In agreement with Sisken & others (1961), the brain GABA concentrations increased during the first 3 weeks of life (Table 1). Moreover the change produced by a standard hypoxic condition was greater in the 22-day old birds than in the 2-day old chicks. The constant ratio (Table 1) indicates that the elevation in GABA is proportional to the anticonvulsant effect (i.e. change in CT50) thereby lending support to the hypothesis of Baumel & others that the elevation in brain GABA concentration is responsible for the anticonvulsant action of hypoxia. However, the evidence is still not unequivocal.

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Triglyceride and precursor concentrations in the fatty liver of rats after chronic administration of ethanol

The most widely held explanation of the induction of fatty liver by ethanol is that an increase in the $NADH_2$:NAD ratio, secondary to the oxidation of ethanol, favours the formation of α-glycerophosphate and impairs the oxidation of fatty acyl-CoA derivatives (Lieber, Rubin & de Carli, 1969). The resulting increase in the concentrations of both precursors should then favour esterification to form triglyceride. In apparent support of this view, a single dose of ethanol has been found to increase the hepatic concentration of α-glycerophosphate and of triglycerides (Nikkilä & Ojala, 1963; Zakim, 1965). We have examined the concentrations of both precursors and product after chronic administration of ethanol in doses which consistently give rise to fatty liver in the rat.

Male Wistar rats, 200–300 g, were given homogenized liquid diets (Khanna, Kalant & Bustos, 1967) for 14 and 21 days. One diet, freely available, provided 35% of the total calories as ethanol, 19% as protein hydrolysate, 5% as sucrose and 41% as fat, and was nutritionally adequate in all other respects. The daily intake of ethanol averaged 10–12 g/kg. In a second diet the pair-fed controls had ethanol replaced by a calorically equivalent concentration of sucrose.

At the end of the treatment the animals were decapitated, the abdomen opened, and a portion of liver frozen instantly *in situ* by Wollenberger tongs precooled in liquid nitrogen. The frozen tissue was ground, deproteinized in 6% (w/v) perchloric acid, and centrifuged at 20 000 *g* for 15 min. The precipitate contained the long-chain fatty acyl-CoA derivatives, which were hydrolysed (Bortz & Lynen, 1963) and assayed for CoA content (Stadtman, 1955). α-Glycerophosphate was measured enzymatically in the neutralized supernatant (Hohorst, 1963). Other portions of the same livers were homogenized in phosphate buffer, and the triglycerides were extracted and measured (Butler, Maling & others, 1961).

Table 1. *Hepatic concentrations of triglycerides, long-chain acyl-CoA derivatives and α-glycerophosphate in rats consuming isocaloric ethanol and sucrose liquid diets.* Values shown represent mean ± s.e.

Time (days)	Group	Triglycerides (mg/g liver, wet weight)	Long-chain Acyl-CoA (n mol/g liver, wet weight)	α-Glycerophosphate (n mol/g liver, wet weight)
14	Ethanol	52·8±2·5 (4)‡	20·50±2·10 (9)‡	1347±124 (9)‡
	Sucrose	15·2±0·86* (4)	18·80±2·20 (9)	1210±58 (9)
21	Ethanol	64·6±1·8 (4)	27·80±3·50 (5)	943±149 (5)
	Sucrose	17·3±1·2* (4)	18·80±3·0† (5)	1195± 59 (5)

* Differs from ethanol group ($P < 0.01$). † Differs from ethanol group ($P < 0.02$).
‡ No. of animals.

An increase in liver triglyceride content in the ethanol groups was found at 14 and 21 days, compared with their respective pair-fed controls (Table 1). The concentration in the 21 day ethanol group was significantly higher than in the 14-day group ($P<0.01$). However, no significant difference was found in α-glycerophosphate levels at either time. The concentration of long-chain fatty acyl-CoA derivatives was increased in the ethanol group only at 21 days, but not at 14 days.

The results do not support the hypothesis that fat accumulation in this situation results from a rise in esterification owing to increase in the concentrations of the precursors. The concentration of long-chain fatty acyl-CoA derivatives was increased at 3 weeks, but this change followed the development of fatty liver, rather than preceding it. Since the secretion of hepatic lipoproteins into the plasma is increased rather than decreased by ethanol (Baraona & Lieber, 1970) three alternative possibilities deserve consideration. (1) Production and turnover of the precursors may be increased during ethanol oxidation, but the equilibrium constant of the esterification reaction is far over to the side of the triglyceride, so that the reaction velocity is not markedly influenced by precursor concentrations. (2) The increased triglyceride concentration does not represent the pool of newly synthesized product, but a sequestered compartment (e.g., cytoplasmic fat droplets), the size of which is independent of the rate of esterification. (3) Both of the above are true.

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