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The Inhibitory Effects *in vitro* of Phenothiazines and Other Drugs on Lipid-Peroxidation Systems in Rat Liver Microsomes, and their Relationship to the Liver Necrosis Produced by Carbon Tetrachloride

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1. The effects of several phenothiazine derivatives on lipid-peroxidation systems in rat liver microsomes were studied and the results are considered in relation to the hepatotoxic action of carbon tetrachloride. 2. The lipid-peroxidation system coupled to $NADPH_2$ oxidation and stimulated by an ADP–Fe^{2+} mixture is strongly inhibited *in vitro* by promethazine (50% inhibition at 29 μM). Chlorpromazine and Stelazine also inhibit the peroxidation system but are less effective than promethazine. 3. The effects of promethazine on three other systems involving oxygen uptake (sulphite oxidation, orcinol oxidation and mitochondrial succinate oxidation) were also studied. Promethazine does not inhibit these systems to the same extent as it does the $NADPH_2$–ADP–Fe^{2+} lipid-peroxidation system. 4. Promethazine also produces an inhibition of the $NADPH_2$–ADP–Fe^{2+} system in liver microsomes after administration *in vivo*. It is concluded that the inhibition involves the interaction of the drug (or a metabolite of it) with the microsomal electron-transport chain. 5. Several other compounds known to protect the rat against liver necrosis after the administration of carbon tetrachloride were tested for inhibitory action on the $NADPH_2$–ADP–Fe^{2+} system. No clear correlation was observed between effectiveness *in vivo* as a protective agent and inhibitory effects on the $NADPH_2$–ADP–Fe^{2+} system *in vitro*. 6. Promethazine was found to inhibit the stimulation of lipid peroxidation produced in rat liver microsomes by low concentrations of carbon tetrachloride. This effect occurs at a concentration similar to that observed *in vivo* after administration of a normal clinical dose.

Suspensions of mitochondria or microsomes prepared from rat liver readily undergo lipid peroxidation in the presence of oxygen and various free radical initiators such as Fe^{2+}, vitamin C or GSH (Schneider, Smith & Hunter, 1964; Hochstein & Ernster, 1963; Wills, 1965). Lipid peroxidation may also be induced to occur in microsomal suspensions under certain conditions as a result of the oxidation of $NADPH_2$ by the enzyme system concerned with the metabolism of foreign compounds, the 'drug-detoxication system' (Hochstein & Ernster, 1963). This latter peroxidative pathway is markedly activated by the addition of ADP or other compounds containing the pyrophosphate group, and by the presence of low concentrations of Fe^{2+} (Beloff-Chain, Catanzaro & Serlupi-Crescenzi, 1963; Hochstein & Ernster, 1963; Hochstein, Nordenbrand & Ernster, 1964). This microsomal system involved in a lipid-peroxidation process is described in the present paper as the $NADPH_2$–ADP–Fe^{2+} system.

A single dose of carbon tetrachloride administered to a rat produces a considerable accumulation of fat in the liver and an extensive centrilobular necrosis (Cameron & Karunaratne, 1936). The necrogenic activity of carbon tetrachloride appears to be dependent on a limited metabolism of the lipophilic solvent in the liver to a more toxic product (Butler, 1961; Wirtschaft & Cronyn, 1964; Ghoshal & Recknagel, 1965; Slater, 1966; McLean & McLean, 1966). It has been suggested that this metabolism or 'activation' occurs in the endoplasmic reticulum, by an interaction of carbon tetrachloride with an endogenous radical. As a consequence of this activation, extraneous free radicals are produced that induce the formation of lipid peroxides in

neighbouring lipid-rich membranes. The suggestion has further been made that one possible route for such an activation stage with subsequent propagation of lipid peroxidation is by the $NADPH_2$–ADP–Fe^{2+} system that is increased shortly after giving a dose of carbon tetrachloride to rats (Slater, 1965).

To test the speculations outlined above, some additional properties of the $NADPH_2$–ADP–Fe^{2+} system were studied in the present investigation. The inhibitory actions on the microsomal peroxidation system of various drugs that are known to protect the liver *in vivo* against the necrosis induced by carbon tetrachloride were also studied.

