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Peroxisomal fatty acid oxidation as detected by H_2O_2 production in intact perfused rat liver

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1. H_2O_2 formation associated with the metabolism of added fatty acids was quantitatively determined in isolated haemoglobin-free perfused rat liver (non-recirculating system) by two different methods. 2. Organ spectrophotometry of catalase Compound I [Sies & Chance (1970) *FEBS Lett.* **11**, 172–176] was used to detect H_2O_2 formation (*a*) by steady-state titration with added hydrogen donor, methanol or (*b*) by comparison of fatty-acid responses with those of the calibration compound, urate. 3. In the use of the peroxidatic reaction of catalase, [^{14}C]methanol was added as hydrogen donor at an optimal concentration of 1mM in the presence of 0.2mM-L-methionine, and $^{14}CO_2$ production rates were determined. 4. Results obtained by the different methods were similar. 5. The yield of H_2O_2 formation, expressed as the rate of H_2O_2 formation in relation to the rate of fatty-acid supply, was less than 1.0 in all cases, indicating that, regardless of chain length, less than one acetyl unit was formed per mol of added fatty acid by the peroxisomal system. In particular, the standard substrate used with isolated peroxisomal preparations ($C_{16:0}$ fatty acid) gave low yield (close to zero). Long-chain monounsaturated fatty acids exhibit a relatively high yield of H_2O_2 formation. 6. The hypolipidaemic agent bezafibrate led to slightly increased yields for most of the acids tested, but the yield with oleate was decreased to one-half the original yield. 7. It is concluded that in the intact isolated perfused rat liver the assayable capacity for peroxisomal β-oxidation is used to only a minor degree. However, the observed rates of H_2O_2 production with fatty acids can account for a considerable share of the endogenous H_2O_2 production found in the intact animal.

The first dehydrogenation step in β-oxidation of fatty acids by peroxisomes involves the reduction of O_2 to H_2O_2, first suggested by Cooper & Beevers (1969), who found that β-oxidation in peroxisomes of castor-bean endosperm (called glyoxisomes) was accompanied by stoichiometric formation of H_2O_2. Peroxisomal β-oxidation has been detected in a variety of cell types, notably rat liver (Lazarow & de Duve, 1976), *Tetrahymena* (Hryb & Hogg, 1976), yeast (Kawamoto *et al.*, 1978), rat brown adipose tissue (Kramar *et al.*, 1978), as well as mouse (Murphy *et al.*, 1979) and human (Bronfman *et al.*, 1979) liver.

Shortly after the detection of hepatic H_2O_2 production, using organ spectrophotometry of the catalase–H_2O_2 intermediate, Compound I, in the intact perfused rat liver (Sies & Chance, 1970), we observed an increased H_2O_2 production during the oxidation of octanoate and suggested that a certain component in the β-oxidation readily reacts with molecular oxygen to produce H_2O_2 (Sies, 1971; Oshino *et al.*, 1973*a*). The catalyst responsible for this reaction, fatty acyl-CoA oxidase, is now characterized and has been purified from rat liver peroxisomes (Osumi & Hashimoto, 1978; Hryb & Hogg, 1979; Inestrosa *et al.*, 1979*a*,*b*, 1980; Osumi *et al.*, 1980). It appears to be the rate-limiting enzyme in the sequence, and recently it was established that H_2O_2 production is a reliable index for the release of acetyl units during β-oxidation in peroxisomal fractions (Thomas *et al.*, 1980).

The debate in the literature on 'relative importance' for palmitoyl-CoA oxidation, carried out largely on data obtained with peroxisomal fractions and liver homogenates, seems to favour a preponderance of the classical mitochondrial system of β-oxidation (Krahling *et al.*, 1978; Shindo & Hashimoto, 1978; Hryb & Hogg, 1979; Inestrosa *et al.*, 1979*b*) rather than the peroxisomal one (Lazarow, 1978). More specific functions such as

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oxidation of very long-chain and/or monounsaturated fatty acids, however, have been ascribed to the peroxisomal system (Christiansen *et al.*, 1978; Osmundsen *et al.*, 1979).

Clearly, it is desirable to examine the function of the peroxisome in more intact systems. By using isolated hepatocytes, Mannaerts *et al.* (1979) calculated that the contribution of the peroxisomes to fatty acid oxidation was less than 10%. In the present work, we have studied the quantitative aspect of H_2O_2 formation during metabolism of several fatty acids in the intact perfused rat liver.

