The Physiological Role of Liver Alcohol Dehydrogenase

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1. Yeast alcohol dehydrogenase was used to determine ethanol in the portal and hepatic veins and in the contents of the alimentary canal of rats given a diet free from ethanol. Measurable amounts of a substance behaving like ethanol were found. Its rate of interaction with yeast alcohol dehydrogenase and its volatility indicate that the substance measured was in fact ethanol. 2. The mean alcohol concentration in the portal blood of normal rats was 0.045 mM. In the hepatic vein, inferior vena cava and aorta it was about 15 times lower. 3. The contents of all sections of the alimentary canal contained measurable amounts of ethanol. The highest values (average 3.7 mM) were found in the stomach. 4. Infusion of pyrazole (an inhibitor of alcohol dehydrogenase) raised the alcohol concentration in the portal vein 10-fold and almost removed the difference between portal and hepatic venous blood. 5. Addition of antibiotics to the food diminished the ethanol concentration of the portal blood to less than one-quarter and that of the stomach contents to less than one-fortieth. 6. The concentration of alcohol in the alimentary canal and in the portal blood of germ-free rats was much decreased, to less than one-tenth in the alimentary canal and to one-third in the portal blood, but detectable quantities remained. These are likely to arise from acetaldehyde formed by the normal pathways of degradation of threonine, deoxyribose phosphate and β -alanine. 7. The results indicate that significant amounts of alcohol are normally formed in the gastro-intestinal tract. The alcohol is absorbed into the circulation and almost quantitatively removed by the liver. Thus the function, or a major function, of liver alcohol dehydrogenase is the detoxication of ethanol normally present. 8. The alcohol concentration in the stomach of alloxan-diabetic rats was increased about 8-fold. 9. The activity of liver alcohol dehydrogenase is generally lower in carnivores than in herbivores and omnivores, but there is no strict parallelism between the capacity of liver alcohol dehydrogenase and dietary habit. 10. The activity of alcohol dehydrogenase of gastric mucosa was much decreased in two out of the three germ-free rats tested. This is taken to indicate that the enzyme, like gastric urease, may be of microbial origin. 11. When the body was flooded with ethanol by the addition of 10% ethanol to the drinking water the alcohol concentration in the portal vein rose to 15 mM and only a few percent of the incoming ethanol was cleared by the liver.

The physiological significance of the high activity of alcohol dehydrogenase in the mammalian liver has been puzzling because it was thought that the substrates of this enzyme do not normally occur, except in mere traces, in the great majority of mammalian species. Waller, Theorell & Sjövall (1965) commented 'The main physiological function of horseliver alcohol dehydrogenase is still unknown. The oxidation of ethanol is probably an occasional activity since ethanol is, as far as is known, not formed in the animal's body'. As alcohol dehydrogenase reacts with many types of alcohol it has been

suggested that the normal substrates are not the alcohols or aldehydes that react most readily with the enzyme but special substances such as vitamin A₂ (Zachman & Olson, 1961) or farnesol (an intermediate in the synthesis of cholesterol) (Christophe & Popják, 1961; Waller, 1965). However, the dehydrogenase that reacts with retinol in the retina is an isoenzyme different from liver alcohol dehydrogenase (Koen & Shaw, 1966). Waller *et al.* (1965) found that certain hydroxy and keto steroids can react with liver alcohol dehydrogenase, but for steroids the enzyme

has a high degree of stereospecificity and reacts only with the 3- β -hydroxy group of 5- β -steroids and their corresponding ketones, and compounds of this type are very rare in animal tissues.

The experiments reported in the present paper show that significant amounts of alcohol are formed in, and absorbed from, the intestinal tract of the rat, and that the liver effectively removes the alcohol from the blood.

EXPERIMENTAL

Collection of blood samples from portal and hepatic veins, aorta and inferior vena cava. Rats were anaesthetized by intraperitoneal injection of Nembutal (60 mg/kg body wt.). Since commercial Nembutal solutions contain ethanol the Nembutal was freshly dissolved in 0.9% NaCl to a concentration of 6%. The rats were also treated with heparin by injecting 0.1 ml of a freshly prepared solution (1000 units/ml of 0.9% NaCl) into the saphenous vein. Commercial heparin solutions proved unsuitable because they gave significant extinction changes in the ethanol determination, possibly because they contained an alcohol. The portal vein was exposed as described by Hems, Ross, Berry & Krebs (1966) and the hepatic vein by cutting the ligament connecting liver and diaphragm; the abdominal aorta and inferior vena cava were exposed by blunt dissection after moving the intestines aside. Blood samples (1 ml) were collected with 1 ml syringes, with the needle pointing against the direction of the blood flow. It was found convenient to bend the needles (25-gauge) into a U-shape. The hepatic vein samples were collected first. The samples were deproteinized with 2 ml of 10% (w/v) HClO_4 .

It was not practicable to collect 1 ml samples from more than two vessels of the same animal. Samples from the aorta and inferior vena cava were collected by similar techniques from a different group of rats.

Treatment of the contents of the intestinal tract. Contents of the stomach, small intestine, caecum and large intestine were collected immediately after sampling the blood, weighed, and deproteinized with 2 vol. of 5% (w/v) HClO_4 . For the calculation of the ethanol content it was assumed that 1 g of intestinal content equalled 1 ml and that the distribution of ethanol between sediment and supernatant was uniform. The samples were centrifuged and neutralized with KOH. An ethanol-free indicator was prepared from Bromothymol Blue and Phenol Red.

