Effect of Maternal Ethanol Consumption on Foetal and Neonatal Rat Hepatic Protein Synthesis

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Effects of maternal ethanol consumption were investigated on the rates of protein synthesis by livers of foetal and neonatal rats both *in vivo* and *in vitro*, and on the activities of enzymes involved in protein synthesis and degradation. The rates of general protein synthesis by ribosomes *in vitro* studied by measuring the incorporation of [^{14}C]leucine into ribosomal protein showed that maternal ethanol consumption resulted in an inhibition of the rates of protein synthesis by both foetal and neonatal livers from the ethanol-fed group. The rates of incorporation of intravenously injected [^{14}C]leucine into hepatic proteins were also significantly lower in the foetal, neonatal and adult livers from the ethanol-fed group. Incubation of adult-rat liver slices with ethanol resulted in an inhibition of the incorporation of [^{14}C]leucine into hepatic proteins; however, this effect was not observed in the foetal liver slices. This effect of externally added ethanol was at least partially prevented by the addition of pyrazole to the adult liver slices. Pyrazole addition to foetal liver slices was without significant effect on the rates of protein synthesis. Cross-mixing experiments showed that the capacity of both hepatic ribosomes and pH5 enzyme fractions to synthesize proteins was decreased in the foetal liver from the ethanol-fed group. Maternal ethanol consumption resulted in a decrease in hepatic total RNA content, RNA/DNA ratio and ribosomal protein content in the foetal liver. Foetal hepatic DNA content was not significantly affected. Ethanol consumption resulted in a significant decrease in proteolytic activity and the activity of tryptophan oxygenase in the foetal, neonatal and adult livers. It is possible that the mechanisms of inhibition of protein synthesis observed here in the foetal liver after maternal ethanol consumption may be responsible for at least some of the changes observed in 'foetal alcohol syndrome'.

The metabolic consequences of acute ethanol administration on the liver have been investigated in the past in several laboratories (Lundquist *et al.*, 1962; Forsander *et al.*, 1965; Krebs, 1968; Veech *et al.*, 1972). A variety of alterations observed in the intermediary metabolism of liver after acute administration of ethanol have been found to be the direct result of hepatic ethanol metabolism (Reboucas & Isselbacher, 1961; Rawat, 1968; Williamson *et al.*, 1969). Although a significant amount of information is now available about the effects of ethanol on the pathways of carbohydrate and lipid metabolism in the liver, effects of ethanol on the metabolism of protein have, however, not been investigated in detail. Some workers have suggested that ethanol impairs the release of lipoproteins from liver (Isselbacher & Greenberger, 1964) and decreases the synthesis of albumin, with polyribosome disaggregation (Oratz *et al.*, 1973). Ethanol has been observed to decrease transferrin and albumin within hours after its oral administration, accompanied by an effect on hepatic

endoplasmic reticulum (Jeejeebhoy *et al.*, 1972; Nadkarni, 1974).

Observations from our laboratory (Rawat, 1975) on the inhibitory effect of ethanol on cerebral protein synthesis in the developing brain have led to our interest in the effects of ethanol on protein synthesis in the developing and adult liver.

There has been widespread interest recently in the 'foetal alcohol syndrome'; however, the biochemical and metabolic mechanisms of this syndrome are not well understood. Human experience suggests that infants born to alcoholic mothers are growth-retarded, may have morphological and cardiac anomalies and may be mentally deficient (Jones *et al.*, 1973).

Since ethanol passes freely from the maternal circulation to the foetus via the placenta and through the breast milk to the newborn, in the present study it was decided to investigate (a) the effect of maternal ethanol consumption on foetal hepatic protein synthesis, (b) hepatic protein synthesis of suckling newborn rats as affected by chronic maternal ethanol

consumption, and (c) the influence of ethanol consumption by pregnant and lactating rats on the enzymes of protein synthesis and degradation in foetal and neonatal livers.

Materials and Methods

Chemicals

ATP, GTP and leucine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{14}C]-Leucine (270mCi/mmol) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Other chemicals were obtained from Fisher Scientific Co. (Livonia, MI, U.S.A.). All chemicals used were of reagent grade. Enzymes and other biochemicals were obtained from Boehringer Mannheim Corp. (New York, NY, U.S.A.).