Experimental

Haemoglobin-free liver perfusion (open system)

Livers from male Wistar rats of 130–180g body wt., fed on stock diet (Altromin), were perfused at 37°C as described by Sies (1978) without recirculation of the perfusate, using the bicarbonate-buffered salt solution described by Krebs & Henseleit (1932), equilibrated with O_2/CO_2 (19:1, v/v). Unless indicated otherwise, the perfusion medium also contained, as sodium salts, L-lactate (0.3mM), pyruvate (0.3mM), DL-β-hydroxybutyrate (0.5mM), acetoacetate (0.3mM) and, in the experiments for $^{14}CO_2$ production from [^{14}C]methanol, also L-methionine (0.2mM). Perfusate flow (4.0–5.5ml/min per g wet wt.) was maintained constant throughout the individual experiment. Stepwise additions of substances were performed by infusion of neutralized stock solutions into the influent perfusate entering the portal vein, by using precision micropumps. The stock solutions were simply aqueous except for the longer-chain fatty acids. These fatty acids (palmitate, oleate and erucoate) were prepared daily at 15mM concentration in 5mM-albumin (bovine serum albumin, fraction V, defatted; Serva, Heidelberg, Germany). The neutralization was performed by first saponifying with a surplus of NaOH under sonification and subsequent retitration to pH 7.6 with HCl.

Assays

H_2O_2 production. This was quantitatively determined (*a*) by organ spectrophotometry of catalase Compound I at 660−640nm (Sies & Chance, 1970; Sies *et al.*, 1973; Oshino *et al.*, 1973*a*) or (*b*) by measuring flux through the peroxidatic reaction of catalase, the system of coupled oxidations described by Keilin & Hartree (1945).

For (*a*), the spectrophotometer used was either the one described previously (Schwab & Sies, 1978) or a dual-wavelength spectrophotometer (Sigma ZWS-11; Biochem. Co., München, Germany) adapted for organ spectrophotometry (T. Bücher, & H. Sies, unpublished work), with no detectable differences between the results obtained with the two types of apparatus. Light modulated at the two wavelengths was directed through the liver lobe by Y-shaped optics consisting of quartz light-guides, and the intensity of light passing through the lobe (thickness, 3mm) was directed to a photomultiplier and further processed to give the signal of absorbance difference ΔA (660−640nm). The details of the quantification procedure for H_2O_2 production are given in the Results section.

For (*b*), peroxisomal H_2O_2 production rates were assayed by measuring oxidation of [^{14}C]methanol to $^{14}CO_2$ and calibrating the method with the peroxisomal uricase reaction. After a period of 15min of perfusion, [^{14}C]methanol (sp. radioactivity 0.3mCi/mol) was added to the influent perfusate at a constant concentration of 1mM unless indicated otherwise, and 20min later a plateau value of effluent $^{14}CO_2$ production was reached. Effluent perfusate samples were then collected at 1min intervals for $^{14}CO_2$ measurement as described by Häussinger *et al.* (1975). Additions, e.g. of fatty acids, were usually made for 15min. After a period of recovery, sodium urate was infused at the appropriate concentration. The rates of $^{14}CO_2$ production (nmol/min per g wet wt. of liver) were calculated for each steady state (mean ± S.D., from 5min to 15min) based on the specific radioactivity of [^{14}C]methanol. A blank value of radioactivity in the phenylethylamine phase, obtained by measuring influent perfusate that had not passed through the liver, was subtracted. The $^{14}CO_2$ production rates were converted into H_2O_2 production rates by use of urate oxidation for calibration. Rates of urate oxidation were determined from the influent/effluent urate concentration difference measured with the uricase assay (cf. Bergmeyer, 1974). A 1:1 stoichiometry between peroxisomal H_2O_2 formation and urate oxidation has been established by Oshino *et al.* (1975*a*).

Metabolite assays. These were carried out for effluent concentrations of L-lactate, pyruvate, D-β-hydroxybutyrate, acetoacetate and urate in enzymic optical tests on the basis of procedures described previously (Bergmeyer, 1974). Non-esterified fatty acids were estimated by a slightly modified version of Duncombe's (1964) method with chloroform/heptane/methanol (80:60:3, by vol.) as solvent for extraction (Laurell & Tibbling, 1967). O_2 concentration in perfusate was measured with a Clark-type platinum electrode (Häussinger *et al.*, 1975).

