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Lipid Peroxide Formation in Microsomes

RELATIONSHIP OF HYDROXYLATION TO LIPID PEROXIDE FORMATION

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1. Aminopyrine strongly inhibits NADPH-induced lipid peroxide formation in rat liver microsomes, but ascorbate-induced peroxidation is inhibited to a smaller extent. 2. Aminopyrine oxidation is stimulated by Mg^{2+} but inhibited by Ca^{2+}. Concentrated solutions (10mM) of iron-chelating agents inhibit aminopyrine oxidation, but the more dilute solutions (0·5mM) of chelators that block lipid peroxide formation do not inhibit aminopyrine oxidation. Microsomes prepared from sucrose–EDTA homogenates rapidly oxidize aminopyrine, but do not form lipid peroxide when incubated with ascorbate or NADPH. 3. Aminopyrine oxidation is strongly inhibited by *p*-chloromercuribenzoate, less by iodoacetamide and weakly by *N*-ethylmaleimide. The site of action of these compounds is considered to be a ferredoxin-type protein. GSH and cysteine also inhibit. 4. Other drugs oxidized by microsomes such as caffeine, phenobarbitone and hexobarbitone had either no or little effect on lipid peroxide formation, but codeine inhibited. 5. Most aliphatic hydrocarbons, alcohols, ketones and aldehydes did not affect lipid peroxide formation, but chloroform and carbon tetrachloride inhibited. 6. Many aromatic compounds inhibited lipid peroxide formation. Only aromatic acids were without any effect and phenols and amines were very strong inhibitors. 7. Induction of lipid peroxide formation in microsomes by incubation with ascorbate or NADPH or by treatment with ionizing radiation leads to a sharp decline in the ability of microsomes to oxidize aminopyrine or hydroxylate aniline. 8. It is considered that the two processes of hydroxylation and lipid peroxide formation are closely linked in microsomes. They probably depend on the same electron-transport chain, and peroxide formation, which involves membrane disintegration, may be part of the normal membrane remodelling process.

For several years now it has been well established that the microsomes of the liver are the site of oxidative metabolism of many aromatic compounds, steroids and drugs (Williams, 1959). This process was early shown to require NADPH as a source of electrons (Brodie, Gillette & La Du, 1958), and more recently (Siekevitz, 1966; Ernster & Orrenius, 1966) it became clear that the oxidation was a result of the operation of a microsomal electron-transport chain. This consists of several ill-defined components but is known to contain a cytochrome, *P*-450, that utilizes gaseous oxygen to oxidize a large number of drugs and convert these into hydroxylated derivatives (Omura, Sato, Cooper, Rosenthal & Estabrook, 1966).

When microsomes are incubated with NADPH there is a rapid production of lipid peroxide (Wills, 1969*a*), and it is therefore likely that the two processes of peroxidation and hydroxylation are closely linked and may be a result of the operation of the same electron-transport chain. In support of this concept Orrenius, Dallner & Ernster (1964) noted that some drugs such as aminopyrine, which are oxidized by microsomes, caused a significant decrease in the rate of lipid peroxide formation in microsomes incubated in the presence of NADPH.

A study of the interrelationship between microsomal lipid peroxide formation and hydroxylation has now been made in an attempt to establish the role of lipid peroxidation in the hydroxylation process.

EXPERIMENTAL

Preparation of microsomes, NADPH and measurements of lipid peroxide, O_2 uptake and protein concentration were made as described by Wills (1969*a*). In addition, the following methods and materials were used.

Materials. Aminopyrine, codeine, caffeine and phenobarbitone were obtained from John Bell and Croyden, London W.1, and all other chemicals from British Drug Houses Ltd. (Poole, Dorset).

Oxidative demethylation of aminopyrine. This oxidation was studied by measuring the rate of formaldehyde production (Ernster & Orrenius, 1966).

Sodium phosphate buffer, pH 7·0 (20 mM), NADPH (40 μM), nicotinamide (20 mM), microsomal suspension (3·0 mg. of protein/ml.) and aminopyrine (10 mM) were incubated at 37° in a total volume of 5–10 ml. Samples (2·0 ml.) were removed at intervals for determination of formaldehyde concentration by using a modified version of the method described by Nash (1953). Trichloroacetic acid (20%, w/v) (1 ml.) was added to the sample and the tube placed in a boiling-water bath for 30 sec. After cooling the mixture was centrifuged and to 2 ml. of the supernatant M-sodium acetate buffer, pH 5·8 (0·4 ml.), and 0·6 ml. of the formaldehyde reagent [containing 0·067% acetylacetone in 15% (w/v) ammonium acetate, and 0·3% acetic acid] was added. The tubes were incubated at 50° for 30 min. to develop the yellow colour and read at 412 nm. Formaldehyde concentration was calculated by using 8000 cm.^{2} m-mole^{-1} as the molar extinction coefficient for formaldehyde (Nash, 1953).