Animals and diets

Pregnant albino rats were obtained from Spartan Research (Haslett, MI, U.S.A.). The animals for short-term experiments were maintained on Purina Rat Chow and water *ad libitum*. In experiments where effects of acute ethanol administration were studied, ethanol (2g/kg of body weight) was given by gastric intubation. For chronic ethanol treatment several pairs of male and female rats were housed in separate cages. These paired animals were divided into control and ethanol-fed groups and were maintained on a liquid Metrecal diet. The male animals from both control and ethanol-fed groups were removed from the cages of their mates once the pregnancy was confirmed. Immediately after birth, the mothers were given liquid Metrecal diet. The newborn were suckled by mothers fed on either a Metrecal/sucrose or Metrecal/ethanol diet for a maximum of 10 days. The liquid Metrecal diet consisted of 61% (v/v) commercial Metrecal (Mead Johnson Co., Evansville, IN, U.S.A.; vanilla-flavoured), 6% (v/v) ethanol or sucrose and water. The daily dietary intake of liquid diet per experimental animal was measured. An equivalent amount of liquid diet containing sucrose was given to the control group. The average daily intake of liquid diet containing sucrose was 150ml per animal. Their survival rate was 100% up to 4 weeks of chronic ethanol feeding. At the end of 4 weeks of treatment the difference in weight gain between the two groups was not significant.

Studies with foetal liver

To study the effects of chronic alcoholism, 18–21-days'-pregnant rats were used. The abdomens of the pregnant mothers after acute ethanol treatment (3h) or after 2 weeks of ethanol administration were opened and the foetuses were dissected out while still connected to the maternal blood supply. The foetal livers were homogenized in 0.25M-sucrose/0.02M-Tris/HCl (pH 7.6)/10mM-magnesium acetate/40mM-

NaCl/100mM-KCl/6mM-2-mercaptoethanol. The homogenate was centrifuged at 0–4°C. Nuclear and mitochondrial fractions were prepared as described (Rawat & Kuriyama, 1972). The supernatant obtained after sedimentation of the mitochondrial fraction was further centrifuged at 45000 rev./min in a refrigerated Beckman L-2B 65 ultracentrifuge for 2h. The supernatant fraction was used for the preparation of pH 5 enzymes as described by Rawat (1975). The resulting pellet was used for the preparation of purified ribosomes as described by Munro *et al.* (1964).

Studies with neonatal and adult livers

The livers of newborn rats from 0 to 5 days after birth were used in the present study. The newborn came from either control or chronic ethanol-fed groups. In the control group the newborn rats were suckled by Metrecal/sucrose-fed mothers, whereas in the alcohol group, the neonatal animals were suckled by mothers fed on a Metrecal/ethanol diet. The adult animals used in the present study were 100–120 days old. Adult and neonatal animals were killed by cervical dislocation, and the livers were removed and ribosomes prepared as described above for foetal livers.

Study of [^{14}C]leucine incorporation into rat liver *in vivo*

Trichloroacetic acid-soluble and -insoluble radioactive samples were prepared as follows, as described by Mørland (1974). Rats that had been chronically fed on ethanol, and control rats, were injected intravenously with [^{14}C]leucine (3 μCi). After 30min the livers were removed, weighed and immediately homogenized in 5ml of ice-cold 10% trichloroacetic acid. The homogenate was centrifuged at 15000 rev./min in a refrigerated Beckman J-21 B centrifuge. The supernatant was extracted with 3 $\times$ 5ml of diethyl ether to remove trichloroacetic acid. Portions of this supernatant were added directly to scintillation vials and the radioactivity of the trichloroacetic acid-soluble material was counted in a Packard Tri-Carb 385 liquid-scintillation counter. The protein precipitate was washed with 3 $\times$ 10ml of ice-cold 5% trichloroacetic acid and then incubated at 80°C for 20min in 5ml of 5% trichloroacetic acid to solubilize the nucleic acids. It was then washed with 2 $\times$ 10ml of 5% trichloroacetic acid at 0°C, once with 10ml of 70% (v/v) ethanol at 0°C, twice with 10ml of 96% ethanol at room temperature and finally once with 10ml of ether at room temperature. Then the precipitate was transferred to filter-paper discs and counted for radioactivity (Mans & Novelli, 1961).

