Effects of Maternal Ethanol Consumption on Hepatic Lipid Biosynthesis in Foetal and Neonatal Rats

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Effects of prolonged maternal ethanol consumption were studied on hepatic lipid content, on the rates of fatty acid synthesis and on the activities of enzymes involved in fatty acid synthesis in the livers of foetal and suckling neonatal rats. Prolonged maternal ethanol consumption resulted in a significant increase in the contents of hepatic total lipids, triacylglycerols and plasma unesterified fatty acids in foetal and neonatal rats. Studies *in vitro* with $^3\text{H}_2\text{O}$ showed that maternal ethanol consumption did not result in a significant change in its rate of incorporation into lipid fractions of foetal and neonatal livers. The rates of fatty acid synthesis showed a pronounced decrease immediately after birth, compared with the foetal stage, but increased in the adult animals. On the other hand, the highest rates of lipid oxidation were observed in the neonatal stage. Maternal ethanol consumption resulted in a significant decrease in the rates of [^{14}C]palmitate oxidation to $^{14}\text{CO}_2$ by both the foetal and neonatal livers. Maternal ethanol consumption did not result in an increase in the activities of any of the lipid-synthesizing enzymes tested throughout the period of development. Although increased fatty acid synthesis does not seem to be the mechanism for the accumulation of these lipids, decreased oxidation of the lipids may be partly responsible for the lipid accumulation.

Recently, the detrimental effect of ethanol on pregnancy has been rediscovered (Jones *et al.*, 1973, 1974; Ferrier *et al.*, 1973). A derangement in certain anatomical, physiological and morphological features has been found in about one-half of the infants of severely alcoholic mothers (Jones & Smith, 1975; Rawat, 1976a; Quелlette & Rosett, 1976). Although there has been a widespread interest recently in the so called 'Foetal Alcohol Syndrome', the extent of derangement in the metabolic and biochemical pathways is not well understood (Rawat, 1977). Other studies from our laboratory have shown that prolonged ethanol consumption by pregnant or lactating rats results in an inhibition in hepatic (Rawat, 1976b) and cerebral (Rawat 1975) protein synthesis in foetal and neonatal rats. Metabolism of ethanol has been suggested to result in alterations in hepatic lipid synthesis (Nikkilä & Ojala, 1963), lipid oxidation (Rawat, 1972) and peripheral lipid mobilization (Brodie *et al.*, 1961). However, no information is available on the effects of prolonged ethanol consumption by pregnant or lactating mothers on hepatic lipid metabolism of the foetal or the neonatal rat. During foetal development rapid growth necessitates the production of large quantities of lipids. This is provided by rapid rates of synthesis in the foetal tissue (Roux, 1966; Taylor *et al.*, 1967), or by transfer across the placenta from

the mother (Portman *et al.*, 1969). Since ethanol also passes freely from maternal circulation to the foetus via the placenta and from milk to the suckling newborn animals (Kessaniemi, 1974; Vorherr, 1974), it was decided to investigate the effects of maternal ethanol consumption on the rate of foetal and neonatal hepatic lipid synthesis. The factors controlling lipid synthesis in the developing liver were studied.

Materials and Methods

Chemicals

NAD^+ , NADH , NADP^+ , CoA , ATP and other enzymes and substrates were obtained from Boehringer Mannheim, New York, NY, U.S.A. $^3\text{H}_2\text{O}$ (100mCi/g) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Other chemicals were obtained from Fisher Scientific (Lavonia, MI, U.S.A.). Analytical-grade absolute ethanol used in this study was redistilled before use.