Materials

Biochemicals and enzymes were from Boehringer (Mannheim, Germany), chemicals were from Merck (Darmstadt, Germany) except for L-lactic acid (Roth, Karlsruhe, Germany), bovine serum albumin and the long-chain fatty acids (Serva, Heidelberg,

Germany) and [^{14}C]methanol (Amersham–Buchler, Braunschweig, Germany). Bezafibrate was a gift from Boehringer (Mannheim, Germany).

Results

Quantitative estimation of H_2O_2 production elicited by fatty acids as detected by catalase Compound I

In examining the H_2O_2 production associated with fatty acid oxidation by organ spectrophotometry, there are two possibilities that we compare below. In the first, an effect of a fatty acid on the steady-state level of catalase Compound I is related to that of urate oxidation, whereas in the second a titration with the hydrogen donor methanol is performed without and with the fatty acid by the method of Oshino *et al.* (1973*a*).

As shown in Fig. 1(*a*), the infusion of urate at three different concentrations into the influent perfusate elicits stepwise increases in the level of catalase Compound I towards new steady states. The increase in H_2O_2 production for the urate infusions of 44, 22 and 11 μM correspond to 154, 77, and 39 nmol of H_2O_2/min per wet wt. g of liver respectively, as determined from the measured rate of urate oxidation (Sies, 1977) and the 1:1 stoichiometry between urate oxidized and H_2O_2 formed (Oshino *et al.*, 1975*a*). Thus it can be read off

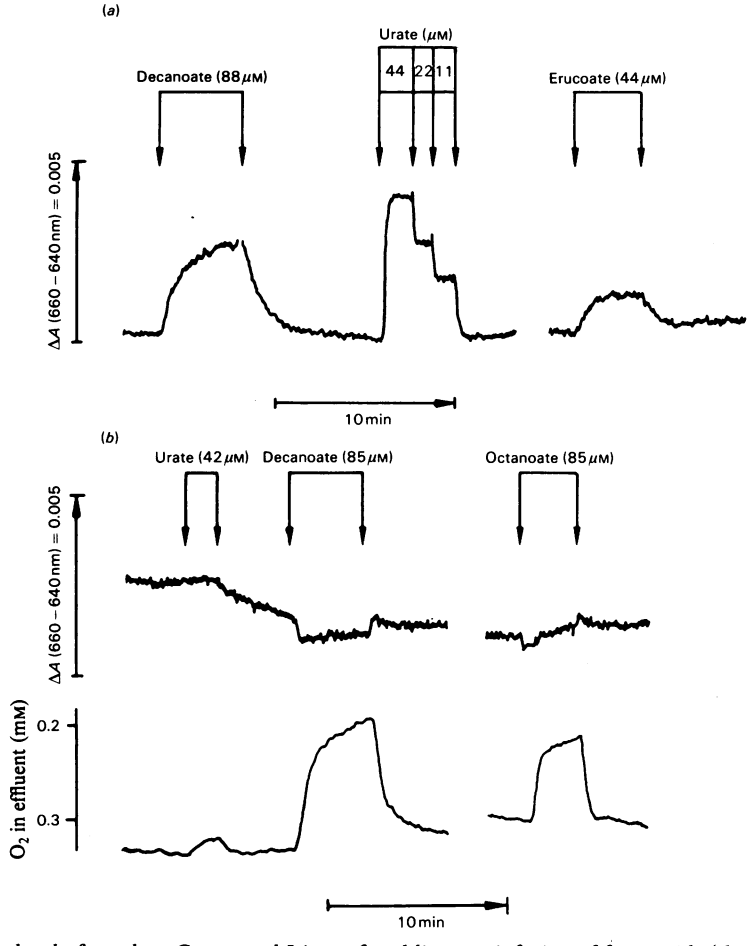

Fig. 1. *Increase in the level of catalase Compound I in perfused liver on infusion of fatty acids (decanoate and erucoate) and urate (a) and lack of significant changes in ΔA (660–640 nm) in liver from rat treated with 3-amino-1,2,4-triazole (b)*
The perfusion medium contained methanol at a concentration of 0.1 mM throughout in (*a*) and in (*b*). In (*a*) perfusate flow was 4.7 ml/min per g wet wt., so that the infusion rate for decanoate (88 μM) was 411 nmol/min per g wet wt. In (*b*) fatty acids (decanoate and octanoate) and urate were infused to liver obtained from aminotriazole-treated rat (1 g/kg body wt., 1 h before the experiment). O_2 concentration in effluent perfusate (lower trace) is also shown to indicate the metabolic response under this condition.