Incubation of hepatic ribosomes with [^{14}C]leucine

The standard incubation mixture (1ml) consisted of 0.25M-sucrose, 0.02M-Tris/HCl (pH 7.6), 0.11M-KCl, 0.04M-NaCl, 0.01M-magnesium acetate,

0.006 M-GTP, 0.1 ml of liver ribosomes (2.5 mg) and 0.1 ml of pH5-enzyme protein (2.0 mg). The final concentration of [U-¹⁴C]leucine was 2.5 μ M (specific radioactivity 270 mCi/mmol, radiochemical purity 99%). Incubations were carried out in a metabolic shaker at 37°C for 30 min in an air atmosphere. The incorporation of [U-¹⁴C]leucine into protein was determined by the filter-paper-disc method (Mans & Novelli, 1961). After adequate washing with 5% trichloroacetic acid and removal of amino acid-charged tRNA and lipid material, the disc was counted for radioactivity in a Packard 385 liquid-scintillation counter. Incorporation into protein was calculated and expressed as c.p.m./mg of microsomal or pH5-enzyme protein. The total protein was determined colorimetrically (Lowry *et al.*, 1951) with bovine serum albumin as protein standard.

Assay of enzymes

The activities of tryptophan oxygenase (EC 1.13.11.11) and tyrosine transaminase (EC 2.6.1.5) were assayed in whole homogenates of foetal, neonatal and adult rat livers. The animals from both ethanol-fed and control groups were used.

Tryptophan oxygenase assay. The activity of tryptophan oxygenase was assayed by measuring the rate of kynurenine formation by the whole homogenates at pH 7.0. For routine analysis of tryptophan oxygenase activity the whole homogenates were incubated with tryptophan in the presence of haemoglobin for 20 min. The amount of kynurenine formed during incubation was measured spectrophotometrically in the neutralized supernatants at 365 nm (Mørland, 1974). The tryptophan oxygenase activity is expressed in units (μ mol of kynurenine formed/h at 37°C per g of wet liver tissue).

Tyrosine transaminase assay. The activity of hepatic tyrosine transaminase was measured by incubating the whole liver homogenate with a buffered medium

(pH 7.4) containing phosphate buffer (0.2 M), pyridoxal phosphate (2.8 mM), 2-oxoglutaric acid (0.1 M), sucrose (0.25 M) and diethyldithiocarbamate (0.5 M). After a preincubation period of 3 min at 38°C, tyrosine (0.01 M) was added and, the incubation was continued for a further 10 min. The reaction was terminated by the addition of 70% trichloroacetic acid. The blanks already included trichloroacetic acid. The samples were filtered and, after filtration, portions were taken and mixed with colour reagent containing ammonium molybdate in 5 M-HCl (3%) and K₂HPO₄ (1%). The colour was left to develop for 60 min at room temperature and was read at 850 nm in a Gilford spectrophotometer. Simultaneous standards were run with each experiment, containing *p*-hydroxyphenylpyruvic acid.

Determination of proteolytic activity

Effect of ethanol consumption by pregnant or lactating rats on the proteolytic activity in the foetal or neonatal livers was determined with casein or haemoglobin as the substrate. Whole liver homogenates or subcellular fractions of liver were used for this study. The spectrophotometric method (Rick, 1965) was used for the measurement of proteolytic activity at pH 7.5.

Results

As shown in Table 1, ethanol (6%, v/v) consumption by pregnant rats for 2 weeks or by lactating mothers resulted in an inhibition of the rate of [¹⁴C]leucine incorporation into hepatic ribosomes. The incorporation of [¹⁴C]leucine into the hepatic ribosomes of adult animals was also significantly inhibited (Table 1). The advantage of the present study over other studies, where ethanol was fed by drinking water or gastric intubation, is that in the present study the animals were pair-fed on liquid

Table 1. *Effect of prolonged maternal ethanol consumption on the rate of [¹⁴C]leucine incorporation into hepatic ribosomal protein in the rat in vitro*

Effects of chronic ethanol consumption by pregnant, lactating and adult animals on the rates of [¹⁴C]leucine incorporation into ribosomal protein in the livers of foetuses, suckling newborn and adult rats were studied. The ribosomes were prepared as described in the Materials and Methods section. The details of [¹⁴C]leucine incorporation into ribosomal protein are given in the text. The results are expressed as means $\pm$ s.e.m., with the numbers of observations in parentheses.

