Promethazine inhibits the formation of aldehydic products of lipid peroxidation but not covalent binding resulting from the exposure of rat liver fractions to CCl₄

Giuseppe POLI,* Kevin H. CHEESEMAN,† Fiorella BIASI,* Elena CHIARPOTTO,* Mario U. DIANZANI,* Hermann ESTERBAUER‡ and Trevor F. SLATER†

*Department of Experimental Medicine and Oncology, University of Turin, Corso Raffaello 30, 10125 Turin, Italy,

†Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex UB8 3PH, U.K.,

and ‡Institute of Biochemistry, University of Graz, Halbarthgasse 5/I, A-8010 Graz, Austria

Promethazine is known to have protective activity in relation to CCl₄-induced liver necrosis. This hepatoprotective property has been investigated with regard to the free radical scavenging and antioxidant properties of promethazine using isolated hepatocytes and microsomal suspensions. CCl₄ is activated in both systems to free radical metabolites that bind covalently to lipid and protein, and initiate lipid peroxidation. A large number of carbonyl products is produced during CCl₄-induced lipid peroxidation; promethazine strongly inhibits the production of all classes of carbonyl compounds in both microsomal suspensions and isolated hepatocytes. In contrast, promethazine is a very weak inhibitor of the covalent binding of metabolites of CCl₄. We conclude that promethazine acts by scavenging the trichloromethylperoxyl radical and lipid peroxyl radicals, and is a weak scavenger of the trichloromethyl radical. These data, when considered together with the hepatoprotective effects of promethazine, suggest that lipid peroxidation is of relatively more importance than covalent binding in the pathogenesis of CCl₄-induced liver necrosis.

INTRODUCTION

Intoxication with CCl₄ leads to well-characterized fatty degeneration and centrilobular necrosis of the liver (see Slater, 1972a, for review), and the pro-oxidant effect of the haloalkane has been invoked to explain, at least in part, the pathogenesis of this specific injury (see Slater, 1966a, 1982; Recknagel, 1967). The biochemical characterization of the CCl₄-induced stimulation of lipid peroxidation was first studied extensively using rat liver microsomes (Slater & Sawyer, 1971a,b,c), and has been investigated more recently using isolated intact hepatocytes (Poli *et al.*, 1979, 1983a). The latter model allows a more physiological but still relatively simple approach to the problem.

The phenothiazine drug promethazine is of special interest among the wide variety of antioxidants tested to investigate the role of lipid peroxidation in the pathogenesis of CCl₄-induced liver cell injury. It was the first antioxidant clearly demonstrated to have hepatoprotective properties against the necrogenic action of CCl₄ (Rees *et al.*, 1961) and against changes produced by CCl₄ in the endoplasmic reticulum *in vivo* (Slater & Sawyer, 1969). This protective effect, confirmed by Reddrop *et al.* (1983), has been connected with the strong inhibition shown by the drug of malonaldehyde (MDA) production stimulated by CCl₄ in rat liver microsomes (Slater & Sawyer, 1971b).

The stimulus for the present study of the antioxidant effects of promethazine on the formation of the aldehydic derivatives of lipid peroxidation is the rapidly increasing interest in the general biological involvement of this class of biochemical intermediates (see Esterbauer, 1985;

Slater, 1987). In this paper we report on the very efficient inhibition exerted by promethazine on CCl₄-induced aldehyde formation in rat liver microsomes and in isolated rat hepatocytes. Moreover, special emphasis is given to the fact that the strong antioxidant effect of the phenothiazine contrasts with its minimal ability to modify the covalent binding of CCl₄ metabolites.

EXPERIMENTAL

Materials

Chemicals were obtained from Boehringer–Mannheim (Mannheim, Germany) and Sigma Chemical Company (St. Louis, MO, U.S.A.).

¹⁴CCl₄ (sp. radioactivity 26.9 mCi/mmol; radiochemical purity 99%) was obtained from The Radiochemical Centre, Amersham (Bucks., U.K.), and promethazine hydrochloride was kindly provided by May & Baker (Dagenham, Essex, U.K.). Solvents, t.l.c. plates and all other chemicals (analytical grade) were purchased from Merck (Darmstadt, Germany).