Animals

Pregnant albino rats were obtained from Spartan Research (Haslett, MI, U.S.A.). The animals were given a chronic ethanol treatment in liquid Metrecal diet. The liquid Metrecal diet consisted of 61 % (w/v)

commercial Metrecal (Mead Johnson Co., Evansville, IN, U.S.A.; vanilla-flavoured), 6% (w/v) ethanol or isocaloric sucrose (10.92%, w/v), and water. Metrecal is a nutritionally complete diet and according to its proximate analysis contains (% w/w): protein, 6.3; fat, 1.3; carbohydrate, 13.0; ash, 1.0; water, 78.0. Among the ingredients are concentrated sweet skim-milk, corn-syrup solids, concentrated sucrose, partially hydrogenated soya-bean oil, sodium caseinate, soya-bean protein isolate. It contained vitamins A, B₆, B₁₂, C, D, E, folic acid, thiamin, niacin, biotin and pantothenic acid. In addition, it contains phosphorus, iodine, iron, magnesium, copper, zinc, manganese, potassium, sodium and choline. The daily intake of liquid diet per experimental animal was measured. An equivalent amount of liquid diet containing sucrose was given to control animals. Metrecal contains concentrated sucrose as one of the major sources of carbohydrates. In the control diets, therefore, sucrose addition to make it isocaloric with ethanol diet was considered most suitable. The average daily intake of liquid diet by pregnant rats was 150 ml per animal. The rats feed well on the Metrecal diet and gain weight. The average daily weight gain by a pregnant animal was 3.1 ± 0.6 g per day (mean $\pm$ S.E.M. for 15 determinations). A significant difference in the weight gain by pregnant rats from ethanol-fed and sucrose-fed groups could not be observed at any stage of gestation. However, the body weights of individual foetuses from the ethanol-fed group were consistently ($P < 0.01$) lower than the corresponding controls. The average weight ($\pm$ S.E.M.) of a foetus at day 18 of gestation from the control group was 5.6 ± 0.8 g and of a foetus from ethanol group was 4.3 ± 0.4 g. There were 20 observations in each group. The number of foetuses in ethanol-fed and sucrose-fed mothers varied; however, no consistent evidence could be obtained to show that ethanol affected the number of foetuses. A generalized prenatal and postnatal growth retardation could be clearly observed in the offspring of the ethanol-fed group. The survival rate of the mothers was 100% up to 4 weeks of chronic ethanol feeding.

Determination of total lipids, triacylglycerols and unesterified fatty acid

To estimate the hepatic contents of total lipids, triacylglycerols and plasma unesterified fatty acids, liver samples from adult, neonatal and foetal animals were taken, under light anaesthesia with diethyl ether, by aluminium clamps pre-cooled in liquid N₂. Foetal hepatic tissue was obtained during various intervals of pregnancy by opening the abdomen of the pregnant mothers from either control or ethanol-fed groups. The abdomen of the foetus was carefully opened while still intact to maternal circulation.

The frozen liver sample was homogenized with a mortar and pestle and with the addition of liquid N₂. Lipids were extracted in a mixture of chloroform/methanol (3:1, v/v). After treatment with ethanolic KOH the triacylglycerols were determined enzymically (Laurell, 1974). For the determination of fatty acids, plasma samples were extracted in chloroform, mixed with silicic acid and fatty acids were determined colorimetrically (Antonis, 1965).

Studies in vitro

In studies where liver slices were used, livers were obtained by the following procedure. Foetal livers were obtained by opening the abdomens of pregnant rats fed on either sucrose/Metrecal or ethanol/Metrecal diets. The neonatal livers were obtained from 4-day-old neonatal rats suckling on either sucrose-fed or ethanol-fed mothers. In both neonatal and adult animals, livers were removed after cervical dislocation. The foetal, neonatal and adult livers were pooled separately and were chilled in ice immediately. Liver slices (0.3 mm thickness) were cut in the cold with a McIlwain-Buddle tissue slicer. The slices were used for ³H₂O incorporation studies.