Age	Treatment	[¹⁴ C]Leucine incorporation	
		(pmol/30 min per mg of protein)	(pmol/30 min per mg of pH5 enzyme)
Foetal (-4 days)	Control	108 $\pm$ 6 (12)	205 $\pm$ 10 (12)
	Ethanol	68 $\pm$ 6 (12)	115 $\pm$ 8 (12)
Neonatal (+10 days)	Control	152 $\pm$ 10 (10)	300 $\pm$ 15 (10)
	Ethanol	100 $\pm$ 8 (10)	208 $\pm$ 10 (10)
Adult (+120 days)	Control	120 $\pm$ 10 (7)	245 $\pm$ 20 (7)
	Ethanol	76 $\pm$ 5 (7)	160 $\pm$ 15 (7)

diets of sucrose or ethanol. This method was chosen to ascertain that while the effect of ethanol on hepatic protein synthesis was being studied any nutritional imbalance was not produced as a result of the method of ethanol administration itself. As shown in Table 1, the prolonged and continuous exposure of either the pregnant mother or the lactating mother to ethanol resulted in a significant effect on both the ribosomal and pH 5-enzyme fractions of the foetal and neonatal livers. This suggests that such exposure to ethanol not only interferes with the capacity of foetal and neonatal hepatic ribosomes to synthesize proteins, but also affects the pH 5-enzyme fraction. Although the possibility remains that administration of ethanol may result in a change in the precursor leucine pools, such changes in precursor leucine pools have not been reported.

Data in Fig. 1 suggest that the prolonged consumption of ethanol by pregnant mothers also results in a significant inhibition of the overall capacity of the foetal liver to synthesize proteins. The rates of [14 C]-leucine incorporation into foetal hepatic proteins were significantly lower in the ethanol-fed group as compared with the corresponding control. For these studies the [14 C]leucine was injected directly into the maternal circulation. The rate of [14 C]leucine incorporation into the trichloroacetic acid-soluble fraction into foetal hepatic proteins in the ethanol-fed group was lower than that observed in the control group (Fig. 2). Total incorporation of the label suggested that the rates of protein synthesis reached a peak in the livers from neonatal animals. The rates of protein

Fig. 1. Effect of maternal ethanol consumption on the incorporation of [14 C]leucine into foetal and neonatal livers

Influence of ethanol consumption by pregnant or lactating rats was investigated on the rates of [14 C]leucine incorporation into total hepatic proteins in the foetal and neonatal livers. The details of incorporation studies are given in the Materials and Methods section. $\circ$, Control mothers; Δ , ethanol-fed mothers. Each point represents the values from six animals; the bars represent the S.E.M.

Fig. 2. Influence of maternal ethanol consumption on the incorporation of [14 C]leucine into the trichloroacetic acid-soluble fraction of the foetal liver

The effect of maternal ethanol consumption was studied on the rate of [14 C]leucine incorporation into the trichloroacetic acid-soluble fraction of the foetal liver. The experimental details are given in the Materials and Methods section. $\circ$, Control group; Δ , ethanol-fed group. Each point represents the values from at least six animals; the bars represent the S.E.M.

synthesis in the foetal livers were lower than those observed in the neonatal and adult livers. The inhibitory effect of ethanol on protein synthesis was much more pronounced in the intact liver preparation than the ribosomal preparations.

As shown in Table 2, the addition of ethanol *in vitro* (10mM) resulted in a significant inhibition of the rate of [14 C]leucine incorporation into the adult liver. Preincubation of adult liver slices with pyrazole (2mM) and subsequent addition of ethanol (10mM) partially prevented the inhibitory effect of ethanol on the rate of [14 C]leucine incorporation into hepatic proteins. The addition of pyrazole alone to the adult liver slices did not significantly affect the rates of [14 C]-leucine incorporation into hepatic proteins *in vitro*. The inhibitory effect of ethanol on the rate of [14 C]-leucine incorporation into hepatic proteins, and the partial prevention of this effect by the addition of pyrazole, was also observed in homogenates of adult livers. The addition of ethanol (10mM) to adult liver ribosomes did not inhibit the rate of [14 C]leucine incorporation into ribosomal protein. Addition of pyrazole to the ribosomes from adult animals did not significantly inhibit the incorporation of [14 C]leucine into ribosomal proteins. Addition of ethanol to the homogenates of normal foetal livers did not significantly change the rates of [14 C]leucine incorporation into foetal hepatic proteins. Consequently the preincubation of foetal liver homogenates with pyrazole and subsequent ethanol addition were also ineffective in changing the rates of [14 C]leucine incorporation