Male Wistar rats (180–220 g body wt.) were used; they were fed on standard laboratory diet (Piccioni, Brescia, Italy) containing 12000 units of vitamin A and 40 units of vitamin E/kg.

Incubation procedures

Microsomes were prepared from rat livers as described by Slater & Sawyer (1971a) after the animals had been deprived of food overnight (16 h). Microsomal incubations were carried out for 15 min at 37 °C in the presence of CCl₄ (8.6 mM) in a defined medium (Slater & Sawyer, 1971a) containing a NADPH-generating system; CCl₄

was omitted from the control incubations. The gas phase was air.

Hepatocytes were prepared as previously described (Poli *et al.*, 1981) and incubated for 60 min (when not otherwise indicated) in closed 50 ml flasks at 37 °C in 3 ml of a defined balanced salt solution (Poli *et al.*, 1979) at 5×10^6 cells/ml in the presence of CCl_4 , which was added to the centre well and allowed to diffuse into the closed system; the gas phase was air. Cell viability was checked before and after the incubation by Trypan Blue exclusion and by the release of lactate dehydrogenase (Poli *et al.*, 1979). When required, promethazine hydrochloride was added directly to the microsomal or cell suspensions at final concentrations of 10 or 100 μM respectively.

The O_2 concentration in the aqueous media used for incubations of microsomal suspensions and hepatocytes was in the range that is known to be appropriate for studies on lipid peroxidation *in vitro* (Lumper *et al.*, 1968; Slater, 1972a; Noll & De Groot, 1984; De Groot & Noll, 1986), and was similar to O_2 concentrations in normal portal venous blood *in situ* (Kessler *et al.*, 1984; Lemasters *et al.*, 1986).

Preparation and extraction of the 2,4-dinitrophenylhydrazine derivatives of carbonyl-compounds

The aldehydic compounds formed during microsomal and hepatocyte incubations in the presence of CCl_4 , with or without the addition of promethazine, were allowed to react with 2,4-dinitrophenylhydrazine (DNPH); the hydrazone derivatives were then extracted according to the procedure previously described (Esterbauer *et al.*, 1982; Poli *et al.*, 1985).

Quantitative determination and h.p.l.c. analysis of the individual carbonyl classes

To separate the complete mixture of dinitrophenylhydrazones into polar and non-polar classes, the aldehydic extract was separated by t.l.c. (silica gel 60, precoated 20 cm $\times$ 20 cm plates; Merck) with dichloromethane as first developer and benzene as a second developer (Esterbauer *et al.*, 1982). All compounds with an R_f greater than or equal to that of 4-hydroxynonenal (HNE) are hydrazones of medium to low polarity and are called non-polar hydrazones from here on; compounds with lower R_f values are called polar hydrazones. The non-polar hydrazones were separated by t.l.c. into individual classes corresponding to 4-hydroxyalkenals (zone I), osazones (zone II) and alkanals, alkenals and ketones (zone III).

The assignment of various bands into certain carbonyl classes was done by comparison with known standards (Esterbauer *et al.*, 1982). The individual fractions were scraped off the plates and eluted three times with 10 ml of methanol, the eluate being collected by centrifugation. The material remaining at the origin was adjusted to 1 ml in methanol, whereas the three non-polar fractions (hydroxyalkenals, osazones and alkanals etc.) were further purified by t.l.c. and collected as in the first step, then adjusted to 1 ml of methanol. The total carbonyl content of each fraction was estimated spectrophotometrically by recording u.v.-visible spectra and calculating the concentration from the absorbance at 365 nm using an average molar absorption coefficient of 25000 litre $\cdot$ mol $^{-1}$ $\cdot$ cm $^{-1}$ (Esterbauer *et al.*, 1982).

The individual hydrazones of each fraction were sepa-

rated by h.p.l.c. with the conditions indicated in the Figure legends. Peak quantification was achieved by separating authentic standards under identical conditions.

