Changes in the content of *n*-6 fatty acids in liver phospholipids in rats as a consequence of partially hydrogenated dietary oils

BY MAGNY S. THOMASSEN*, TORILL RØRTVEIT, ERLING N. CHRISTIANSEN AND KAARE R. NORUM

Institute for Nutrition Research, University of Oslo, Blindern, Oslo, Norway

(*Received 18 October 1983 – Accepted 31 October 1983*)

1. The effects of unhydrogenated and partially-hydrogenated marine and rapeseed oils on the relative content of *n*-6 fatty acids in rat liver phospholipids were studied by gas–liquid chromatography.

2. The type of dietary oil strongly affected the pattern of *n*-6 fatty acids in the liver phospholipids even after a relatively short feeding period (3 weeks).

3. With diets deficient in linoleic acid, accumulation of the 20:3 *n*-9 fatty acid was observed in the animals receiving partially hydrogenated oils, but not in the animals receiving the unhydrogenated marine oil which contained relatively high amounts of polyunsaturated fatty acids of the *n*-3 family.

4. The results presented are in line with reports in the literature that dietary *trans* fatty acids may inhibit the desaturation–elongation enzyme systems of rat liver. The observed effects did not appear to be directly correlated to the dietary content of very-long-chain monounsaturated fatty acids.

Partially-hydrogenated oils of vegetable as well as marine origin are commonly used in margarine production. Such oils contain a wide range of positional and geometrical fatty acid isomers (Hølmer & Aaes-Jørgensen, 1969; Conacher *et al.* 1972; Ackman, 1982) and when fed to rats in relatively large doses these oils seem to intensify the symptoms of essential fatty acid (EFA) deficiency (Holman & Aaes-Jørgensen, 1956; Aaes-Jørgensen & Hølmer, 1969; Jensen, 1976). This effect has been associated with the inhibition of linoleic acid desaturation by octadecenoic acid isomers observed in vitro (Mahfouz *et al.* 1980), and recently Hill *et al.* (1982) reported that rats given diets containing partially-hydrogenated soya-bean oil (PHSO) were found to exhibit decreased Δ5-and Δ6-desaturase activities in the liver. The content of *trans* fatty acids in partially-hydrogenated oils has consequently been a matter of concern in general food recommendations (Dhopeshwarkar, 1981; Kinsella *et al.* 1981).

Most studies so far have been focused on the effects of C_{18} fatty acid isomers on the metabolism of the essential fatty acids and little is at present known about the possible effects of *trans*, as well as *cis* isomers of the very-long-chain fatty acids (20–24 carbon atoms) also present in some dietary oils.

In a previous study we observed that partially-hydrogenated marine oil (PHMO) diets, containing both 20:1 and 22:1 fatty acids, influenced the product:precursor values in the linoleate sequence in rat liver phospholipids (Thomassen *et al.* 1982).

PHMO has also been found to give a twofold increase in the mitochondrial 4-enoyl-CoA reductase (acyl-CoA: $NADP^+$ oxidoreductase, *EC* 1.3.1.8) in rat liver (Borrebæk *et al.* 1980). Moreover, we have found that PHMO and partially-hydrogenated rapeseed oil (PHRO), containing 22:1 fatty acids, induce an extensive increase in peroxisomal chain-shortening of long-chain fatty acids in the liver (Thomassen *et al.* 1982). These effects may possibly have bearings on the catabolism or retroconversion of EFA in the liver, or both.

In the present study, we have investigated the effect of PHMO and PHRO diets on the content of *n*-6 fatty acids in liver phospholipids, compared with the effects of corresponding

* Present address: Norwegian Food Research Institute, PO Box 50, N-1432 Aas-NLH, Norway.

