Biochemical Pharmacology, Vol. 26, pp. 1417–1421. Pergamon Press, 1977. Printed in Great Britain.

THE EFFECT OF DIETARY LIPID PEROXIDES, STEROLS AND OXIDISED STEROLS ON CYTOCHROME P450 AND OXIDATIVE DEMETHYLATION IN THE ENDOPLASMIC RETICULUM

LUCY LAMBERT and E. D. WILLS

Department of Biochemistry, The Medical College of St. Bartholomew's Hospital Charterhouse Square, London, EC1M 6BQ, England

(*Received* 13 *December* 1976; *accepted* 4 *February* 1977)

Abstract—The effects of dietary linoleate, oxidised linoleate, lipid peroxides, cholesterol, cholesterol hydroperoxides and squalene have been examined on the concentration of cytochrome P450 and rate of oxidative demethylation in the endoplasmic reticulum. Addition of methyl linoleate (5%) to a diet containing 5% lard caused a large increase in the rate of oxidative demethylation, oxidised methyl linoleate (5%) was much less effective and also depressed the rate of oxidation when added to a diet containing corn oil. A supply of dietary linoleic acid or other polyunsaturated fatty acids are essential for maximum rate of oxidative demethylation in the endoplasmic reticulum and for maximum induction by phenobarbitone. Dietary lipid peroxide did not increase the rate of oxidative metabolism in the endoplasmic reticulum or its induction. Feeding a diet which included 10% herring oil, and which contains only 1.7% linoleic acid caused a very rapid rate of oxidative demethylation and a high concentration of cytochrome P450 in the liver endoplasmic reticulum. Induction by phenobarbitone was also very effective after feeding this diet. The unsaponifiable fraction of herring oil, added to the lard diet, very effectively increased the rate of oxidative demethylation to a value similar to that observed after feeding a diet containing 10% herring oil. Addition of cholesterol (2 g/kg diet) or of squalene (1 g/kg diet) to diets containing 10% lard were less effective than the unsaponifiable fraction of herring oil but caused a large increase in the content of cytochrome P450 and rate of oxidative demethylation in the endoplasmic reticulum. Induction of phenobarbitone was also enhanced. Cholesterol hydroperoxide was not more effective than cholesterol. It may be concluded that a supply of dietary sterols is essential for maximum activity of oxidative demethylation in the liver endoplasmic reticulum, especially when linoleic acid is in restricted supply and for maximum induction by phenobarbitone. The non-saponifiable fraction of herring oil cannot be completely replaced in the diet by pure cholesterol, cholesterol hydroperoxide or squalene.

Several investigations have demonstrated that dietary lipids can play an important role in the regulation of the activity of drug metabolising enzymes in the liver endoplasmic reticulum. A lipid deficient diet causes a reduction in enzyme activity [1–3] and it has been demonstrated that the unsaturated linoleic acid is especially important [3]. The role of linoleic acid is also substantiated by the finding that during enzyme induction by phenobarbitone, more linoleic acid is incorporated into the phospholipids of the endoplasmic reticulum [4]. Brown *et al.* [5] demonstrated that organic peroxides added to the diet enhanced the inducing effect of phenobarbitone and other drugs. Oxidised sterols were especially effective in enhancing induction and this was confirmed by Marshall and McLean [1] but oxidised sterols had little inducing power of their own [1, 5].

Oxidation of unsaturated fatty acids leads to rapid destruction of the double bonds, the formation of peroxides and more complex degradation products such as aldehydes [6, 7]. It would thus appear that oxidation of lipids of diets may have opposing effects on the regulation of liver drug metabolisim enzymes. On the one hand, destruction of the linoleic acid by oxidation would lead to a reduction in the activity of the enzymes whilst the oxidation of fatty acids or sterols could lead to an enhanced inductive effect.

In the experiments described in this paper the relation between dietary linoleic acid, other polyunsaturated fatty acids, lipid peroxides formed from unsaturated fatty acids, sterols and oxidised sterols have been studied with special reference to their effects on oxidative drug metabolism in the liver.

MATERIALS AND METHODS

Rats. Male albino rats 6–7 weeks old, 120–150 g were used in all experiments. Food and water were available *ad lib.*

Diets. Purified diets were prepared as described by Rowe and Wills [3].

