Biochem. J. (1981) **196**, 149–159
Printed in Great Britain

Effects of high-fat diets on hepatic fatty acid oxidation in the rat

Isolation of rat liver peroxisomes by vertical-rotor centrifugation by using a self-generated, iso-osmotic, Percoll gradient

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(*Received 13 October 1980/Accepted 4 November 1980*)

1. Rat liver peroxisomal fractions were isolated in iso-osmotic Percoll gradients by using vertical-rotor centrifugation. The fractions obtained with rats given various dietary treatments were characterized. 2. The effect on peroxisomal β-oxidation of feeding 15% by wt. of dietary fat for 3 weeks was investigated. High-fat diets caused induction of peroxisomal β-oxidation, but diets rich in very-long-chain mono-unsaturated fatty acids produced a more marked induction. 3. Peroxisomal β-oxidation induced by diets rich in very-long-chain mono-unsaturated fatty acids can oxidize such acids. *Trans*-isomers of mono-unsaturated fatty acids are oxidized at rates that are faster than, or similar to, those obtained with corresponding *cis*-isomers. 4. Rates of oxidation of [14-^{14}C]erucic acid by isolated rat hepatocytes isolated from rats fed on high-fat diets increased with the time on those diets in a fashion very similar to that previously reported for peroxisomal β-oxidation [see Neat, Thomassen & Osmundsen (1980) *Biochem. J.* **186**, 369–371]. 5. Total liver capacities for peroxisomal β-oxidation (expressed as acetyl groups produced per min) were estimated to range from 10 to 30% of mitochondrial capacities, depending on dietary treatment and fatty acid substrate. A role is proposed for peroxisomal β-oxidation in relation to the metabolism of fatty acids that are poorly oxidized by mitochondrial β-oxidation, and, in general, as regards oxidation of fatty acids during periods of sustained high hepatic influx of fatty acids.

Chain shortening of long-chain mono-unsaturated fatty acids is increased when rats are fed on diets containing partially hydrogenated marine oils (Thomassen *et al.*, 1979; R. Z. Christiansen *et al.*, 1979). We have previously described the effects of feeding high-soya-bean-oil diets and marine-oil diets on β-oxidation by liver peroxisomes isolated by using a vertical rotor and iso-osmotic Percoll gradients (Neat *et al.*, 1980). Ishii *et al.* (1980*a*) have also reported that high-fat diets cause induction of peroxisomal β-oxidation. In the present paper we report further experiments incorporating data with two other dietary fats, ground-nut oil and rape-seed oil. Some properties of the peroxisomal β-oxidation system induced are described. Attempts are made to correlate changes in abilities of hepatocytes, isolated from rats fed on some of the diets, to oxidize [14-^{14}C]erucic acid with extent of induction of peroxisomal β-oxidation. We also further describe the characteristics of peroxisomal fractions isolated in a Percoll gradient.

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Experimental

Materials

Brassidic acid (*trans*-$C_{22:1(13)}$), elaidic acid (*trans*-$C_{18:1(9)}$), palmitoelaidic acid (*trans*-$C_{16:1(9)}$), myristoleic acid (*cis*-$C_{14:1(9)}$) and myristoelaidic acid (*trans*-$C_{14:1(9)}$) were purchased from Nu-Check Preps., Elysian, MN, U.S.A. Coenzyme A (grade IL) and bovine serum albumin (essentially fatty acid-free) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Fatty acyl-CoA esters were synthesized and characterized as previously described (Osmundsen *et al.*, 1979). Clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropionate] was obtained from Weiders Farmasøytiske A/S, Oslo, Norway. Details of other reagents have been given elsewhere (Osmundsen & Neat, 1979).

Animals and diets

Weanling male Wistar rats (approx. 60g body

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wt.) were purchased from Vætrinær Møllegårds Avlsstasjon, Havdrup, Denmark. Semi-synthetic diets were prepared as described previously (Thomassen *et al.*, 1979), and contained 15% by wt. of one of the following oils: soya-bean oil, groundnut oil, rape-seed oil and partially hydrogenated marine oil. A diet containing 5% by wt. of soya-bean oil, and correspondingly more of corn starch, was used as a low-fat control diet. The rats were fed on a standard pelleted diet for the initial 5 days after arrival from the supplier, and were subsequently fed on one of the semi-synthetic diets with free access to food and water. Animals fed on these diets showed weight gains of about 44g per week similar to, or better than, the supplier's suggested normal value. The fatty acid composition of the dietary oils has been described elsewhere (Houtsmuller, 1978; Thomassen *et al.*, 1979).

Some animals were treated with clofibrate as described by Osmundsen *et al.* (1979).

Preparation of peroxisomes and mitochondria

Peroxisomal fractions were prepared from livers of rats fed on semi-synthetic diets as described by Neat *et al.* (1980). Peroxisomal fractions from clofibrate-treated rats were prepared in a similar fashion, centrifugation now being carried out at $79000 g_{av.}$ for 30min. The resulting distribution of enzyme marker activities did not differ markedly from that obtained with rats fed on partially hydrogenated marine-oil diet (see Neat *et al.*, 1980), excepting a shift of all peaks one fraction down the gradient.

Enzyme assays

Peroxisomal β-oxidation can conveniently be measured in gradient fractions. When required most of the Percoll in the peak peroxisomal fractions was removed as described by Neat *et al.* (1980). The recoveries of enzyme activities in pellet and supernatant were 95–100%. Preliminary experiments suggested that Percoll had no significant effect on the enzyme activities measured (results not shown). Enzyme marker activities were assayed as described previously (Neat & Osmundsen, 1979).

Peroxisomal β-oxidation was assayed as acyl-CoA-dependent NAD^+ reduction. The rate of NAD^+ reduction was found to increase linearly with increasing protein concentrations up to about 300μg of protein per ml of assay (results not shown). The inclusion of KCN in this assay (to inhibit mitochondrial β-oxidation) is not required, as contaminating mitochondria also would be solubilized by added Triton X-100. Mitochondrial β-oxidation does not function under solubilized conditions (Stewart *et al.*, 1973). All acyl-CoA esters were included in the assay at a concentration of 10μM, and assays were carried out at 37°C.

Estimation of liver capacities for peroxisomal β-oxidation

Total liver capacities for peroxisomal β-oxidation were estimated as described by Neat *et al.* (1980).