Other analytical procedures

MDA was measured by the thiobarbituric acid assay (Slater & Sawyer, 1971a) which, under these conditions, gives results corresponding closely to direct h.p.l.c. measurements of MDA (Esterbauer & Slater, 1981). $^{14}\text{CCl}_4$ covalent binding to liver microsomal proteins and to total lipid or protein of isolated hepatocytes was measured using procedures described elsewhere (Cheeseman, 1982a; Poli *et al.*, 1983b).

Fig. 1. U.v.-visible spectra of zone I carbonyls (4-hydroxyalkenals) from liver microsomal suspensions

B, Reagent blank; 1, time zero; 2, control; 3, 10 μM -promethazine; 4, 8.6 mM- CCl_4 ; 5, promethazine plus CCl_4 . For the analytical details, see the Materials and methods section. The results shown are representative of four similar experiments.

Table 1. Effect of promethazine on the formation of MDA and HNE by rat liver microsomes incubated in the presence or in the absence of CCl_4

Liver microsomal suspensions were incubated for 60 min at 37 °C. Values are means $\pm$ S.E.M. of four or five experiments. Values for the CCl_4 -treated group are significantly increased compared with the corresponding control value as evaluated by the Wilcoxon test ($P < 0.01$).

	MDA (nmol/mg of protein)	HNE (nmol/mg of protein)
Control	0.76 $\pm$ 0.09	0.94 $\pm$ 0.57
Promethazine (10 μM)	0.23 $\pm$ 0.57	0.55 $\pm$ 0.19
CCl_4 (8.6 mM)	1.74 $\pm$ 0.55	1.46 $\pm$ 0.58
CCl_4 + promethazine	0.38 $\pm$ 0.04	0.54 $\pm$ 0.30

RESULTS

Microsome suspensions

The addition of promethazine to microsomal suspensions led to a very strong reduction in the CCl₄-stimulated generation of both polar and non-polar aldehydes. This is illustrated in Fig. 1 for the hydroxyalkenal fraction (zone I carbonyls) separated by t.l.c. On the basis of the absorbance at 365 nm, CCl₄ stimulates the formation of hydroxyalkenals about 2-fold over the level found in microsomes incubated in the absence of CCl₄

Fig. 2. Inhibition by promethazine of aminopyrene demethylase activity (▲), CCl₄-dependent MDA production (●) and covalent binding of ¹⁴CCl₄ to protein (○) in rat liver microsomes

Results are means ± S.D. of four or five experiments.

but in the presence of NADPH. Promethazine, at a concentration of 10 μM, completely suppressed the CCl₄-stimulated production of hydroxyalkenals, as well as that due to endogenous (NADPH-stimulated) lipid peroxidation. Promethazine also totally inhibited the formation of zone II carbonyls, and partially inhibited the production of zone III carbonyls. The inhibitory effect of promethazine on zone I carbonyls is also seen when one particular hydroxyalkenal, HNE, is measured directly by h.p.l.c. (Table 1) and is comparable with the strong inhibition that is produced by promethazine of CCl₄-stimulated MDA production (Table 1; Slater & Sawyer, 1971c).

Although promethazine shows a strong inhibition of the lipid peroxidation process triggered by CCl₄, this compound is a poor inhibitor of the haloalkylation of protein that occurs simultaneously with lipid peroxidation in CCl₄ poisoning. In fact, the same concentration of promethazine that was found to be strikingly effective as an antioxidant (10 μM) reduces covalent binding of CCl₄ metabolites to microsomal protein by no more than 10–20 %, and the extent of inhibition of covalent binding was only about 30 % in the presence of 100 μM-promethazine (Fig. 2).

Isolated hepatocytes

The formation of a wide range of carbonyl products of lipid peroxidation in isolated hepatocytes poisoned with CCl₄ has been previously demonstrated by us (Poli *et al.*, 1985) and is confirmed here and, in addition, the effects of promethazine are shown (Table 2). The stimulation by CCl₄ of the production of MDA and of polar and non-polar carbonyls was effectively suppressed by the addition of promethazine to the cell incubation medium at a concentration of 100 μM (Table 2). This concentration was chosen on the basis of previous results achieved in the intact hepatocyte model showing a complete inhibition by this phenothiazine of CCl₄-stimulated MDA production at concentrations between 50 and 100 μM (Poli *et al.*, 1978; Poli & Gravela, 1982). As was found with liver microsomes, the stimulation of hydroxyalkenal production by CCl₄ was totally prevented by promethazine as measured by the absorbance at 365 nm of the hydrazone derivatives separated by t.l.c. (Table 2). This strong inhibition was confirmed in the case of HNE