Dietary lipid components. Lard, free from added antioxidants, was supplied by Unigate Ltd., Mazola pure corn oil was used as a source of corn oil and refined herring oil was supplied by Marfleet Refining Co., Hull. The unsaponifiable matter used to supplement the lard was the material remaining after saponification of an equal quantity of herring oil, Squalene (2, 6, 10, 15, 19, 23, Hexamethyl 2, 6, 10,

14, 18, 22, Tetracosahexene) Grade 1 was obtained from Sigma Chemical Co.

A commercial sample of methyl linoleate (Koch-Light Ltd.) was found to have undergone considerable autoxidation, and was used for supplementation of the dietary lipids as oxidised methyl linoleate (OML). A pure sample of methyl linoleate was prepared by fractional distillation of the partially oxidised methyl linoleate under vacuum. The fraction distilling over at 134° was collected and stored under nitrogen.

Oxidised lard was prepared by irradiation in air of a sample with an ultraviolet lamp for 48 hr or until the lipid peroxide content of the lard reached 40 m-mole peroxide/kg.

Preparation of cholesterol hydroperoxide. Cholesterol (BDH) was oxidised to cholesterol 7-α-hydroperoxide by photoxidation based on the method of Schenck *et al.* [8, 9] and its purity assessed by t.l.c. [10].

Diet analysis. The diets were analysed for lipid peroxides using an iodometric method [11]. The aldehyde breakdown products formed by peroxidation of dietary lipids were also determined by the thiobarbituric acid method [12]. The unsaponifiable content of the dietary lipids was estimated by saponification in the presence of an antioxidant β-hydroxyacetanilide using the diethyl ether extraction method (American Oil Chemists Soc. 1946) and the cholesterol content of unsaponifiable matter was determined [13]. The squalene content of the dietary lipid was estimated by separation of squalene by column chromatography [14]. An accurately weighed sample of lipid in hexane was put on a Florisil (Floridin and Co.) 60–100 mesh column and the squalene eluted from the column with a further 25 ml of hexane. The eluate was evaporated to dryness over a boiling water bath and the remaining sample of squalene dried over sodium sulphate and weighed.

Enzyme induction. Induction of drug metabolising enzymes was achieved by giving the animals 1 mg sodium phenobarbitone/ml in the drinking water [1].

Preparation of microsomal fraction. Each rat was killed by cervical fracture, the liver removed and rinsed in ice cold 0.25 M sucrose. The microsomal fraction was recovered by centrifugation of a 10% liver homogenate in 0.25 M sucrose at 9000 *g* for 25 min and the microsomes precipitated from the supernatant with a calcium salt [15]. The microsomal pellet was resuspended in 125 mM KCl (0.5 g liver/ml) to make a 50% microsomal fraction.

Determination of oxidative demethylation and cytochrome P450. The rate of oxidative demethylation of aminopyrine and concentration of cytochrome P450 were determined as previously described [3].

Experimental procedure. Diets containing the various lipids were fed to groups of 30 rats for 21 days. After this period 15 rats were given oral phenobarbitone (10 mg phenobarbitone/100 g body weight/day) in the drinking water for 10 days whilst continuing to feed the diet. Two animals were killed by cervical fracture 10, 12, 16, 18 and 21 days after starting feeding the diet and then 3, 4, 7, 8 and 10 days after administration of phenobarbitone.

RESULTS

Diet analysis. Diets were stored at 4° and the peroxide and malondialdehyde contents measured at intervals during the period of feeding. The main fatty acid components of the diet were those recorded by Rowe and Wills [3]. Herring oil contained 29% saturated fatty acids, 12.6% oleic acid, 1.7% linoleic acid, 1.2% of 18:3, 5.4% of 18:4, 15.8% of 20:1, 21% of 22:1, 2.4% of 22:5 and 6.4% of 22:6. Its total polyunsaturated fatty acid content was 17.1% and the peroxide content of the diet is shown in Table 1.

The diet containing lard, which consisted mainly of saturated or monounsaturated fat, contained low levels of oxidised lipids, while the diet containing herring oil which consisted of highly unsaturated fatty acids and more prone to peroxidation contained nearly three times the quantity of lipid peroxides of the lard diet (Table 1). The fatty acids of the corn oil diet were nearly 50% polyunsaturated, but a high concentration of the natural antioxidant Vitamin E

