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Ethanol Administration and the Relationship of Malonyl-Coenzyme A Concentrations to the Rate of Fatty Acid Synthesis in Rat Liver

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1. The effect of ethanol on liver fatty acid synthesis was studied *in vivo* in 24h-starved and 'meal-fed' rats (i.e. fed for 3h per day and not *ad libitum*). 2. In the fed animal ^3H_2O was incorporated into fat at a rate of 0.46μmol of C_2 units/min per g wet wt. of liver. Administration of either ethanol (3.2g/kg) or equicaloric amounts of glucose had no effect on the rate of ^3H_2O incorporation into lipid. 3. In the 24h-starved animal, administration of the same dose of ethanol produced an increase in the rate of ^3H_2O incorporation from 0.06 to 0.12μmol of C_2 units/min per g fresh wt. after 3h whereas [malonyl-CoA] increased from 0.006 to 0.009μmol/g. Glucose given in amounts equicaloric to ethanol was significantly more lipogenic, increasing both the ^3H_2O incorporation from 0.06 to 0.20μmol of C_2 units/min per g and the malonyl-CoA content from 0.006 to 0.013 μmol/g wet wt. at 3h. 4. The decrease in the redox state of free cytoplasm NAD or NADP couples or the changes in content of citrate, glucose 6-phosphate and pyruvate of liver after ethanol administration had no measurable effect on the rate of fatty acid synthesis *in vivo*. 5. Under the conditions of the experiments there was no significant difference, among any of the groups, in the activity of liver fatty acid synthetase measured *in vitro*. A double-reciprocal plot of the rate of ^3H_2O incorporation and the total tissue malonyl-CoA concentrations showed a striking relationship. It has been concluded that the rate of fatty acid synthesis *in vivo* is determined principally by the $V_{max.}$ of fatty acid synthetase and the concentration of free malonyl-CoA. 6. It has also been concluded that under the conditions of the present study, the synthesis of fatty acids *de novo* is unlikely to be an important factor in the increased liver lipid content associated with ethanol administration.

A single dose of ethanol has been shown to increase triglyceride content in rat liver as early as 1h after administration (Mallov & Bloch, 1956; Mallov, 1957; DiLuzio, 1958). However, the significance of fatty acid synthesis *in vivo* in this accumulation has been controversial. Many previous reports suggest that fatty acid synthesis is increased (Lieber & Schmid, 1961; Lieber & Spritz, 1966; Gordon, 1972). Some workers have even suggested that an increased rate of fat synthesis after ethanol might be a means of disposing of the excess of reducing equivalents generated in liver during the metabolism of ethanol (Lieber, 1973). Other reports, however, deny that increased fat synthesis is a significant cause of the accumulation of fat in the liver after ingestion of alcohol (Lundquist *et al.*, 1962; Reboucas & Isselbacher, 1961).

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Part of the reason for these discrepancies may be technical. Much of the previous work suggesting that ethanol-stimulated fatty acid synthesis was performed *in vitro* in slices where cellular redox states and metabolite contents are grossly distorted compared with those *in vivo* (Krebs & Gascoyne, 1968). Another source of these discrepant results stems from the use of the incorporation of radioactive acetate (or ethanol) as a measure of the rate of fat synthesis. Since acetate is incorporated into fat via acetyl-CoA synthetase (EC 6.2.1.1), which in rat liver has an activity (Kornacker & Lowenstein, 1965; Aas & Bremer, 1968; Iliffe & Myant, 1970; Barth *et al.*, 1971) similar to or lower than that of fatty acid synthetase (Hicks *et al.*, 1965; Diamant *et al.*, 1972; Craig *et al.*, 1972*a*; Bruckdorfer *et al.*, 1972), the rate of acetate incorporation may reflect limitations at the acetyl-CoA synthetase or subsequent steps rather than in the overall rate of fatty acid synthesis. These methodological difficulties may