Hydroxylation of aniline. The incubation mixture was essentially the same as that used for the study of aminopyrine oxidation, but 6·7 mM-aniline added in acetone replaced the aminopyrine. Samples (2 ml.) were removed for the determination of *p*-aminophenol and added to 1 ml. of trichloroacetic acid (20%). The precipitated proteins were filtered off, and to 1 ml. of filtrate phenol [1% (w/v) in 0·5 M-NaOH] (1 ml.) and M-Na_2CO_3 (1 ml.) were added. The tubes were left for 15 min. for development of the colour, which was read at 630 nm. Recovery of added *p*-aminophenol by this method was greater than 99%.

Irradiation. Irradiation of microsomal suspensions was carried out with a 15 Mev linear accelerator as described by Wills & Wilkinson (1967).

Unless otherwise stated all determinations are means of three separate experiments.

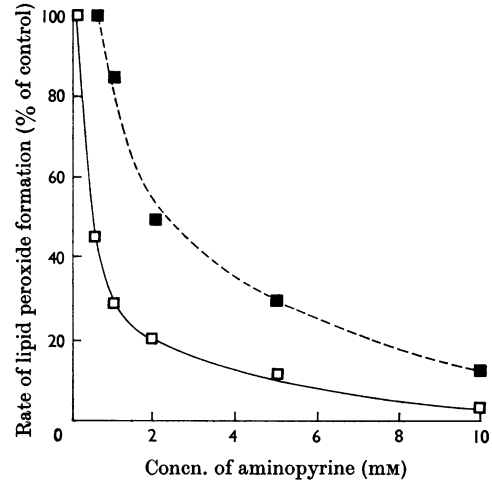


Fig. 1. Effect of aminopyrine on lipid peroxide formation by microsomes induced by ascorbate or by NADPH. Suspensions of microsomes (1·5 mg. of protein/ml.) were incubated at 37° in a medium (total volume 2 ml.) containing 20 mM-sodium phosphate buffer, pH 7·0, KCl (90 mM) and either ascorbate (0·5 mM) or NADPH (40 μM) + nicotinamide (50 mM). Suitable volumes of aminopyrine (0·1 M) were added to give the concentration specified. Samples (0·5 ml.) were removed at intervals during the 15 min. incubation period for the determination of lipid peroxide. Rates are expressed as percentages of the control rates in the absence of aminopyrine, which, as nmoles of malonlaldehyde/min., were 8·6 for the NADPH system and 5·0 for the ascorbate system. ■, Ascorbate system; □, NADPH system.

RESULTS

Effect of aminopyrine on lipid peroxide formation. At low concentrations (1 mM) aminopyrine inhibited NADPH - stimulated peroxide formation very strongly and had little effect on ascorbate-induced lipid peroxidation, but at higher concentrations (10 mM) aminopyrine strongly inhibited whichever inducer of peroxide was used (Fig. 1).

The rate of oxygen uptake of microsomal suspensions incubated in the presence of NADPH was decreased by aminopyrine, but although lipid peroxide formation was decreased to zero by 10 mM-aminopyrine oxygen consumption continued at about 30% of the control rate (Fig. 2).

Effects of some drugs on lipid peroxide formation. To establish whether the inhibitory effect of aminopyrine on lipid peroxidation was a property unique to this particular drug, other drugs oxidized by microsomes such as caffeine, codeine, phenobarbitone and hexobarbitone were tested under similar conditions, but all these drugs were much less effective than aminopyrine. Hexobarbitone and caffeine had no effect and only codeine caused a large decrease in the rate of peroxide formation (Fig. 3).

Effects of aliphatic and aromatic compounds on lipid peroxide formation. In view of the fact that several drugs oxidized by microsomes possessed different capacities for decreasing the rate of lipid peroxide formation and that the effect appeared to depend on the structure of the molecule, a large number of simple aliphatic and aromatic hydrocarbons, alcohols, phenols, ketones, aldehydes, acids, halides and amines were tested in the system. Table 1 shows that, in general, aliphatic compounds, with the exception of chloroform and carbon tetrachloride, did not affect the rate. Many aromatic compounds did, however, markedly inhibit the rate of lipid peroxide formation by microsomes, but aromatic acids were not effective. Benzene was much more effective than aliphatic hydrocarbons, but phenols and amines were strongest inhibitors, and substituted or polyhydric phenols more effective than phenol itself, which was more effective than benzene (Fig. 4).