Table 1. Lipid peroxide and malonaldehyde content of diets

Diet	Additions	Malonaldehyde equiv. μmoles/kg diet	Lipid peroxide m-moles/kg diet
10% Lard	—	60 ± 4	1.07 ± 0.07
10% Oxidised Lard	—	461 ± 40	2.63 ± 0.24
5% Lard	5% OML	310 ± 5	3.96 ± 0.17
5% Lard	5% ML	114 ± 8	0.99 ± 0.06
10% Lard	Unsaponifiable matter*	340	1.59
10% Lard	Cholesterol† (2 g/100 g)	102	1.37
10% Lard	Oxidised cholesterol† (2 g/100 g)	135	3.50
10% Lard	Squalene‡ (1 g/100 g)	58.4	1.55
10% Corn oil	—	90 ± 8	0.27 ± 0.05
5% Corn oil	5% OML	284 ± 12	3.00 ± 0.05
10% Herring oil	—	816 ± 4	3.16 ± 0.1
Fat Fee	—	1.1 ± 0.1	0.39 ± 0.07

Results expressed as Mean ± S.E.M.

OML = oxidised methyl linoleate contained 80 m-moles lipid peroxide and 371 μmoles malonaldehyde/kg.

ML = methyl linoleate (distilled) contained 2.75 m-moles lipid peroxide and 245 μmoles malonaldehyde/kg.

* Unsaponifiable matter prepared from a weight of herring oil equivalent to the weight of lard used.

† 2 g Cholesterol and oxidised cholesterol/kg diet.

‡ 1 g Squalene/kg diet.

Table 2. Unsaponifiable matter and sterols in the dietary lipids

	Herring oil	Lard
Unsaponifiable matter (%)	2.32	0.78
Cholesterol (mg/100 g fat)	745	90
Squalene (mg/100 g fat)	900	<10

(100 mg/kg) [3] kept the lipid peroxide concentration at a low level (Table 1).

Irradiation of the lard with u.v. caused the lipid peroxide value of the diet containing lard to increase from 1.07 m-mole peroxide/kg to 2.63 m-mole peroxide/kg, or by 40%, but the irradiation increased the aldehyde breakdown products to 750% of the unoxidised lard diet (Table 1).

Herring oil contained a high percentage of unsaponifiable matter mostly comprised of cholesterol and squalene while lard contained very little unsaponifiable matter and almost no squalene (Table 2).

Both cholesterol and squalene are readily oxidised to the hydroperoxides and the supplementation of the lard diet with 0.8% cholesterol hydroperoxide considerably increased the concentration of lipid peroxides and, to a much smaller extent, aldehydes in the diet. Addition of pure cholesterol did not significantly alter the peroxide content (Table 1). Herring oil contained a high concentration of oxidised lipids and when the unsaponifiable matter prepared from this oil was added to the lard diet, it caused only a small increase in peroxide but a large increase in aldehydes (Table 1). This indicated that the peroxides in the herring oil were mainly in the saponifiable fraction and that only a small proportion were steroid or squalene peroxides.

Effect of oxidised lipids on oxidative demethylation and cytochrome P450. The concentration of cytochrome P450 and the rate of animopyrine demethylation as indicated by the V_{max}, in the microsomal fraction prepared from rats fed a lard diet were both significantly less than the values obtained from the fractions prepared from rats fed the corn oil or herring oil diet (Table 3). The effect of corn oil was almost certainly due to the high linoleic acid content [3] and this was substantiated by addition of methyl linoleate to the lard diet when a greatly increased rate of demethylation was observed (Table 3).

Herring oil, however, contains only 1.7% linoleic acid and its effect must be due to other factors. Other polyunsaturated fatty acids in herring oil such as 22:6 (6.4%) may be able to replace linoleic acid but in view of the importance ascribed to oxidised lipids and oxidised sterols in the induction process [1, 5], it was considered possible that the peroxide content of the oil might be of major importance.

The importance of lipid peroxide was studied by oxidation of the lard before adding to the diet or by addition of oxidised methyl linoleate (OML) to lard or corn oil diets.

Addition of unoxidised methyl linoleate to the lard diet significantly increased the concentration of cytochrome P450 in the microsomal fraction and the rate of aminopyrine demethylation, and enhanced slightly the inducing effect of phenobarbitone, but the high levels of dietary lipid peroxides resulting from adding OML to the diet containing lard or corn oil did not cause any significant increases in the rate of oxidative demethylation or concentration of cytochrome P450 in the endoplasmic reticulum over and above those observed after feeding the lard + ML or corn oil diets (Table 3).

It is thus unlikely that the effect of dietary herring oil on the liver enzyme system was a result of its lipid peroxide content or aldehyde degradation products of peroxidation.

Effects of unsaponifiable fractions of herring oil, cholesterol and cholesterol hydroperoxide. In view of the fact that the peroxide content of herring oil did not appear important for the high rate of oxidative demethylation observed, the unsaponifiable fraction which formed a relatively large proportion of herring oil (Table 2), was prepared and studied.

