group 2 the cytochrome *c* oxidase activity was similar to that of the patients of group 1. Hence in these five patients (three of whom were biopsied on two or three occasions) a specific defect in the oxidation of palmitoylcarnitine was demonstrated. This is shown in Fig. 1, where the oxidation of palmitoylcarnitine is expressed per unit of cytochrome *c* oxidase activity to compensate for any decrease in the numbers of mitochondria. The results for the patients in group 1 are almost identical with those for normal subjects, whereas those for the patients in group 2 show very low activities.

The oxidation of palmitoylcarnitine depends on a number of enzymes, including long-chain acylcarnitine translocase, internal carnitine palmitoyltransferase, the β-oxidation pathway, tricarboxylic acid-cycle enzymes and components of the electron-transport chain. In the patients of group 2 a possible defect in the tricarboxylic acid cycle and the electron-transport chain was excluded because pyruvate oxidation was measurable in all the patients. In patients D and E (Fig. 1) the carnitine palmitoyltransferase activity was 43% and 13% of normal respectively. Hence in these patients low rates of oxidation of palmitoylcarnitine could be attributable to diminished carnitine palmitoyltransferase activity. In patients A and B the carnitine palmitoyltransferase activity was in the normal range but the rate of oxidation of palmitoyl-CoA was less than 6% of those of three normal subjects and three patients of group 1 in whom this activity was measured. This suggests a defect in the β-oxidation pathway, probably in the activity of palmitoyl-CoA dehydrogenase. The defect in patient C remains to be elucidated.

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Comparative kinetics of human and rat liver alcohol dehydrogenase

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Alcohol dehydrogenase (EC 1.1.1.1) is the major location of initial ethanol oxidation. Although this enzyme has been detected in various tissues in humans (Moser *et al.*, 1968) and in rats (Raskin & Sokoloff, 1972; Sjöblom & Morland, 1979), the liver has always been considered to contain the greatest alcohol dehydrogenase activity and to be responsible for the major ethanol-oxidation capacity in the body. Acetaldehyde and NADH are formed by alcohol dehydrogenase action, and these two products are responsible for most of the toxic effects and metabolic disturbances produced by ethanol ingestion. The enzyme is a dimer which in human liver occurs in multiple molecular forms (Berger *et al.*, 1971; Li, 1977). In most individuals liver alcohol dehydrogenase has optimum pH 10.5 for catalysis, but in some cases there is an 'atypical' form with optimum pH 8.8 (Von Wartburg *et al.*, 1965). In rat liver, the kinetic characteristics of the enzyme appear similar to those of the human enzyme, but direct comparison studies with fresh preparations have not been reported. In the present work we performed this comparison, using samples of fresh human liver biopsies taken for diagnosis during abdominal surgery (cholecystectomy). We studied only liver samples which proved to be histophathologically unaffected. Livers from female Wistar rats killed under Nembutal anaesthesia (33 mg/100 g body wt.) were also examined. Immediately after excision, liver samples were placed in ice-cold 50 mM-sodium phosphate buffer, pH 7.4, and, after homogenization in a glass Potter vessel with Teflon pestle in an ice bath and sonication in an MSE sonifier (set at 12 μM, for 1 min) they were centrifuged at 56000 ***g*** for 20 min, at 4°C. Supernatants were then centrifuged at 143000 ***g*** for 60 min, and final supernatants were immediately used for the assay, performed at 25°C in cubettes containing 66.7 mM-sodium phosphate (pH 7.4) or -glycine (pH 10.5) buffers, 1.66 mM-NAD^+ and 0.8–1.0 mg of enzyme protein in 3 ml. The reaction was initiated by the addition of ethanol to give 16.7 mM (final concn,) for measurement of total activity, and 0.02, 0.05, 0.1, 1 and 16.7 mM for K_m and $V_{max.}$ determinations. Kinetic measurements were made with a Beckman DU-8B (Kinetics II) spectrophotometer at 340 nm, and a unit of alcohol dehydrogenase corresponds to 1 μmol of substrate transformed/min. Proteins were determined (Wang & Smith, 1975), and statistical comparisons between groups were made by Student's *t* test.

As shown in Table 1, alcohol dehydrogenase activity at physiological pH (7.4) was significantly higher in human liver than in rat liver. In both species, activity was increased by raising the pH in the assay medium, and the optimum pH was 10.5 (changes produced by different pH are not shown). The percentage increase in enzyme activity at pH 10.5 compared with that at pH 7.4 was much higher for the human enzyme (424 ± 33) than for the rat preparations (293 ± 23), and absolute values of total activity were significantly higher in human than in rat liver (Table 1). At the optimum pH, both $V_{max.}$ and K_m values for the human liver enzyme were also significantly higher than for the rat enzyme (Table 1). Pyrazole is known to be a specific and effective competitive inhibitor of alcohol dehydrogenase (Theorell *et al.*, 1969), with a K_i value about one-hundredth of the K_m for ethanol. At pH 10.5, 50 μM-pyrazole in the cuvette produced approx. 50% inhibition of human liver alcohol dehydrogenase (Table 1), whereas the rat

Table 1. *Alcohol dehydrogenase activity in human and rat liver*

Total activity was measured in fresh liver preparations in the presence of 16.7 mM-ethanol and 66.7 mM-sodium phosphate (pH 7.8) or glycine (pH 10.5) buffers. Other methodological details are given in the text. Values are means ± S.E.M. for four subjects per group, and *P* values refer to the statistical comparison between human and rat liver.