Effect of metal ions on oxidation of aminopyrine. It has been shown (Wills, 1969*a*) that the rate of

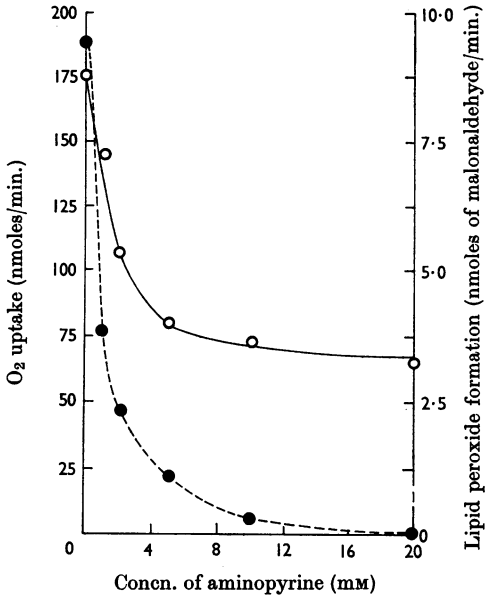


Fig. 2. Comparison of the effects of aminopyrine on lipid peroxide formation and O_2 uptake by microsomes in the presence of NADPH. Experimental details were as described for Fig. 1, but the experiments were carried out in the oxygen electrode for a period of 1–2min. Peroxide determinations were carried out on the whole suspension (2·0ml.) immediately after removal from the reaction vessel. ○, O_2 uptake; ●, lipid peroxide formation.

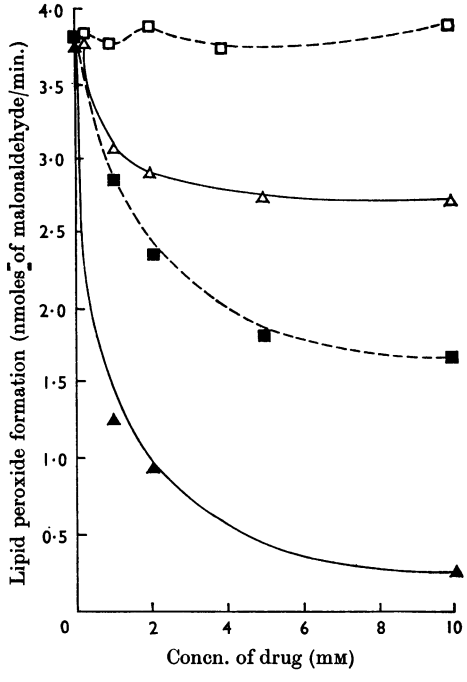


Fig. 3. Comparison of the effects of aminopyrine, phenobarbitone, codeine and hexobarbitone on lipid peroxide formation by microsomes oxidizing NADPH. Experimental details were as described for Fig. 1. □, Hexobarbitone; △, phenobarbitone; ■, codeine; ▲, aminopyrine.

lipid peroxide formation in microsomes is stimulated by Mg^{2+} and inhibited by Ca^{2+}. Mg^{2+} also stimulated the rate of oxidation of aminopyrine but to a greater extent than lipid peroxide formation, and Ca^{2+} inhibited in equivalent concentrations. The percentage stimulation when an optimum concentration of Mg^{2+} (20mM) was added varied with the preparations of microsomes. It was sometimes as much as 8 times, but was normally within the range 1·5–3·0 times (Fig. 5).

Lipid peroxide formation in microsomes incubated with NADPH or ascorbate is blocked by addition of EDTA, *o*-phenanthroline or desferrioxamine (Wills, 1969*b*). EDTA (1mM), but not *o*-phenanthroline (1mM) or desferrioxamine (1mM), strongly inhibited aminopyrine oxidation. That this inhibition was due to chelation of Mg^{2+} and not Fe^{2+} was proved by addition of Mg^{2+} (10mM) (Table 2). More concentrated solutions of desferrioxamine (10mM) did, however, inhibit aminopyrine oxidation even when the concentration of Mg^{2+} was adequate, but this inhibition was overcome by the addition of Fe^{2+}. These experiments indicate that a non-haem iron component is essential for oxidative demethylation of aminopyrine, but that this iron is less available to, or less readily bound by, the chelating agents that inhibit lipid peroxide formation.