Table 2. *Effects of addition of ethanol and pyrazole in vitro on [¹⁴C]leucine incorporation into hepatic proteins in adult and foetal rat liver*

Effects of pyrazole addition on the inhibitory effect of ethanol on the rate of [¹⁴C]leucine incorporation into adult or foetal liver slices, homogenates or ribosomes were investigated. Hepatic preparations from normal adult or foetal livers were incubated at 37°C in Krebs-Henseleit (1932) buffer (pH 7.4), with the additions indicated in the Table. The details of [¹⁴C]leucine incorporation into hepatic preparations have been described in the text. The results are expressed as means $\pm$ s.e.m. with the numbers of experiments in parentheses. N.S., Not significant.

Liver preparations	Additions	Rates of [¹⁴ C]leucine incorporation (pmol/30 min per mg of protein)	
		Adult	Foetal
Slices	Control	1250 $\pm$ 105 (6)	950 $\pm$ 86 (4)
	Ethanol (10 mM)	790 $\pm$ 80 (6)	900 $\pm$ 48 (4)
	Ethanol (10 mM)+pyrazole (2 mM)	1190 $\pm$ 130 (6)	938 $\pm$ 40 (4)
	Pyrazole (2 mM)	1200 $\pm$ 145 (6)	943 $\pm$ 72 (4)
Homogenates	Control	805 $\pm$ 90 (6)	672 $\pm$ 50 (4)
	Ethanol (10 mM)	609 $\pm$ 60 (6)	585 $\pm$ 40 (4)
	Ethanol (10 mM)+pyrazole (2 mM)	785 $\pm$ 65 (6)	648 $\pm$ 60 (4)
	Pyrazole (2 mM)	800 $\pm$ 85 (6)	680 $\pm$ 65 (4)
Ribosomes	Control	130 $\pm$ 20 (7)	108 $\pm$ 6 (12)
	Ethanol (10 mM)	100 $\pm$ 10 (7)	108 $\pm$ 8 (12)
	Ethanol (10 mM)+pyrazole (2 mM)	120 $\pm$ 10 (7)	100 $\pm$ 6 (12)
	Pyrazole (2 mM)	120 $\pm$ 20 (7)	110 $\pm$ 6 (12)

Table 3. *Effect of mixing hepatic ribosomes and pH5-enzyme fractions of foetuses from ethanol-fed and control groups on protein synthesis*

Hepatic pH5-enzyme fractions and ribosomes were prepared from the foetuses from control or ethanol-fed rats. The rates of [¹⁴C]leucine incorporation by hepatic ribosomes from both control and ethanol-fed groups were studied in the presence of hepatic pH5-enzyme fractions from foetuses of control and ethanol-fed groups. The details of the experimental procedure are given in the Materials and Methods section. The results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses.

Source of foetal liver pH5 enzyme	Source of foetal liver ribosomes	...	[¹⁴ C]Leucine incorporated (pmol/30 min per mg of protein)	
			Control	Ethanol-fed
Control			110 $\pm$ 9 (8)	86 $\pm$ 7 (8)
Ethanol-fed			90 $\pm$ 8 (8)	60 $\pm$ 5 (8)

into foetal liver proteins. Pyrazole alone did not significantly change the rate of [¹⁴C]leucine incorporation into the proteins of foetal liver homogenates.

Studies by mixing hepatic ribosomal and pH5-enzyme fractions from the livers of foetuses from ethanol- or sucrose-fed mothers were conducted to determine the site of ethanol action. As shown in Table 3, the lowest rates of [¹⁴C]leucine incorporation were observed when the hepatic ribosomes and pH5-enzyme fractions from the ethanol-fed group were incubated together. Both mixing of pH5-enzyme fraction from the control group and ribosomes from the ethanol-fed group, and mixing of pH5-enzyme fraction from the ethanol group and ribosomes from

the control group gave intermediate rates of protein synthesis (Table 3).