METHODS

Adult albino female rats, body wt. approx. 150g., were used except where otherwise stated. They were killed by cervical dislocation. The liver was rapidly removed, weighed, homogenized in ice-cold 0·25M-sucrose and centrifuged for 10min. at 15000*g*. The supernatant was removed and centrifuged again under the same conditions; the pellets were rejected after both centrifugations. The supernatant so obtained was centrifuged at 200000*g* for 30min. The microsomal pellet was carefully rinsed with ice-cold 0·15M-KCl to remove any adhering supernatant, and then suspended in ice-cold 0·15M-KCl; the volume used was such that the microsomes obtained from 1g. wet wt. of liver were present in a volume of 1ml. Microsomal suspensions from 2-day-old albino rats, and from adult chickens of the Rhode Island strain, were prepared in an identical manner.

The $NADPH_2$–ADP–Fe^{2+} system was measured in microsomal suspensions by following oxygen uptake with a platinum electrode assembly of the Clark type (assemblies were obtained both from the Yellow Springs Instrument Co. Ltd., Yellow Springs, Ohio, U.S.A., and from Rank Bros., Bottisham, Cambs., and gave identical results). The electrode chamber contained 2·0ml. of 0·22M-KCl, 0·5ml. of 0·1M-tris–HCl buffer, pH8·0, and 0·1–0·3ml. of microsomal suspension. $NADPH_2$ (0·36μmole), ADP (2·3μmoles) and $FeSO_4$ (4μg.) were added with Agla microsyringes (Burroughs Wellcome Ltd., Beckenham, Kent). Uptake of O_2 was monitored with a Kent 2 mv recorder and is expressed as mμmoles of O_2/min./mg. of liver protein. Uptake of O_2 during the experiments with promethazine was also measured with a normal Warburg flask assembly. The simultaneous appearance of lipid peroxide was demonstrated by the thiobarbituric acid reaction (Bieri & Anderson, 1960; Saslow, Corwin & Waravdekar, 1966) and a colorimetric iodometric procedure (Swoboda & Lea, 1958).

The activity of the microsomal drug-detoxication system was measured by the procedure described by Orrenius (1965). Formaldehyde was measured by the method of Nash (1953).

Uptake of O_2 coupled to the oxidation (i) of orcinol (Slater, 1961) and (ii) of Na_2SO_3 (Fridovich & Handler, 1961) was measured in the oxygen electrode assembly with the following components: (i) orcinol, 21mM; 0·012N-NH_3 (final vol. 3·3ml.); (ii) sodium sulphite, 11mM; tris–HCl buffer, pH8·0, 300μmoles; $NADPH_2$, 0·36μmole; 0·2ml. of microsomal suspension (final vol. 3·6ml.). Succinate oxidation was measured with the following system: tris–HCl buffer, pH7·4, 200μmoles; sodium succinate, pH7·4, 35mM; 0·1ml. of mitochondrial suspension in 0·25M-sucrose–mM-EDTA (final vol. 2·8ml.).

The stimulatory effects of carbon tetrachloride on lipid peroxidation were studied in a cell-sap-plus-microsome suspension prepared as described above for the preparation of the microsomal fraction but with the final centrifuging at 200000*g* omitted. Portions (2·5ml.) of the following mixture were placed in the main compartments of Warburg flasks: 0·1M-tris, pH7·4, 12ml.; 0·1M-KCl, 18ml.; 0·1M-glucose 6-phosphate, 1·8ml.; nicotinamide, 200mg.; NADP, 6mg.; glucose 6-phosphate dehydrogenase (Boerhinger; 1mg./ml.) 0·06ml.; cell sap plus microsomes, 6ml. The drug was added as an aqueous solution (0·5ml.). The side arm either had no addition (control), or 2μl. of a carbon tetrachloride–liquid paraffin mixture (1:1, v/v) was added with a Hamilton syringe. The flasks were stoppered and incubated for 60min. at 37°. At the end of the reaction, 2ml. of the mixture was removed and mixed with 4ml. of 10% (w/v) trichloroacetic acid and left for 10min. in ice. After the mixture had been centrifuged, 2ml. of supernatant solution was mixed with 2ml. of 0·67% thiobarbituric acid and the mixture was placed in a boiling-water bath for 10min. After the mixture had cooled in ice the volume was adjusted to 5ml. by the addition of water and E_{535} was determined.