Preparation of rat hepatocytes

Parenchymal liver cells were prepared and purified by the method of Seglen (1973). Cell viability was routinely checked by using the Trypan Blue exclusion procedure, and only preparations showing 90%, or more, of unstained cells were used experimentally. With cells isolated from rats fed on partially hydrogenated-marine-oil diet it was, in particular, essential to include 1mM-$CaCl_2$ in the suspension medium to achieve this high proportion of unstained cells. Before use the cells were preincubated for 30min at 37°C in the presence of 1mM-L-carnitine to ensure optimal concentrations of intracellular carnitine (Christiansen & Bremer, 1976).

Measurements of rates of oxidation of [14-^{14}C]-erucic acid by isolated rat hepatocytes

Oxidation of [14-^{14}C]erucic acid was measured essentially as described previously (Christiansen, 1978; Christiansen *et al.*, 1974). Incubations were initiated by the addition of 1ml of cell suspension (containing about 10^7 cells) to 1ml of Krebs–Ringer phosphate buffer, also containing defatted 0.54mM-bovine serum albumin and 1.5mM-[14-^{14}C]erucic acid. The cells were incubated for 60min at 37°C. Rates of oxidation of [14-^{14}C]erucic acid were calculated from the sum of radioactivity in $^{14}CO_2$ and in the acid-soluble fraction.

Measurements of chain-shortening of [14-^{14}C]-erucic acid by isolated rat hepatocytes

Samples (0.5ml) from hepatocyte incubations were pipetted into 10ml of chloroform/methanol (2:1, v/v). Triacylglycerols were separated out from the resulting lipid by t.l.c. as described by Thomassen *et al.* (1979). The distribution of radioactivity in the constituent fatty acids was measured by radio-g.l.c., using a column of 10% SP-2340 on 100/120 Chromosorb W AW as described previously (Osmundsen *et al.*, 1979).

Measurements of rates of oxidation of some acyl-CoA esters by isolated rat liver mitochondria

Rates of oxidation of acyl-CoA esters by isolated rat liver mitochondria were measured polarographically by using a Clark-type oxygen electrode. The incubation medium contained 130mM-KCl, 10mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 0.1mM-EGTA, 10mM-malonate, 1.5mg of defatted bovine serum albumin per ml, 3mM-ADP, 2mM-P_i, pH 7.2, and 4–6mg of mitochondrial protein in a final volume of 2ml. Acyl-

CoA esters were included to a concentration of 40 μM, and mitochondrial β-oxidation was initiated by the addition of 2 mM-L-carnitine. The mitochondria appeared functionally intact as judged by respiratory control ratios in excess of 3. The measurements were carried out at 30°C.

Assay of protein

Proteins were assayed as described previously (Neat & Osmundsen, 1979).

Statistical analysis

The Mann–Whitney U-test or one-way analysis of variance was used to test the significance of differences between population means. Probabilities greater than 0.05 were taken to be non-significant.

Results

Isolation of peroxisomes

The isopycnic densities of organelles fractionated in an iso-osmotic Percoll gradient were found markedly different from those observed in hyperosmotic sucrose gradients (see Rickwood, 1978; Neat & Osmundsen, 1979; Neat *et al.*, 1980). In the Percoll gradient peroxisomes showed a lower density (about 1.075–1.085 g/ml) than mitochondria (about 1.095 g/ml). In a sucrose gradient peroxisomes band at a higher density compared with mitochondria.

In the Percoll gradient endoplasmic reticulum banded at a density range of 1.055–1.075 g/ml, which is sufficiently close to that of peroxisomes to impair complete separation of these organelles. The microsomal contamination was, however, very low, as only about 2% of the microsomal marker enzyme activity of the starting homogenate reached the L fraction, and was layered on to the gradient.

Recoveries of marker enzyme activities in gradient fractions from clofibrate-treated rats

The removal of Percoll from the pooled peak fractions of peroxisomal activity resulted in a loosely packed pellet, presumably due to remaining Percoll. The supernatant could thus only be completely removed with some loss of sedimented material. Hence recoveries of marker enzyme activities in the pellet were usually less than that previously achieved with peroxisomes isolated in sucrose gradients (see Neat & Osmundsen, 1979). Only 60% of urate oxidase (EC 1.7.3.3) activity was recovered (see Table 1) compared with up to 95% when sucrose gradients were used (Neat & Osmundsen, 1979). On the other hand 49% of catalase (EC 1.11.1.6) activity was now recovered, compared with 55% when sucrose was used. The ratios of recoveries, from the 10% homogenate, of urate oxidase to catalase activities was 1.3 for Percoll preparations (see Table 1) and 5 for preparations from sucrose gradients (Neat & Osmundsen, 1979). Similarly about 50% of β-oxidative activity was recovered in the pellet after removal of Percoll (results not shown). As judged by the decreased loss of catalase, the peroxisomes prepared by the present Percoll procedure appear less damaged. This may be due to the use of iso-osmotic conditions throughout the isolation procedure. Yokota & Fahimi (1978) have reported that poly(vinylpyrrolidone) appears to stabilize the peroxisomal membrane, and even 250 mM-sucrose caused membrane disruption. It is therefore possible that the poly(vinylpyrrolidone) component of Percoll may have a stabilizing effect on the peroxisomal membrane.

Purity of peroxisomal preparations

Estimates of the composition of peroxisomal preparations can be obtained from measurements of recoveries of marker enzyme activities. These can be expressed in terms of percentage contribution of various organelles to the total protein using the values of Leighton *et al.* (1968) (i.e. 20% for mitochondria, 20% for endoplasmic reticulum and 2% for lysosomes).

Results showing the composition of the peroxisomal fractions isolated from rats fed on the various

Table 1. *Recoveries of enzyme marker activities resulting from the use of the Percoll-density-gradient procedure with livers from clofibrate-treated rats*

Peroxisomal fractions were prepared as described in the Experimental section. The pooled peak peroxisomal fractions and the peroxisomal fraction obtained after removal of Percoll (by dilution and centrifugation) were assayed for enzyme marker activities as described in the Experimental section. The tabulated values represent mean percentage recoveries (± S.D.) indicated for three animals.