Table 2. Effect of promethazine on the formation of MDA and other carbonyl compounds by isolated rat hepatocytes incubated in the presence or in the absence of CCl₄

Hepatocytes were incubated at 37 °C for 60 min. Promethazine and CCl₄ concentrations were 100 μM and 129 μM respectively. Values are means ± S.D. of four to seven experiments. *, †, significant differences compared with control and CCl₄ groups respectively by Student's *t* test; *P* < 0.01.

Compound	Carbonyl compounds (nmol/10 ⁸ cells)			
	Zero time	Control	CCl ₄	CCl ₄ + promethazine
MDA	7 ± 1	32 ± 5	87 ± 16*	30 ± 3†
Alkanals, ketones etc. (III)	150 ± 33	155 ± 34	303 ± 37*	196 ± 32†
Osazones (II)	35 ± 16	28 ± 14	46 ± 7*	36 ± 15
Hydroxyalkenals (I)	35 ± 7	46 ± 15	91 ± 20*	42 ± 28†
Total non-polar carbonyls	220 ± 39	229 ± 42	440 ± 62*	274 ± 71†
Polar carbonyl compounds	322 ± 130	556 ± 137	404 ± 112	153 ± 24†

Fig. 3. Zone I carbonyls from liver cell suspensions poisoned with CCl_4 in the absence (a) and in the presence (b) of promethazine

The final concentrations of the two compounds in the suspension were $129 \mu\text{M}$ - CCl_4 and $100 \mu\text{M}$ -promethazine. A, 4-OH-hexenal; B, 4-OH-nonanal.

Table 3. Effect of promethazine on CCl_4 covalent binding to total protein and lipid of intact rat hepatocytes

Hepatocytes were incubated at 37°C for 60 min in the presence of CCl_4 plus or minus promethazine. Values are means $\pm$ s.d. of four experiments. Values from (CCl_4 + promethazine)-treated hepatocytes are not significantly modified compared with the corresponding control value as evaluated by Student's *t* test.

	Protein binding (d.p.m./ 10^6 cells)	Lipid binding (d.p.m./ 10^6 cells)
CCl_4 ($129 \mu\text{M}$)	392 ± 27	225 ± 24
CCl_4 ($129 \mu\text{M}$) + promethazine ($100 \mu\text{M}$)	329 ± 30	243 ± 37

by h.p.l.c. analyses (Fig. 3). The CCl_4 -induced increase in carbonyls yielding osazones with DNPH (i.e. α -dicarbonyls and 2-hydroxyaldehydes) and in the alkanals, 2-alkenals and ketones was markedly and almost completely prevented by promethazine (Table 2).

Promethazine, even at a concentration of $100 \mu\text{M}$, was consistently ineffective in preventing the covalent binding of CCl_4 metabolites to both protein and lipid in whole hepatocytes (Table 3). Higher concentrations of promethazine cannot be used because of the cytotoxicity of this amphipathic material at concentrations greater than about $150 \mu\text{M}$ (see Slater, 1972b).

DISCUSSION

Promethazine was reported by Rees *et al.* (1961) to substantially reduce the extent of liver necrosis in rats given CCl_4 , but to have little effect on the increased content of triacylglycerols that is another feature of this type of liver injury. The effects of promethazine in the rat in relation to CCl_4 -induced liver injury were further studied by Reddrop *et al.* (1983) who described a number of contributory mechanisms for protective action. These

included a significant decrease in body temperature when promethazine and CCl_4 were given together to rats, a decreased rate of uptake of CCl_4 into the blood and liver in the presence of promethazine, an increased respiratory rate and exhalation of CCl_4 , and scavenging of reactive free radicals by promethazine. In addition, Reddrop *et al.* (1983) reported the concentrations of promethazine that occur in the liver 1–2 h after administration to rats to be in the range of 10 – $50 \mu\text{M}$.