Incorporation of ³H₂O into hepatic lipids in vitro

To study the effects of maternal ethanol consumption on the rates of hepatic lipid synthesis in foetal and neonatal livers the following procedure was used. The livers of the foetuses from sucrose-fed or ethanol-fed mothers were removed after opening of the abdomens of the pregnant rats. The neonatal livers were obtained from neonatal rats suckling on either sucrose-fed or ethanol-fed mothers. The pooled livers were sliced as described above and were incubated with Krebs & Henseleit (1932) bicarbonate buffer, pH 7.4, containing ³H₂O. The slices were incubated for 15 or 60 min at 37°C, then the reaction was terminated by the addition of 10% (w/v) KOH. The lipids were saponified (Folch *et al.*, 1957) with 3 ml of 10% (w/v) KOH and tubes were put in a boiling-water bath for 2 h. After saponification the extract was acidified with 10 M-H₂SO₄. Fatty acids and non-saponifiable lipids were extracted with 3 $\times$ 10 ml of light petroleum (b.p. 40–60°C). The ratio of ³H incorporation into non-saponifiable lipids/fatty acids was about 1:20 in the adult liver. In the foetal and neonatal livers no measurable amount of radioactivity could be detected in the cholesterol fraction. The extract was washed with 3 $\times$ 30 ml of 5% (w/v) acetic acid, then dried over anhydrous Na₂SO₄. A 10 ml portion of the organic extract was evaporated to dryness in a glass vial containing 10 ml of toluene-based scintillation fluid (Jones & Firmin, 1976) in added benzene. The radioactivity was counted in a Packard Tri-Carb liquid-scintillation counter model 3385.

Oxidation of [U-¹⁴C]palmitate to ¹⁴CO₂ by liver slices

Liver slices (0.2–0.3 g) from foetuses, neonatal or adult rats from ethanol-fed or control groups were incubated in Krebs–Henseleit bicarbonate buffer, pH 7.4. The flasks were sealed with rubber serum stoppers equipped with hanging polyethylene cups. Each flask contained 10 mM-[U-¹⁴C]palmitate. The incubation was carried out for 60 min at 37°C. After incubation, 0.3 ml of Hyamine 10X was injected into the plastic cups and 0.5 ml of 10 M-H₂SO₄ was injected into flasks to terminate the reaction and release ¹⁴CO₂. The incubation was continued for an additional 40 min to ensure complete liberation and trapping of ¹⁴CO₂. The contents of the cups were transferred to vials that contained a mixture of toluene, 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene (Rawat, 1974) for the measurements of ¹⁴CO₂. Radioactivity was measured in a Packard Tri-Carb 3385 scintillation spectrometer. Counting efficiency was determined by internal standardization and all counts were corrected to 100% efficiency on this basis.

Assay of hepatic enzymes

The foetal, neonatal or adult livers were homogenized in 0.25 M-sucrose, or in a given homogenizing medium. Homogenates were centrifuged at 2°C for 60 min at 100 000 g in a Beckman L-2-65B ultracentrifuge with 65 fixed-angle rotor. High-speed supernatant fractions were used for the enzyme assays.

Fatty acid synthetase complex. The supernatant fraction was used for the measurement of the enzyme activity by following the oxidation of NADPH at 340 nm. The reaction mixture contained the following components: 100 μmol of potassium phosphate, pH 6.5; 0.5 μmol of NADPH; 50 nmol of acetyl-CoA; 5 μmol of dithiothreitol. The total volume of the reaction mixture was 1.5 ml and it contained 3.0 mg of supernatant protein. The background oxidation of NADPH was monitored for 3 min and then the fatty acid synthetase reaction was initiated by the addition of 75 nmol of malonyl-CoA. Enzyme activity is expressed as nmol of malonyl-CoA incorporated/min per mg of supernatant protein, assuming 2 mol of NADPH oxidized/mol of malonyl-CoA incorporated (Lynen, 1969). Protein content of enzyme preparation was determined by the method of (Lowry *et al.*, 1951), with bovine serum albumin as standard.