Table 1. *Fatty acid composition (mmol/mol) of dietary oils**

Fatty acid†	Soya-bean oil		Rapeseed oil		Marine oil	
	Unhydro-genated	Partially-hydrogenated‡	Unhydro-genated	Partially-hydrogenated†	Unhydro-genated	Partially-hydrogenated†
$C_{14:0}$	—	—	—	—	67	68
$C_{16:0}$	109	106	49	41	147	128
$C_{16:1}$	—	—	—	—	95	99
$C_{18:0}$	38	140	10	63	10	30
$C_{18:1}$	243	738	135	325	161	151
$C_{18:2}$	525	—	200	—	16	25
$C_{18:3}$	75	—	79	—	—	—
$C_{18:4}$	—	—	—	—	54	—
$C_{20:0}$	—	—	—	17	—	24
$C_{20:1}$	—	—	62	65	128	164
$C_{20:2}$	—	—	—	—	—	43
$C_{20:5}$	—	—	—	—	90	—
$C_{22:0}$	—	—	—	56	—	30
$C_{22:1}$	—	—	435	427	135	145
$C_{22:2}$	—	—	—	—	—	19
$C_{22:6}$	—	—	—	—	62	—
Proportion of *trans* double bonds		0·55		0·65		0·55

* Only major fatty acids (> 10 mmol/mol) are included. The marine oils were obtained from capelin (*Mallotus villosus*).

† Analysis performed by the supplier.

‡ The partially-hydrogenated oils were commerical and not laboratory-scale hydrogenated from the unhydrogenated oils listed in this table.

unhydrogenated oils (marine oil (MO) and rapeseed oil (RO)) and PHSO and unhydrogenated soya-bean oil (SO).

EXPERIMENTAL

Materials

Dietary oils, including analytical details, were obtained from DeNoFa and Lilleborg Fabriker A/S, Fredrikstad, Norway, except for the unhydrogenated rapeseed oil which was supplied by AB Karlshamns Oljefabriker, Karlshamn, Sweden. The fatty acid compositions are given in Table 1. In the partially-hydrogenated oils there was no 18:2 (9, 12) all *cis*, 18:3 (9, 12, 15) all *cis* or their polyunsaturated elongation derivatives as judged by gas–liquid chromatographic analysis of the fatty acid content of the oils. The isomeric distribution of 18:2 and 18:3 in the unhydrogenated oils has been thoroughly analysed; in fish oil almost 90% of 18:2 is the all *cis* *n*-6 isomer and 90% of 18:3 is the all *cis* *n*-3 isomer (Ackman, 1982), in rapeseed oil (Ackman & Loew, 1977) and in soya-bean oil (Houtsmüller, 1978) almost 100% are of the all *cis* *n*-6 and *n*-3 isomers respectively. The 20:5 and 22:6 in fish oils are exclusively of the all *cis* *n*-3 isomers (Ackman, 1982).

Vitamin and salt mixtures were from ICN Pharmaceuticals, Cleveland, Ohio, USA (vitamin diet fortification mixture, catalogue no. 904654; and U.S.P. XVII, catalogue no. 904610).

Animals and diets

Weanling male rats of the Wistar strain (body-weight approximately 60 g) were purchased from Møllegård breeding laboratory, Ejby, Denmark. The rats were fed on a standard

Table 2. *Food consumption and weight gain for rats given the various semi-synthetic diets for 3 weeks with free access to food and water*

(Values are means and standard deviations for four animals in each group)

	Food consumption (g/d)		Weight gain (g/d)	
Diet†	Mean	SD	Mean	SD
SO	13·9	1·2	6·5	1·0
SO 50	15·7*	1·9	5·8	0·5
PHSO	15·5*	1·8	5·6	0·8
RO	11·5**	0·8	4·5*	0·7
PHRO	17·2**	0·4	4·7**	0·5
MO	12·3*	0·1	5·6	0·4
PHMO	13·0	0·1	5·0*	0·9
PHMO 50	13·5	0·2	5·1*	0·3

SO, soya-bean oil; SO 50, 50 g SO/kg; PHSO, partially-hydrogenated SO; RO, rapeseed oil; PHRO, partially-hydrogenated RO; MO, marine oil; PHMO, partially-hydrogenated MO; PHMO 50, 50 g PHMO/kg.
Values were significantly different from those for the SO group: * $0{\cdot}05 > P > 0{\cdot}01$, ** $P < 0{\cdot}01$.
† 250 g dietary oil/kg.