directly from the trace in Fig. 1(*a*) that the extra H_2O_2 production observed with the infusion of decanoate at 88 μM concentration corresponds to approx. 80 nmol of H_2O_2/min per g wet wt. of liver. A control for the selectivity of the spectrophotometric readout is given by Fig. 1(*b*), where the result of a similar experiment is also shown in which the animal had been treated with 3-amino-1,2,4-triazole to inactivate catalase (cf. Sies & Chance, 1970). It can be seen that, except for a slight baseline shift, there is little effect on ΔA (660 − 640 nm).

An experiment in which the second approach is used is shown in Fig. 2. The methanol titration is performed during the time from 40 to 56 min in Fig. 2(*a*) without addition of fatty acid for an estimation of the endogenous H_2O_2 formation, and then the methanol titration is repeated in the presence of decanoate in the influent perfusate; later, the initial titration in the endogenous condition is repeated. The quantitative evaluation is performed by determining the half-maximal methanol concentration, $a_{\frac{1}{2}}$, from a double logarithmic plot of the fractional saturation of catalase Compound I versus the methanol concentration. As shown in Fig. 2(*b*), $a_{\frac{1}{2}}$ for the endogenous condition is 0.12 mM-methanol, in agreement with our previous results (Oshino *et al.*, 1973*a*), whereas that observed in the presence of decanoate is 0.24 mM. The extra H_2O_2 formation due to decanoate is derived from these data to be 75 nmol of H_2O_2/min per g of liver (Table 1), in agreement with the rate obtained with the first approach described above (Fig. 1).

Quantitative estimation of H_2O_2 production elicited by fatty acids as detected by peroxidatic oxidation of [^{14}C]methanol to $^{14}CO_2$

Owing to the competition between the catalatic and peroxidatic pathways the efficiency of the peroxidatic pathway, i.e. the ratio of the rate through the peroxidatic indicator reaction to the rate of H_2O_2 production, is less than 1. The efficiency index F is increased with the concentration of hydrogen donor, and it is also increased when the steady-state catalytic-centre activity of catalase decreases (Oshino *et al.*, 1973*b*). As shown in Fig. 3, we have determined the efficiency index F in perfused liver by measuring the yield of $^{14}CO_2$ from [^{14}C]methanol at a given rate of H_2O_2 production from urate. In agreement with previous work on the system *in vitro* (Oshino *et al.*, 1973*b*) and with isolated adipocytes where the $^{14}CO_2$ production from [^{14}C]formate was studied (May & de Haen, 1979), we find an increase in F as the hydrogen donor concentration is increased. In view of the relatively low rates of H_2O_2 production, the experimental scatter markedly increases as the methanol concentration is increased. Therefore, we chose to use routinely an optimum concentration of methanol (1 mM). The dependence