Determination of ethanol. Ethanol was determined with yeast alcohol dehydrogenase as described by Krebs, Freedland, Hems & Stubbs (1969). Because of the low alcohol concentration in the blood it was necessary to use relatively large samples. The main extinction change occurred within 5 min of adding the enzyme, but with larger samples this was often followed by a drift of up to 0.003 E unit/min, the drift being linear for at least 30 min. It was assumed that the drift was not due to ethanol, and in calculating the ethanol content the non-specific component was eliminated by extrapolation to zero time.

A direct determination of ethanol proved impractical in some extracts of the contents of the intestinal tract, especially of the caecum and large intestine, because the extracts showed high extinctions at 340 nm. In these

cases the ethanol was separated by distillation. The neutralized HClO_4 extract was placed in the side-arm of a 15 ml Thunberg tube of the shape described by Bartley (1953) and frozen in liquid N_2 , with rapid swirling in order to spread the material over a large area. The tube was then rapidly evacuated by an oil pump to about 0.5 mmHg and closed. The body of the tube was immersed in liquid N_2 so that the ethanol distilled into it. The distillation was accelerated by warming the side-arm with an infrared lamp. Warming of the liquid N_2 was avoided by covering it with a sheet of polystyrene foam, 3 cm thick, which also served as a support of the tube. When the distillation was complete (0.5–2 h) the vacuum was released and the distillate was allowed to thaw. The alcohol was determined in the distillate. Recovery of known amounts of aqueous ethanol was 85% and recovery of known amounts added to samples of intestinal content was 83%.

Specificity of the ethanol determination. Many alcohols are known to react with yeast alcohol dehydrogenase, but the rates vary greatly: the time-course of a reaction can provide information about which alcohols are present. Data on K_m values (Aono, 1958) and reaction rates (van Eys & Kaplan, 1957) indicate that the alcohols most likely to interfere with the determination of ethanol are propan-1-ol and butan-1-ol. Under the present assay conditions the times required for 50% of 0.045 μmol of alcohol to react were 0.5 min for ethanol, 1.3 min for propan-1-ol and 6.9 min for butan-1-ol; reaction was complete at 2.0 min for ethanol and at 6.9 min for propan-1-ol, whereas the reaction of butan-1-ol was only 90% complete at 15.7 min. The time-courses in the present assays indicated that the bulk of the 'alcohol' was ethanol. Nevertheless the term 'alcohol' is used in this paper when referring to the results of measurements made with yeast alcohol dehydrogenase; the term 'ethanol' is used when the alcohol was known to be ethanol, e.g. when used in recovery tests.

Experimental animals. Adult female Wistar rats weighing about 200 g were used. They were maintained on a commercial standard diet (Oxoid pasteurized breeding diet for rats and mice).

Germ-free rats (Strain A/GUS) were obtained from the Medical Research Council Laboratory Animals Centre, Carshalton, Surrey, U.K., by courtesy of Dr D. K. Blackmore. They originated from the laboratory of Professor B. E. Gustafsson, Carolinska Institute, Stockholm, Sweden, and belonged to the Long-Evans strain; the strain had been germ-free for over 30 generations. The samples of blood and intestinal contents were collected immediately after the removal of the rats from their germ-free environment. Control rats of the Long-Evans strain (pathogen-free) were also obtained from the Centre; these had been derived from the germ-free strain about 6 months before the experiments.

Modifications of diet. To test whether the alcohol concentration of the intestinal contents depended on the nature of the food, a diet high in soluble carbohydrate was given for 3 days. It consisted of 75% sucrose, 22.5% casein and 2.5% mineral-plus-vitamin supplement. In some experiments antibiotics were added to the standard diet. Neomycin sulphate (Glaxo Laboratories Ltd., Greenford, Middx., U.K.) was given in the drinking water (0.75%), the mean dose being about 140 mg/rat per day.

Table 1. *Alcohol content of portal blood and hepatic venous blood in rats under various conditions*

For experimental details see the text. The mean percentage decrease of the alcohol content of the blood during the flow through the liver was calculated from the difference in individual rats and not from the mean alcohol concentrations in the portal veins and hepatic veins. The values are expressed as means $\pm$ s.e.m. with numbers of observations in parentheses.

Condition of rats	Alcohol concentration (mm)		% decrease of blood alcohol concentration on flow through liver
	Portal vein	Hepatic vein	
Normal, well fed	0.045 $\pm$ 0.004 (10)	0.0033 $\pm$ 0.002 (10)	91.9 $\pm$ 4.6% (10)
Treated with neomycin and nystatin	0.010 $\pm$ 0.002 (4)	0.0018 $\pm$ 0.0006 (4)	80.3 $\pm$ 7.8% (4)
Diet low in roughage and high in soluble carbohydrate	0.019 $\pm$ 0.013 (3)	0.0028 $\pm$ 0.001 (3)	82.6 $\pm$ 5.6% (3)
Continuous pyrazole infusion for 19 h	0.46 $\pm$ 0.037 (7)	0.43 $\pm$ 0.046 (7)	6.5 $\pm$ 11.7% (7)

Nystatin (Nystan for oral suspension; E. R. Squibb and Sons, Twickenham, Middx., U.K.) was mixed with the powdered standard diet, which was supplied in the form of a thick paste, by adding a little water to the powder. The mean dose of nystatin was about 15 mg/rat per day. This regimen was also given for 3 days.