pelleted diet for 5 d and then given the experimental, semi-synthetic diets for 3 weeks. The compositions of the semi-synthetic diets were (g/kg diet): sucrose, 200; maize starch, 278 (high fat) or 478 (low fat); casein (with 20 g methionine/kg), 200; cellulose, 10; vitamin mixture, 22; salt mixture, 40; dietary oil, 250 (high fat) or 50 (low fat). The high- and low-fat diets had a calculated energy density of 18·0 (4·3) and 15·9 (3·8) kJ/g (kcal/g) respectively. The rats were housed in grid-bottomed cages, two in each cage, and had free access to food and water. The climatic conditions were: 23°, 60% relative humidity and a 12 h light period (07.00–19.00 hours).

Lipid extraction and gas–liquid chromatographic (GLC) analysis

The methods employed for lipid extraction and determination of fatty acid distribution in total liver phospholipids have been described previously (Thomassen *et al.* 1982). GLC was performed with a non-polar (SE30) vitreous silica capillary column; the column temperature was programmed to rise rapidly from 50 to 220° and then by a slow gradient (0·5°/min) to 260°.

Statistical analysis

Dunnett's multiple comparison test (Dunnett, 1955) was used to evaluate the significance of differences between population means; $P > 0{\cdot}05$ was taken to be not significant.

RESULTS AND DISCUSSION

The animals in all dietary groups thrived and seemed healthy throughout the 3 week experimental period. The weight gain curves were fairly linear with no marked deviations in any animal or groups of animals (results no shown).

Reduced weight gain as compared with the SO-fed animals was, however, observed in the rats receiving RO and PHRO diets (Table 2). This is a well documented effect of diets containing unhydrogenated RO (Beare-Rogers, 1977) and has been ascribed to a relatively poor digestibility of this oil due to its content of erucic acid (22:1 *n*-9, *cis*). For the RO group, the lowered food consumption (Table 2) may also have contributed to this effect.