Addition of cycloheximide *in vitro* resulted in an inhibition in the rates of [¹⁴C]leucine incorporation by the ribosomes in newborn rats from both control and ethanol-fed groups (Fig. 3). This inhibition by cycloheximide was studied at 10 min intervals up to a maximum of 30 min. Cycloheximide addition gave an inhibition at all the intervals tested. The observations recorded in Fig. 3 also showed a progressive incorporation with time of [¹⁴C]leucine into ribosomal proteins *in vitro* without cycloheximide addition.

As shown in Table 4, maternal ethanol consumption for 2 weeks resulted in a significant decrease in the foetal hepatic content of total RNA as measured

Fig. 3. Effect of cycloheximide addition on $[^{14}\text{C}]$ leucine incorporation into hepatic ribosomal protein

The influence of cycloheximide addition *in vitro* was studied on the rate of $[^{14}\text{C}]$ leucine incorporation into ribosomal protein by liver preparations of newborn rats suckled by control (●) or ethanol-fed (▲) mothers. ○, Ribosomes from the control group with cycloheximide; △, ribosomes from the ethanol-fed group with cycloheximide. The details of $[^{14}\text{C}]$ leucine incorporation into ribosomal protein are given in the Materials and Methods section. Each point represents the mean values from at least four experiments.

Fig. 4. Influence of maternal ethanol consumption on the activity of tryptophan oxygenase in foetal and neonatal livers

The effect of ethanol consumption by pregnant or lactating rats was investigated on the activity of tryptophan oxygenase in foetal and neonatal livers. The details of tryptophan oxygenase assay are given in the Materials and Methods section. ●, Control group; ○, ethanol-fed group. Each point represents the values from at least six animals; the bars represent the S.E.M.

by RNA phosphorus content. Maternal ethanol consumption did not result in a significant change in the foetal hepatic content of total DNA. However, maternal ethanol consumption resulted in a significant decrease in foetal hepatic RNA/DNA ratio.

Table 4. Effect of maternal ethanol consumption on foetal hepatic RNA, DNA and protein contents

Effect of prolonged ethanol consumption by pregnant rats was investigated on the foetal hepatic contents of total RNA, total DNA and total protein. The livers were homogenized in 0.25 M-sucrose. The ribosomal fractions were prepared as described in the text. The whole liver fractions or ribosomes were taken for the extraction of RNA or DNA. The details of RNA and DNA determinations are given in the Materials and Methods section. The values are expressed as means $\pm$ S.E.M. for six observations. N.S., Not significant.

Treatment	Total RNA (mg/g fresh wt. of liver)	Total DNA (mg/g fresh wt. of liver)	RNA/DNA	Total protein (mg/g fresh wt. of liver)	RNA/protein	Ribosomal protein (mg/g fresh wt. of liver)
Control	5.00 ± 0.25	1.90 ± 0.20	2.63	95.85 ± 5.0	0.052	0.75 ± 0.1
Ethanol	3.80 ± 0.20	1.93 ± 0.20	1.96	86.70 ± 5.0	0.044	0.53 ± 0.1
	$P < 0.02$	N.S.	$P < 0.02$	$P < 0.02$	N.S.	$P < 0.02$

Fig. 5. Effect of maternal ethanol consumption on the activity of tyrosine aminotransferase in foetal and neonatal livers

The influence of ethanol consumption by pregnant or lactating rats was investigated on the activity of tyrosine aminotransferase in foetal and neonatal livers. The details of tyrosine aminotransferase assay are given in the Materials and Methods section. ●, Control group; ○, ethanol-fed group. Each point represents the values from at least six animals; the bars represent the S.E.M.

Ethanol feeding to the pregnant rats resulted in a decrease in total protein in the foetal livers; however, the RNA/protein ratio was not significantly affected by this treatment. A decrease in ribosomal protein content was also observed in foetal livers from ethanol-fed mothers compared with the corresponding control group (Table 4).