largely be overcome by combining the measurement of tissue malonyl-CoA concentrations with measurements of the rate of ^3H_2O incorporation into fatty acids (Guynn *et al.*, 1972). Measurement of the tissue content of malonyl-CoA, the product of acetyl-CoA carboxylase (EC 6.4.1.2) and the immediate precursor of fatty acids, appears to give a more reliable estimate of the capacity of acetyl-CoA carboxylase *in vivo* to meet the demands of the fatty acid synthetase multienzyme complex than can be arrived at by measurement of the activity of these two enzymes *in vitro* in the absence of the functionally important inhibitors of acetyl-CoA carboxylase *in vivo*. The use of ^3H_2O as a measure of the rate of fatty acid synthesis *in vivo* (Fain & Scow, 1966; Lowenstein, 1971) has the advantage of adding a non-exchangeable label to fatty acids during the final reductive step of fatty acid synthesis and is thus subject to control at no other enzymic step than that imposed by fatty acid synthetase itself.

In the present study the combined techniques of measuring both the ^3H_2O incorporation and the malonyl-CoA concentrations have been applied to the question of the effect of ethanol administration on fat synthesis *in vivo*. In addition, changes in other metabolite concentrations and redox states have been measured and examined to see what effects they have on the measured rates of fatty acid synthesis.

Experimental

Animals and diets

Male Wistar rats (200–225g) were adapted for 2 weeks to a meal-feeding schedule by allowing them to feed for 3h a day from 8:00 to 11:00h. The diet was Wayne Lablox, which consisted of 24% crude protein, 4% crude fat and 52% carbohydrate (largely starch); water was allowed *ad libitum*. Groups of eight animals were housed in plastic cages fitted with wire-mesh floors and air filter caps. The temperature was 24°C and the lights were on from 7:30 to 19:30 daily. All animals were meal-fed on the same diet for 2 weeks before the experiment so that the concentrations of lipogenic enzymes would be uniform within the group. On the day of the experiment part of the group of animals was fed as usual (the 'fed' group) and another part of the group received no food (the '24h-starved' group). The rats were killed 3 to 3.5h after the time scheduled for the beginning of the meal.

Chemicals and enzymes

All the chemicals used were of reagent grade. Dextrose, anhydrous (Baker Chemical Co., Phillipsburg, N.J., U.S.A.), dehydrated ethanol, 99.5% by volume (Health Services and Mental Health Administration, Social Security Administration, Perry Point, Md., U.S.A.), [^{14}C]bicarbonate (sodium salt; 17.8mg/mCi; New England Nuclear Corp., Boston, Mass., U.S.A.) and other biochemicals and enzymes (Boehringer Mannheim Corp., New York, N.Y., U.S.A.) were used.

Administration of materials in vivo

Ethanol (32g/100ml), NaCl (0.9g/100ml) or glucose equicaloric with the ethanol (61g/100ml) were given intraperitoneally at 1ml/100g body wt. The ethanol and glucose solutions were prepared in NaCl (0.9g/100ml).

Table 1. *Activity of acetyl-CoA carboxylase and fatty acid synthetase in the liver of 24h-starved rats or 3h after the beginning of the last meal*

The activities of acetyl-CoA carboxylase (38°C, pH7.4) and fatty acid synthetase (38°C, pH7.0) were measured in the liver of meal-fed rats either after 24h starvation or 3h after the beginning of the last meal. For the measurement of fatty acid synthetase activity the rats were intraperitoneally injected with 1ml of NaCl (0.9g/100ml), ethanol (32g/100ml) or glucose (61g/100ml)/100g body wt. For other details see the Experimental section. Numbers of observations are given in parentheses. The values are reported as μmol of C_2 units/min per g wet wt. of liver, ±S.E.M. *P* values testing the significance of the difference between means were calculated with Student's *t* test.

		Enzyme activity (μmol of C_2 units/min per g wet wt.)		
Liver enzyme	Treatment	Rats starved for 24h	Rats 3h after beginning of meal	*P*
Acetyl-CoA carboxylase		0.75±0.12 (6)	0.64±0.09 (6)	0.7
Fatty acid synthetase	NaCl	0.52±0.07 (5)	0.64±0.04 (5)	0.2
	Glucose	0.55±0.14 (5)	0.59±0.05 (5)	0.3
	Ethanol	0.49±0.08 (5)	0.67±0.07 (5)	0.1
Fatty acid synthetase (combined groups)		0.52±0.05 (15)	0.64±0.03 (15)	0.1

Measurement of incorporation of ^3H into fatty acids

The methods used were the same as previously described (Guynn *et al.*, 1972).