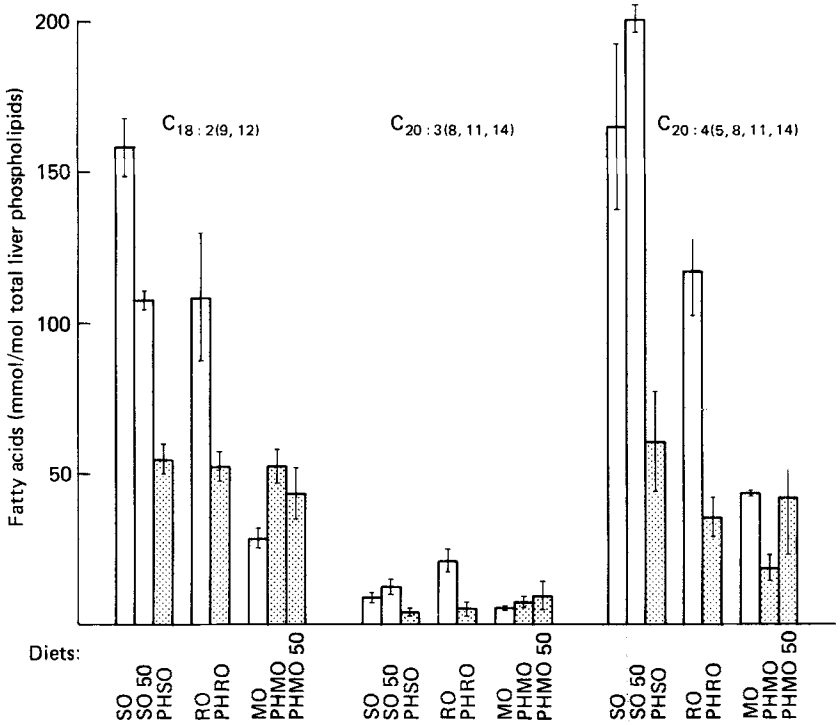

Fig. 1. Effects of different dietary oils on *n*-6 fatty acids in total liver phospholipids. Rats were fed on the following semi-synthetic diets containing (*a*) 50 g dietary oil/kg for 3 weeks: soya-bean oil (SO 50), partially-hydrogenated marine oil (PHMO 50), or (*b*) 250 g dietary oil/kg for 3 weeks: soya-bean oil (SO), partially-hydrogenated soya-bean oil (PHSO), rapeseed oil (RO), partially-hydrogenated rapeseed oil (PHRO), marine oil (MO), partially-hydrogenated marine oil (PHMO). Liver phospholipids were isolated and analysed by gas–liquid chromatography as described on p. 317. The results are mean values (standard deviations represented by vertical bars). ▨, Partially-hydrogenated oils; □, unhydrogenated oils.

A slightly lower weight gain was observed also in the group given PHMO diets. This may possibly be related to the content of 22:1 fatty acids in this oil.

Feeding the different dietary oils for 3 weeks was found to have substantial effects on the relative content of *n*-6 fatty acids in total liver phospholipids. This is illustrated in Fig. 1 where the amounts of 18:2, 20:3 and 20:4 *n*-6 fatty acids are expressed relative to the total phospholipid fatty acids. Since all experimental groups except the ones receiving SO were nearly devoid of longer-chain *n*-6 metabolites (i.e. 22:4 and 22:5), these are not included.

Low levels of *n*-6 fatty acids were observed in all animals receiving partially-hydrogenated oils (PHSO, PHRO and PHMO), and also the unhydrogenated MO. This reflects the near absence of linoleic acid (18:2 *n*-6) in these diets (Table 1). The value of 20:3 *n*-9:20:4 *n*-6 fatty acids, usually regarded as an index of EFA deficiency, was also determined in the liver phospholipids, and the results are presented in Table 3. As would be expected with partially-hydrogenated oils, deficient in linoleic acid, this value had increased above the level of 0·4 which is considered as an upper limit of the range of normality (Holman, 1968). In the animals given the unhydrogenated MO, however, this value was not increased above the level found in the SO-fed animals in spite of the low level of *n*-6 fatty acids both in the diet and liver phospholipids. This observation is in accordance with other reports

Table 3. *Essential fatty acid deficiency index with diets containing different dietary oils, both partially-hydrogenated and unhydrogenated*

(Values are means and standard deviations for the no. of animals indicated in parentheses)

Diet*	$C_{20:3}$ *n*-9 (mmol/mol total fatty acids in liver phospholipids)		$\frac{C_{20:3}\ n\text{-}9}{C_{20:4}\ n\text{-}6}$	
	Mean	SD	Mean	SD
SO (5)	< 0·1	—	< 0·01	—
SO 50 (4)	2·3	0·9	0·01	0·005
PHSO (4)	49·5	5·7	0·80	0·17
RO (4)	1·7	0·2	0·01	0·005
PHRO (4)	25·2	3·8	0·71	0·07
MO (4)	< 0·1	—	< 0·01	—
PHMO (4)	19·0	4·0	0·99	0·14
PHMO 50 (4)	40·6	8·3	1·02	0·12

SO, soya-bean oil; SO 50, 50 g SO/kg; PHSO, partially-hydrogenated SO; RO, rapeseed oil: PHRO, partially-hydrogenated RO; MO, marine oil; PHMO, partially-hydrogenated MO; PHMO 50, 50 g PHMO/kg.
* 250 g dietary oil/kg.

indicating that the 20:3 *n*-9:20:4 *n*-6 fatty acid value does not always reflect the status of EFA (or more precisely linoleic acid and its metabolites) in an organism (Kinsella *et al.* 1981). This seems to be especially important when diets containing *n*-3 fatty acids are used, since members of this family are reported to prevent any significant conversion of oleic acid (18:1 *n*-9) to 20:3 *n*-9 (Lundberg, 1980).