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49). Liver was homogenized in 3 vol. of 154 mM-KCl, pH 7.0, containing 0.60 mM-EDTA (hydrated disodium salt) and it was diluted 1:10 with triethanolamine buffer, pH 7.5. The activity of glucose 6-phosphate-NADP⁺ oxidoreductase was assayed in the supernatant by incubating in 50 mM-triethanol-

amine buffer, pH 7.5, containing 5 mM-EDTA, 0.5 mM-NADP⁺ and 0.66 mM-glucose 6-phosphate and by following the reduction of NADP⁺. The total incubation mixture was 2.5 ml and contained about 3.0 mg of supernatant protein. The enzyme activity was corrected for initial NADPH production (Jones & Ashton, 1976); 1 unit is the amount of enzyme that catalyses the transformation of 1 μmol of substrate/min.

'Malic' enzyme (EC 1.1.1.40). High-speed supernatant was used for the spectrophotometric determination (Hsu & Lardy, 1969) of L-malate-NADP⁺ oxidoreductase (decarboxylating) activity (1 unit is the amount of enzyme that catalyses the transformation of 1 μmol of substrate/min. The total volume of the assay mixture was 3.0 ml and it contained about 3.0 mg of supernatant protein.

Results*Effects of maternal ethanol consumption on lipids of foetal and neonatal rats*

The content of hepatic total lipids showed a 2.5-fold increase from foetal (–4 days) to neonatal (+4 days) stage (Table 1) and then increased from 47 mg/g of liver in the 4-day-old rat to 65 mg/g of liver in the adult animal (120 days). The hepatic content of triacylglycerols also showed an increase of about 2-fold from foetal to neonatal stage. The hepatic triacylglycerols continued to increase after birth until adult values were reached. The most pronounced developmental change was observed in the concentrations of plasma unesterified fatty acids, which increased by about 7-fold from the foetal to neonatal stage. The highest plasma unesterified fatty acid concentrations were observed in the adult animal (Table 1).

Ethanol consumption by pregnant rats resulted in a significant ($P < 0.001$) increase in the foetal hepatic content of total lipids compared with the corresponding sucrose-fed animals. Increase in total hepatic lipid content was also observed in neonatal rats suckling on ethanol-fed mothers, compared with those suckling on sucrose-fed animals (Table 1). Chronic ethanol consumption also resulted in an increase ($P < 0.001$) in the hepatic total lipids in the adult animals. As shown in Table 1, hepatic triacylglycerol contents and the concentrations of plasma unsaturated fatty acids were significantly higher in foetal, neonatal and adult rats from the ethanol-fed group compared with the corresponding sucrose-fed group.

Effects of maternal ethanol consumption on ³H₂O incorporation into foetal and neonatal hepatic fatty acids

The rates of ³H₂O incorporation into fatty acids by liver slices of foetal, neonatal and adult rats were not

Table 1. *Effects of maternal ethanol consumption on foetal or neonatal hepatic and plasma lipids*

Hepatic total lipids, triacylglycerols and plasma unesterified fatty acids were determined in the foetal (−4 days), neonatal (+4 days) and adult (+120 days) rats. The foetuses were obtained from chronically (at least 2 weeks) ethanol- or sucrose-fed rats after abdominal operation. The neonatal rats were suckling on ethanol- or sucrose-fed mothers. The details of hepatic and plasma-lipid determination are given in the Materials and Methods section. The results are expressed as means±S.E.M. with the numbers of observations in parentheses. Significance was determined by Student's *t* test.

Age	Treatment	Hepatic total lipids (mg/g wet wt. of liver)	Hepatic triacylglycerols (mg/g wet wt. of liver)	Plasma unesterified fatty acids (mM)
Foetal (−4 days)	Control	18.5±2.30 (8)	3.36±0.30 (8)	42±4 (8)
	Ethanol	24.2±3.0 (8) (<i>P</i> <0.0001)	4.30±0.50 (8) (<i>P</i> <0.05)	58±4 (8) (<i>P</i> <0.01)
Neonatal (+4 days)	Control	46.6±3.0 (8)	5.80±0.80 (8)	280±15 (8)
	Ethanol	59.3±4.0 (8) (<i>P</i> <0.001)	12.3±2.0 (8) (<i>P</i> <0.001)	695±40 (8) (<i>P</i> <0.001)
Adult (+120 days)	Control	65.3±5.0 (8)	7.2±1.8 (8)	325±10 (8)
	Ethanol	90.1±10.0 (8) (<i>P</i> <0.001)	15.1±2.6 (8) (<i>P</i> <0.001)	875±50 (8) (<i>P</i> <0.001)