Preparation of microsomes from sucrose–EDTA homogenate. If the original liver homogenate used for preparation of the microsomes is made in 0·25M-sucrose containing EDTA (1mM) instead of in sucrose alone, no lipid peroxide is formed on incubation with NADPH or ascorbate (Wills, 1969*a*). When aminopyrine was added to a suspension of microsomes prepared from a sucrose–EDTA homogenate and incubated in the presence of NADPH, an increase of oxygen uptake results. This is in sharp contrast with the decrease in the rate of oxygen utilization that occurs when microsomes prepared with sucrose alone are used (Fig. 6). The oxygen taken up by the microsomes prepared with sucrose–EDTA in the presence of aminopyrine is utilized almost exclusively for the oxidative demethylation of aminopyrine and this continues at the same rate as when microsomes prepared with sucrose alone are used. Aminopyrine therefore is clearly able to regulate the rate of utilization of oxygen by the microsomes.

Effects of thiol reagents, GSH and cysteine on

Table 1. *Effect of some typical aliphatic and aromatic compounds on lipid peroxide formation in microsomes*

Microsomal suspensions were incubated in the presence of sodium phosphate buffer, pH 7·0, and NADPH for 10 min. at 37°. Experimental details were as described for Fig. 1. Aromatic phenols and acids, aniline and acetanilide were dissolved in water before addition. Benzaldehyde, *p*-dichlorobenzene, *p*-dibromobenzene, thymol, nitrophenol, *p*-aminobenzoic acid and 1-chloro-2,4-dinitrobenzene were dissolved in ethanol. All other compounds were added as pure liquids.

Addition	Quantity (m-mole/ml. of suspension)	Rate of lipid peroxide formation (%)
None	—	100
Methanol	1·0	97
Ethanol	1·0	97
Propan-1-ol	1·0	98
Propan-2-ol	1·0	94
Hexane	1·0	79
Acetone	1·0	95
Acetamide	0·05	100
Butan-2-one	1·0	83
Diethyl ether	1·0	95
Chloroform	1·0	9
	0·5	12
Carbon tetrachloride	1·0	12
	0·5	73
Benzene	0·5	12
	1·0	6
Toluene	0·5	59
	1·0	46
Phenol	0·02	0
Catechol	0·02	0
p-Cresol	0·02	0
Thymol	0·02	2
Naphthol	0·02	0
p-Nitrophenol	0·02	5
Benzyl alcohol	1·0	0
Benzaldehyde	0·05	70
Benzoic acid	1·0	110
Salicylic acid	1·0	91
Phenylacetic acid	1·0	70
	0·5	82
Sulphanilic acid	1·0	6
	0·5	60
Aniline	0·01	0
Acetanilide	0·025	80
	0·05	50
p-Dichlorobenzene	0·025	10
p-Dibromobenzene	0·025	30
1-Chloro-2,4-dinitrobenzene	0·025	5

aminopyrine oxidation. The effects of thiol reagents on aminopyrine oxidation were very similar to those observed on lipid peroxide formation in the presence of NADPH (Wills, 1969*a*). *p*-Chloromercuribenzoate inhibited very strongly but iodoacetamide had no effect. *N*-Ethylmaleimide inhibited provided that it was kept in contact with the microsomal suspension for 15–30 min. before the experiment was started. A comparison of the effects on lipid peroxide formation and aminopyrine oxidation is shown in Table 3.

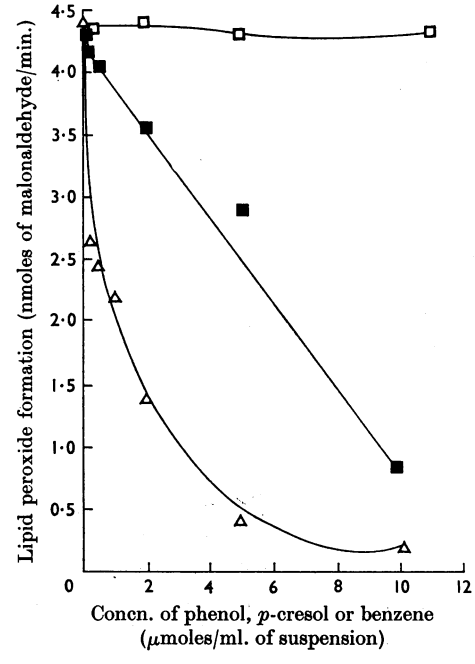


Fig. 4. Comparison of the effects of benzene, phenol and *p*-cresol on the rate of lipid peroxide formation by microsomes. Experimental details were as described for Fig. 1. □, Benzene; ■, phenol; △, *p*-cresol.