Fig. 1 also shows that the amounts of 18:2 *n*-6 fatty acid were remarkably similar in all animals receiving partially-hydrogenated oils. This may be taken as an indication that the marked increased peroxisomal chain-shortening capacity observed with the PHMO and PHRO diets (Thomassen *et al.* 1982) does not result in any increased degradation of this acid. The amounts of both 20:3 *n*-6 and 20:4 *n*-6 were, however, different depending on the type of partially-hydrogenated oil. The diets giving the strongest increase in peroxisomal chain-shortening (i.e. 250 g PHMO or PHRO/kg) also resulted in the lowest amount of 20:4 *n*-6. Thus, it cannot be totally excluded that the reduced amount of 20:4 *n*-6 in these two groups as compared with the PHSO group may, at least in part, reflect an increase in peroxisomal or mitochondrial degradation capacity, or both. These results may also be related to the increase in 4-enoyl-CoA reductase activity found with PHMO diets (Borrebæk *et al.* 1980). It has been suggested that the enzyme might play a significant role during the β-oxidation of unsaturated fatty acids containing double bond(s) at even-numbered carbon atoms (as 20:4 *n*-6) (Hiltunen *et al.* 1983).

As normally observed, the amount of 20:3 *n*-6 was relatively low. The lowest level was found in the animals given PHSO, PHRO and MO (4, 5 and 5 mmol fatty acid/mol total liver phospholipids, respectively). The relative amount of this acid, as compared with the amount of 18:2 *n*-6 and 20:4 *n*-6 was, however, lowest in the animals given the high-fat SO diet and highest in the animals given the PHMO diet. This can be seen from Table 4 where the product:precursor values in the *n*-6 fatty acid sequence are presented. The values, in fact, illustrate that when compared with the SO-fed animals, all other dietary oils result in a decrease in the fatty acid 20:4 *n*-6:20:3 *n*-6 value. The animals given RO, MO and PHMO also revealed an increase in the 20:3 *n*-6:18:2 *n*-6 value. With PHMO this effect was clearly established with as little as 50 g/kg in the diet. Thus, there seems to be an accumulation of 20:3 *n*-6 relative to 20:4 *n*-6 in liver phospholipids after feeding all the test

Table 4. *Product:precursor values in the* n-6 *fatty acid sequence in liver phospholipids after feeding diets containing different dietary oils, both partially-hydrogenated and unhydrogenated*

(Values are means and standard deviations for the no. of animals indicated in parentheses)

	Ratio, product:precursor					
	$C_{20:4}$ *n*-6 / $C_{20:3}$ *n*-6		$C_{20:3}$ *n*-6 / $C_{18:2}$ *n*-6		$C_{20:4}$ *n*-6 / $C_{18:2}$ *n*-6	
Diet†	Mean	SD	Mean	SD	Mean	SD
SO (5)	19·6	4·3	0·06	0·02	1·1	0·2
SO 50 (4)	16·5	3·3	0·12	0·02	1·9**	0·1
PHSO (4)	10·6**	2·9	0·08	0·02	0·8	0·1
RO (4)	5·9**	2·3	0·19**	0·02	1·1	0·4
PHRO (4)	7·2**	1·7	0·10	0·03	0·7*	0·1
MO (4)	8·0**	0·4	0·19**	0·02	1·6*	0·2
PHMO (4)	2·8**	0·6	0·18**	0·03	0·5**	0·05
PHMO 50 (4)	5·0**	1·6	0·21**	0·11	1·1	0·3

SO, soya-bean oil; SO 50, 50 g SO/kg; PHSO, partially-hydrogenated SO; RO, rapeseed oil; PHRO, partially-hydrogenated RO; MO, marine oil; PHMO, partially-hydrogenated MO; PHMO 50, 50 g PHMO/kg.
Values were significantly different from those for the SO group: * $0{\cdot}05 > P > 0{\cdot}01$, ** $P < 0{\cdot}01$.
† 250 g dietary oil/kg.

oils compared with the results obtained after SO feeding. We have previously reported on such effects with PHMO-feeding (Thomassen *et al.* 1982) and the results obtained in the present study confirm this finding. The results further reveal effects on the pattern of *n*-6 fatty acids by other partially-hydrogenated (PHSO and PHRO) and unhydrogenated (MO and RO) oils. This is also evident from Table 4, which shows the values for the main product 20:4 *n*-6 (arachidonic acid) relative to the dietary-derived substrate 18:2 (linoleic acid). The hydrogenated oils all seem to result in lower 20:4 *n*-6:18:2 *n*-6 values compared with the unhydrogenated SO diets, while unchanged or higher values were found after feeding the unhydrogenated oils.