CCl_4 has been shown (see Slater, 1982) to damage the liver by a variety of mechanisms. One mechanism of considerable importance is the pro-oxidant action of CCl_4 resulting in a stimulation of lipid peroxidation (Comporti *et al.*, 1965; Ghoshal & Recknagel, 1965; Slater, 1966b). It is of interest to note, therefore, that promethazine inhibits this CCl_4 -mediated lipid peroxidation both *in vivo* (Jose & Slater, 1972) and *in vitro* (Slater & Sawyer, 1971a) at concentrations compatible with those known to occur in the whole animal after dosing (Reddrop *et al.*, 1983). Another contributory mechanism to the overall injury produced in the liver by CCl_4 is known to be covalent binding (see Reynolds & Yee, 1968). However, promethazine, whilst protecting the liver against necrosis *in vivo*, has very little effect on covalent binding of CCl_4 to microsomes (Cheeseman, 1982a,b). In earlier studies it was difficult to understand these different effects of promethazine on lipid peroxidation and covalent binding, since both phenomena were thought to be due to the same reactive species, the trichloromethyl radical $\cdot\text{CCl}_3$. The discovery (Packer *et al.*, 1978), that $\cdot\text{CCl}_3$ reacts very rapidly with molecular O_2 to give the much more reactive $\text{CCl}_3\text{OO}\cdot$ species allows the interpretation that it is this peroxy species that initiates lipid peroxidation, whilst $\cdot\text{CCl}_3$ is largely responsible for covalent binding (Slater, 1982). Subsequent studies showed that $\text{CCl}_3\text{OO}\cdot$ reacts very rapidly with polyunsaturated fatty acids to initiate lipid peroxidation (Forni *et al.*, 1983) and with promethazine (the second-order rate constants for the interactions of $\text{CCl}_3\text{OO}\cdot$ with oleic, linoleic, linolenic and arachidonic acids were respectively 1.7 , 3.9 , 7.0 and $7.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$; the corresponding value for the interaction of $\text{CCl}_3\text{OO}\cdot$

with promethazine was $4.5 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$; Packer *et al.*, 1980); the interaction of $\cdot\text{CCl}_3$ with promethazine is relatively very slow.

The main effect of promethazine on CCl_4 -mediated damage in microsomal suspensions appears to be by scavenging $\text{CCl}_3\text{OO}\cdot$ -free radicals that would otherwise initiate lipid peroxidation and most probably also by scavenging lipid peroxy radicals, since promethazine also has antioxidant effects in other lipid peroxidation systems (Slater, 1968; Cheeseman, 1982a,b). An alternative possibility is that promethazine essentially blocks metabolic activation of CCl_4 to $\cdot\text{CCl}_3$ by the cytochrome P_{450} system, but this is ruled out for three main reasons. Firstly, promethazine does not significantly affect covalent binding of CCl_4 to microsomes (Fig. 2), a process dependent on the production of $\cdot\text{CCl}_3$. Secondly, the effects of promethazine on CCl_4 -stimulated lipid peroxidation in microsomes occur at a much lower concentration than that causing inhibitory effects on cytochrome P_{450} -linked drug metabolism (Fig. 2). Thirdly, promethazine ($50 \mu\text{M}$) has no significant effect on the formation of the spin adduct of $\cdot\text{CCl}_3$ with phenyl-*t*-butyl nitron, as demonstrated by e.s.r. spectroscopy (results not shown).

Another possible mechanism for promethazine action is related to the inhibitory action of phenothiazines on calmodulin and hence on Ca^{2+} movements (Uzanov *et al.*, 1974; Norman *et al.*, 1979). Although this is probably not of significance in the microsomal studies, it is a factor to consider in isolated hepatocytes where disturbances of Ca^{2+} transport have been proposed to be of significance to cell injury (Moore *et al.*, 1976; Schanne *et al.*, 1979), but this still remains controversial (see Smith & Sandy, 1985). A remote possibility that promethazine exerts its effects on the production of aldehydes by combining directly with them can be unequivocally ruled out for two reasons. Firstly, promethazine inhibits lipid peroxidation at very low final concentrations (EC_{50} $0.6 \mu\text{M}$; Slater & Sawyer, 1971c; see also Fig. 2), much lower than the concentration of aldehydes produced in its absence. Secondly, direct addition of promethazine to 4-hydroxy-alkenals has no effect on the u.v. absorption spectra, or on t.l.c. separations.

