldehyde/ethanol ratio and free NADH/free NAD^+ ratio in this cell compartment. The free NADH/free NAD^+ ratio expresses the ratio between nucleotides, which are not bound to proteins. The lactate/pyruvate ratio is generally employed as representing the free NADH/free NAD^+ ratio in the cytosol.

The equilibrium of the ethanol–acetaldehyde reaction favours the reduction of acetaldehyde to ethanol.[19] This suggests the importance of the acetaldehyde/ethanol ratio upon the oxidation rate of ethanol. Lindros *et al.*[8] have shown that addition of acetaldehyde to the perfused liver decreased the ethanol oxidation rate. However, the acetaldehyde/ethanol ratio is not considered as a normal regulating factor for ethanol oxidation owing to the rapid oxidation of the acetaldehyde formed. The higher acetaldehyde output and higher ethanol oxidation rate in the perfused female rat livers compared to the male rat livers of respective strains, taken in conjunction with the increase in acetaldehyde output during perfusion by about 100 per cent in all groups of rats without any diminution in ethanol oxidation rate (rather a slight increase), shows clearly that the differences in the rate of ethanol oxidation could not be explained by any regulative effect of the acetaldehyde/ethanol ratio.

Positive correlations were noted between the ethanol oxidation rate and the oxygen consumption. The mitochondrial oxidations, which include the oxidation of NADH, are in turn reflected in the oxygen consumption. This oxidation of NADH, affecting NADH/NAD^+ ratio, has been proposed as the most important regulator of the ethanol oxidation,[8,20,21] which agrees with the respiration data obtained here (Table 7). However, it is difficult to state whether the sex and strain differences in ethanol oxidation rates are due to differences in the NADH reoxidation or to a combined effect of differences in NADH reoxidation and alcoholdehydrogenase activity.

Acetaldehyde oxidation. The second step in ethanol metabolism is the oxidation of acetaldehyde to acetate. This oxidation has also been found to be NAD^+-dependent, and enzymes have been located in both the cytoplasmic and mitochondrial compartments of the liver cells.[22–24] On the basis of these results, it seems difficult to establish whether this reaction *in vivo* proceeds in the cytosol, mitochondria or in both compartments. However, some recent studies give greater importance to the mitochondria in the oxidation of acetaldehyde.[8,25] The basis for these indications lay in the small effects of acetaldehyde upon the lactate/pyruvate ratio in liver perfusions. In this work a connection between liver acetaldehyde (Table 3) and mitochondrial NADH/NAD^+ ratios, as represented by the 3-hydroxybutyrate/acetoacetate ratio (Table 4) was observed. Both sexes of the ANA strain have a higher acetaldehyde concentration and

lower 3-hydroxybutyrate/acetoacetate ratio during ethanol oxidation compared to those of the AA strain. Comparisons cannot be drawn between the sexes, because of the completely different states of mitochondrial oxidation in male and female which was observed in the perfusion experiments (Table 7). The lower mitochondrial redox ratio in the females could be explained by their greater oxygen consumption. The 3-hydroxybutyrate/acetoacetate ratios enable some conclusions to be drawn in regard to the causes of the strain differences in acetaldehyde metabolism. If acetaldehyde oxidation were regulated only by the mitochondrial redox state (which includes deficiency of NAD^+ or excess of NADH), it would be kinetically impossible to obtain higher acetaldehyde oxidation rates in rat groups with a higher mitochondrial NADH/NAD^+ ratio. These strain differences must be explained by differences in the activity of the aldehyde dehydrogenase. Thus, AA, compared to the ANA strain, should have a higher activity; this induces more rapid oxidation of acetaldehyde, which in turn brings about a greater increase in the mitochondrial NADH/NAD^+ ratio.

Acetaldehyde and ethanol preference. In an excellent review by Truitt and Walsh[26] of the role of acetaldehyde in the actions of ethanol, the most important effect of acetaldehyde was assumed to be an interaction with the biogenic amine metabolism of the brain. With rat-brain mitochondrial aldehyde dehydrogenase, Lahti and Majchrowicz[27] demonstrated a competitive inhibition between acetaldehyde and 5-hydroxyindole aldehyde. The K_i value was found to be of the order of 10^{-6} M. Consequently the blood acetaldehyde concentrations obtained here (Table 2) were within a range that would influence the oxidation of 5-hydroxyindole aldehyde to the corresponding acid. The higher acetaldehyde output during the ethanol oxidation in the ANA strain as compared with the AA strain, suggests a greater inhibitory influence upon the metabolism of the brain, which could decisively affect behaviour in respect of ethanol preference.

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