Table 3. The effect of dietary lipid peroxide, methyl linoleate and oxidised methyl linoleate on oxidative demethylation of aminopyrine and cytochrome P450 concentrations

Diet	Dietary lipid peroxide m-moles/kg	Cytochrome P450 nmoles/mg protein		Demethylation of aminopyrine K_m (mM)		V_{max} (nmoles/min/mg protein)	
		Basic	Induced	Basic	Induced	Basic	Induced
10% Lard	1.07	0.47 ±0.01 (12)	1.15 ±0.06 (12)	0.49 ±0.02 (12)	0.41 ±0.01 (12)	4.45 ±0.01 (12)	10.95 ±0.74 (12)
10% Oxidised Lard	2.63	0.51 ±0.04	1.26 ±0.07	0.59 ±0.03	0.47 ±0.03	4.66 ±0.19	11.60 ±0.58
5% Lard + 5% OML	3.96	0.42 ±0.03 (7)	1.06 ±0.09 (7)	0.60 ±0.06 (7)	0.43 ±0.02 (7)	6.46 ±0.51 (7)	14.28 ±0.34 (7)
5% Lard + 5% ML	0.99	0.74 ±0.05 (9)	1.44 ±0.10 (10)	0.50 ±0.08 (9)	0.41 ±0.04 (10)	7.75 ±0.48 (9)	14.89 ±0.32 (10)
10% Corn oil	0.27	0.62 ±0.03 (14)	1.48 ±0.26 (14)	0.55 ±0.01 (14)	0.46 ±0.02 (14)	6.06 ±0.28 (14)	13.20 ±0.78 (14)
5% Corn oil + 5% OML	6.00	0.42 ±0.05 (6)	1.19 ±0.12 (7)	0.71 ±0.05 (6)	0.44 ±0.03 (7)	5.73 ±0.74 (6)	13.52 ±1.02 (7)
10% Herring oil	3.16	0.83 ±0.04	1.63 ±0.04	0.59 ±0.02	0.38 ±0.01	8.36 ±0.41	17.93 ±0.89

Figures in parenthesis are the number of animals used and results are expressed as Mean ± S.E.M.
OML = oxidised methyl linoleate.
ML = methyl linoleate.

Table 4. Effect of unsaponifiable matter and dietary sterols on microsomal aminopyrine demethylase activity and levels of cytochrome P450

Diet	Cytochrome P450 nmoles/mg protein		Aminopyrine demethylation			
			K_m (mM)		V_{max} (nmoles/min/mg protein)	
	Basic	Induced	Basic	Induced	Basic	Induced
10% Lard	0.47 ± 0.01	1.15 ± 0.06	0.49 ± 0.02	0.41 ± 0.01	4.45 ± 0.10	10.95 ± 0.74
10% Lard + cholesterol†	0.69 ± 0.03	2.08 ± 0.17	0.58 ± 0.02	0.41 ± 0.02	5.83 ± 0.24	13.08 ± 0.83
10% Lard + unsaponifiable matter*	0.70 ± 0.04	1.87 ± 0.11	0.44 ± 0.02	0.40 ± 0.01	8.84 ± 0.40	15.34 ± 0.63
10% Lard + squalene‡	0.63 ± 0.03	1.78 ± 0.12	0.52 ± 0.03	0.46 ± 0.02	5.79 ± 0.29	15.05 ± 0.73
10% Herring oil	0.83 ± 0.04	1.63 ± 0.14	0.59 ± 0.02	0.38 ± 0.01	8.36 ± 0.41	17.93 ± 0.87

Results are expressed as Mean ± S.E.M.
* Unsaponifiable matter of herring oil equivalent to weight of dietary lard.
† 2 gm cholesterol/kg diet.
‡ 1 gm squalene/kg diet.

Saponifiable matter was prepared from herring oil and a quantity added to lard so that each gram of lard contained the unsaponifiable matter from 1 g herring oil. This diet would thus be exactly equivalent to herring oil in respect of the unsaponifiable fraction.

The supplementation of the lard diet with unsaponifiable matter from herring oil increased the levels of both cytochrome P450 and rate of aminopyrine demethylation close to the values observed after feeding the herring oil. Addition of cholesterol and squalene to the lard diet also increased considerably the values of these parameters, but neither was as effective as the unsaponifiable fractions. This is shown most clearly in the un-induced animals where the V_{max} after addition of saponifiable matter to the lard was considerably more than that after feeding lard + cholesterol or lard + squalene (Table 4).