GSH, which inhibits lipid peroxide formation induced by ascorbate (Christophersen, 1968) or by NADPH (Wills, 1969*a*), also inhibited aminopyrine oxidation but to a much smaller extent than lipid peroxidation. Cysteine on the other hand inhibited aminopyrine oxidation more strongly than peroxidation (Table 4).

Aminopyrine oxidation by microsomal fractions. The microsomal fraction was subfractionated into a sediment composed mainly of ribosomes, fluffy layer composed mainly of membranous vesicles and supernatant, by a method based on that of Dallner (1963) as described by Wills (1969*a*).

Each fraction was used in place of the microsome fraction for the study of the rate of aminopyrine oxidation. Like lipid peroxidation, aminopyrine oxidation was associated mainly with the fluffy layer of membranous vesicles (Table 5). Higher concentrations of deoxycholate than those used in the fractionation inhibited both peroxidation and aminopyrine oxidation (Fig. 7).

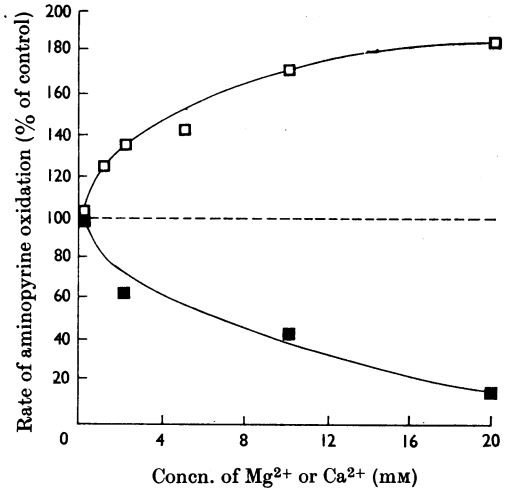


Fig. 5. Effect of Mg^{2+} and Ca^{2+} on the rate of oxidation of aminopyrine by microsomes. Suspensions of microsomes (3mg. of protein/ml.) were incubated in a total volume of 5–10ml. at 37° for 15–30min. in a medium containing sodium phosphate buffer or tris–HCl buffer (20mM), pH 7·0, NADPH (40μM), nicotinamide (50mM), aminopyrine (10mM), KCl (125mM) and Mg^{2+} or Ca^{2+} as shown. Samples (2·0ml.) were removed at intervals for determination of the concentration of formaldehyde. Results are expressed as percentages of the control rate (8·95nmoles of formaldehyde/min.). □, Mg^{2+}; ■, Ca^{2+}.

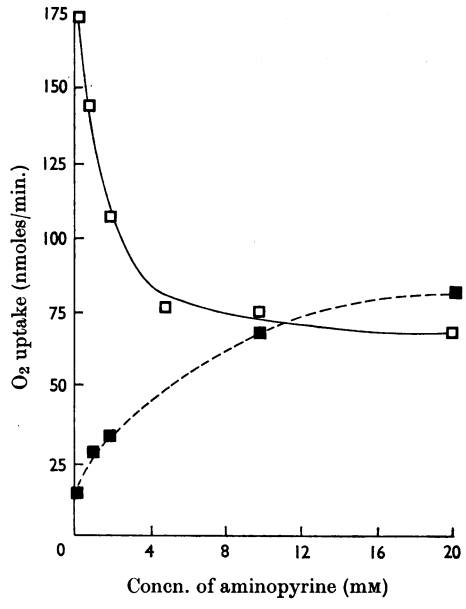


Fig. 6. Comparison of the effects of aminopyrine on O_2 uptake by microsomes prepared from sucrose homogenate or from sucrose–EDTA homogenate. Rat liver (10g.) was divided into two equal portions. A 5g. portion was homogenized in 0·25M-sucrose and the other 5g. in 0·25M-sucrose containing EDTA (1mM). □, Sucrose homogenate; ■, sucrose–EDTA homogenate.

Table 2. *Effect of metal ion-chelating agents on the rate of oxidative demethylation of aminopyrine by microsomes*

Microsomes were incubated with EDTA or desferrioxamine for 5min. before addition of Mg^{2+} or of Fe^{2+}. Experimental details were as described for Fig. 5.