All animals were injected with ^3H_2O at the same time of day (10:25h) and killed 70min later. If 35min after the injection of ^3H_2O is taken to be zero time, the fed group of animals received their meal at −3h (i.e. 8:00h). On the same time scale the injection of NaCl, glucose or ethanol were −1h or −3h (i.e. 10:00 or 8:00h).

Metabolite measurements

Metabolites were measured in a duplicate set of rats. On the above time-scale these rats were killed at zero time (11:00h). Reagents and methods of tissue processing and calculation of free nucleotide ratios were as described by Veech *et al.* (1972).

Measurement of enzyme activity in vitro

The activity of acetyl-CoA carboxylase was measured at 38°C, pH7.4, after gel filtration of the 40000*g* supernatant through Sephadex G-25 (Chang *et al.*, 1967), by incorporation of [^{14}C]bicarbonate into malonyl-CoA (Moss *et al.*, 1972). The scintillation fluid consisted of 5g of 2,5-diphenyloxazole, 100g of naphthalene and 1 litre of dioxan. A liquid-scintillation counter (Beckman) was used.

The activity of fatty acid synthetase *in vitro* was measured directly in the 40000*g* supernatant at 38°C by the method of Burton *et al.* (1968) except that the malonyl-CoA-dependent oxidation of NADPH was followed spectrophotometrically at 340nm.

Results

Activity of fatty acid synthetase and acetyl-CoA carboxylase in vitro

Under the conditions of our experiments, the measured activities of acetyl-CoA carboxylase were unchanged 3h after the beginning of a meal (Table 1). These findings are not surprising, since earlier work indicates that the half-life of acetyl-CoA carboxylase is about 30h (Majerus & Kilburn, 1969; Nakanishi & Numa, 1970). Likewise the measured activity of fatty acid synthetase was unchanged by the treatments used (Table 1). This finding agrees with earlier work showing that the activity of fatty acid synthetase is essentially unchanged up to 3h after the beginning of feeding (Burton *et al.*, 1969).

Malonyl-CoA content and the rate of ^3H_2O incorporation into liver fatty acids

In the 24h-starved animal, equicaloric amounts of either glucose or ethanol increased the tissue content of malonyl-CoA (Table 2) and the rate of ^3H_2O

Table 2. *Liver concentration of malonyl-CoA and the rate of ^3H_2O incorporation into fatty acids in vivo in 24h-starved rats*

Numbers of observations are given in parentheses. All mean values are in terms of g wet wt. of liver, ±S.E.M. ** $P < 0.01$ as judged by Student's *t* test. For NaCl, glucose and ethanol treatments see legend of Table 1.

Treatment ...	NaCl	Glucose	Glucose	Ethanol	Ethanol
Time after treatment ...		1h	3h	1h	3h
Malonyl-CoA (μmol/g wet wt.)	0.006 ± 0.001 (15)	0.009 ± 0.002 (6)	0.013 ± 0.001 (15)**	0.007 ± 0.001 (6)	0.009 ± 0.001 (15)**
^3H_2O incorporation (μmol of C_2 units/min per g wet wt.)	0.052 ± 0.006 (12)	0.112 ± 0.013 (6)**	0.199 ± 0.046 (5)**	0.083 ± 0.016 (5)	0.125 ± 0.030 (5)**

Table 3. *Liver concentration of malonyl-CoA and the rate of ^3H_2O incorporation into fatty acids in vivo in meal-fed rats*

Numbers of observations are given in parentheses. All values are in terms of g wet wt. of liver, ±S.E.M. Administration of NaCl, glucose and ethanol are as indicated in the legend of Table 1. All the rats were killed 1 h after injection. * indicates $P < 0.05$ as judged by Student's t test.