RESULTS

Alcohol in the portal and hepatic veins. The alcohol concentrations in the hepatic veins (Table 1) were of the same order of magnitude (0.0012–0.019 mm) as those reported in the literature (reviewed by Harger & Forney, 1963) for normal mammalian blood not exposed to an intake of ethanol. The normal portal veins contained about 15 times more alcohol than the hepatic veins. On average 92% of the alcohol present in the portal vein was removed from the blood by the liver. Determination of alcohol in the inferior vena cava and abdominal aorta gave values [0.0017 $\pm$ 0.0005 mm (5) and 0.0043 $\pm$ 0.0013 mm (5) respectively] that were not significantly different from those of the hepatic vein in view of the limits of the method at the very low alcohol concentrations. Treatment of the rats with neomycin and nystatin decreased the mean alcohol content of the portal blood to 22% of the normal values (Table 1). The livers of the treated animals still removed 80% of the alcohol so that the alcohol content of the hepatic veins became virtually identical with that of the untreated rats.

These observations indicate that the alcohol in the portal blood originated from the intestinal tract, presumably formed by microbial fermentations. Since roughage tends to create favourable conditions for such fermentations, the crude fibre content was varied by replacing the standard diet containing 2.7% roughage by a diet containing 75% sucrose and no roughage instead of material derived from grains. The rapid absorption of the

nutrients of such a diet would decrease microbial fermentation. As shown in Table 1, the alcohol content of the portal veins was significantly lower in this group of rats than in the controls ($P < 0.01$).

Effect of pyrazole on the concentration of alcohol in the blood. If alcohol is continually formed in the intestinal tract and removed by the alcohol dehydrogenase of the liver it is to be expected that inhibition of the dehydrogenase would cause a rise of the blood alcohol concentration. To test this, pyrazole, a relatively non-toxic powerful inhibitor of alcohol dehydrogenase (Theorell & Yonetani, 1963), was continually infused into the circulation over a period of about 19 h. The rats were anaesthetized with ether and given an initial intraperitoneal dose of 20 μ mol of pyrazole (0.2 ml of an aqueous 0.1 M solution). A hypodermic needle (25-gauge) was inserted into the tail vein and kept in position by adhesive tape. The rats were then placed in restraining cages and the needle was connected with a reservoir containing aqueous 0.067 M-pyrazole. A Delta pump (Watson-Marlow Ltd., Falmouth, Cornwall, U.K.) was inserted in the connecting tubing to give a mean delivery of 0.32 ml/h. Blood samples were taken from the portal and hepatic veins immediately after the infusion had been stopped. Recovery tests showed that the presence of pyrazole in the blood samples did not interfere with the determination of ethanol. Pyrazole treatment had the expected effect (Table 1). The removal of alcohol from the portal blood was almost completely inhibited. The hepatic vein contained about 130 times more alcohol than the normal control and the difference between alcohol concentrations in the portal and hepatic veins was not significant.

Alcohol in the contents of the gastro-intestinal tract. Measurable amounts of alcohol were found in the contents throughout the gastrointestinal tract (Table 2). In the normal animals the highest

Table 2. *Alcohol in the gastro-intestinal tract*

Rats were generally killed in the morning. In normal rats the total quantities of alcohol were calculated only when the concentrations had been measured throughout the tract. The values are expressed as means $\pm$ S.E.M. with numbers of observations in parentheses, except when only two observations were made when the mean is given.

Condition of rats	Stomach			Small intestine			Caecum			Large intestine			Total alcohol content of the whole tract (μ mol)
	Concn. of alcohol (μ mol/g)	Total alcohol content (μ mol)	Concn. of alcohol (μ mol/g)	Concn. of alcohol (μ mol/g)	Total alcohol content (μ mol)	Concn. of alcohol (μ mol/g)	Concn. of alcohol (μ mol/g)	Total alcohol content (μ mol)	Concn. of alcohol (μ mol/g)	Concn. of alcohol (μ mol/g)	Total alcohol content (μ mol)	Concn. of alcohol (μ mol/g)	
Normal, well fed	3.71 ± 1.59 (11)	2.39 ± 0.91 (5)	0.39 ± 0.08 (5)	0.64 ± 0.12 (5)	0.89 ± 0.17 (18)	0.89 ± 0.32 (5)	0.34 ± 0.06 (5)	0.20 ± 0.05 (5)	4.13 ± 1.13 (5)				
Treated with neomycin and nystatin	0.07 ± 0.03 (4)	0.09 ± 0.03 (4)	0.15 ± 0.08 (4)	0.32 ± 0.20 (4)	0.37 ± 0.05 (4)	3.30 ± 0.59 (4)	0.21 ± 0.09 (4)	0.33 ± 0.18 (4)	4.03 ± 0.80 (4)				
Diet low in roughage and high in soluble carbohydrate	0.86 ± 0.39 (3)	2.01 ± 1.15 (3)	0.33 ± 0.09 (3)	0.22 ± 0.07 (3)	1.08 ± 0.23 (3)	1.36 ± 0.20 (3)	1.32 ± 0.52 (3)	0.16 ± 0.07 (3)	3.75 ± 1.33 (3)				
Diet supplemented with 2% (w/w) fresh baker's yeast	16.4 (mean of 2)	26.2 (mean of 2)	0.26 (mean of 2)	0.72 (mean of 2)	0.67 (mean of 2)	2.53 (mean of 2)	0.58 (mean of 2)	0.45 (mean of 2)	29.9 (mean of 2)				
Diet supplemented with 2% (w/w) fresh baker's yeast and 10% glucose	14.9 ± 7.7 (4)	20.2 ± 12.2 (4)	0.81 ± 0.22 (4)	0.99 ± 0.22 (4)	0.92 ± 0.11 (4)	2.14 ± 0.16 (4)	0.48 ± 0.15 (4)	0.45 ± 0.16 (4)	23.8 ± 12.2 (4)				