These results demonstrate that the *n*-6 fatty acid pattern of liver phospholipids is strongly affected by the type of dietary oil, even after relatively short feeding periods. Many factors may be responsible for the observed effects; changes in enzyme levels and enzyme activities, competing substrates, both for the desaturation–elongation enzyme reactions and for incorporation into phospholipids, and also changes in retroconversion or degradation of the *n*-6 fatty acids. Evidence is, however, accumulating that different dietary oils may exert a strong influence on the desaturation–elongation systems in liver microsomes. The elongation process in this system is thought to be very rapid compared with the desaturation steps, and normally the Δ6-desaturation is regarded as being rate-limiting in the conversion of linoleic acid to arachidonic acid (Brenner, 1974). The relative accumulation of 20:3 *n*-6 fatty acid, however, is indicative of a sequence where the Δ5-desaturation step is rate-limiting which again may be due to a reduced conversion at this step, or to a stimulated conversion at the preceding Δ6-desaturation step.

An increased Δ6-desaturase activity has been observed in EFA-deficient animals (Brenner, 1981) and could possibly explain the results found in the groups given partially-hydrogenated oils. However, Kurata & Privett (1980) found no increase in Δ6-desaturation

activity in animals where the EFA-deficiency had been induced by diets containing *trans* fatty acids. Moreover, Mahfouz *et al.* (1980) demonstrated that Δ9-, Δ6-and Δ5-desaturases are inhibited by *trans* octadecenoic acids in vitro. Thus, an inhibition of the Δ5-desaturase seems to be the most likely explanation. An inhibition of Δ6-and Δ5-desaturase has also been observed in vitro after PHMO-feeding (Hølmer *et al.* 1982; Svensson, 1982; Kirstein *et al.* 1983). Notably, these studies were conducted with diets containing an adequate amount of linoleic acid. Their findings are, therefore, in good agreement with our previous observation that the effect of PHMO feeding on the *n*-6 fatty acid pattern was not markedly alleviated by addition to the diet of 50 g SO/kg (Thomassen *et al.* 1982). Hill *et al.* (1982) also reported an inhibition of Δ5-and Δ6-desaturases after feeding PHSO.

A decrease in the 20:4 *n*-6:18:2 *n*-6 value in the groups given partially-hydrogenated oils (Table 4) may also be indicative of an inhibited flux through the desaturation–elongation system. Our results may indicate a stronger inhibition by PHMO and PHRO than by PHSO. An explanation of this finding is not readily evident. There seems to be no direct relationship with the dietary content of very-long-chain fatty acids since the PHMO giving the greatest inhibition, as judged from our results, had a much lower content of such fatty acids (300 mmol/mol total fatty acids) than the PHRO (500 mmol/mol total fatty acids).

In the rats given the unhydrogenated RO and MO an increase in the Δ6- rather than an inhibition of Δ5-desaturase may possibly account for the observed results. This is indicated by the increased 20:4 *n*-6:18:2 *n*-6 value in the MO group and is in accordance with the suggestion made by Kirstein *et al.* (1983) that the presence of *n*-3 fatty acids may induce increased Δ6-desaturase levels as a compensation for competitive effects.

In conclusion, great changes in the pattern of *n*-6 fatty acids in rat liver phospholipids can be observed after relatively short feeding periods due to different dietary oils. The results are in line with reports in the literature that dietary oils containing *trans* fatty acids may inhibit the desaturation–elongation enzyme system. Consequently, this work contributes to the accumulating evidence that oils containing *trans* fatty acids may have effects on specific enzyme systems or reaction sequences, even though no untoward effects on growth, longevity or reproductive capacity have been observed so far (Duthie & Barlow, 1982; Kritchevsky, 1982; Svaar, 1982). There were no indications in the present study of any direct relationship of the effects observed on the *n*-6 fatty acid pattern to the dietary content of very-long-chain fatty acids.