	Recoveries of protein and marker enzyme activities from 10% homogenate (%)					
	Protein	Urate oxidase	Catalase	Cytochrome *c* oxidase	β-*N*-Acetyl-D-glucosaminidase	Rotenone-insensitive NADPH:cytochrome *c* reductase
Pooled peak peroxisomal fractions	2.2 ± 0.9	22.0 ± 4.7	19.6 ± 6.7	1.1 ± 0.6	3.0 ± 0.6	3.0 ± 0.5
Peroxisomal preparation after removal of Percoll	1.0 ± 0.2	12.7 ± 2.1	9.7 ± 3.0	0.6 ± 0.4	1.4 ± 0.4	0.5 ± 0.0

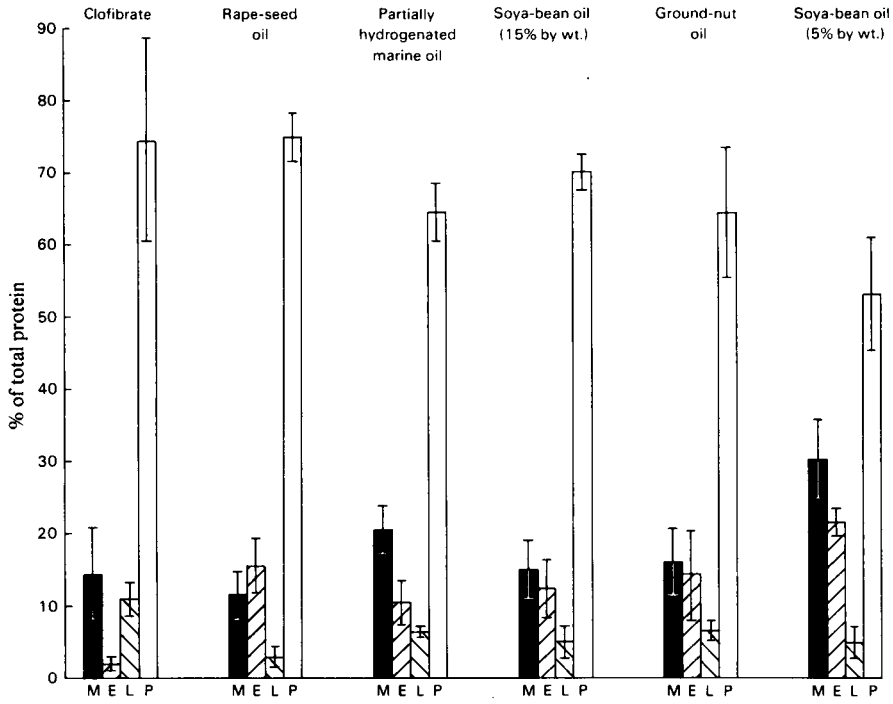

Fig. 1. *Composition of peroxisomal fractions isolated from livers of rats given different dietary treatment*
The relative contributions of the various subcellular organelles to the total protein were calculated for purified peroxisomal preparations. Data represent means ± S.D. for three animals, excepting the group fed on soya-bean-oil diet (5% by wt.), where four animals were used. The following abbreviations are used: E, microsomal fractions ('microsomes') (▨); L, lysosomes (▧); M, mitochondria (■); P, peroxisomes (□). The data show the percentage contribution of the various organelles to the total protein of the peroxisomal fractions (set to 100%).

diets are presented in Fig. 1. It is assumed that the relative proportions of the various organelles in the liver does not change markedly with the treatment used. This may not always be the case, but these results should at least be a guide to the purity of the peroxisomal preparations.

As can be seen from Fig. 1, contamination by endoplasmic reticulum was higher in peroxisomal fractions isolated from rats fed on semi-synthetic diets compared with animals treated with clofibrate. This may be due to the fact that the pelleted L fraction obtained from such animals was relatively loosely packed. It was therefore difficult to remove sedimented microsomes lying on the top of the pellet, without losing a large amount of underlying material.

Table 2 shows the changes in specific activities of the various marker enzymes during isolation of the peroxisomal fractions from the liver of a clofibrate-treated rat. Urate oxidase shows a 12.5-fold increase in specific activity. With catalase, after allowing for differential losses, a 12-fold increase was observed (see Tables 1 and 2). About 74% of the protein in the preparations from clofibrate-treated rats was peroxisomal (see Fig. 1). The maximum possible increase in specific activity was therefore 17-fold. This would be equivalent to peroxisomal protein accounting for about 6% of total liver protein, which is about 2.4-fold more than the value for control rats (Blouin *et al.*, 1977; Leighton *et al.*, 1968). Lazarow & de Duve (1976) reported a 2.5-fold increase in liver peroxisomal protein after clofibrate treatment.

Estimates for peroxisomal protein content in livers of animals fed on the various semi-synthetic diets (see Table 4) were derived in the same way. These ranged from 3.8 to 4.5% for all rats fed on semi-synthetic diets, excepting those fed on rape-seed-oil diet, which had the significantly higher value of $5.9\% \pm 0.8\%$ ($P < 0.05$). This value is similar to that obtained with clofibrate-treated rats. The purity of the peroxisomal fraction isolated from rats fed on rape-seed-oil diet was also higher than that of the other dietary groups.

The lower purity of the latter groups leads to less-precise estimates of percentages of peroxisomal protein, and may also be the cause of our failure to detect significant differences between these dietary

Table 2. *Specific activities of enzyme marker activities in starting homogenate and final peroxisomal fraction derived from rats treated with clofibrate*

The specific activities of various marker enzyme activities were measured both in the starting 10% (w/v) liver homogenate from clofibrate-treated rats and in the final peroxisomal preparation as described previously (Neat & Osmundsen, 1979). The specific activities are means ± S.D. for five animals. The unit of cytochrome *c* oxidase activity is defined by Wharton & Tzagoloff (1967), and that of catalase by Chance & Maehly (1955).

	Urate oxidase (nmol/min per mg of protein)	Catalase (units/mg of protein)	Cytochrome *c* oxidase (units/mg of protein)	Rotenone-insensitive NADPH : cytochrome *c* reductase (nmol/min per mg of protein)	β-*N*-Acetyl-D-glucosaminidase (nmol/min per mg of protein)	Palmitoyl-CoA-dependent NAD^+ reduction (nmol/min per mg of protein)
10% homogenate	11.4 ± 1.6	0.57 ± 0.06	0.13 ± 0.08	131 ± 16	11.6 ± 5.8	—
Peroxisomal preparation	141.0 ± 23.0	5.2 ± 0.4	0.7 ± 0.01	74 ± 4	16.1 ± 3.4	21.3 ± 9.0

Table 3. *Effect of different diets on some parameters of liver metabolism*

Rats were fed on the diets indicated for 17 days. The numbers of animals in each dietary group are indicated by the numbers in parentheses. Values represent means ± S.D. Values significantly less ($P < 0.05$) than the corresponding ones for the group fed on partially hydrogenated marine oil are indicated by *. The unit of catalase activity is defined by Chance & Maehly (1955). The animals in the group fed on rape-seed-oil diet had a higher starting body weight (about 15 g) than the animals in the other dietary groups.