concentrations and the highest total amounts were found in the stomach (mean concentration $3.7 \mu\text{mol/g}$). The next highest values were in the caecum (mean concentration $0.9 \mu\text{mol/g}$). As expected, antibiotics greatly decreased the alcohol concentration throughout the tract. The effect was most marked in the stomach where the alcohol concentration fell to 2% of the normal value. Despite the fall in the alcohol concentration, there was a large increase in the total amount of alcohol in the caecum. This was due to an increase in the wet weight of the contents from 1.1–2.9 g for the normal animals to 7.6–10.4 g for the treated animals. The antibiotics did not affect in a major way the alcohol contents of small and large intestines and the total content was about the same as in the normal rats. The diet low in roughage and high in soluble carbohydrate increased the alcohol concentration in the caecum and large intestine, presumably because the concentrations of fermentable sugars were higher than normal in these regions. However, the total alcohol content was similar to that of normal animals. Addition of 2% (w/w) of fresh baker's yeast to the standard diet or to the standard diet supplemented with 10% (w/w) of glucose increased the alcohol concentration in the stomach about four-fold; addition of glucose doubled the mean alcohol concentration in the small intestine. To prevent alcohol accumulation in the diet, the food was prepared freshly each day by adding the yeast cake (and glucose) to the dry powdered standard diet. Neither the alcohol concentrations in the portal vein [0.048 mm (mean of 2) and $0.043 \pm 0.006 \text{ mm}$ (4) respectively] nor in the hepatic veins [0.0095 mm (mean of 2) and $0.0062 \pm 0.003 \text{ mm}$ (4) respectively] were significantly affected by the addition of yeast.

Alcohol formation by the contents of the gastro-intestinal tract. To obtain information on the rate of alcohol formation within the gastro-intestinal tract the contents of the stomach, caecum and large intestine were removed from fed rats immediately after death, weighed, suspended with the help of a glass rod in 4 vol. of diluent and incubated with glucose at 37°C under anaerobic conditions. For incubation of the stomach contents the diluent was 25 mm-glucose; for the contents of the caecum and large intestine it was a solution containing 25 mm- NaHCO_3 , 130 mm- NaCl and 25 mm-glucose. For convenience Warburg flasks were used for the incubations. These contained 1 ml of the suspension in the main compartment of conical vessels, a stick of yellow phosphorus in the centre-well and 0.5 ml of 5% (w/v) perchloric acid in the side-arm. The gas space was filled with $\text{CO}_2 + \text{N}_2$ (5:95). Enzyme activities were stopped by adding the perchloric acid solution from the side-arm after 10, 20 and 40 min. The rate of increase of the ethanol con-

Table 3. *Alcohol formation by the contents of the gastro-intestinal tract of rats*

The contents of stomach, caecum or large intestine were suspended in 4 vol. of glucose-containing medium and were incubated anaerobically. Details are given in the text. Normal well-fed rats on the standard diet were used. Each mean value is based on observations on three different rats.

Material incubated	Initial alcohol concentration ($\mu\text{mol/g}$)		Rate of alcohol formation ($\mu\text{mol/h per g}$)	
	Range	Mean	Range	Mean
Contents of stomach	0.9–12.6	5.1	1.0–2.9	1.7
Contents of caecum	0.5–1.0	0.7	1.1–2.7	2.1
Contents of large intestine	0.4–0.6	0.5	2.3–3.0	2.5

Table 4. *Alcohol in the blood and the contents of the gastro-intestinal tract of 'conventional' and germ-free Long-Evans rats*

For experimental details see the text. The values are expressed as means $\pm$ S.E.M. with numbers of observations in parentheses.

Condition of rats ...	'Conventional'	Germ-free
Blood alcohol concentration (mm)		
Portal vein	0.051 ± 0.012 (7)	0.018 ± 0.006 (3)
Hepatic vein	0.015 ± 0.005 (7)	0.023 ± 0.003 (3)
Difference between portal and hepatic veins	0.036 ± 0.009 (7)	-0.005 ± 0.004 (3)
Percentage decrease on flow through liver	71.0 $\pm$ 8.0 (7)	
Alcohol concentration in gastro-intestinal contents ($\mu\text{mol/g}$)		
Stomach	1.16 ± 0.45 (7)	0.10 ± 0.05 (3)
Small intestine	0.42 ± 0.09 (7)	0.035 ± 0.010 (3)
Caecum	1.62 ± 0.25 (7)	0.024 ± 0.011 (3)
Large intestine	0.46 ± 0.13 (7)	0.018 ± 0.008 (3)

centration was approximately linear over the period tested.

As shown in Table 3, alcohol was formed in all three samples. There were wide individual variations in the stomach contents. The mean rates were of the same order of magnitude, in the region of $2 \mu\text{mol/h per g}$.