This work was supported by the Royal Norwegian Council for Scientific and Industrial Research.

REFERENCES

Aaes-Jørgensen, E. & Hølmer, G. (1969). *Lipids* **4**, 501–506.
Ackman, R. G. (1982). In *Nutritional Evaluation of Long-chain Fatty Acids in Fish Oil*, pp. 25–88 [S. M. Barlow and M. E. Stansby, editors]. London: Academic Press.
Ackman, R. G. & Loew, F. M. (1977). *Fette, Seifen, Anstrichmittel* **79**, 1–21.
Beare-Rogers, J. L. (1977). *Progress in the Chemistry of Fats and Other Lipids* **15**, 29–56.
Borrebæk, B., Osmundsen, H., Christiansen, E. N. & Bremer, J. (1980). *FEBS Letters* **121**, 23–24.
Brenner, R. R. (1974). *Molecular and Cellular Biochemistry* **3**, 41–52.
Brenner, R. R. (1981). *Progress in Lipid Research* **20**, 41–47.
Conacher, H. B. S., Page, B. D. & Chadha, R. K. (1972). *Journal of the American Oil Chemist Society* **49**, 520–523.
Dhopeshwarkar, G. A. (1981). *Progress in Lipid Research* **19**, 107–118.
Dunnett, C. W. (1955). *American Statistical Association Journal* **50**, 1096–1121.
Duthie, I. F. & Barlow, S. M. (1982). In *Nutritional Evaluation of Long-chain Fatty Acids in Fish Oil*, pp. 185–214 [S. M. Barlow and M. E. Stansby, editors]. London: Academic Press.
Hill, E. G., Johnson, S. B., Lawson, L. D., Mahfouz, M. M. & Holman, R. T.(1982). *Proceedings of the National Academy of Sciences, USA* **79**, 953–957.
Hiltunen, J. K., Osmundsen, H. & Bremer, J. (1983). *Biochimica et Biophysica Acta* **752**, 223–232.

Holman, R. T. (1968). *Progress in the Chemistry of Fats and Other Lipids* **9**, 275–348.
Holman, R. T. & Aaes-Jørgensen, E. (1956). *Proceedings of the Society for Experimental Biology and Medicine* **93**, 175–179.
Hølmer, G. & Aaes-Jørgensen, E. (1969). *Lipids* **4**, 507–514.
Hølmer, G., Høy, C.-E & Kirstein, D. (1982). *Lipids* **17**, 585–593.
Houtsmüller, U. M. T. (1978). *Fette, Seifen, Anstrichmittel* **80**, 162–169.
Jensen, B. (1976). *Lipids* **11**, 179–188.
Kinsella, J. E., Bruckner, G., Mai, J. & Shimp, J. (1981). *American Journal of Clinical Nutrition* **3**, 2307–2318.
Kirstein, D., Høy, C.-E. & Hølmer, G. (1983). *British Journal of Nutrition* **31**, 749–756.
Kritchevsky, D. (1982). *Federation Proceedings* **41**, 2813–2817.
Kurata, N. & Privett, O. S. (1980). *Lipids* **15**, 1029–1036.
Lundberg, W. O. (1980). *Nutrition Reviews* **38**, 233–235.
Mahfouz, M. M., Johnson, S. & Holman, R. T. (1980). *Lipids* **15**, 100–107.
Svaar, H. (1982). In *Nutritional Evaluation of Long-chain Fatty Acids in Fish Oil*, pp. 163–184 [S. M. Barlow and M. E. Stansby, editors]. London: Academic Press.
Svensson, L. (1982). *Lipids* **18**, 171–178.
Thomassen, M. S., Christiansen, E. N. & Norum, K. R. (1982). *Biochemical Journal* **206**, 195–202.

Printed in Great Britain