Intraperitoneal injection ...	NaCl	Glucose	Ethanol
Malonyl-CoA (μmol/g wet wt.)	0.021 ± 0.002 (9)	0.024 ± 0.002 (9)	0.016 ± 0.001 (9)*
^3H_2O incorporation into lipid (μmol of C_2 unit/min per g wet wt.)	0.461 ± 0.024 (6)	0.464 ± 0.034 (6)	0.496 ± 0.071 (6)

incorporation into fatty acids. However, it is clear that glucose increased both tissue malonyl-CoA content and the rate of fatty acid synthesis *de novo* to a greater extent than did equicaloric amounts of ethanol. In contrast with the findings in starved animals, administration of glucose or ethanol had no effect on the rate of ^3H_2O incorporation into fat in the fed animals (Table 3). Ethanol produced a small but statistically significant decrease in the tissue malonyl-CoA content, but administration of glucose to the fed animal had no effect.

Neither malonyl-CoA concentrations (changes from 0.006 to 0.024 μmol/g wet wt.; see Tables 2 and 3), nor the rates of fatty acid synthesis (changes from 0.052 to 0.496 μmol of C_2 units/min per g wet wt.; Tables 2 and 3), correlate with the observed constancy (Table 1) of the activity of either acetyl-CoA carboxylase (0.64 and 0.75 μmol of C_2 units/min per g wet wt.) or fatty acid synthetase (0.49–0.67 μmol of C_2 units/min per g wet wt.). The relatively large changes in the rates of synthesis in the face of constant enzymic capacity emphasizes the importance of the so-called 'short-term control' in determining the rate of fatty acid synthesis (Masoro & Korchak, 1962; Srere & Foster, 1967; Lowenstein, 1968).

Content of intermediary metabolites in the freeze-clamped liver of 24 h-starved or meal-fed rats after administration of either ethanol or glucose

The injection of glucose into the 24 h-starved animal produced an increase in the concentrations of lactate, pyruvate, glycerol phosphate, citrate, 2-oxoglutarate and CoA and a decrease in the concentrations of 3-hydroxybutyrate, acetoacetate and acetyl-CoA (Table 4). Injection of ethanol produced qualitatively similar changes in the concentrations of lactate, glycerol phosphate, citrate, 2-oxoglutarate, 3-hydroxybutyrate and acetoacetate. In contrast with the effects of glucose, however, ethanol produced a decrease in [pyruvate] at 1 h, a decrease in [glucose 6-phosphate] and no change in total tissue content of either CoA or acetyl-CoA.

In the fed animals (Table 5) glucose administration increased the concentration of pyruvate but not lactate, increased [glycerol phosphate] and [glucose 6-phosphate], whereas [citrate], [2-oxoglutarate] and [3-hydroxybutyrate] were decreased. Again ethanol administration produced similar changes except that [pyruvate] was not increased and [3-hydroxybutyrate] and [glucose 6-phosphate] remained unchanged. The total tissue acetyl-CoA and CoA content was unchanged by either glucose or ethanol administration in the fed animals.

Changes in the redox states of freeze-clamped livers of 24 h-starved or meal-fed rats after administration of either ethanol or glucose

In the starved animals the administration of glucose resulted in an increase in the cytoplasmic free [NAD^+]/[NADH] ratio and a transient decrease in the cytoplasmic free [$NADP^+$]/[NADPH] ratio (Table 6). The same table shows that administration of ethanol caused a decrease in both these ratios. Neither glucose nor ethanol changed the mitochondrial [NAD^+]/[NADH] ratio.

In the fed animals (Table 7) both glucose and ethanol decreased the cytoplasmic free [$NADP^+$]/[NADPH] and increased the mitochondrial [NAD^+]/[NADH]. The cytoplasmic free [NAD^+]/[NADH] ratio rose after glucose and fell after ethanol administration, as was expected.

Discussion

Under the conditions of our experiments, ethanol has no effect on the rate of fat synthesis *in vivo* in the fed animal. Ethanol was less lipogenic in the starved animal than was an equicaloric amount of glucose. We are unable to substantiate the report in perfused liver (Gordon, 1972) or in slices (Lieber & Schmid, 1961) that the decrease in the redox state of the NAD^+ couple associated with ethanol ingestion is responsible for an increased rate of fat synthesis. It follows that our findings do not support the hypothesis that an increased rate of fat synthesis serves as a mechanism for disposal of excess of reducing power generated by ethanol (Lieber, 1973).