Alcohol in germ-free rats. If the alcohol found in the blood and intestinal contents stems solely from microbial fermentations it is to be expected that germ-free rats would be free from alcohol. To test this postulate alcohol in the portal and hepatic veins and in the contents of the intestinal tract was measured. Control Long-Evans rats that had been kept under 'conventional' conditions for 6 months were available for comparison. The alcohol concentrations in the portal vein of rats of the 'conventional' Long-Evans strain were about the same as in rats of the Wistar strain. The alcohol concentrations in the hepatic vein were somewhat higher than in the Wistar strain; the average

removal on passage through the liver was 72%. This removal was slightly less than in the Wistar strain, which may be related to the lower alcohol dehydrogenase activity of the Long-Evans rats (see Table 4). The alcohol concentration in the stomach was rather lower in the Long-Evans rats than in the Wistar strain, but on the whole there were no major differences between the Wistar and the 'conventional' Long-Evans strains.

As expected, very much less alcohol was found in all specimens collected from the germ-free rats except in the hepatic veins. The concentration in the portal veins of the germ-free rats was about one-third of that in the 'conventional' rats. There was no significant difference between the alcohol concentrations in the portal and hepatic veins of the germ-free rats. In the contents of the intestinal tract the alcohol concentrations were less than 10% of that of the conventional rats; they were still lower than in the Wistar rats treated with antibiotics. However, measurable amounts of 'alcohol'

Table 5. *Alcohol in the portal and hepatic veins and in the contents of the intestinal tract in acute alloxan-diabetes*

The rats were killed 48 h after intravenous injection of alloxan. For details see the text. The values are expressed as means $\pm$ S.E.M. with numbers of observations in parentheses.

Concn. of blood alcohol (mm)		
Portal vein	0.081 $\pm$	0.019 (9)
Hepatic vein	0.036 $\pm$	0.010 (9)
Difference between portal and hepatic veins	0.045 $\pm$	0.014 (9)
Percentage decrease on flow through liver	61 $\pm$	10 (9)
Alcohol concentration in gastro-intestinal contents (μ mol/g)		
Stomach	30 $\pm$	6 (8)
Small intestine	0.81 $\pm$	0.33 (7)
Caecum	0.12 $\pm$	0.03 (7)
Large intestine	0.27 $\pm$	0.08 (4)

were still found in the germ-free animals; whether the 'alcohol' measured was ethanol is discussed below.

Alcohol in diabetic rats. The raised glucose concentration in diabetic tissues may be expected to be associated with raised glucose concentration in the gastro-intestinal contents because the absorption of glucose may be decreased on account of the higher concentration gradients. The alcohol content of the portal and hepatic veins and of the intestinal tract were therefore measured in alloxan-diabetic rats. Two groups of rats were made diabetic as described by Williamson, Lund & Krebs (1967). The rats of one group were killed at 48 h. Those of the other were maintained for from 3 to 6 weeks by a daily subcutaneous injection of 1 i.u. of protamine zinc insulin; insulin was withheld for 24 h before the animals were killed, and only if the blood glucose concentrations were greater than 30 mm were the rats included in the experiment.

In the rats killed at 48 h, a doubling ($P < 0.05$) of the mean portal vein alcohol concentration and a considerable increase in the hepatic venous blood alcohol concentration were found (Table 5). The mean absolute difference between the concentrations was similar to that in the normal rats, but the percentage decrease of the alcohol concentration on passage of the blood through the liver was much smaller. The mean alcohol concentration in the stomach was about eight times that in the controls, but there were no significant increases elsewhere in the intestinal tract. In the caecum the alcohol concentration was well below the normal value. Hochheuser, Weiss & Wieland (1964) have reported an increased acetate concentration in the blood of chronic alloxan-diabetic rats. Ethanol is a major precursor of acetate, but whether the increased alcohol content of the stomach in diabetic rats is related to the increased acetate concentration in the blood remains an open question.

In the chronic diabetic rats treated with insulin, there was no increase in the portal venous blood alcohol concentrations.

Liver alcohol dehydrogenase activity in various species. If it is the main function of liver alcohol dehydrogenase to dispose of ethanol formed in the intestinal tract, it may be expected that the capacity of the enzyme is higher in herbivorous than in carnivorous species. A survey of several species (Table 6) reveals no strict parallelism between the activity of the enzyme and dietary habits, though several of the species differences are in the expected direction. The lowest values (below 10 units) occur in carnivores, though cattle are an exception. The high capacities of human and horse liver alcohol dehydrogenases are striking. The present values for cattle, guinea pig, rat and mouse agree reasonably well with those obtained by Moser, Papenburg & von Wartburg (1968).

The two rat strains show differences on the borderline of significance ($P < 0.05$), but there was no difference between germ-free and conventional rats. Strain differences have also been reported for the alcohol dehydrogenase activity of mice (Wilson, Respass, Hollifield & Parson, 1961; Rodgers, McClearn, Bennett & Herbert, 1963).

Alcohol dehydrogenase in gastric mucosa. Schmidt & Schmidt (1960) have reported that human gastric mucosa possesses a higher alcohol dehydrogenase activity than any other human organ tested except liver, the activity of the gastric mucosa being about 3% of that of the liver (see also Moser *et al.* 1968). Rat gastric mucosa also contains measurable quantities of alcohol dehydrogenase, especially in the fundus region (Krebs *et al.* 1969). The fact that at least one enzyme found in the gastric mucosa, namely urease, is known to be of bacterial origin (Kornberg, Davies & Wood, 1954a,b) raises the question of whether gastric alcohol dehydrogenase may also stem from parasitic micro-organisms such

Table 6. *Liver alcohol dehydrogenase activity in various species*

Activity was determined as described by Krebs *et al.* (1969) at pH 7.2. The specimens of human liver were biopsy samples: the material had a normal appearance. The value for horse liver is based on measurements by Moser *et al.* (1968). The values are expressed as means $\pm$ S.E.M. with number of observations in parentheses, except when only one or two observations were made.