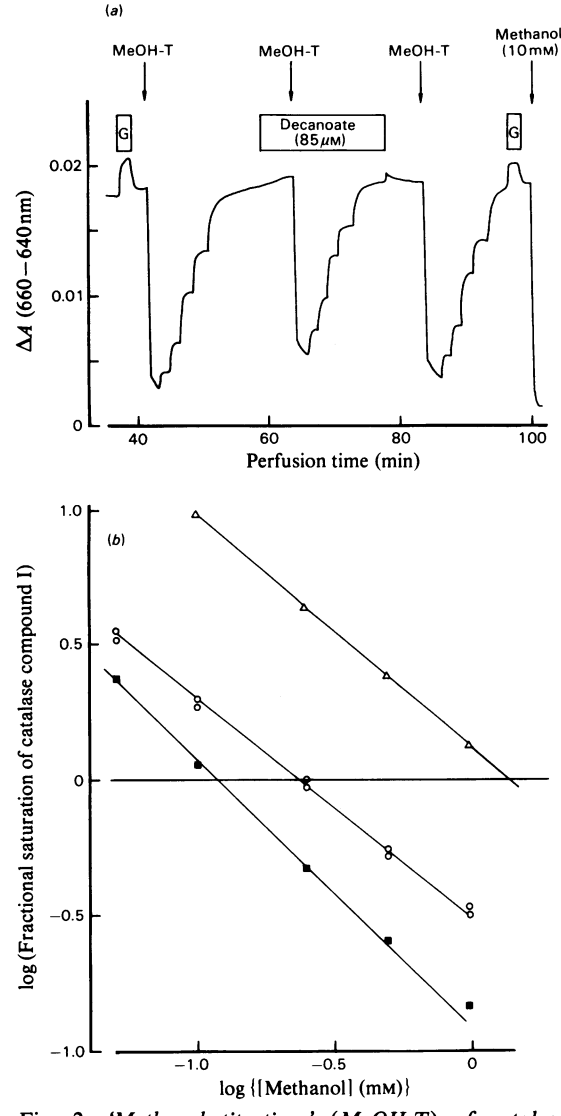

Fig. 2. *'Methanol titrations' (MeOH-T) of catalase Compound I in perfused liver in the absence and presence of decanoate (a) and determination of $a_{\frac{1}{2}}$ (indicated by the arrows in a) for the methanol titrations (b)*

The methanol concentrations in the titrations were, from left to right in (*a*), 1.0, 0.5, 0.25, 0.10 and 0.05 mM respectively. Maximal haem occupancy was estimated by infusing 1.0 mM-glycolate (G) in the absence of methanol, and zero haem occupancy was estimated by infusing methanol at high concentration (10 mM). Note the optical stability over the period of 1 h. The widths of the boxes indicate the time of presence of glycolate and decanoate. (*b*) Shows data from (*a*) plus an additional titration obtained in the presence of glycolate (0.42 mM). Further details and evaluation are given in Table 1. Slight deviation from the slope of −1.0 for decanoate may result from a small baseline shift (cf. Fig. 1*b*). ■, Endogenous substrates; ○, decanoate (85 μM); △, glycolate (0.42 mM).

Table 1. *H_2O_2 formation in perfused liver as detected by catalase Compound I*

The rates of H_2O_2 formation are calculated based on the equation:

$$dx_n/dt \cdot \frac{1}{e} = 31.5\ a_{\frac{1}{2}} \text{ (Oshino } et\ al.\text{, 1975}a\text{)}$$

$a_{\frac{1}{2}}$ is the methanol concentration required for half-maximal saturation of the catalase–H_2O_2 intermediate Compound I, e is the catalase content of liver (20nmol of catalase haem/g wet wt.), and dx_n/dt is rate of H_2O_2 formation. The data are obtained from the experiment shown in Fig. 2.

Addition	$a_{\frac{1}{2}}$ (mM)	Steady-state catalytic-centre activity or $dx_n/dt \cdot \frac{1}{e}$ (min^{-1})	Rate of H_2O_2 formation (nmol/min per g wet wt.)
None ('endogenous')	0.12	3.8	76
Decanoate (85μM; equivalent to 458nmol/min per g wet wt.)	0.24	7.6	151
Glycolate (0.42mM; equivalent to 2.26μmol/min per g wet wt.)	1.40	44.1	889

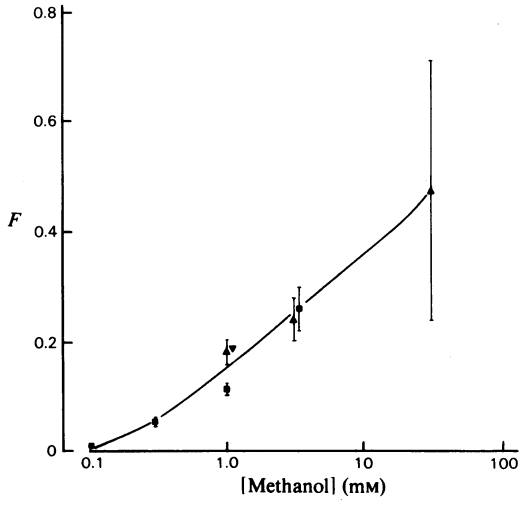

Fig. 3. *Apparent efficiency of the peroxidatic reaction of catalase with methanol as hydrogen donor in perfused rat liver*

Efficiency was determined at a given rate of peroxisomal H_2O_2 production from added urate (378 ± 6nmol of H_2O_2/min per g wet wt. of liver) by observing extra $^{14}CO_2$ production from [^{14}C]-methanol at various influent methanol concentrations. The efficiency index (F) = (rate of extra methanol oxidation)/(rate of extra H_2O_2 formation from urate). ■, ▲ and ▼ indicate results from different perfusion experiments.