Table 2. *Effects of maternal ethanol consumption on the rates of ³H₂O incorporation into lipids of liver slices from foetal and neonatal rats*

Liver slices were prepared from the pooled livers of foetal, neonatal and adult rats. The details of liver-slice preparation are given in the Materials and Methods section. The slices were incubated in the presence of ³H₂O (2mCi) in Krebs–Henseleit bicarbonate buffer, pH 7.4, at 37°C in the presence of O₂/CO₂ (19:1). The reaction was stopped after 60 min of incubation. The lipids were extracted as described in the Materials and Methods section. The results are expressed as means±S.E.M. with the numbers of observations in parentheses. Abbreviation: N.S., not significant.

Age	Treatment	³ H ₂ O incorporation into hepatic lipids (μmol/min per g fresh wt. of liver)	
Foetal (−4 days)	Control	0.606±0.04 (6)	(N.S.)
	Alcohol	0.553±0.04 (6)	
Neonatal (+6 days)	Control	0.16±0.009 (6)	(N.S.)
	Alcohol	0.14±0.009 (6)	
Adult (+120 days)	Control	1.42±0.12 (6)	(N.S.)
	Alcohol	1.27±0.12 (6)	

significantly affected by maternal ethanol consumption (Table 2). Foetal livers from both sucrose/Metrecal- and ethanol/Metrecal-fed groups showed about 4-fold higher rates of ³H₂O incorporation into hepatic lipids compared with the corresponding suckling neonatal rats. The livers from adult animals showed the highest (*P*<0.001) ³H₂O incorporation into lipids (Table 2).

Oxidation of [¹⁴C]palmitate to ¹⁴CO₂ by foetal and neonatal liver slices

As shown in Table 3, effects of maternal ethanol consumption during pregnancy or during lactation were studied on the capacity of foetal and neonatal liver-slice preparations to oxidize [¹⁴C]palmitate to

Table 3. *Effect of maternal ethanol consumption on the rate of [¹⁴C]palmitate oxidation to ¹⁴CO₂ by foetal and neonatal rat liver slices*

The liver slices of foetal or neonatal rats from ethanol-fed or sucrose-fed mothers were incubated with [U-¹⁴C]palmitate (10mM) in Krebs–Henseleit bicarbonate buffer, pH 7.4. The incubation was carried out at 37°C for 60 min. The details of ¹⁴CO₂ trapping are given in the Materials and Methods section. The results are expressed as means±S.E.M. with the numbers of animals observed in parentheses. Significance was determined by Student's *t* test.

Age	Treatment	[U- ¹⁴ C]Palmitate oxidation to ¹⁴ CO ₂ (μmol of substrate oxidized to CO ₂ /h per g wet wt. of liver)
Foetal (−4 days)	Sucrose-fed	0.85±0.1 (10)
	Ethanol-fed	0.68±0.08 (10) (<i>P</i> <0.01)
Neonatal (+4 days)	Sucrose-fed	6.9±0.5 (8)
	Ethanol-fed	5.1±0.5 (8) (<i>P</i> <0.01)
Adult (+120 days)	Sucrose-fed	4.2±0.3 (8)
	Ethanol-fed	2.0±0.2 (8) (<i>P</i> <0.001)

¹⁴CO₂. Maternal ethanol consumption both during pregnancy and during lactation resulted in significant (*P*<0.01) inhibition in the rates of [¹⁴C]palmitate oxidation to ¹⁴CO₂ compared with the corresponding sucrose-fed group. Prolonged ethanol consumption by adult rats also leads to a marked inhibition (*P*<0.001) in the rates of [¹⁴C]palmitate oxidation to ¹⁴CO₂.