Addition	Concn. (mM)	Aminopyrine oxidation (nmoles of formaldehyde/min.) No Mg^{2+} added	Aminopyrine oxidation (nmoles of formaldehyde/min.) +Mg^{2+} (10mM)
None (control)	—	13·3	21·2
EDTA	1	0·5	18·6
Desferrioxamine	1	14·9	18·3
	4	—	10·6
	100	—	0
Desferrioxamine Fe^{2+}	4, 10	—	21·7

Effect of peroxidation in microsomes on hydroxylation. The effect of lipid peroxide formation in microsomes on their capacity to carry out hydroxylation was studied by inducing lipid peroxide formation, either by incubating microsome suspensions at 37° for 1–2hr. or more effectively by incubating them for short periods in the presence of NADPH or ascorbate. At the end of the incubation period aminopyrine was added with NADPH and nicotinamide, and the rate of oxidation of the drug compared with that added to a control suspension of microsomes kept at 0°. Lipid peroxide formation was also induced by ionizing radiation by giving doses of electrons within the range 10–100 krads to microsomal suspension (Wills & Wilkinson, 1967). By whatever method lipid peroxide formation was induced it always caused a decrease in the capacity of the microsomes to hydroxylate aniline or demethylate aminopyrine (Table 6 and Fig. 8).

The loss of capacity to hydroxylate could have been due to membrane disintegration or to the production of toxic products. If microsomes containing a high content of lipid peroxide were added to fresh microsomes they did not alter the capacity of the fresh microsomes to oxidize aminopyrine, indicating that toxic products are unlikely to be formed and that the effect of peroxidation is associated with membrane disorganization.

Table 3. *Comparison of the effects of thiol reagents on aminopyrine oxidation and on lipid peroxide formation*

N-Ethylmaleimide was left in contact with the microsomes for different periods before the experiment was started. Rates are expressed as percentages of control values.

Addition	Concn. (mM)	Rate of aminopyrine oxidation (%)	Rate of lipid peroxide formation (NADPH system) (%)
None (control)	0	100	100
p-Chloromercuribenzoate	0·1	89	66
	0·2	44	42
	0·5	0	21
	1·0	6	9
Iodoacetamide	1·0	97	103
	2·0	100	100
N-Ethylmaleimide	1·0 (0 min.)	100	94
	(15 min.)	83	87
	(30 min.)	80	71
	2·0 (0 min.)	82	78
	(15 min.)	54	67
	(30 min.)	47	60

Table 4. *Effect of GSH and cysteine on aminopyrine oxidation and lipid peroxide formation by microsomes*

Addition	Concn. (mM)	Aminopyrine oxidation (nmoles of formaldehyde/min.)	Lipid peroxide formation (nmoles of malonaldehyde/min.)
None (control)	0	22·3	3·95
GSH	0·1	23·8	2·30
	0·2	19·4	1·40
	0·5	20·1	1·45
	1·0	13·4	1·50
	2·0	6·8	1·50
Cysteine	0·2	11·6	3·40
	0·5	7·6	3·20
	1·0	3·6	3·22
	2·0	3·5	3·04
	4·0	3·3	—

DISCUSSION

These experiments lead to the conclusion that there is a close link between lipid peroxidation and hydroxylation in microsomes. As a basis for discussion possible relationships are summarized in Scheme 1. Scheme 1(*a*) assumes the presence of two entirely separate electron-transport systems both requiring NADPH, the linked Scheme 1(*b*) assumes that the oxidation system is similar in part but then diverges at a specific point to permit drug oxidation or lipid peroxide formation in lipids, and Scheme 1(*c*) assumes that lipid peroxides are an obligatory part of, and take part directly in, the hydroxylation process.

Most of the experimental evidence leads to the rejection of Scheme 1(*a*), two completely separate systems. The facts that both processes are located in the membranous vesicles (Table 5) and that both processes are destroyed by deoxycholate (Fig. 7) and inhibited by GSH and *p*-chloromercuribenzoate (Tables 3 and 4) weigh against Scheme 1(*a*). Further, it is clear that many substances metabolized by the microsomes such as aminopyrine or aniline can powerfully inhibit lipid peroxide formation while at the same time being oxidized themselves. Aminopyrine especially can regulate the rate of oxygen uptake, diverting it from generation of lipid peroxide to oxidation of the drug (Figs. 1 and 6). A simple interpretation of this effect would be to assume that both processes depend on the same electron-transport chain and that if a drug such as aminopyrine is not available oxygen is utilized to oxidize lipids of the membrane. In support of this concept, May & McCay (1968) have demonstrated that polyunsaturated fatty acids, components of phosphatidylethanolamine and phosphatidylcholine, are oxidized as a result of the operation of the NADPH electron-transport chain. If, however, an oxidizable compound is available, then it is oxidized preferentially, possibly because of its favourable spacial alignment within the transport chain. Such evidence is in favour of Scheme 1(*b*).