It is significant that phenobarbitone induction was most effective after feeding diets in which cholesterol or squalene was mixed with lard. Increases of 300% in cytochrome P450 after addition of cholesterol and 283% after addition of squalene were observed (Table 4). Increases in the rates of oxidative demethylation were 224% for cholesterol addition and 260% for squalene addition.

Dietary cholesterol or squalene thus directly induces the concentration of cytochrome P450 in the endoplasmic reticulum and enhances the inductive effect of phenobarbitone, particularly on cytochrome P450 (Table 4).

The incorporation of cholesterol hydroperoxide in the diet was not more effective than cholesterol in increasing the concentration of cytochrome P450 or activity of aminopyrine demethylation, either before or after phenobarbitone treatment (Table 5).

DISCUSSION

In an earlier investigation [3] we demonstrated that the strong enhancement of the rate of oxidative demethylation in the liver endoplasmic reticulum after feeding corn oil could be accounted for by its linoleic acid and Vitamin E contents. Vitamin E was effective in increasing the rate of oxidative demethylation when added to a lard diet [3].

Pure re-distilled methyl linoleate, when added to a lard diet caused a large stimulation of the rate of oxidative demethylation and concentration of cytochrome P450. This, together with results obtained using corn oil, clearly establish the important role played by dietary linoleic acid in inducing a high rate of oxidative demethylation.

Previously, some investigators [1, 5] have reported that organic peroxides or oxidised lipids in the diet enhanced the induction of cytochrome P450 and microsomal hydroxylation. Formation of peroxide in the lard diet before feeding caused no significant increase in the cytochrome P450 concentration and rate of oxidative demethylation beyond that observed after feeding the lard diet (Table 3). Addition of oxidised methyl linoleate to the corn oil diet did not change the basic or induced rate of oxidative demethylation and even decreased the levels of cytochrome P450 (Table 3). The results of these experiments do not therefore support the importance of dietary lipid peroxides or their degradation products for activation or induction of enzymes involved in oxidative demethylation.

Of the dietary fats so far studied herring oil has been found to be most effective in causing a very high rate of oxidative demethylation and a maximum induction after phenobarbitone treatment.

Table 5. Effect of dietary oxidised sterols on microsomal aminopyrine demethylase activity and levels of cytochrome P450

Diet	Dietary lipid peroxides m-moles/kg diet	Cytochrome P450 (nmoles/mg protein)		Aminopyrine demethylation			
				K_m (mM)		V_{max} (nmoles/min/mg protein)	
		Basic	Induced	Basic	Induced	Basic	Induced
10% Lard + cholesterol	1.37	0.69 ± 0.03	2.08 ± 0.17	0.58 ± 0.02	0.41 ± 0.02	5.83 ± 0.24	13.08 ± 0.35
10% Lard + cholesterol hydroperoxide	3.50	0.62 ± 0.04	1.75 ± 0.16	0.57 ± 0.08	0.45 ± 0.03	6.32 ± 0.15	12.42 ± 0.35

2 gm cholesterol or cholesterol hydroperoxide/kg diet.
All results are expressed as Mean ± S.E.M.

The effect of herring oil is unlikely to be due to its linoleic acid content which is only 1.7%, much less than corn oil (50%), and even less than lard (5.9%).

Several possible explanations could be advanced to explain the great efficiency of herring oil: its content of more highly unsaturated fatty acids such as of 22:6 (6.4%) or 18:4 (5.7%), its very high peroxide content (Table 1) or its high content of unsaponifiable matter including squalene and especially cholesterol (Table 2), could be involved separately or additively.

Although the peroxide and aldehyde concentrations of the herring oil were very high and much higher than corn oil (Table 1), it would appear very unlikely that these were important factors because, as discussed above, oxidation of lard or addition of oxidised methyl linoleate did not increase the rate of oxidative demethylation (Table 3).

The highly unsaturated fatty acids, such as 18:4 or 22:6 could be incorporated into the membrane directly and convert it into a configuration which is very effective in activation of the enzymes. We have shown recently* that rats fed a diet of herring oil incorporated much more highly unsaturated acid such as 22:6 into their endoplasmic reticulum lipid than those animals fed a normal diet. Degradation and saturation of these highly unsaturated fatty acids to form linoleic acid may occur but as the total content of these acids is only 17% of the total fatty acid content of herring oil it is very unlikely that this oil would be more effective than corn oil with its 50% content of linoleic acid.