Ethanol consumption by pregnant or lactating animals resulted in an inhibition of tryptophan oxygenase activity (EC 1.13.11.11) in both the foetal and neonatal livers (Fig. 4). Ethanol consumption also resulted in an inhibition of tryptophan oxygenase activity in the adult liver. This effect of ethanol was rather specific on the activity of tryptophan oxygenase, since maternal ethanol consumption did not result in a significant change (Fig. 5) in the activity of hepatic tyrosine aminotransferase (EC 2.6.1.5). The developmental changes in the activities of hepatic tryptophan oxygenase and tyrosine aminotransferase are shown in Figs. 4 and 5 respectively.

Chronic ethanol consumption by adult animals resulted in a decrease in hepatic proteolytic activity tested against haemoglobin (Table 5). Proteolytic activity of liver against casein also decreased in the ethanol-fed group. Ethanol consumption by pregnant or lactating rats resulted in a small but significant decrease in the hepatic proteolytic activity in the foetal and neonatal livers. Hepatic proteolytic activity showed a progressive increase from foetal to adult stage. The developmental changes in the hepatic proteolytic activity are expressed as percentages of that of the adult control.

Table 5. Effect of ethanol consumption on the development of proteolytic activity in rat liver

Effect of ethanol consumption was investigated on the hepatic proteolytic activity of the adult rat, and the effect of maternal ethanol consumption was investigated on the proteolytic activity of newborn rats and foetuses. Liver homogenates were incubated with haemoglobin in a buffered medium (pH 7.6). The details of the procedure are given in the Materials and Methods section. The results are expressed as percentages of activity of the adult control. The values are means $\pm$ S.E.M., with the numbers of observations in parentheses.

Age	Treatment	Hepatic proteolytic activity (% of adult control)	
Adult	Control	100.0 $\pm$ 9.0 (8)	$P < 0.02$
	Ethanol	83.6 $\pm$ 7.0 (8)	
Newborn	Control	85.5 $\pm$ 6.3 (8)	$P < 0.02$
	Ethanol	68.5 $\pm$ 5.2 (8)	
Foetus	Control	70.5 $\pm$ 6.3 (12)	$P < 0.001$
	Ethanol	48.5 $\pm$ 5.2 (12)	

Discussion

Although depressive effects of prolonged ethanol administration on the rates of protein synthesis in adult liver (Mørland, 1974), brain (Lamar, 1972; Nobel & Tewari, 1973; Rawat, 1975), pancreas (Tsuzuki *et al.*, 1965) and heart (Schreiber *et al.*, 1972) have been observed, no information is available in the literature about the effect of ethanol on protein synthesis in the developing liver. Our laboratory has reported that prolonged maternal ethanol consumption by pregnant or lactating rats resulted in a significant inhibition of the rate of [14 C]leucine incorporation into foetal and neonatal cerebral ribosomes (Rawat, 1975). In the present study it was observed that prolonged maternal ethanol consumption also results in an inhibition of the rate of [14 C]leucine incorporation into ribosomes from foetal and neonatal livers. The inhibitory effect of ethanol on [14 C]leucine incorporation into foetal hepatic ribosomes was observed on the basis of both ribosomal protein and pH 5-enzyme proteins. It suggests that maternal ethanol consumption interferes not only with the capacity of foetal ribosomes to synthesize proteins, but it also affects pH 5 enzymes. In an attempt to investigate if the inhibitory effect of maternal ethanol consumption was also exerted on the developing foetus *in situ*, the experiments *in vivo* were designed. The observations recorded in Fig. 1 show that ethanol consumption by pregnant rats can also inhibit the incorporation of [14 C]leucine into trichloroacetic acid-precipitable and trichloroacetic acid-non-precipitable ninhydrin-positive material of the foetal liver.

Although ethanol addition to adult rat liver slices has been observed to inhibit the incorporation of [14 C]leucine into hepatic proteins (Perin *et al.*, 1974),

the addition of ethanol *in vitro* to foetal liver slices or foetal hepatic ribosomes was ineffective in altering the rates of protein synthesis. The inhibitory effect of externally added ethanol on the rate of protein synthesis in the adult-rat liver slices and the absence of this effect on the foetal liver slices suggests that at least part of this effect is mediated through the changes in hepatic NADH/NAD⁺ ratio through alcohol dehydrogenase. It has been shown that in the early stages of foetal development the activity of alcohol dehydrogenase in the liver is either absent or very low (Räihä *et al.*, 1967; Rawat, 1976).