Species	Dietary habit	Liver alcohol dehydrogenase activity (μ mol of NADH oxidized/min per g wet wt. of tissue)
Horse	Herbivorous	220
Sheep	Herbivorous	19.8 $\pm$ 5.2 (4)
Guinea pig	Herbivorous	17.1 (one value)
Cattle	Herbivorous	2.8 $\pm$ 0.3 (7)
Man	Omnivorous	43.7 $\pm$ 6.8 (9)
Rat (Wistar)	Omnivorous	18.7 $\pm$ 3.7 (8)
Rat (Long-Evans, 'conventional')	Omnivorous	11.0 $\pm$ 0.8 (7)
Rat (Long-Evans, germ-free)	Omnivorous	8.6 $\pm$ 2.2 (3)
Mouse	Omnivorous	17.0 $\pm$ 1.6 (3)
Dog	Carnivorous	8.6 (mean of 2 values)
Cat	Carnivorous	5.2 $\pm$ 0.6 (3)
Common shrew	Carnivorous	4.3 $\pm$ 1.3 (4)
Ferret	Carnivorous	2.4 $\pm$ 0.2 (3)
Water shrew	Carnivorous	<0.1 (one value)

Table 7. *Alcohol dehydrogenase activity of gastric mucosa*

The enzyme assay was carried out as described by Krebs *et al.* (1969). The values are expressed as means $\pm$ S.E.M. with numbers of observations in parentheses. A value of <0.001 means not detectable.

Type of rat	Alcohol dehydrogenase activity (μ mol of NADH oxidized/min per g)	
	Fundus	Corpus
Wistar, adult	0.30 $\pm$ 0.14 (3)	0.20 $\pm$ 0.03 (3)
Long-Evans ('conventional')	0.38 $\pm$ 0.13 (7)	<0.001 (6)
		0.49 (1)
Long-Evans (germ-free)	<0.001 (2)	<0.001 (3)
	0.44 (1)	

as *Sarcina ventriculi*, which obtains almost all of its energy from an alcoholic fermentation and is known to be a common inhabitant of the gastric mucosa (Goodsir, 1842; Smit, 1930; Kluyver, 1931; Bauchop & Dawes, 1959; Arbutnott, Bauchop & Dawes, 1960). The activity of alcohol dehydrogenase was therefore measured in the gastric mucosa of three germ-free rats (Table 7).

The Wistar control rats showed a much lower activity than that previously found in this laboratory, especially in the fundus region. The 'conventional' Long-Evans rats had the same activity as the Wistar strain in the fundus, but six of these had no detectable activity in the corpus whereas one gave a 'normal' value. The 'conventional' strain had been derived from germ-free ancestry only 6 months

before the experiments, so their microbial flora might still have been subnormal.

Two of the germ-free rats showed no detectable enzyme activity, but in one other rat the fundus gave a value as high as in the 'conventional' animals. But for this one result the experiment suggests that gastric alcohol dehydrogenase was of microbial origin. The variable, erratic, enzyme activities of the conventional rats also supported this conclusion. The enormous differences between the results of the assays in the intestinal mucosa obtained by Mistilis & Birchdale (1969) and Krebs *et al.* (1969) may also be connected with microbial contamination of the tissue.

Alcohol in body fluids after ethanol intake. In the experiments on normal rats recorded in Tables 1,

Table 8. *Alcohol in blood and gastro-intestinal contents after ethanol consumption*

The animals had free access to aq. 10% (v/v) ethanol for 1 h before they were killed. For experimental details see the text.

	Rat 1	Rat 2
Decrease in volume of drinking fluid (ml)	2.8	4.0
Blood alcohol concentration (mM)		
Portal vein	15.8	11.1
Hepatic vein	15.0	10.3
Difference between portal and hepatic veins	0.81	0.82
Percentage decrease on flow through liver	5.1	7.4
Alcohol in gastro-intestinal contents ($\mu\text{mol/g}$)		
Stomach	239	199
Small intestine	12.1	8.1
Caecum	9.0	5.5
Large intestine	10.5	4.2

4 and 5, almost all the alcohol present in the incoming blood was removed by the liver. To measure the maximal capacity of the liver for 'clearing' alcohol from the blood, rats were deprived of water overnight and then provided through their drinking bottle with an aqueous 10% (v/v) ethanol solution. After 1 h, when the volume of the drinking fluid had decreased by a few millilitres, the animals were killed for ethanol determinations (Table 8). The concentrations of the alcohol in the stomach contents was 0.24 and 0.20 M (compared with 1.7 M in the drinking fluid). It was very much lower in the small intestine, caecum and large intestine (about 0.01 M). Most probably this dilution was caused by secretion into the intestinal tract rather than by selective absorption of alcohol. The portal vein contained relatively high concentrations of ethanol (15 and 11 mM), and the percentage decrease of the alcohol concentration during the passage through the liver was only 5 and 7%. This was partly due to the dilution of portal blood by the hepatic arterial blood.