Diet	Body weight (g)	Liver weight as % of body weight	Total liver protein (mg)	Protein (mg/g of liver)	Liver catalase (units)	Catalase (units/g of liver)
Partially hydrogenated marine oil (7)	207 ± 10	5.5 ± 0.3	1880 ± 175	165 ± 18	1450 ± 260	128 ± 29
Ground-nut oil (7)	192 ± 17*	4.9 ± 0.7*	1800 ± 325	194 ± 42	1000 ± 310*	109 ± 23
Soya-bean oil (7) (15% by wt.)	186 ± 14*	5.0 ± 0.8*	1820 ± 240	192 ± 65	1020 ± 200*	111 ± 16
Soya-bean oil (4) (5% by wt.)	178 ± 5*	5.1 ± 0.2*	1620 ± 05	180 ± 13	1050 ± 190*	116 ± 20
Rape-seed oil (4)	220 ± 3	5.1 ± 0.1	1614 ± 87*	144 ± 10*	1184 ± 162	105 ± 10

groups. The recoveries of urate oxidase activity, from the 10% liver homogenates of rats fed the semi-synthetic diets, were 2–3 times higher than those of catalase (results not shown). This is greater than the ratio of 1.3 for clofibrate-treated rats, and indicates that peroxisomes from animals fed on the semi-synthetic diets were more fragile than those from clofibrate-treated rats.

Effects of various diets on some parameters of liver metabolism

Table 3 gives data on the effect of the various diets on body weight after 17 days of feeding. The mean body weights of the groups fed on partially hydrogenated marine-oil diet and on rape-seed-oil diet were significantly higher than those of any one of the other groups ($P < 0.05$). The low soya-bean-oil diet had a caloric content that was 10% less per g of diet than any of the other diets, and also a slightly different distribution of calories between fats and carbohydrates (see the Experimental section). These differences were probably responsible for the lower body weight of this dietary group.

Liver weight expressed as a percentage of body weight was, for the group fed on partially hydrogenated-marine-oil diet, significantly higher than the values for any one of the other dietary groups ($P < 0.05$). This increase in liver weight parallels that observed with clofibrate (Lazarow & de Duve, 1976).

Liver protein per g of liver was significantly lower in the rape-seed-oil group compared with any one of the other groups. The liver protein content of the dietary groups that did not show marked peroxisomal induction (soya-bean-oil- and ground-nut-oil-fed groups) were close to the value of 200 mg of protein/g of liver considered normal (Leighton *et al.*, 1968). Liver catalase activity was significantly higher in the partially-hydrogenated-marine-oil-fed group compared with all the other groups except the rape-seed-oil-fed group. This difference was, however, not significant when expressed per g of liver,

Table 4. *Effect of different semi-synthetic diets on total liver capacity for peroxisomal β-oxidation*

Rats were fed on the various semi-synthetic diets for 17 days. Peroxisomal fractions were prepared and assayed for palmitoyl-CoA-dependent NAD^+-reduction as described in the Experimental section. Values are means ± S.D. for the numbers of animals indicated in parentheses on the left-hand side of the Table. Values that are significantly less than the corresponding value for the group fed on partially hydrogenated-marine-oil diet ($P < 0.05$) are indicated by *. All means for the group fed on soya-bean-oil diet (5% by wt.) were significantly less ($P < 0.05$) than the corresponding values for the group fed on soya-bean-oil diet (15% by wt.). The percentages of peroxisomal protein are based on the numbers of animals indicated on the right-hand side of the Table in parentheses.

	Palmitoyl-CoA-dependent NAD^+ reduction				
Diet	Total activity in liver (nmol/min)	(nmol/min per g body wt.)	(nmol/min per g of liver)	(nmol/min per mg of liver protein)	Peroxisomal protein (% of total liver protein)
Partially hydrogenated marine oil (7)	7010 ± 670	34 ± 3	593 ± 55	3.95 ± 0.4	4.1 ± 0.6 (4)
Ground-nut oil (4)	3710 ± 570*	19 ± 2*	409 ± 76*	2.26 ± 0.3*	3.8 ± 0.8 (3)
Soya-bean oil (15% by wt.) (4)	3990 ± 540*	21 ± 3*	433 ± 57*	2.35 ± 0.3*	4.3 ± 0.2 (3)
Soya-bean oil (4) (5% by wt.)	2760 ± 265*	15 ± 2*	230 ± 30*	1.65 ± 0.2*	3.8 ± 0.7 (4)
Rape-seed oil (4)	6768 ± 878	31 ± 4	594 ± 67	4.1 ± 0.5	5.9 ± 0.8 (4)

and probably reflected the increased size of the liver. When expressed per mg of liver protein, the values for the partially-hydrogenated-marine-oil- and rape-seed-oil-fed groups were significantly higher than those of the other two high-fat dietary groups (results not shown).

Effects of diet on relative capacities for peroxisomal β-oxidation

We have previously reported that after 17 days of feeding the total liver capacity for peroxisomal β-oxidation was higher in rats fed on a diet containing 15% by wt. of soya-bean oil than in animals fed on a diet containing 5% by wt. of soya-bean oil (Neat *et al.*, 1980). Results presented in Table 4 show that animals fed on ground-nut-oil diet (15% by wt.) showed a liver capacity similar to the group fed on soya-bean-oil diet (15% by wt.). These results also show that feeding of a diet containing rape-seed oil (15% by wt.) induced peroxisomal β-oxidative capacity that was not significantly different from that obtained with animals fed on partially hydrogenated-marine-oil diet (15% by wt.). The capacities obtained with the two latter diets were, however, higher than those obtained with any of the other diets, whether this was expressed per g of body wt., per g of liver or as per mg of liver protein (see Table 4). Both the rape-seed oil diet and the partially hydrogenated-marine-oil diet gave per g of liver about 2.5-fold higher capacity than the soya-bean-oil diet (5% by wt.). Similarly the ground-nut-oil diet and soya-bean-oil diet (15% by wt.) gave 1.9- and 1.8-fold increases respectively.