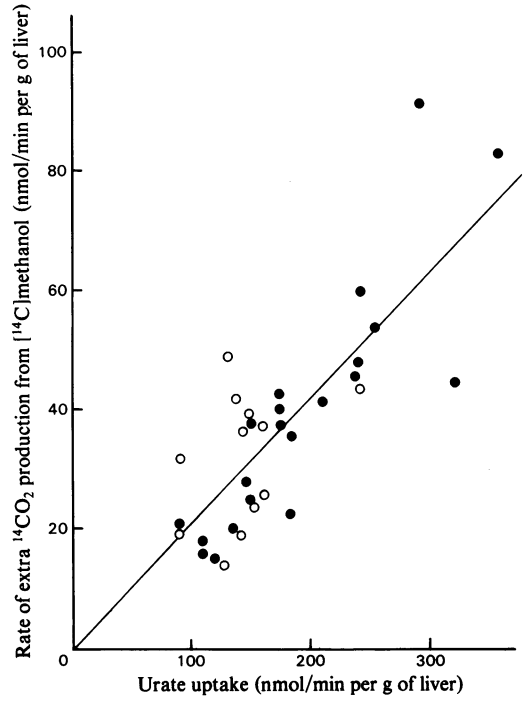

Fig. 4. *Dependence of extra $^{14}CO_2$ production from [^{14}C]methanol on urate uptake*

Methanol concentration in perfusate was 1mM. Each point represents one separate perfusion experiment, and measurements refer to steady states of urate uptake. The efficiency index (F) is 0.2, determined from the slope of the regression line. ●, Control; ○, bezafibrate-treated.

of F on the steady-state catalytic-centre activity is not critical within the range from 0 to 300nmol of extra H_2O_2/min per g wet wt. of liver; as shown in Fig. 4, the extra $^{14}CO_2$ production is approximately linearly related to the rate of urate uptake.

As shown in Table 2 and in Fig. 5, the results obtained for decanoate are in agreement with those obtained by organ spectrophotometry (Table 1 and Fig. 1*a*).

Properties of peroxisomal fatty-acid oxidation in perfused liver

As found with the saturated (Fig. 5) and mono-unsaturated (not shown) fatty acids, there is an

Table 2. *Yield of H_2O_2 from added fatty acids in perfused rat liver*

Data from experiments such as those shown in Fig. 5 are collected for comparison of the yield of H_2O_2 at a rate of fatty acid supply of about 300nmol/min per g wet wt. of liver. The rate of H_2O_2 formation was calculated from $^{14}CO_2$ production from [^{14}C]methanol using the efficiency index F as indicated in the Experimental section. The yield is calculated as the rate of extra H_2O_2 formation divided by the rate of fatty-acid supply. Thus no assumption is made on the fate of the carbon skeleton for the various saturated and unsaturated fatty acids. Treatment of rats with bezafibrate was by intraperitoneal injection of 50mg/kg body wt. daily for three consecutive days before the experiment. Data are given as means ± S.E.M. for the numbers of separate experiments shown in parentheses.

	Control			Bezafibrate treatment		
Fatty acid	Rate of supply (nmol/min per g wet wt. of liver) (*a*)	H_2O_2 formation (nmol/min per g wet wt. of liver) (*b*)	Yield of H_2O_2 [(*b*)/(*a*)]	Rate of supply (nmol/min per g wet wt. of liver) (*a*)	H_2O_2 formation (nmol/min per g wet wt. of liver) (*b*)	Yield of H_2O_2 [(*b*)/(*a*)]
Octanoate	309 ± 59 (6)	20 ± 4 (6)	0.06	300 ± 52 (5)	34 ± 5 (5)	0.11
Decanoate	342 ± 19 (5)	61 ± 5 (5)	0.18	290 ± 12 (4)	85 ± 3 (4)	0.29
Palmitate	280 (2)	None	None	293 ± 97 (3)	9 ± 3 (3)	0.03
Oleate	326 ± 36 (4)	62 ± 5 (4)	0.19	302 ± 17 (4)	28 ± 2 (4)	0.09
Erucoate	297 ± 34 (4)	91 ± 15 (4)	0.31	288 ± 39 (4)	103 ± 13 (4)	0.36