Table 4. *Effects of glucose or ethanol on the metabolite concentrations in the livers of rats starved for 24h*

Numbers of animals are given in parentheses. Mean values are given as μmol/g wet wt., ±S.E.M. For NaCl, glucose and ethanol administration see legend of Table 1. * Indicates $P < 0.05$ and ** $P < 0.01$ as judged by Student's *t* test.

	Concn. of metabolite (μmol/g)				
Treatment ...	NaCl (15)	Glucose (6)	Glucose (15)	Ethanol (6)	Ethanol (15)
Time after treatment ...		1h	3h	1h	3h
Metabolite					
Lactate	0.194±0.023	0.803±0.165**	0.688±0.064**	0.430±0.090*	0.399±0.036**
Pyruvate	0.019±0.001	0.099±0.030*	0.159±0.013**	0.012±0.002**	0.016±0.001
Glycerol phosphate	0.153±0.014	0.300±0.040**	—	0.725±0.127**	—
Citrate	0.302±0.020	0.450±0.019**	0.506±0.035**	0.503±0.055**	0.597±0.086**
2-Oxoglutarate	0.114±0.010	0.136±0.007*	0.252±0.020**	0.112±0.008	0.160±0.020*
3-Hydroxybutyrate	1.01±0.04	0.233±0.105**	0.216±0.076**	0.595±0.161*	0.864±0.145
Acetoacetate	0.289±0.035	0.095±0.027**	0.073±0.019**	0.136±0.020**	0.176±0.014**
Glucose 6-phosphate	0.187±0.010	0.214±0.047	0.214±0.060	0.096±0.014**	0.118±0.008**
Ethanol	—	—	—	51.6±3.0	40.0±1.3
Acetyl-CoA	0.074±0.001	0.036±0.009**	0.036±0.006**	0.066±0.006	0.074±0.001
CoA	0.080±0.004	0.105±0.011*	0.093±0.009	0.089±0.007	0.080±0.004

Our data suggest that the rate of fat synthesis is largely insensitive to changes in the redox state of either the NAD or the NADP couple.

The insensitivity of the rate of fat synthesis *de novo* is somewhat surprising, since [pyruvate] is known to decrease as a result of the decrease in cytoplasmic [NAD^+]/[NADH] ratio that accompanies ethanol administration. Recent work with perfused rat liver suggests that ethanol inhibits the rate of fat synthesis owing to a decrease in steady-state pyruvate concentrations (Scholz *et al.*, 1972). Comparison of the data in Table 5 with those in Table 3 shows that there is no change in the rate of fat synthesis *in vivo* even though [pyruvate] falls from 0.26μmol/g with glucose to 0.07μmol/g with ethanol. It is tempting to speculate that the small but statistically significant fall in [malonyl-CoA] from 0.024μmol/g after glucose to 0.016μmol/g after ethanol treatment (Table 3) might be related to the lowered [pyruvate]; however, we have no other evidence to support this hypothesis. Guynn *et al.* (1972) showed that of the proposed modifiers of acetyl-CoA carboxylase activity such as citrate or glycerol phosphate only the inhibition by long-chain acyl-CoA (Numa *et al.*, 1965) appeared to correlate with the malonyl-CoA contents and the rate of fatty acid synthesis *in vivo*.

Ethanol administration produced no significant change in the total liver acetyl-CoA content in either the fed or the starved state (Tables 4 and 5). Several authors have reported an increase in liver acetyl-CoA content on administration of ethanol (Forsander & Lindros, 1967; Lindros & Aro, 1969; Williamson *et al.*, 1969; Lindros, 1970), although Rawat (1968) reported a fall. We have no explanation for these divergent reports, but in the absence of information on the specific changes of acetyl-CoA and CoA in the mitochondria and cytoplasmic compartments, where these substrates serve important but separate functions, interpretation of these changes is difficult.