Chain-length specificity of peroxisomal β-oxidation

In Fig. 2 results showing the chain-length specificities of peroxisomal preparations isolated from animals fed on various diets are presented. The specificity of peroxisomes from animals fed on normal pelleted diet for oxidizing acyl-CoA esters of various chain lengths (results not shown) was similar to that of preparations from rats that had been fed on soya-bean-oil diet (5% by wt.), in that both preparations showed a broad peak of specific activity with CoA esters of chain lengths C_{14} and C_{16} (see Fig. 2). Also *trans*-mono-unsaturated acyl-CoA esters of the same chain lengths were oxidized at rates that were similar to those of the corresponding saturated derivatives. The corresponding *cis*-mono-unsaturated derivatives were, however, oxidized at lower rates than the corresponding *trans*-isomers, the exceptions being $C_{18:1(9)}$ and $C_{22:1(13)}$, in which case the two isomers were oxidized at similar rates (see Fig. 2).

Data for peroxisomes isolated from animals fed on ground-nut-oil diet (shown in Fig. 2) and from rats fed on soya-bean-oil diet (15% by wt.) (results not shown) were, apart from a general increase in specific activity, similar to those described above. Rates of oxidation of $C_{20:1s}$- and $C_{22:1}$-CoA esters were detectable, although this was not so with every preparation tested.

The chain-length specificity for peroxisomal preparations from animals fed on partially hydrogenated-marine-oil diet is also shown in Fig. 2. These preparations showed a distinct peak of specific activity with acyl-CoA esters with C_{16} chain lengths. For acyl-CoA esters of chain lengths C_{14} and C_{16} the *trans*-ω9 isomers were also now oxidized faster than the corresponding *cis*-isomers, as were also the corresponding saturated acyl-CoA esters (see Fig. 2). With longer acyl-CoA esters there were no

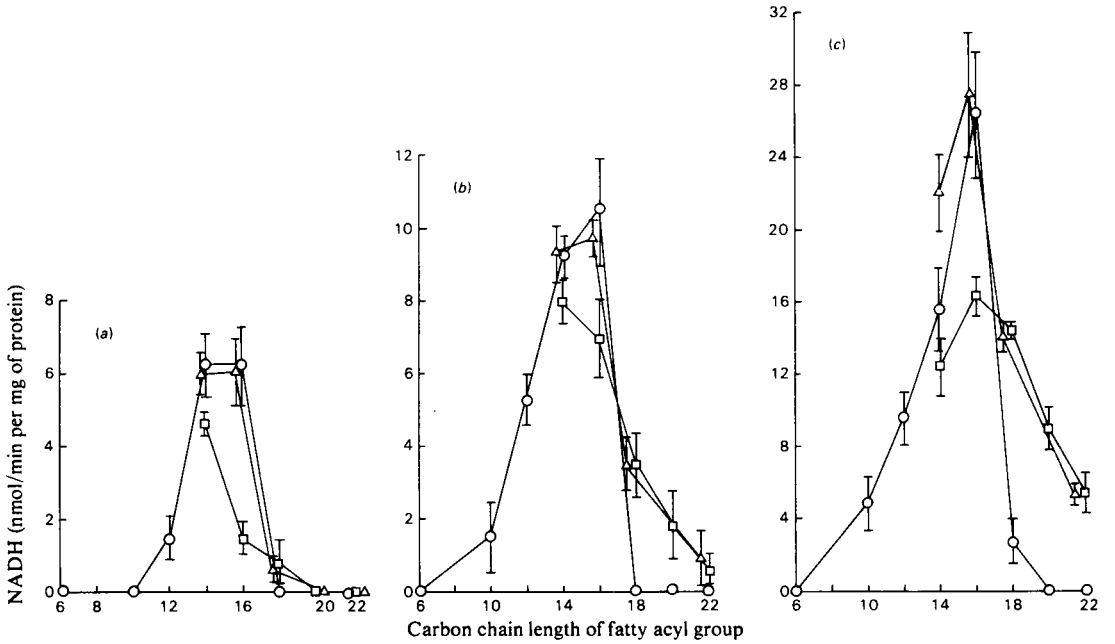

Fig. 2. *Acyl-CoA ester chain-length specificity of peroxisomal β-oxidation with rats fed on different semi-synthetic diets*
Rates of peroxisomal β-oxidation were assayed as acyl-CoA ester-dependent reduction of NAD^+, as described in the Experimental section, with peroxisomes isolated from rats fed on soya-bean-oil diet (5% by wt.) (*a*), ground-nut-oil diet (*b*) and partially hydrogenated-marine-oil diet (*c*). The data represent means ± S.D. for at least three animals. ○, Saturated acyl-CoA; □, *cis*-mono-unsaturated isomers; △, the corresponding *trans*-isomers. All mono-unsaturated acyl groups were derived from fatty acids with the double bond in position-9 (from the carboxy end), excepting *cis*-$C_{22:1}$ fatty acid, which was erucoyl-CoA, *trans* -$C_{22:1}$ fatty-acid, which was brassidoyl-CoA, and $C_{20:1}$ fatty acid, which was gadeoyl-CoA. Cetoleoyl-CoA (*cis*-$C_{22:(11)}$) was oxidized at a rate similar to that of erucoyl-CoA.

marked differences in rates of oxidation between *cis*- and *trans*-isomers. For saturated acyl-CoA esters of chain lengths longer than C_{18} it was not possible to detect β-oxidative activity. This could be due to formation of acyl-CoA ester micelles, which may not be substrates for the enzymes. The chain-length specificity of this preparation is similar to that previously reported for peroxisomes from clofibrate-treated animals, although peaks of specific activities were then found with myristoyl-CoA ($C_{14:0}$) and oleoyl-CoA (*cis*-$C_{18:1(9)}$) (Osmundsen *et al.*, 1979).

Oxidation of some acyl-CoA esters by isolated liver mitochondria

Most *trans*-isomers of $C_{18:1}$ fatty acid are oxidized slower than the corresponding *cis*-isomers by isolated heart mitochondria (Lawson & Kummerow, 1979*a*,*b*). Elaidic acid is usually found to be oxidized at about 50% of the rate obtained with oleic acid.