It would thus appear that the unsaponifiable, or sterol fraction of the oil is of major importance.

Addition of the unsaponifiable fraction from herring oil to the lard diet produced values for cytochrome P450 concentration and rate of oxidative demethylation close to those observed after feeding herring oil (Table 4). This demonstrates that the unsaponifiable fraction of the herring oil is of major importance in producing the increased rate of oxidative demethylation. Addition of pure cholesterol or pure squalene to the diet also increased the rates and although these additions were less effective than the unsaponifiable fraction before induction they permitted a very effective induction of cytochrome P450 concentration and rate of oxidative demethylation by aminopyrine (Table 4).

Addition of pure cholesterol to the diet had previously been found to have little effect on levels of hepatic cytochrome P450 and demethylation but that "aged" or oxidised cholesterol induced drug metabolism and particularly enhanced the inducing effect of phenobarbitone [1, 5].

Both the pure cholesterol and pure cholesterol hydroperoxide induced the levels of cytochrome P450 and *N*-demethylation to an approximately equal extent (Table 5). Thus the effects observed for "aged" cholesterol are unlikely to be ascribed to a preformed cholesterol hydroperoxide. The enhancing effect of cholesterol on the drug metabolising enzyme system could be a result of a specific inductive effect similar to that caused by phenobarbitone. 7α-hydroxylation of cholesterol, is an important step in its metabolism to bile acids and may be dependent on a cytochrome P450 which is similar to, but not identical with the cytochrome P450 essential for drug hydroxylation [16].

Alternatively, a direct incorporation of the dietary cholesterol into the membranes cannot, however be excluded. Rapid proliferation of membranes following phenobarbitone induction requires much extra cholesterol and internal synthesis may not be able to keep pace with demand.

It may be concluded that a supply of dietary sterols is essential for maximum activity of oxidative demethylation and especially when linoleic acid is in restrictive supply and for maximum induction by phenobarbitone. Cholesterol hydroperoxide is not more effective than cholesterol but the non-saponifiable fraction of herring oil appears to contain other, so far unidentified components, which increase the activity of the system.

Acknowledgements—The authors wish to express their thanks to the Cancer Research Campaign for a grant which supported the investigation, to Messrs Unigate for a supply of casein and antioxidant-free lard, to the Marfleet Refining Co., for a supply of herring oil, to Roche for the supply of vitamins A/D_3, and to Bovril Ltd., for a supply of dried yeast used for preparation of the diet and to Professor E. M. Crook for useful advice in the preparation of this paper.

* C. E. Hammer and E. D. Wills, unpublished observations.

REFERENCES

1. W. J. Marshall and A. E. M. McLean, *Biochem. J.* **122,** 569 (1971).
2. A. E. M. McLean and W. J. Marshall, *Scand. J. clin. Lab. Invest.* **25,** Suppl. No. 113, 9 (1970).
3. L. Rowe and E. D. Wills, *Biochem. Pharmac.* **25,** 175 (1976).
4. S. C. Davison and E. D. Wills, *Biochem. J.* **140,** 461 (1974).
5. R. R. Brown, J. A. Miller and E. C. Miller, *J. biol. Chem.* **209,** 211 (1954).
6. E. D. Wills and J. Rotblat, *Int. J. Radiat. Biol.* **8,** 551 (1964).
7. R. Marcuse and L. Johansson, *J. Ann. Oct. Chem. Soc.* **50,** 387 (1973).
8. G. O. Schenk, K. Gollnick and O. A. Neumuller, *Liebigs Ann. Chem.* **603,** 46 (1957).
9. G. O. Schenk, O. A. Neumuller and W. Eisfield, *Annln. Chem.* **618,** 202 (1958).
10. J. R. Milton, N. A. Scholan and G. S. Boyd, *Eur. J. Biochem.* **20,** 569 (1971).
11. P. A. T. Swoboda and C. H. Lea, *Chem. Ind.* 1090 (1958).
12. E. D. Wills, *Biochem. J.* **99,** 667 (1966).
13. G. V. Mann, *Clin. Chem.* **7,** 275 (1961).
14. K. K. Carrol, *J. Lipid Res. Commun.* **2,** 135 (1961).
15. S. A. Kamath and E. Rubin, *Biochem. biophys. Res. Commun.* **49,** 52 (1972).
16. S. D. Atkin, E. D. Palmer, P. D. English, B. Morgan, M. A. Cawthorne and J. Green, *Biochem. J.* **128,** 237 (1972).