The situation with malonyl-CoA is quite different, however, because this is produced and utilized almost entirely in the cytoplasm. Our data indicate that, in the presence of a constant $V_{max.}$ of fatty acid synthetase, malonyl-CoA concentration is the most important single factor in determining the rate of fatty acid synthesis. This may be seen more strikingly in a double-reciprocal plot of the steady-state malonyl-CoA concentration and the rate of incorporation of ^3H into fatty acids (Fig. 1). Though the plot is not linear, a relationship between the malonyl-CoA concentration and the incorporation of ^3H is clear and shows that under conditions where the assayed activity of fatty acid synthetase is constant, the major determinant of the rate of ^3H_2O incorporation is the malonyl-CoA concentration. The relationship between the malonyl-CoA concentration and the rate of ^3H_2O

Table 5. *Effects of glucose or ethanol on the metabolite concentrations in the livers of meal-fed rats*

Numbers of animals are given in parentheses. Mean values are given as μmol/g wet wt., ±S.E.M. * indicates $P<0.05$ and ** indicates $P<0.01$ as judged by Student's t test. For NaCl, glucose and ethanol injection see legend of Table 1.

	Concn. of metabolite (μmol/g)		
Treatment ...	NaCl (6)	Glucose (6)	Ethanol (6)
Time after injection ...	1 h	1 h	1 h
Metabolite			
Lactate	1.43 ± 0.16	1.79 ± 0.08	1.91 ± 0.30
Pyruvate	0.134 ± 0.018	0.257 ± 0.008**	0.073 ± 0.021
Glycerol phosphate	0.190 ± 0.020	0.315 ± 0.029**	0.370 ± 0.045**
Citrate	0.454 ± 0.012	0.302 ± 0.028*	0.319 ± 0.020**
2-Oxoglutarate	0.284 ± 0.036	0.105 ± 0.006**	0.135 ± 0.014**
3-Hydroxybutyrate	0.118 ± 0.017	0.051 ± 0.006**	0.115 ± 0.021
Acetoacetate	0.066 ± 0.009	0.096 ± 0.012	0.091 ± 0.012
Glucose 6-phosphate	0.120 ± 0.023	0.535 ± 0.080**	0.151 ± 0.022
Ethanol	—	—	47.8 ± 3.0
Acetyl-CoA	0.033 ± 0.003	0.027 ± 0.002	0.039 ± 0.006
CoA	0.066 ± 0.011	0.078 ± 0.006	0.095 ± 0.013

incorporation was not seen in previous studies (Guynn *et al.*, 1972) because the activity of fatty acid synthetase was not constant, owing to the different dietary histories of the animals (Burton *et al.*, 1969; Gibson & Hubbard, 1960; Korchak & Masoro, 1962; Lundquist *et al.*, 1962; Butterworth *et al.*, 1966). In the present experiments, conditions were so chosen as to maintain the activity of fatty acid synthetase roughly constant among the groups studied. Under these conditions the direct relationship between the malonyl-CoA concentration and the rate of ^3H incorporation was apparent. The shape of the curve resembles the curve for substrate activation, though there is no evidence from enzyme studies *in vitro* to suggest that malonyl-CoA activates fatty acid synthetase. A reasonable explanation can be offered if the simple assumption is made that some 4 nmol of malonyl-CoA/g wet wt. of liver is bound to tissue constituents. The assumption that there is some binding of malonyl-CoA to tissue components is reasonable, especially since the amount of fatty acid synthetase itself can be estimated to be in the range of 1–5 nmol/g wet wt. [calculated from its molecular weight of 450000 (Burton *et al.*, 1968), turnover number of 0.5 μmol of malonyl-CoA/min per mg of enzyme protein (Collins *et al.*, 1971) and the observed range of activity in tissue in the fed state (Iliffe & Myant, 1970; Hicks *et al.*, 1965; Diamant *et al.*, 1972; Craig *et al.*, 1972*a*; Bruckdorfer *et al.*, 1972)]. From Table 1 it can be estimated that the concentration of fatty acid synthetase in the animals of the current study is 3–4 nmol/g wet wt. of liver. With the assumption of binding of 4 nmol of malonyl-CoA/g wet wt., the Lineweaver–Burk plot of the values for the starved animals becomes linear (Fig. 2) with a K_m of 13 μM. This K_m value is in good agreement with the reported K_m of 8 μM observed *in vitro* both for the yeast (Lynen, 1969) and pigeon liver enzymes (Chesterton *et al.*, 1968). It should be noted that, even if the entire 4 nmol of malonyl-CoA/g wet wt. of liver were bound to fatty acid synthetase itself, it would still be the unbound malonyl-CoA that would determine the steady-state rate of fatty acid synthetase.