Evidence so far obtained is against Scheme 1(*c*) because peroxidized microsomes are much less, not more, effective in hydroxylating aminopyrine or aniline (Table 6 and Fig. 8). However, it should be emphasized that the method used for measurement of peroxidation, the thiobarbituric acid method, determines a breakdown product of peroxidized unsaturated fatty acids, malonaldehyde (Wills,

Table 5. *Aminopyrine oxidation and lipid peroxide formation in subfractions of microsomes*

Microsomes were fractionated (Wills, 1969*a*) into a sediment, fluffy layer and supernatant. The sediment and fluffy layer were suspended in 0·125M-KCl and the supernatant was used as prepared. Rates of lipid peroxide formation and aminopyrine oxidation were measured with 1 ml. of microsomal suspension, or resuspended fraction, in a total volume of 5 ml.

Fraction	Concn. of protein (mg./ml.)	Lipid peroxide formation (nmoles of malonaldehyde/min./mg. of protein)	Aminopyrine oxidation: Rate (nmoles of formaldehyde/min.)	Aminopyrine oxidation: Rate in relation to protein content (nmoles of formaldehyde/min./mg. of protein)
Whole microsomes	1·84	4·10	23·50	12·7
Supernatant	1·20	0·96	1·83	1·5
Fluffy layer	0·89	4·82	17·70	19·9
Sediment	0·70	0·95	0	0

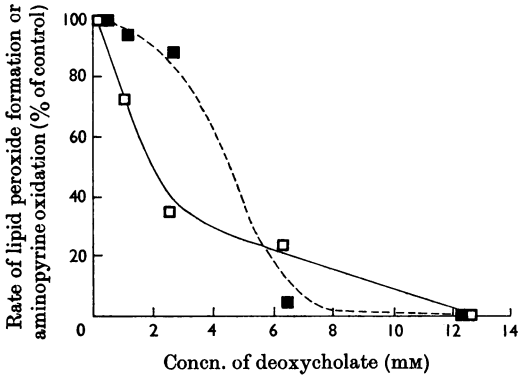


Fig. 7. Comparison of the effects of deoxycholate on the oxidation of aminopyrine and lipid peroxide formation by microsomes. Rates are expressed as percentages of control values, which were 19·7 nmoles of formaldehyde/min. for the oxidation of aminopyrine and 4·06 nmoles of malonaldehyde/min. for lipid peroxide formation. □, Aminopyrine oxidation; ■, lipid peroxide formation.

Table 6. *Effect of lipid peroxide formation on the oxidative demethylation of aminopyrine by microsomal suspensions*

A suspension of microsomes (20 ml. containing 8·4 mg. of protein/ml.) in a mixture containing KCl (125 mM), and sodium phosphate buffer, pH 7·0 (40 mM), was divided into four equal parts. One was kept at 0°, another incubated at 37° without addition, a third incubated with NADPH (40 μM) + nicotinamide (50 mM) and a fourth incubated with ascorbate (0·5 mM). At the end of the incubation period (20 min.) lipid peroxide content and aminopyrine oxidation were measured.

Treatment of microsome suspension (20 min.)	Lipid peroxide content (nmoles of malonaldehyde/mg. of protein)	Aminopyrine oxidation (nmoles of formaldehyde/min.)
0° (no addition)	1·62	37·7
37° (no addition)	2·55	33·2
[NADPH (40 μM)]	15·25	17·0
[ascorbate (0·5 mM)]	17·68	14·4

1966). The actual peroxide concentration is not really measured and lipid peroxide may be produced as an unstable intermediate when a compound is undergoing hydroxylation as suggested in Scheme 1(*c*). There are some difficulties in accepting Scheme 1(*b*) in its simple form. One problem is the precise role of the non-haem iron that is essential for the lipid peroxide formation but not for aminopyrine oxidation (Fig. 6) (Wills, 1969*b*). A possible explanation is that cytochrome *P*-450 and an iron–protein complex present alternative pathways in the electron-transport chain, the cytochrome *P*-450 pathway leading to hydroxylation and the iron–protein complex leading to lipid peroxide formation. If such an iron–protein complex were available to ascorbate it would explain an important fact that ascorbate readily induces lipid peroxide formation but is an ineffective electron donor for hydroxylation (Ernster & Orrenius, 1966). However, it is possible to block aminopyrine oxidation by adding desferrioxamine in a very high concentration (Table 2), so that a component of the system, containing iron in a form that is more firmly bound to protein and less readily available to chelating agents than the iron complex necessary for lipid peroxide formation (Wills, 1969*b*), appears to be essential for hydroxylation. The pattern of inhibition by thiol reagents (Table 3) strongly resembles those observed for bacterial and plant ferredoxins (Malkin & Rabinowitz, 1967), so that this more firmly bound non-haem iron may be a