To compare some properties of mitochondria and peroxisomal β-oxidation we measured rates of oxidation of selected acyl-CoA esters by rat liver mitochondria isolated from animals fed on standard pelleted diet, and from animals fed on partially hydrogenated-marine-oil diet for 14 days. With both groups the *trans*-isomers of $C_{16:1(9)}$ and $C_{18:1(9)}$ fatty acids were oxidized at rates that were about 50% of the corresponding *cis*-isomers. With $C_{14:1(9)}$ the *cis*- and *trans*-isomers were oxidized at similar rates. With mitochondria isolated from rats fed on partially hydrogenated marine-oil diet, the absolute rates of oxidation were about 50% higher, compared with those of the control group. Palmitoyl-CoA gave a mean (±S.D.) rate of 47 ± 4 ng-atoms of O/min per mg of protein ($n = 3$), whereas the corresponding value for animals fed on partially hydrogenated-marine-oil diet was 74 ± 5 ($n = 3$).

Neither feeding on soya-bean-oil diet (15% by wt.) nor feeding on partially hydrogenated-marine-oil diet altered the pattern of oxidation of acylcarnitines, with various chain lengths, by isolated liver mitochondria, as examined by using a spectrophotometric assay selective for mitochondrial β-oxidation (see Osmundsen & Bremer, 1977). The resulting pattern (results not shown) was not significantly different from that observed with animals fed on standard pelleted diet (see Christiansen *et al.*, 1978).

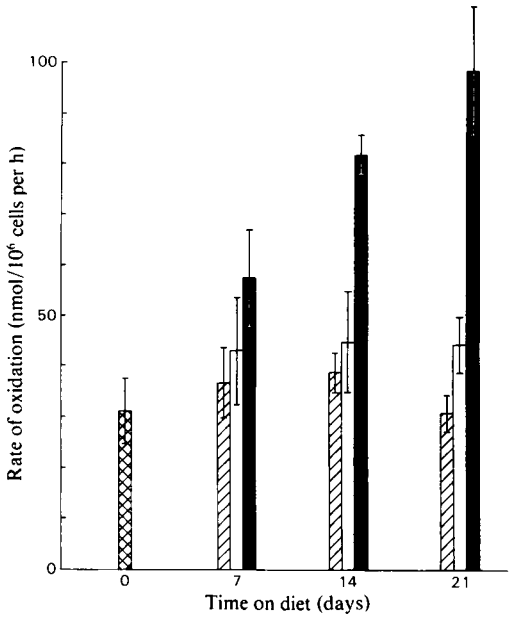

Fig. 3. *Effect of duration of feeding on various semi-synthetic diets on rates of oxidation of [14-^{14}C]erucic acid by isolated rat hepatocytes*

Rates of oxidation of [14-^{14}C]erucic acid by isolated hepatocytes were measured as described in the Experimental section with hepatocytes prepared from rats that had been fed on the various diets for different time intervals. The data represent means ± S.D. for four animals (at each time interval) fed on soya-bean-oil diet (5% by wt.). (▨), soya-bean-oil diet (15% by wt.) (□) or partially hydrogenated-marine-oil diet (■).

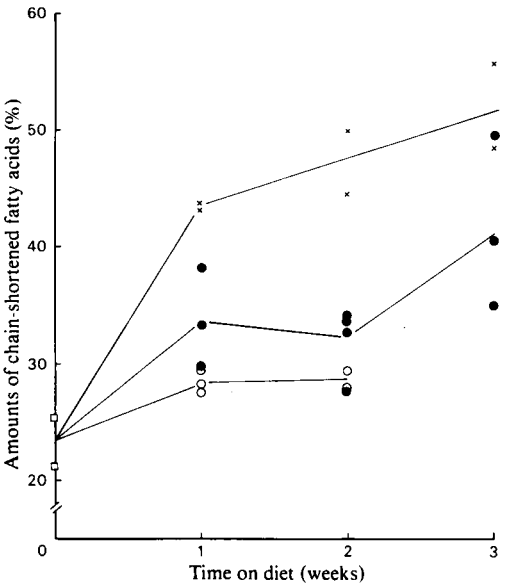

Fig. 4. *Effect of duration of feeding on various semi-synthetic diets on the fraction of chain-shortened fatty acids derived from [14-^{14}C]erucic acid*

Hepatocytes were prepared from rats that had been fed on soya-bean-oil diet (5% by wt.) (O), soya-bean-oil diet (15% by wt.) (●) and partially hydrogenated-marine-oil diet (×) for the different periods of time shown. Amounts of chain-shortened fatty acids in triacylglycerol fractions after 60 min of incubation were measured as described in the Experimental section, and are expressed as percentages of total radioactivity in all fatty acid peaks. Each point represents results derived from one animal.

Effect of high-fat diets on oxidation of [14-^{14}C]-erucic acid by isolated rat hepatocytes

Results presented in Fig. 3 show that the rate of oxidation of [14-^{14}C]eurcic acid by isolated hepatocytes was influenced by the amount, and composition, of the dietary oils. With rats fed on the partially hydrogenated-marine-oil diet an increase was apparent after 5 days on the diet, and after 3 weeks this amounted to about 200%. This latter observation correlates well with that of R. Z. Christiansen *et al.* (1979). The increase observed with rats fed on soya-bean oil (15% by wt.) was, in contrast, only about 25% higher than that of the control group fed on soya-bean oil (5% by wt.). Further time on the diet did not lead to additional increase in rates of oxidation. These time courses also correlate well with those observed previously for induction of peroxisomal β-oxidation after feeding on these diets (see Neat *et al.*, 1980). It is therefore likely that induction of peroxisomal β-oxidation is a necessary prerequisite for hepatocytes to attain enhanced ability to oxidize erucic acid.

Hepatocytes isolated from animals fed on partially hydrogenated-marine-oil diet for 21 days showed about 50% increase in rates of palmitate oxidation (results not shown). This correlates with the observed higher rate of oxidation of palmitoyl-CoA by mitochondria isolated from rats fed on this diet.

Effect of some diets on chain-shortening of [14-^{14}C]erucic acid by isolated hepatocytes

We have previously shown that isolated rat liver peroxisomes will chain-shorten [14-^{14}C]erucoyl-CoA (Osmundsen *et al.*, 1979). Measurements of chain-shortening by isolated hepatocytes may therefore serve as a guide to the activity of peroxisomal oxidation of [14-^{14}C]erucic acid in the hepatocyte.