Though there was no significant net synthesis of new fatty acid synthetase during the 3 h of feeding (Table 1), the small deviations from the theoretical curve that occur at high malonyl-CoA concentrations found in the fed groups could reflect a slight increase in the activity of fatty acid synthetase, perhaps by conversion of an apoenzyme (Craig *et al.*, 1972*b*). On the other hand, the type of deviation shown in Fig. 2 may be produced when two enzymes compete for the same substrate. The activity of the malonyl-CoA-dependent microsomal chain-lengthening system is 20–25% of the activity of fatty acid synthetase (Abraham *et al.*, 1961; Lorch *et al.*, 1963; Nugteren, 1965; Guchhait *et al.*, 1966; Donaldson *et al.*, 1970) and apparently has a higher K_m for malonyl-CoA than does fatty acid synthetase (Chesterton *et al.*, 1968). The competition between the two enzyme systems could also account for the deviation seen in the fed groups, but it must be admitted that this is speculative.

There are several implications which can be drawn from this '*in vivo*' plot. First, because of the dependence of the ^3H incorporation on the malonyl-CoA concentration, it is unlikely that there is any association of fatty acid synthetase with

Table 6. *Redox states in liver of 24h-starved rats given ethanol or glucose*

Values for the free nucleotide ratios are the means of individual animals ±S.E.M. Numbers of animals are given in parentheses. * indicates $P < 0.05$ and ** indicates $P < 0.01$ as judged by Student's *t* test. For method of calculation, see the text. The data used for calculations were taken from Table 4.

Treatment ...	NaCl (15)	Glucose (6)	Glucose (15)	Ethanol (6)	Ethanol (15)
Time after treatment ...		1h	3h	1h	3h
Free cytoplasmic $[NAD^+]/[NADH]$	945±135	1310±400	2060±119**	300±56**	381±35**
Free cytoplasmic $[NADP^+]/[NADPH]$	0.0055±0.0001	0.0046±0.0003*	0.0070±0.001	0.0035±0.0004**	0.0033±0.0001**
Free mitochondrial $[NAD^+]/[NADH]$	5.88±0.78	12.2±4.0	11.2±4.3	6.61±1.52	4.88±0.94

acetyl-CoA carboxylase to form a micro-environment *in vivo*. Secondly, it is unlikely that there is a significant allosteric effector of fatty acid synthetase *in vivo*. This conclusion is suggested by the ability to plot all the points from a wide variety of metabolic conditions (fed, starved and after glucose or ethanol administration) in a relatively simple relationship (Figs. 1 and 2). The relationship holds in spite of large changes in concentration of a large