Alcohol in cattle rumen. The relatively high alcohol content in the rat stomach prompted us to test the alcohol content of cattle rumen, where alcohol has been known to arise regularly as a very minor product of carbohydrate fermentation (Allison, Dougherty, Bucklin & Snyder, 1964; Cunningham & Brisson, 1955; Krogh, 1959; Moomaw & Hungate, 1963). The experiments were carried out on four fistulated cows made available to us by Dr J. D. Sutton at the National Institute for Research in Dairying, Shinfield, Reading, U.K. Between 1 and 5 h after feeding, samples of rumen fluid (5 ml) were withdrawn from the centre of the rumen through cannulae provided with filters. In two cows, fed on mainly dairy concentrates, the alcohol concentration in the rumen fluid reached 2 mM after 3 and 4 h. Addition of glucose and sucrose to the feed did not increase the concentration of alcohol. In two other cows, fed on either hay or

rolled barley, the alcohol concentration in the rumen fluid remained below 0.1 mM. Thus the alcohol concentrations were lower than those found in the stomach content of the rat. As the amounts present in the stomach are the resultant of the rates of formation and absorption the steady-state concentrations cannot be regarded as a measure of the rate of formation.

DISCUSSION

Alcohol in the body fluids and its origin. The experiments show conclusively that a volatile substance behaving like ethanol in relation to yeast alcohol dehydrogenase is present in the gastro-intestinal tract of rats not exposed to ethanol. Although alcohol dehydrogenase reacts with alcohols other than ethanol, the rate of reaction of the alcohol in the body fluids was specific for ethanol. The conclusion is thus justified that ethanol is formed in, and absorbed from, the intestinal tract. The substance reaches the liver through the portal vein and largely (80–90%) disappears in the liver. Most of the ethanol must be of microbial origin because the concentration of ethanol within the intestinal tract and in the portal vein is greatly decreased in germ-free rats and in rats given antibiotics with their food.

Apart from *Sarcina ventriculi*, a common inhabitant of the gastric mucosa that is known to derive its energy mainly by alcoholic fermentations (Goodsir, 1842; Bauchop & Dawes, 1959; Arbutnot et al. 1960), there are several intestinal parasites that produce alcohol (see von Brand, 1966). These include Cestodes (*Echinococcus*, *Taenia*) and Acanthocephala (*Moniliformis dubius*). The last-named organism inhabits the alimentary tract of rats and according to Ward & Crompton (1969) ferments glucose mainly to ethanol and CO₂, with succinate, acetate, butyrate and lactate as minor by-products.

There are many reports indicating that animals under their normal living conditions may become exposed to large amounts of alcohol. This applies for example to African elephants (Carrington, 1958) and to warthogs (Moorehead, 1962) when feeding on the decaying fruit of the marula tree (*Sclerocarya schweinfurthii*; Anacardiaceae) and to the ring tailed lemur, *Lemur catta* (Jolly, 1966) when feeding on the fermenting pods of the tamarind tree (*Tamarindus indica*). Ethanol has also been found (McBee & West, 1969) as a major product of caecal fermentation in Willow ptarmigans (*Lagopus lagopus*), reaching concentrations of 130 mm in the caecal contents. Bullfinches and hawfinches can become intoxicated if they feed on willow catkins, which contain a readily fermenting nectar (D. Lack personal communication). Cows have been reported to become intoxicated on feeding on windfall apples (Frost, 1923).

Physiological significance of liver alcohol dehydrogenase. It may now be taken as established that the major function of liver alcohol dehydrogenase is to remove from the circulation a potentially toxic substance, ethanol, and channel it into the pathway of energy supply. The relatively high activity of the enzyme can hardly be regarded as excessive because it does not even protect the body effectively from intoxication under some naturally occurring circumstances. The amounts of ethanol that are normally formed are almost entirely disposed of by the liver enzyme.

Endogenous sources of ethanol. Although the experiments show that most of the alcohol in the blood is of microbial origin, measureable quantities of alcohol were still present in the blood of germ-free rats. Thus there must be a non-microbial and non-parasitic source of ethanol. Several degradation reactions are in fact known to form acetaldehyde, and any aldehyde formed may be expected to be rapidly reduced to ethanol because the equilibrium in the alcohol dehydrogenase system favours the formation of ethanol. Threonine can be split into glycine and acetaldehyde (Braunstein & Vilenkina, 1949; Meltzer & Sprinson, 1952; Greenberg, 1961). Deoxyribose phosphate is split to form glyceraldehyde phosphate and acetaldehyde (Racker, 1952). A third precursor is β -alanine, which forms acetaldehyde via malonic aldehyde and subsequent decarboxylation (Pihl & Fritzson, 1955).

Microsomal oxidation of alcohol. It has been suggested (Orme-Johnson & Ziegler, 1965; Lieber & DeCarli, 1968; Rubin & Lieber, 1968; Roach, Reese & Creaven, 1969) that the oxidation of alcohol by the microsomal 'mixed-function' oxidase system (Mason, 1957) involving cytochrome P-450, and NADPH may play a major role in the metabolism of alcohol. This system is not inhibited by pyrazole (H. A. Krebs & M. Stubbs, unpublished work),

and, since the present experiments show that the removal of physiological concentrations of alcohol by the liver in normal rats was almost completely inhibited by pyrazole, the microsomal oxidase system cannot have played a significant role under the experimental conditions (see also Goldberg & Rydberg, 1969). It is not impossible that the system may operate at high ethanol concentrations, but this is unlikely because appreciable activity can be demonstrated only in the presence of relatively high concentrations of NADPH. The 'mixed-function' oxidase system, which can oxidize a large number of substrates, is inducible, but even when maximally induced its potential activity is still low compared with that of alcohol dehydrogenase, at least in the rat. The capacity to oxidize alcohol is in all probability connected with the lack of specificity of the system.