Effect on lipid-synthesizing enzymic activities

The developmental patterns of hepatic fatty acid synthetase, glucose 6-phosphate dehydrogenase and 'malic' enzyme activities are shown in Figs. 1(a), 1(b) and 1(c) respectively. Prolonged maternal ethanol consumption either during pregnancy or during lactation did not significantly affect the activities

Fig. 1. Effects of maternal ethanol consumption on the activities of fatty acid synthetase (a), glucose 6-phosphate dehydrogenase (b) and 'malic' enzyme (c) in foetal and neonatal livers

- (a) ●, Sucrose-fed group; ▲, ethanol-fed group. Values are means $\pm$ S.E.M. for at least four animals.
 (b) ●, Sucrose-fed group; ▲, ethanol-fed group. Values are means $\pm$ S.E.M. for at least six animals.
 (c) ●, Sucrose-fed group; ▲, ethanol-fed group. Values are means $\pm$ S.E.M. for at least six animals.

of these enzymes (Fig. 1), when expressed as per g of liver or per liver.

Discussion

Although studies conducted in the past with adult humans and experimental animals have shown that ethanol interferes with the pathways of lipid metabolism (Mallov, 1961; Gordon, 1972; Abrams & Cooper, 1976), no studies are available in the literature on the effects of maternal ethanol intake on these pathways in the developing foetal or suckling neonatal animals. In the present study the effects of maternal ethanol consumption by both the pregnant and lactating rats were studied on the

metabolism of hepatic lipids in the developing foetus and the suckling neonatal rat. Table 1 shows that prolonged ethanol consumption by pregnant and lactating rats leads to an increase in the hepatic total lipids and triacylglycerols in the livers of offspring. In the adult ethanol affects accumulation of lipids, which may lead to fatty liver and liver cirrhosis. However, the role of such lipid accumulation in the liver of the offspring in causing fatty infiltration and cirrhosis is not clear.

The contents of hepatic lipids at a given stage of development reflect a net balance among lipid-synthesizing activity, the lipid-oxidizing capacity of the liver, lipid uptake by the liver and lipid secretion from the liver as lipoproteins. However, as discussed below, the increase in the hepatic lipid content does not necessarily reflect an increase in the hepatic lipid-synthesizing activity after ethanol administration at different stages of development. It is possible that lipid transfer across the placenta from the mother to the foetus is at least in part responsible for the observed increase in the contents of lipids in the foetal liver from the ethanol-fed group. Similarly, there exists a possibility of transfer of dietary lipids through milk to suckling neonatal rats. Indeed Table 2 demonstrates that prolonged maternal ethanol consumption during either pre-natal or postnatal development does not increase fatty acid synthesis in the foetal and neonatal livers as measured by $^3\text{H}_2\text{O}$ incorporation. The study with adult animals also showed (Table 2) that prolonged ethanol intake did not result in an increased lipid-synthesizing activity of the liver. Observations with adult animals agree with previous findings (Gwynn *et al.*, 1973). The rates of $^3\text{H}_2\text{O}$ incorporation into hepatic lipids ($78\mu\text{mol/h}$ per g of liver) observed in the adult liver-slice preparation in this study are lower than those reported ($186.2\mu\text{mol/h}$ per g of liver) for the perfused livers (Windmueller & Spaeth, 1966). Obviously perfused liver is a better preparation for such study; however, the difficulty of working with foetal livers led to the choice of the present system. The liver-slice preparation does, however, respond to conditions that alter rates of fatty acid synthesis such as starvation. Liver slices from starved (12h) rats showed an incorporation of only $5\mu\text{mol/h}$ per g of liver.