The data in Fig. 4 show the amounts of chain-shortened fatty acids (mainly $C_{18:1}$) in triacylglycerols, expressed as a percentage of total radioactivity in triacylglycerol fatty-acid moieties. In parallel with results obtained from measurements of

[14-^{14}C]erucic acid oxidation, animals fed on partially hydrogenated-marine-oil diet showed the highest increase in the amounts of chain-shortened fatty acids (from 25 to 55% after 1 week on the diet). Animals fed on the soya-bean-oil diets showed a much lower increase (from 25% to about 30%), and there is no marked difference between those fed on the diet containing 15% by wt. of soya-bean oil and those fed on the 5% by wt. diet. This similarity appears contradictory to the observed higher induction of peroxisomal β-oxidation by the diet containing more soya-bean oil (see Table 4). This may be due to competition between products of peroxisomal β-oxidation derived from [14-^{14}C]erucic acid and endogenous fatty acids for incorporation into triacylglycerols.

Discussion

Isolation procedure

One advantage of this isolation procedure was that it allowed analysis of samples from up to eight animals without requiring time-consuming centrifugation. Removal of Percoll from the peroxisomal fractions was not strictly required. This was nevertheless, thought worthwhile with fractions used in studies of peroxisomal β-oxidation of various acyl-CoA esters. Although the peroxisomal yield was decreased by 50% by this procedure, about 90% of contaminating endoplasmic reticulum was also removed (see Table 1).

Effects of diet on liver peroxisomes

The marked increases in specific activities of peroxisomal β-oxidative activity, but not for catalase, found with animals fed on partially hydrogenated-marine-oil diet and on rape-seed-oil diet (see Tables 3 and 4) suggest that a selective induction of the enzymes of peroxisomal β-oxidation had taken place. Ishii *et al.* (1980*a*) also reported that a high-fat diet leads to peroxisomal proliferation, as judged by electron microscopy. It is, however, also possible that the original peroxisomal population is replaced by another larger population, possessing enhanced β-oxidative capacity.

We have also found that the calculated extent of increase in peroxisomal β-oxidation appears higher when expressed per mg of peroxisomal protein rather than per mg of liver protein for animals fed on partially hydrogenated-marine-oil diet, and on rape-seed-oil diet. The reason for this is not clear. We have, however, found that the cytochrome *c* oxidase (EC 1.9.3.1) activity per g of liver is increased by 10–15% on feeding of partially hydrogenated marine-oil diet, as compared with ground-nut- or soya-bean-oil diet (results not shown). This suggests that mitochondrial changes have taken place, as may be deduced from the increased rates of fatty acid oxidation observed with mitochondria isolated from rats fed on partially hydrogenated-marine-oil diet.

Although feeding of all high-fat diets led to inductions of peroxisomal β-oxidation, this was by far the most marked with diets containing larger amounts of fatty acids, which are poorly oxidized by mitochondrial β-oxidation (see Osmundsen & Bremer, 1978), i.e. partially hydrogenated-marine-oil and rape-seed-oil diet. These findings suggest that there is a general inductive effect of high-fat diets, and that the extent of induction is also determined by the fatty acid composition of the diet.

Capacities of peroxisomal and mitochondrial β-oxidation

Estimates of peroxisomal β-oxidative capacity relative to that of the mitochondria are variable. Lazarow (1978) has suggested that peroxisomal β-oxidation in control rats has a capacity equivalent to about 50% of the mitochondrial capacity for oxidation of palmitate. Mannaerts *et al.* (1979), however, have concluded that peroxisomal β-oxidation can account for less than 10% of the mitochondrial capacity, both in control and clofibrate-treated rats.

It is possible to make estimates of these capacities from the results presented here. Data in Table 4 suggest a mean value of liver protein per g of liver wt. of about 180mg. It is estimated that about 20% of this is due to mitochondrial protein (Leighton *et al.*, 1968; Kurup *et al.*, 1970). Further, our data suggest that about 4–6% of liver protein in the animals fed on the various diets is due to peroxisomal protein (see the Results section). Hence from measurements of peroxisomal and mitochondrial rates of oxidation of e.g. palmitate oxidation it is possible to estimate total liver capacities.

The rate of palmitoylcarnitine oxidation, measured spectrophotometrically (Osmundsen & Bremer, 1977), using liver mitochondria from rats fed on hydrogenated-marine-oil diet for 17 days was about 74nmol of acetyl groups/min per mg of mitochondrial protein (results not shown). This gives an estimated hepatic capacity of about 2.7μmol of acetyl groups/min per g of liver wt. The corresponding value for peroxisomal β-oxidation is about 0.7μmol of acetyl groups/min per g of liver wt. (see Table 4). Therefore, in animals that have had their hepatic capacity for peroxisomal β-oxidation increased by about 2.5-fold (see Table 4), the peroxisomal β-oxidative capacity is about 22% of the mitochondrial capacity. By using rates of erucoylcarnitine and erucoyl-CoA oxidation the peroxisomal capacity is approaching 30% of that of the mitochondria. The corresponding estimates for animals fed on soya-bean-oil diet (15% by wt.) are 17% and 12% respectively, and for those fed on soya-bean-oil diet (5% by wt.) the peroxisomal

capacity was about 10% with respect to palmitate oxidation. With this latter dietary group peroxisomal oxidation of erucoyl-CoA was not detectable (see Fig. 2).

These estimates are based on rates of β-oxidation by intact liver mitochondria, whereas peroxisomal rates represent rates of enzymic activity measured in an assay where the particles have been solubilized by Triton X-100. The measured rate is probably governed by the acyl-CoA oxidase, thought to be rate limiting for peroxisomal β-oxidation (Inestrosa *et al.*, 1979). This does not, however, necessarily imply that the measured activities are absolutely correlated to rates of β-oxidation by intact peroxisomal particles. Our finding that peroxisomal β-oxidation may be regulated by added CoA both quantitatively and qualitatively (Osmundsen & Neat, 1979) underlines this point.