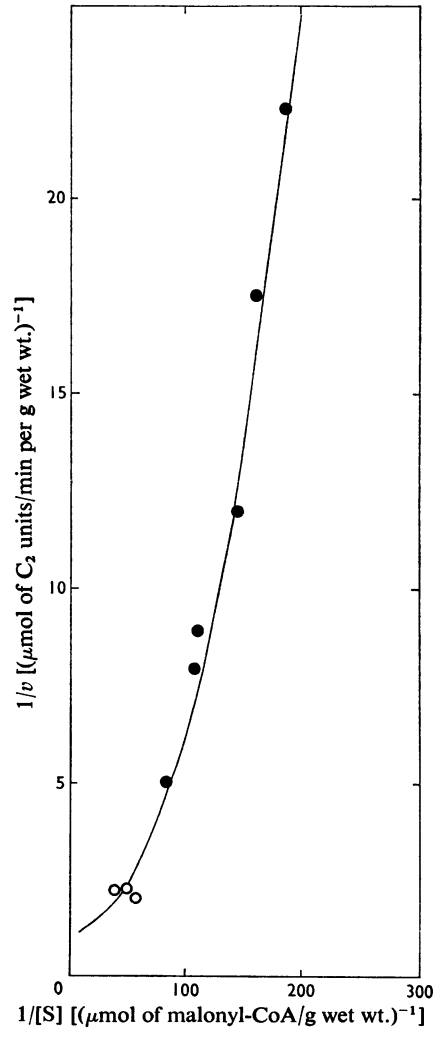

Fig. 1. *Double-reciprocal plot of liver malonyl-CoA content (μmol/g wet wt.) versus rate of ^3H_2O incorporation into fatty acids in vivo (expressed as μmol of C_2 units/min per g wet wt.)*

All animals are included from both starved (●) and fed (○) groups.

Table 7. *Redox states in liver of meal-fed rats given ethanol or glucose*

Values for the free nucleotide ratios are the means of individual animals ± S.E.M. Numbers of animals are given in parentheses. All injections were given 1h before the liver was freeze-clamped. * indicates $P < 0.05$ and ** indicates $P < 0.01$ as judged by Student's t test. The data used for calculations were taken from Table 5.

Treatment ...	NaCl (9)	Glucose (9)	Ethanol (9)
Free cytoplasmic [NAD^+]/[NADH]	854 ± 66	1300 ± 30**	341 ± 45**
Free cytoplasmic [$NADP^+$]/[NADPH]	0.0092 ± 0.0011	0.0044 ± 0.0003**	0.0063 ± 0.0006*
Free mitochondrial [NAD^+]/[NADH]	11.7 ± 1.3	42.8 ± 9.2**	16.9 ± 2.0*

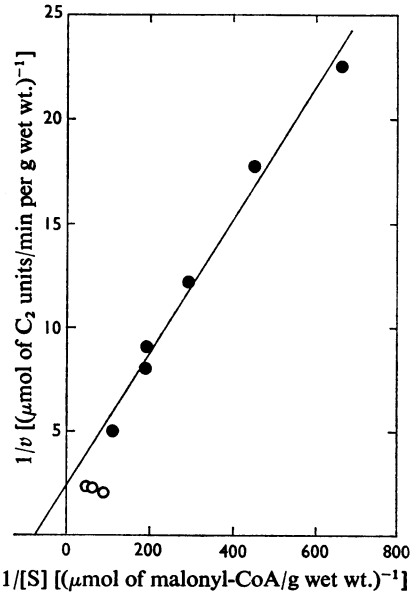

Fig. 2. *Data of Fig.* 1 *replotted with the assumption that 4 nmol of malonyl-CoA/g wet wt. of liver is bound to tissue constituents, and therefore not involved in the determination of the rate of fatty acid synthesis*

$V_{max.}$ is 0.42 μmol of C_2 units/min per g wet wt. and $K_m = 13$ μM. The units and symbols are as explained in the legend of Fig. 1.

number of metabolites produced by ethanol or glucose administration. From these conclusions it follows that the rate of fatty acid synthesis *in vivo* follows Michaelis–Menten kinetics and is largely a function of the $V_{max.}$ of the enzyme fatty acid synthetase and the concentration of free malonyl-CoA. In turn, the malonyl-CoA concentration is largely determined by the rate of malonyl-CoA utilization by fatty acid synthesis and the rate of its production by acetyl-CoA carboxylase. The most important factors which appear to govern the production of malonyl-CoA are the $V_{max.}$ (i.e. amount) of acetyl-CoA carboxylase and its inhibition, possibly by long-chain acyl-CoA (Guynn *et al.*, 1972).

Since the rate of fat synthesis does not appear to be sensitive to the redox state of the NAD^+ or $NADP^+$, within the limits of variation found *in vivo*, and since ethanol does not appear to have induced a major rise in either malonyl-CoA concentrations or the $V_{max.}$ of fatty acid synthetase, it follows that increased fatty acid synthesis *de novo* is unlikely to be an important factor in producing the fatty liver associated with ethanol ingestion under the conditions of these experiments.

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