	Total activity at pH 7.4 (units/mg of protein)	Kinetics at pH 10.5			Inhibition of total activity at pH 10.5 (%)	
		Total activity (munits/mg of protein)	K_m (mM)	$V_{max.}$ (munits/mg of protein)	50 μM-pyrazole	200 μM-pyrazole
Human liver	12.9 ± 0.9	54.6 ± 2.7	1.8 ± 0.3	72.7 ± 4.5	52.0 ± 8.8	78.2 ± 3.4
Rat liver	7.1 ± 1.8	20.8 ± 1.8	0.8 ± 0.1	20.8 ± 1.8	93.9 ± 2.3	98.5 ± 0.6
P	<0.05	<0.001	<0.05	<0.001	<0.01	<0.01

enzyme appeared practically inactive in the same conditions (Table 1). This different inhibitory effect of pyrazole in the two species was also observed with a concentration of 200 μM (Table 1), which produced a partial inhibition of the human enzyme and complete inhibition of the rat enzyme. These findings demonstrate that human, but not rat, liver contains a pyrazole-insensitive activity. The presence of pyrazole-insensitive alcohol dehydrogenase activity in human liver has been reported in another study (Li *et al.*, 1977), and, since it exhibits a high K_m for ethanol, it may influence the ethanol-elimination rates *in vivo* when blood ethanol concentrations are very high. Present results indicate that this pyrazole-insensitive activity is absent from rat liver. At the same time, the lower enzyme activity and K_m and $V_{max.}$ values found for rat liver alcohol dehydrogenase indicate that in this species the enzyme system becomes saturated at lower ethanol concentrations than in humans, which would favour either its greater elimination in unoxidized form or its greater transformation through the alternate pathway, e.g. the microsomal ethanol-oxidizing system (Orme-Johnson & Ziegler, 1965) and/or catalase (De Duve & Baudhuin, 1966). This question is as yet unresolved, and the species-specific effects described should be considered when using the rat as an experimental model for alcohol effects in humans.

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Liver glycogen and glyceride glycerol formation as compared with glucose synthesis in 24h-starved virgin and pregnant rats

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Enhanced liver glucose production during starvation is initially sustained by augmented glycogenolysis, which causes a rapid decrease in glycogen stores (Herrera & Freinkel, 1968; Phan *et al.*, 1974). An increase in liver gluconeogenesis also occurs soon after the onset of starvation (Schimmel & Knobil, 1970; Llobera & Herrera, 1980), not only contributing to the net glucose release by the liver but avoiding complete depletion of its glycogen content. In this way, liver glycogen synthesis in starvation represents a gluconeogenic process. A similar mechanism seems to occur in the synthesis of glyceride glycerol from gluconeogenic substrates, which is also enhanced with starvation (Soley *et al.*, 1983). To determine whether there is a constant relation between the synthesis of either liver glycogen or glyceride glycerol and glucose production independent of the gluconeogenic substrate used, in the present work we administered intravenously 1 μCi of [U-^{14}C]alanine, [3-^{14}C]pyruvate or [U-^{14}C]glycerol (from The Radiochemical Centre, Amersham, Bucks., U.K.) containing 0.2mmol/200g body wt. to 24h-starved virgin rats. As gluconeogenesis is known to be greatly increased during starvation at late gestation in the rat (Herrera *et al.*, 1969), the same experiment was performed in 24h-starved 21-day pregnant rats, to determine also whether differences in the gluconeogenetic activity alter such relations. Animals were decapitated 10min after injection and blood [^{14}C]glucose, liver [^{14}C]glycogen and [^{14}C]glyceride glycerol were purified and determined as described elsewhere (Herrera *et al.*, 1969; Chaves & Herrera, 1980; Carmaniu & Herrera, 1980). As shown in Table 1, the ratio of liver [^{14}C]glycogen to blood [^{14}C]glucose, corrected to 200g body wt., was the same with all three substrates and in both pregnant and virgin animals. The ratio of liver [^{14}C]glyceride glycerol to blood [^{14}C]glucose was always higher than that of [^{14}C]glycogen to [^{14}C]glucose and, although values did not differ among substrates, they were consistently higher for pregnant than for virgin animals.

The present results show that in the starved condition there is a constancy in the proportional amount of newly synthesized glucose which is converted into liver glycogen or released to

Table 1. *Ratios of liver [^{14}C]glycogen or [^{14}C]glyceride glycerol to circulating [^{14}C]glucose synthesized from gluconeogenic substrates in the 24h-starved virgin or 21-day-pregnant rat*

Rats were intravenously injected with 1 μCi of [U-^{14}C]alanine, [3-^{14}C]pyruvate or [U-^{14}C]glycerol containing 0.2mmol/200g body wt. and killed 10min thereafter. Determinations were performed as previously described (Herrera *et al.*, 1969; Chaves & Herrera, 1980; Carmaniu & Herrera, 1980) and ratios were calculated for individual animals and expressed as means ± S.E.M. for five to ten rats per group. *P* refers to the statistical comparison between virgin and pregnant animals for each ^{14}C-labelled substrate (N.S. = not significant, $P > 0.05$). Statistical comparisons between values from different substrates were never significant ($P > 0.05$).

^{14}C-labelled substrate Rats ...	Liver [^{14}C]glycogen/blood [^{14}C]glucose			Liver [^{14}C]glyceride glycerol/blood [^{14}C]glucose		
	Virgin	Pregnant	*P*	Virgin	Pregnant	*P*
L-[U-^{14}C]Alanine	0.44 ± 0.05	0.54 ± 0.09	NS	2.33 ± 0.45	6.99 ± 0.59	<0.001
[3-^{14}C]Pyruvate	0.55 ± 0.07	0.62 ± 0.05	NS	2.64 ± 0.25	5.60 ± 0.41	<0.001
[U-^{14}C]Glycerol	0.42 ± 0.07	0.54 ± 0.13	NS	2.90 ± 0.31	7.57 ± 1.22	<0.01