Role of peroxisomal β-oxidation in cellular fatty acid oxidation

Peroxisomal β-oxidation, unlike mitochondrial β-oxidation, does not oxidize fatty acids to completion (Lazarow, 1978). All fatty acids tested are subjected to three to four β-oxidation cycles (Osmundsen *et al.*, 1979, 1980), i.e. chain-shortened. Although the peroxisomal capacities are markedly less than those of the mitochondria in terms of acetyl groups produced per min, these can be substantial in terms of abilities to chain-shorten fatty acids.

The findings reported in the present paper suggest a strong correlation between induction of peroxisomal β-oxidation, chain-shortening and oxidation of erucic acid. These findings therefore can explain the previously reported increased chain shortening of erucic acid by perfused livers taken from rats fed on partially hydrogenated-marine-oil diet or rape-seed-oil diet (Thomassen *et al.*, 1979; E. N. Christiansen *et al.*, 1979). The present findings may also explain the transient nature of the cardiac lipidosis observed with animals fed on these diets (Rocquelin *et al.*, 1971; Beare-Rogers *et al.*, 1972).

It is also apparent that peroxisomal β-oxidation, when induced by partially hydrogenated-marine-oil diet, can oxidise some *trans*-fatty acids at rates that are up to 50% faster than those of the corresponding *cis*-isomers, and that *trans*-isomers never appear to be oxidized at lower rates than the *cis*-isomers (see Fig. 3). As far as mitochondrial β-oxidation is concerned some *trans*-fatty acids are oxidized at a rate that is about half that of the corresponding *cis*-isomers, and this is not significantly changed in rats fed on partially hydrogenated marine-oil diet.

These findings suggest that peroxisomal β-oxidation may be of significance as regards metabolism of *trans*-fatty acids.

A plausible hypothesis is therefore that one main metabolic function of peroxisomal β-oxidation is to supplement mitochondrial β-oxidation when the liver is faced with a high influx of fatty acids, as during feeding of high-fat diets, or during starvation (Ishii *et al.*, 1980*b*). It is of particular significance when the diet contains appreciable quantities of fatty acids that are poorly oxidized by mitochondrial β-oxidation. It would therefore make metabolic sense not to have this process directly linked to oxidative phosphorylation, even though this appears less energy-effective.

This work was supported by The Royal Norwegian Council for Scientific and Industrial Research. We thank Mr. L. Bohne and Miss T. Rørtveit for excellent technical assistance. C. E. N. was a visiting pre-doctoral research associate.

References

Beare-Rogers, J. L., Nera, E. A. & Craig, E. M. (1972) *Lipids* **7**, 548–554

Blouin, A., Bolender, R. P & Weibel, E. R. (1977) *J. Cell Biol.* **72**, 441–455

Chance, B. & Maehly, A. C. (1955) *Methods Enzymol.* 764–768

Christiansen, E. N., Thomassen, M. S., Christiansen, R. Z., Osmundsen, H. & Norum, K. C. (1979) *Lipids* **14**, 829–835

Christiansen, R. Z. (1978) *Biochim. Biophys. Acta* **530**, 314–324

Christiansen, R. Z. & Bremer, J. (1976) *Biochim. Biophys. Acta* **488**, 562–577

Christiansen, R. Z., Borrebæk, B. & Bremer, J. (1974) *FEBS Lett.* **62**, 313–317

Christiansen, R. Z., Osmundsen, H., Borrebæk, B. & Bremer, J. (1978) *Lipids* **13**, 487–491

Christiansen, R. Z., Christiansen, E. N. & Bremer, J. (1979) *Biochim. Biophys. Acta* **573**, 417–429

Houtsmuller, U. M. T. (1978) *Fette Seifen Anstrichm.* **80**, 162–169

Inestrosa, N. C., Bronfman, M. & Leighton, F. (1979) *Biochem. J.* **182**, 779–788

Ishii, H., Fukomori, N., Horie, S. & Suga, T. (1980*a*) *Biochim. Biophys. Acta* **617**, 1–11

Ishii, H., Horie, S. & Suga, T. (1980*b*) *J. Biochem. (Tokyo)* **87**, 1855–1858

Kurup, C. K. R., Aithal, H. N. & Ramasarma, T. (1970) *Biochem. J.* **116**, 773–779

Lawson, L. D. & Kummerow, F. A. (1979*a*) *Biochim. Biophys. Acta* **573**, 245–254

Lawson, L. D. & Kummerow, F. A. (1979*b*) *Lipids* **14**, 501–503

Lazarow, P. B. (1978) *J. Biol. Chem.* **253**, 1522–1528

Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046

Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J. W., Fowler, S. & de Duve, C. (1968) *J. Cell Biol.* **37**, 482–513

Mannaerts, G. P., Debeer, L. J., Thomas, J. & Schepper, P. J. (1979) *J. Biol. Chem.* **256**, 4585–4595

Neat, C. E. & Osmundsen, H. (1979) *Biochem. J.* **180**, 445–448

Neat, C. E., Thomassen, M. S. & Osmundsen, H. (1980) *Biochem. J.* **186**, 369–371

Osmundsen, H. & Bremer, J. (1977) *Biochem. J.* **164**, 621–633

Osmundsen, H. & Bremer, J. (1978) *Biochem. J.* **174**, 379–386

Osmundsen, H. & Neat, C. E. (1979) *FEBS Lett.* **107**, 81–84

Osmundsen, H., Neat, C. E. & Norum, K. R. (1979) *FEBS Lett.* **99**, 292–296

Osmundsen, H., Neat, C. E. & Borrebæk, B. (1980) *Int. J. Biochem.* **12**, 625–630

Rickwood, D. (1978) in *Centrifugation: A Practical Approach* (Rickwood, D., ed.), p. 139, Information Retrieval, London and Washington

Rocquelin, J. L., Sergiel, J. P., Martin, B. & Leclerc, J. (1971) *J. Am. Oil Chem. Soc.* **48**, 728–732

Seglen, P. O. (1973) *Exp. Cell Res.* **82**, 391–396

Stewart, H. B., Tubbs, P. K. & Stanley, K. K. (1973) *Biochem. J.* **132**, 61–76

Thomassen, M. S., Strøm, E., Christiansen, E. N. & Norum, K. R. (1979) *Lipids* **14**, 58–65

Wharton, D. C. & Tzagoloff, A. (1967) *Methods Enzymol.* **10**, 245–246

Yokota, S. & Fahimi, H. D. (1978) *International Congress on Electron Microscopy 9th (Toronto)*, vol. 11, pp. 244–245