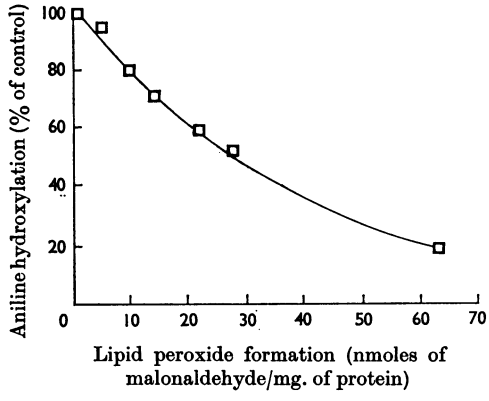
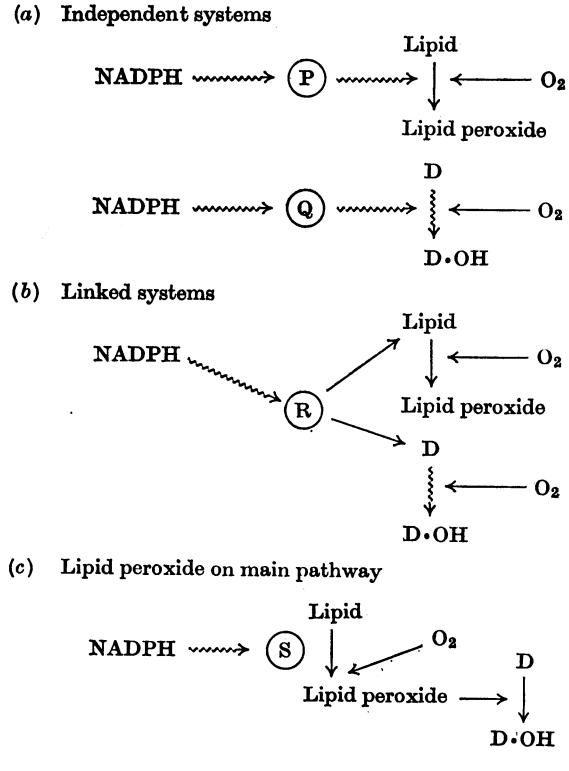


Fig. 8. Relation between lipid peroxide formation and the rate of aniline hydroxylation by irradiated microsomes. Suspensions of microsomes in KCl (125mM) containing 6·25mg. of protein/ml. were irradiated with 0, 5, 10, 20, 50 and 100 krads. Each suspension was then incubated for 60min. at 37°.



Scheme 1. Diagram of possible relationships between lipid peroxide formation and hydroxylation by microsomes. P, Q, R and S are unspecified components of the electron-transport chain; D is a compound undergoing hydroxylation.

protein of this type and be essential for both peroxidation and hydroxylation.

An additional difficulty in interpretation is provided by the wide variety of effects of different compounds on lipid peroxide formation. Thus aminopyrine and phenobarbitone are both readily oxidized by microsomes, and yet aminopyrine strongly inhibits peroxide formation whereas phenobarbitone is relatively ineffective (Fig. 3). Codeine is intermediate in its effect on lipid peroxide formation. Clearly it does not follow that substances that undergo hydroxylation necessarily inhibit lipid peroxide formation. A study of the list of compounds most strongly inhibiting lipid peroxide formation shows that phenols and aromatic amines are the most effective. Many of these compounds are well established as antioxidants and presumably are able to prevent oxidation of unsaturated lipids of the membranes, and other compounds such as benzene are metabolized to an antioxidant, phenol, and would therefore act in a similar manner. The different effects observed on lipid peroxide formation may therefore depend mainly on the antioxidant capacity of the particular compound rather than on the capacity of the microsomes to metabolize it.

It is well established (Ernster & Orrenius, 1966) that injection of several drugs can cause proliferation of microsome membranes and an increased rate of hydroxylation, and that subsequently this is followed by involution of the membranes. It is virtually certain that lipid peroxide formation leads to membrane disintegration, and possibly this is a normal metabolic process that follows a pathway similar to that shown in Scheme (1*b*) before membrane remodelling when high concentrations of drugs or other compounds are unavailable. The possibility that lipid peroxides are essential for actual hydroxylation cannot, however, be excluded at this stage.

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