Lipid peroxidation is an important feature of CCl_4 -mediated damage to microsomal suspensions, to isolated hepatocytes and to liver *in vivo*. Important products of lipid peroxidation are the 4-hydroxy-alkenals; these have many important biological properties (see Esterbauer, 1985). It has been proposed that the formation of such aldehydes in the endoplasmic reticulum, followed by diffusion into the cytosol and extracellular space, permits a widespread diffusion of damaging events (Slater, 1976). In consequence, any attempt to attenuate the damaging effects of CCl_4 and similar compounds should be aimed at decreasing the production of these biologically reactive carbonyls.

In this study we have shown that promethazine has a very strong inhibitory action on the production of carbonyl products of lipid peroxidation in general and of 4-hydroxyalkenals in particular, both in microsomes and in isolated hepatocytes. This is the first study to show that a protective antioxidant decreases the production of cytotoxic aldehydes in liver cells exposed to a hepatotoxic agent (CCl_4). Evaluation of the results obtained suggests that this effect of promethazine is via a scavenging action on the reactive peroxy intermediate $\text{CCl}_3\text{OO}\cdot$

that can initiate lipid peroxidation followed by breakdown of lipid hydroperoxides to carbonyls, as well as by scavenging lipoperoxyl radicals that carry the chain reaction. In both microsomes and isolated hepatocytes there was no significant effect of promethazine on covalent binding, which suggests that this aspect of CCl_4 metabolism is of relatively lesser significance in the production of necrosis *in vivo* than is lipid peroxidation.

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REFERENCES

- Cheeseman, K. H. (1982a) in *Free Radicals, Lipid Peroxidation and Cancer* (McBrien, D. C. H. & Slater, T. F., eds.), pp. 196–214, Academic Press, London
- Cheeseman, K. H. (1982b) Ph.D. thesis, Brunel University, Uxbridge, U.K.
- Comporti, M., Saccocci, C. & Dianzani, M. U. (1965) *Enzymologia* **29**, 185–204
- De Groot, H. & Noll, T. (1986) *Biochem. Pharmacol.* **35**, 15–19
- Esterbauer, H. (1985) in *Free Radicals in Liver Injury* (Poli, G., Cheeseman, K. H., Dianzani, M. U. & Slater, T. F., eds.), pp. 149–158, IRL Press, Oxford
- Esterbauer, H. & Slater, T. F. (1981) *IRCS Med. Sci.* **9**, 749–750
- Esterbauer, H., Cheeseman, K. H., Dianzani, M. U., Poli, G. & Slater, T. F. (1982) *Biochem. J.* **208**, 129–140
- Forni, L. G., Packer, J. E., Slater, T. F. & Willson, R. L. (1983) *Chem.-Biol. Interact.* **45**, 171–177
- Ghoshal, A. K. & Recknagel, R. O. (1965) *Life Sci.* **4**, 1521–1530
- Jose, P. J. & Slater, T. F. (1972) *Biochem. J.* **128**, 141P
- Kessler, M., Höper, J., Harrison, D. K., Skolasinska, K., Klövekora, W. P., Sebening, F. & Volksholz, H. J. (1984) in *Oxygen Transport to Tissue* (Lübbers, D. W., Acker, H., Leniger-Follert, E. & Goldstick, T. K., eds.), vol. 5, pp. 69–80, Plenum, New York
- Lemasters, J. J., Ji, S. & Thurman, R. G. (1986) in *Regulation of Hepatic Metabolism: Intra- and Intercellular Compartmentation* (Thurman, R. G., Kauffman, F. C. & Jungermann, K., eds.), pp. 159–184, Plenum, New York
- Lumper, von L., Plock, H. J. & Standinger, H. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1185–1190
- Moore, L., Davenport, G. R. & London, E. J. (1976) *J. Biol. Chem.* **251**, 1197–1201
- Noll, T. & De Groot, H. (1984) *Biochim. Biophys. Acta* **795**, 356–362
- Norman, J. A., Drummond, A. H. & Moser, P. (1979) *Mol. Pharmacol.* **16**, 1089–1094
- Packer, J. E., Slater, T. F. & Willson, R. L. (1978) *Life Sci.* **23**, 2617–2620
- Packer, J. E., Willson, R. L., Bahnemann, D. & Asmus, K.-D. (1980) *J. Chem. Soc. Perkin Trans. 2*, 295–299
- Poli, G. & Gravela, E. (1982) in *Free Radicals, Lipid Peroxidation and Cancer* (McBrien, D. C. H. & Slater, T. F., eds.), pp. 215–242, Academic Press, London
- Poli, G., Poli-Chiono, M., Slater, T. F., Dianzani, M. U. & Gravela, E. (1978) *Biochem. Soc. Trans.* **6**, 589–591
- Poli, G., Gravela, E., Albano, E. & Dianzani, M. U. (1979) *Exp. Mol. Pathol.* **30**, 116–127
- Poli, G., Cheeseman, K. H., Slater, T. F. & Dianzani, M. U. (1981) *Chem.-Biol. Interact.* **37**, 13–24
- Poli, G., Chiarpotto, E., Albano, E., Biasi, F., Cecchini, G., Gravela, E. & Dianzani, M. U. (1983a) *Chem.-Biol. Interact.* **43**, 253–261