The developmental changes (Table 2) show that the capacity of liver tissue to incorporate $^3\text{H}_2\text{O}$ into hepatic lipids is markedly decreased immediately after birth, when the neonatal rats start suckling, perhaps owing to a high fat content of the milk (Taylor *et al.*, 1967; Jones & Ashton, 1976). The developing rat liver provides a particularly suitable system for studying the influence of nutritional factors on hepatic fatty acid metabolism. The pattern of hepatic fatty acid synthesis and oxidation observed in this study agrees with other studies on this topic.

Several good studies have appeared in recent years (Taylor *et al.*, 1967; Angenfeld & Fritz, 1970; Lockwood & Bayley, 1970; Foster & Bailey, 1976) on the developmental changes in hepatic lipid biosynthesis and oxidation. The observations in Table 2 support the view that increased fatty acid synthesis is not a significant cause of lipid accumulation in the liver after ethanol administration (Guynn *et al.*, 1973; Lundquist *et al.*, 1962; Reboucas & Isselbacher, 1961).

It is widely accepted that during ethanol metabolism liver has a reduced state, which is reflected by an increase in hepatic $[NADH]/[NAD^+]$ ratio (Krebs, 1968; Rawat, 1968; Rawat & Lundquist, 1968). Since an increase in hepatic $[NADH]/[NAD^+]$ ratio has been suggested (Forsander *et al.*, 1965) to be responsible for an inhibition of the tricarboxylic acid cycle, it was considered relevant to investigate the effect of maternal ethanol consumption on the oxidation capacity of foetal and neonatal livers. Liver slices from foetal, neonatal and adult rats (Table 3) of the ethanol-fed groups showed significantly ($P < 0.01$) lower rates of $[^{14}C]$ palmitate oxidation.

Ethanol is preferentially metabolized in the liver. Its metabolism monopolizes at least 75% of hepatic oxidation capacity, resulting in a significant decrease in the oxidation of other substrates in the liver. During the metabolism of ethanol a decrease in respiratory CO_2 is also observed. Since an inhibition of $^{14}CO_2$ production has also been observed from non-fatty acid substrates (Rawat, 1972), it could not be suggested that the observed inhibition of $^{14}CO_2$ production from $[^{14}C]$ palmitate (Table 3) was entirely a consequence of an increased fatty acid pool, during ethanol metabolism. The most pronounced inhibition was, however, observed in the adult liver (Table 3). Although the effect of maternal ethanol consumption on the capacity of foetal and neonatal livers for lipid oxidation has been observed for the first time, this effect in the adult liver has been observed already (Rawat, 1972). Although the production of CO_2 from palmitate in the adult liver is inhibited by about 50% by ethanol, it is not possible on this basis to suggest that the catabolism of fatty acids also decreased by the same percentage, particularly in view of the fact that the steady-state concentration of ketones (Rawat, 1968) or their synthesis is not greatly affected by ethanol in the adult liver. The developmental changes in hepatic lipid oxidation capacity show that, although the foetal liver oxidizes lipids at the lowest rates, the lipid-oxidation capacity of the neonatal liver is highest (Table 3). Although the activities of certain enzymes such as phosphatidate phosphohydrolase (Lamb & Fallon, 1977) and the enzymes of fatty acid esterification are increased by ethanol in the microsomal and supernatant fractions of the liver,

it was observed in the present study that activities of other enzymes that affect fatty acid biosynthesis remain unchanged (Fig. 1).

It is concluded from these studies that maternal ethanol consumption both during pregnancy and lactation is capable of resulting in lipid accumulation in the livers of developing foetal and suckling neonatal rats. This effect of alcohol in leading to lipid accumulation was similar to that observed in adult animals. No experimental evidence could be obtained from 3H_2O incorporation studies to suggest that increase in fatty acid synthesis is an important mechanism for the accumulation of hepatic lipids during ethanol metabolism. Ethanol also did not affect either the activities or the developmental pattern of hepatic fatty acid-synthesizing enzymes.

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