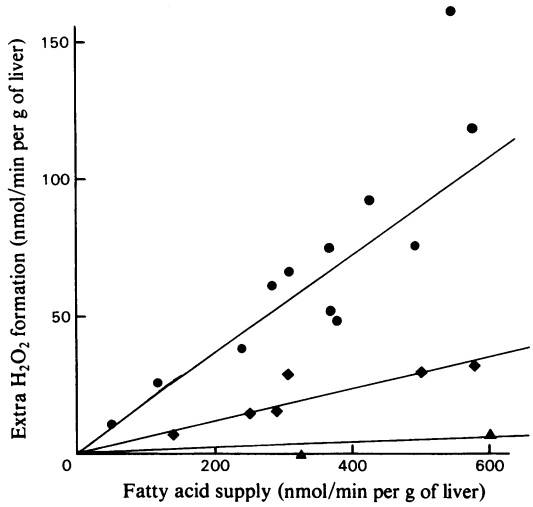

Fig. 5. *Relationship between extra H_2O_2 formation and fatty acid supply*

Each point represents a steady-state condition of fatty acid infusion, and H_2O_2 formation was calculated from $^{14}CO_2$ production from [^{14}C]-methanol (1mM) and the efficiency index F as described in the Experimental section. ♦, Octanoate; ●, decanoate; ▲, palmitate.

approximately linear increase in the steady-state H_2O_2 production with the rate of fatty-acid infusion. The yield of H_2O_2 formed per mol of fatty acid infused, however, varied between the different fatty acids. There was no H_2O_2 formation with butyrate, hexanoate, and also negligible formation with heptanoate (not shown), whereas there was a significant rate of H_2O_2 formation with octanoate and decanoate (Table 2) as well as with laurate and myristate (not shown). The rate increased with chain length up to the maximum found with laurate. Interestingly, the infusion of palmitate at the palmitate/albumin ratio of 3.0 did not elicit any significant extra H_2O_2 formation (Table 2). In these experiments, venous effluent perfusate analyses indicated that two-thirds of the added palmitate was taken up by the liver, in agreement with the observation by Kondrup (1979) for similar conditions. Regarding the unsaturated fatty acids that, like the medium-chain fatty acids, were taken up by 85–90% in the steady state, oleate led to a rate of H_2O_2 formation similar to that of decanoate, whereas erucoate led to even higher rates (Table 2).

Owing to the nature of the indicator system, only H_2O_2 reaching catalase is detected. As the metabolism of urate did not lead to an increased release of oxidized glutathione from liver (Sies *et al.*, 1974), the generation of H_2O_2 by peroxisomal fatty-acid oxidation likewise is not expected to increase the release of oxidized glutathione; indeed, in perfusions with decanoate (85 μM) as substrate, we observed no increase of oxidized glutathione release (not shown). Based on the stoichiometric recovery of H_2O_2 formed by peroxisomal urate oxidase (Oshino *et al.*, 1975*a*) and on the peroxisomal matrix localization of catalase, it is concluded that the observed H_2O_2 production reflects peroxisomal β-oxidation.

H_2O_2 formation in livers from rats pretreated with the hypolipidaemic agent bezafibrate

Bezafibrate, like clofibrate, was shown to lead to an increase in peroxisomal β-oxidation capacity. Lazarow (1979) demonstrated that the administration of bezafibrate is probably more specific in

increasing β-oxidation than is clofibrate, since urate oxidase and catalase activities were largely unaltered. In Table 2, we have compared the steady-state yields of H_2O_2 formation from various fatty acids with those obtained from control rats. As expected, there are increases with octanoate and decanoate and erucoate, but it is noteworthy that there is a decreased yield (one-half of the control value) with oleate; this is unexpected since it is known that treatment with a hypolipidaemic agent, e.g. clofibrate, increases the oxidation of perfused [^{14}C]oleate (Laker & Mayes, 1979). However, the partitioning between mitochondrial and peroxisomal flux through the β-oxidation pathways was not determined in these previous studies, so that the observations by Laker & Mayes (1979) may be explained by increased mitochondrial β-oxidation of oleate after clofibrate treatment. As shown in Table 2, the yield with palmitate is very low also after bezafibrate treatment.