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REFERENCES

- Allison, M. J., Dougherty, R. W., Bucklin, J. A. & Snyder, E. E. (1964). *Science, N.Y.*, **144**, 54.
- Aono, Y. (1958). *Osaka Daig. Igaku Zasshi*, **10**, 619; quoted from *Chem. Abstr.* (1958), **52**, 15620k.
- Arbuthnott, J. P., Bauchop, T. & Dawes, E. A. (1960). *Biochem. J.* **76**, 12r.
- Bartley, W. (1953). *Biochem. J.* **53**, 305.
- Bauchop, T. & Dawes, E. A. (1959). *Biochim. biophys. Acta*, **36**, 294.
- Braunstein, A. E. & Vilenkina, G. Y. (1949). *Dokl. Akad. Nauk SSSR*, **66**, 243.
- Carrington, R. (1958). *Elephants*, p. 68. London: Chatto & Windus.
- Christophe, J. & Popják, G. (1961). *J. Lipid Res.* **2**, 244.
- Cunningham, H. M. & Brisson, G. J. (1955). *Can. J. agr. Sci.* **35**, 511.
- Frost, R. (1923). In *Selected Poems*, p. 3. New York: Henry Holt and Co.
- Goldberg, L. & Rydberg, U. (1969). *Biochem. Pharmac.* **18**, 1749.
- Goodsir, J. (1842). *Edinb. Med. Surg. J.* **57**, 430.
- Greenberg, D. M. (1961). In *Metabolic Pathways*, vol. 2, p. 92. Ed. by Greenberg, D. M. New York and London: Academic Press.

- Harger, R. N. & Forney, R. B. (1963). In *Progress in Chemical Toxicology*, vol. 1, p. 53. Ed. by Stolman, A. New York and London: Academic Press.
- Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966). *Biochem. J.* **101**, 284.
- Hochheuser, W., Weiss, H. & Wieland, O. (1964). *Z. klin. Chem.* **2**, 175.
- Jolly, A. (1966). In *Lemur Behavior*, p. 82. Chicago: University of Chicago Press.
- Kluyver, A. J. (1931). In *The Chemical Activities of Micro-organisms*, p. 39. London: University of London Press.
- Koen, A. L. & Shaw, C. S. (1966). *Biochim. biophys. Acta*, **128**, 48.
- Kornberg, H. L., Davies, R. E. & Wood, D. R. (1954a). *Biochem. J.* **56**, 355.
- Kornberg, H. L., Davies, R. E. & Wood, D. R. (1954b). *Biochem. J.* **56**, 363.
- Krebs, H. A., Freedland, R. A., Hems, R. & Stubbs, M. (1969). *Biochem. J.* **112**, 117.
- Krogh, N. (1959). *Acta Vet. scand.* **1**, 74.
- Lieber, C. S. & DeCarli, L. M. (1968). *Science, N.Y.*, **162**, 917.
- McBee, R. H. & West, G. C. (1969). *Condor*, **71**, 54.
- Mason, H. S. (1957). *Adv. Enzymol.* **19**, 79.
- Meltzer, H. L. & Sprinson, D. B. (1952). *J. biol. Chem.* **197**, 461.
- Mistilis, S. P. & Birchdall, A. (1969). *Nature, Lond.*, **223**, 199.
- Moomaw, C. R. & Hungate, R. E. (1963). *J. Bact.* **85**, 721.
- Moorehead, A. (1962). In *No Room in the Ark*, p. 26. London: Penguin Books Ltd.
- Moser, K., Papenburg, J. & von Wartburg, J. P. (1968). *Enzym. biol. Clin.* **9**, 447.
- Orme-Johnson, W. H. & Ziegler, D. M. (1965). *Biochem. biophys. Res. Commun.* **21**, 78.
- Pihl, A. & Fritzson, P. (1955). *J. biol. Chem.* **215**, 345.
- Racker, E. (1952). *J. biol. Chem.* **196**, 347.
- Roach, M. K., Reese, W. N. & Creaven, P. J. (1969). *Biochem. biophys. Res. Commun.* **36**, 596.
- Rodgers, D. A., McClearn, G. E., Bennett, E. L. & Herbert M. (1963). *J. comp. physiol. Psychol.* **56**, 666.
- Rubin, E. & Lieber, C. S. (1968). *Science, N.Y.*, **162**, 690.
- Schmidt, E. & Schmidt, F. W. (1960). *Klin. Wschr.* **38**, 957.
- Smit, J. (1930). *Die Gärungssarcine: Monographien der Pflanzenforschung*, Heft, 14. Jena: Gustav Fisher.
- Theorell, H. & Yonetani, T. (1963). *Biochem. Z.* **338**, 537.
- van Eys, J. & Kaplan, N. O. (1957). *J. Am. chem. Soc.* **79**, 2782.
- von Brand, T. (1966). *Biochemistry of Parasites*, pp. 112–114, 116–117, 126–127. New York and London: Academic Press.
- Waller, G. R. (1965). *Nature, Lond.*, **207**, 1389.
- Waller, G. R., Theorell, H. & Sjövall, J. (1965). *Archs Biochem. Biophys.* **111**, 671.
- Ward, P. F. V. & Crompton, D. W. T. (1969). *Proc. R. Soc. B*, **172**, 65.
- Williamson, D. H., Lund, P. & Krebs, H. A. (1967). *Biochem. J.* **103**, 514.
- Wilson, E. C., Respass, J. C., Hollifield, G. & Parson, W. (1961). *Gastroenterology*, **40**, 807.
- Zachman, R. D. & Olson, J. A. (1961). *J. biol. Chem.* **236**, 2309.