- Poli, G., Albano, E., Biasi, F., Chiarpotto, E. & Dianzani, M. U. (1983*b*) *FEBS Lett.* **160**, 187–190
- Poli, G., Dianzani, M. U., Cheeseman, K. H., Slater, T. F., Lang, J. & Esterbauer, H. (1985) *Biochem. J.* **227**, 629–638
- Recknagel, R. O. (1967) *Pharmacol. Rev.* **19**, 145–208
- Reddrop, C. J., Cheeseman, K. H. & Slater, T. F. (1983) *Cell Biochem. Funct.* **1**, 55–63
- Rees, K. R., Sinha, K. P. & Spector, W. G. (1961) *J. Pathol. Bacteriol.* **81**, 107–111
- Reynolds, E. S. & Yee, A. G. (1968) *Lab. Invest.* **19**, 273–281
- Schanne, F. A. X., Kane, A. B., Young, E. E. & Farber, J. L. (1979) *Science* **206**, 700–702
- Slater, T. F. (1966*a*) *Nature (London)* **209**, 36–40
- Slater, T. F. (1966*b*), *Biochem. J.* **101**, 16*p*
- Slater, T. F. (1968) *Biochem. J.* **106**, 155–160
- Slater, T. F. (1972*a*) *Free Radical Mechanisms in Tissue Injury*, Pion Ltd., London
- Slater, T. F. (1972*b*), in *A Companion to Biochemistry* (Bull, A. T., Lagnado, J. R., Thomas, J. O. & Tipton, K. F., eds.), pp. 511–551, Longmans Green, London.
- Slater, T. F. (1976) in *Recent Advances in Biochemical Pathology: Toxic Liver Injury* (Dianzani, M. U., Ugazio, G. & Sena, L. M., eds.), pp. 99–108, Minerva Medica, Torino
- Slater, T. F. (1982) in *Free Radicals, Lipid Peroxidation and Cancer* (McBrien, D. C. H. & Slater, T. F., eds.), pp. 243–270, Academic Press, London
- Slater, T. F. (1987) *Agents Actions* **22**, 333–334
- Slater, T. F. & Sawyer, B. C. (1969) *Biochem. J.* **111**, 317–324
- Slater, T. F. & Sawyer, B. C. (1971*a*) *Biochem. J.* **123**, 805–814
- Slater, T. F. & Sawyer, B. C. (1971*b*) *Biochem. J.* **123**, 815–821
- Slater, T. F. & Sawyer, B. C. (1971*c*) *Biochem. J.* **123**, 823–828
- Smith, M. T. & Sandy, M. S. (1985) *Toxicol. Appl. Pharmacol.* **81**, 213–219
- Uzanov, P., Shein, H. M. & Weiss, B. (1974) *Neuropharmacology* **13**, 377–391

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