Discussion

Quantitative assay of H_2O_2 formation as detected by catalase Compound I in intact perfused liver

The agreement of the organ-spectrophotometric determination of H_2O_2 formation associated with fatty-acid oxidation with that of the measurement of flux through the peroxidatic indicator reaction using [^{14}C]methanol is satisfactory and provides mutual support for these two different methods.

Further, the agreement of the results obtained by the organ-spectrophotometric analysis in Figs. 1 and 2 permits a conclusion on a cytotopical problem. For some species, a significant amount of extraperoxisomal catalase has been reported to occur even in the intact cells, and for rat liver the problem is not settled in the literature (see Masters & Holmes, 1977). Compound I estimations of fatty-acid-dependent H_2O_2 formation were found similar when calibrated on the basis of flux through the urate oxidase reaction (Fig. 1), a peroxisomal marker, and when a methanol titration of Compound I *in toto* is performed (Fig. 2), the latter including any potential extraperoxisomal catalase. Thus a share of extraperoxisomal catalase that might exist in the intact perfused rat liver can be considered insignificant in terms of a possible metabolic contribution here.

Comparison of H_2O_2 yield with data from isolated peroxisomes

The yield of extra H_2O_2 formation (Table 2) was relatively low, 0.06 and 0.18 with octanoate and decanoate, and surprisingly it was zero with palmitate. This indicates that in the intact perfused liver the peroxisomal β-oxidation system is utilized to only a fraction of its maximal capacity as assayed with isolated peroxisomal fractions. Factors that govern the partitioning between peroxisomal and mitochondrial pathways will have to be further identified. These include the transport problem (Osmundsen & Neat, 1979) arising for the CoA-esters to reach the peroxisomal matrix by the carrier system described by Appelkvist & Dallner (1980) or the possible rate-limitation by peroxisomal acyl-CoA synthetases (Shindo & Hashimoto, 1978; Krisans *et al.*, 1980). Partitioning will also involve endoplasmic-reticulum esterification activity, and also within the peroxisome some competition for acyl-CoA between β-oxidation and dihydroxyacetone phosphate esterification (Hajra *et al.*, 1979; Jones & Hajra, 1980) may occur; possibly there is even a role for $NADP^+$ (Osmundsen *et al.*, 1980).

However, this does not fully explain why the standard substrate for the assay of β-oxidation with isolated peroxisomes, $C_{16:0}$ fatty acid (Lazarow & de Duve, 1976; Osmundsen *et al.*, 1979; Inestrosa *et al.*, 1979*a*,*b*), is not active in the intact organ. An explanation might be given in the observation by Osumi *et al.* (1980) of an exceedingly low K_i for 3-oxohexadecanoyl-CoA (0.47 μM) with the isolated acyl-CoA oxidase from rat liver. This competitive inhibition may be more effective here compared with incubations of isolated peroxisomes. Lower-chain-length fatty acids, as well as longer-chain-length fatty acids that are unsaturated, would be preferred as substrates, since they do not lead to the intermediate formation of this potent inhibitor of the rate-limiting enzyme of the system.

The low yield of H_2O_2 in the intact liver is in approximate agreement with low numbers found with isolated hepatocytes (Mannaerts *et al.*, 1979). Taken together, these data indicate that the β-oxidation system of the peroxisome will be utilized preferentially for the first cycle, i.e. the release of the first acetyl moiety as derived from the carboxy end of the fatty acid, whereas the subsequent cycles of peroxisomal β-oxidation are subject to varying inhibition by accumulating products such as the 3-oxo derivatives. The system appears to be more equipped to oxidize the rare long-chain monounsaturated fatty acids (Osmundsen *et al.*, 1979); it is suggested that the 3-oxoenoic acyl-CoA esters may be less inhibitory for the acyl-CoA oxidase.

It would be of interest to examine whether the low rates of H_2O_2 production from fatty acids, compared with the rate of fatty-acid uptake, as they were observed in the present paper with livers from fed rats, are modified in other physiological states and whether they can be extrapolated to the intact animal. Oshino *et al.* (1975*b*) measured a rate of H_2O_2 production of 0.38 μmol/min per g wet wt. of liver in the intact exposed liver of the anaesthetized rat using the organ-spectrophotometric method; at physiological fatty acid concentrations, the H_2O_2

production according to Fig. 5 would account for a considerable share of this rate in the intact animal.

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