The observation that in liver slices or liver homogenates the inhibitory effect of ethanol on the rate of protein synthesis, at least in part, is a consequence of ethanol-mediated changes in hepatic NADH/NAD⁺ ratio is further documented by the experiments with pyrazole, an inhibitor of alcohol dehydrogenase. Preincubation of pyrazole (2 mM) with adult-rat liver slices or homogenates was able to relieve at least in part the inhibitory effect of ethanol on protein synthesis. However, in foetal liver slices, where the activity of alcohol dehydrogenase is either very low or absent, pyrazole was ineffective in relieving the ethanol-mediated inhibition. A lack of effect of pyrazole has also been observed in the guinea-pig kidney slices, a tissue where alcohol dehydrogenase is not present to any significant extent. With the development of hepatic alcohol dehydrogenase, the effectiveness of pyrazole in relieving the ethanol-mediated inhibition of protein synthesis also increases, as observed in the adult liver (Table 2).

Since the addition of ethanol *in vitro* to adult ribosomes does not significantly affect the rates of [¹⁴C]leucine incorporation into protein (Table 2) it becomes logical to suggest that the inhibitory effect of ethanol is exerted through two different mechanisms. The first mechanism, which is alcohol dehydrogenase-dependent, operates in the whole liver, is sensitive even to the addition of ethanol *in vitro* and can be inhibited by the addition of pyrazole. The second mechanism, which is not dependent on alcohol dehydrogenase, operates in the ribosomal and pH5-enzyme fractions, is affected only on continuous and prolonged exposure to ethanol and is not sensitive to the addition of pyrazole.

It has been suggested that the effect of prolonged ethanol consumption on hepatic protein synthesis in adult mice may be a consequence of its effect on the concentrations of corticosteroids (Kuriyama *et al.*, 1971). In the present study therefore the effect of prolonged ethanol consumption by pregnant or lactating rats was investigated on the activities of two hepatic enzymes namely tryptophan oxygenase and tyrosine aminotransferase, both of which are induced by corticosteroids (Knox & Auerbach, 1955). These two enzymes in liver have a very rapid turnover (Feigelson *et al.*, 1959; Lin & Knox, 1958) and their activities

can therefore be easily influenced by changes in enzyme synthesis or breakdown. The developmental pattern of hepatic tryptophan oxygenase (Fig. 4) showed that, during early stages of development, maternal ethanol consumption did not significantly affect the activity of tryptophan oxygenase. However, the activity of tryptophan oxygenase decreased in the livers of newborn rats suckled by ethanol-fed mothers. Adult animals feeding on an ethanol diet showed a significant decrease in the activity compared with the corresponding controls. A decrease in hepatic tryptophan oxygenase activity has also been observed (Mørland, 1974) in perfused rat liver preparations from chronic ethanol-fed animals. Although hepatic activity of tyrosine aminotransferase showed a steady linear increase with age (Fig. 5), it was not significantly affected at any stage of development by ethanol. These observations suggest that effect of ethanol on the activity of tryptophan oxygenase, tyrosine aminotransferase or the rate of protein synthesis is not an indirect effect of ethanol exerted through corticosteroids. The latter have been shown to increase transiently in the plasma after ethanol administration (Ellis, 1966).

A small but significant decrease in the contents of total hepatic protein in foetal, neonatal and adult livers from the ethanol-fed group led us to investigate the effect of ethanol consumption on the proteolytic activity in liver. Observations in Table 5, however, suggest that the effect of ethanol on hepatic protein content was not a consequence of increased protein breakdown, since the proteolytic activity in the foetal, neonatal and adult livers did not increase in the ethanol-fed group. It can be assumed that the effect of ethanol on hepatic protein content is a consequence of decreased protein synthesis or increased leakage from the liver of the newly synthesized protein. The inhibitory effect of ethanol on the rates of protein synthesis observed in the present study may be of central importance in the pathogenesis of alcoholic liver cirrhosis and associated abnormal morphology of the liver frequently observed in chronic alcoholics.

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