Estimations of the concentration of promethazine in liver microsomes were performed by the spectrofluorimetric method of Ragland & Kinross-Wright (1964).

The drugs used in this study were: chlordiazepoxide (Roche Products Ltd., Welwyn Garden City, Herts.; Librium) and the phenothiazine derivatives, promethazine, trifluoperazine (Smith, Kline and French Ltd., Welwyn Garden City, Herts; Stelazine), and chlorpromazine, which are used clinically as tranquillizers; phenobarbitone sodium, a hypnotic; cinchocaine (Nupercaine), a local anaesthetic; and cetrimide (Cetab), used as an antiseptic material.

RESULTS

The general conclusions reported by Hochstein & Ernster (1963) for the activity of the microsomal $NADPH_2$–ADP–Fe^{2+} system were confirmed; $NADPH_2$ cannot be replaced by $NADH_2$, and both a pyrophosphate group (as in ADP) and Fe^{2+} are necessary. The rapid utilization of oxygen by this system is accompanied by the production of material giving a positive thiobarbituric acid reaction, and by peroxide as measured iodometrically. The production of material yielding a positive thiobarbituric acid reaction was directly proportional to the production of peroxide as measured by the iodometric titration procedure.

With a microsomal suspension that had been 'aged' by standing in ice for approx. 2hr., the reaction rate (as measured by oxygen uptake) was linear with respect to the concentration of microsomes present in the final incubation mixture (range studied: 0–0·3ml. of microsome suspension added to a final volume of 3·3ml.).

The activity of microsomal suspensions often increased slightly on 'aging' in ice for several hours

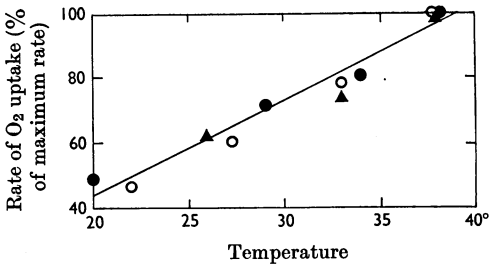

Fig. 1. Effect of temperature on oxygen uptake by the microsomal $NADPH_2$–ADP–Fe^{2+} system. The results are plotted as percentages of the maximum rate obtained at 39°, the highest temperature studied. The different symbols indicate experiments carried out on fresh microsomal suspensions on different days. The line shown is the regression line (regression coefficient r 0·95).

Table 1. *Activity of the $NADPH_2$–ADP–Fe^{2+} system at 22° in rat and chicken liver microsomes*

Mean values ± S.E.M. are given in terms of the oxygen uptake/min. by the microsomal equivalent of 1 g. wet wt. of whole liver.

	No. of animals	Uptake of O_2 (mμmoles/min./g. wet wt. of liver)
Rat liver		
Adult	8	780 ± 33
2-day-old	7*	44
Chicken liver	2	87

* Pooled sample from seven newborn rat livers.

but then decreased; considerable activity remained after 24hr. storage at 4°. In a few cases, however, with microsomes isolated from rats previously treated with carbon tetrachloride or promethazine, activity was virtually absent from the fresh suspension but then rapidly appeared about 1–2hr. after suspension of the microsomes in the cold 0·15M-potassium chloride medium. For comparative purposes, assays were performed on suspensions that had been 'aged' for 2hr. in ice after resuspension in cold 0·15M-potassium chloride.

The activity of the microsomal system, measured by oxygen uptake or by the production of thiobarbituric acid-positive material, displayed a small temperature coefficient. Fig. 1 shows the results found in three separate experiments.

With normal microsome suspensions, after 2hr. aging in ice and measuring activity at 22°, the oxygen consumption was 780 mμmoles/min./microsomal equivalent of 1 g. wet wt. of liver. The corresponding activities found in liver microsomes prepared from 2-day-old rats and from chickens were much lower (Table 1).

Promethazine strongly inhibited oxygen uptake by the microsomal system *in vitro* when present in

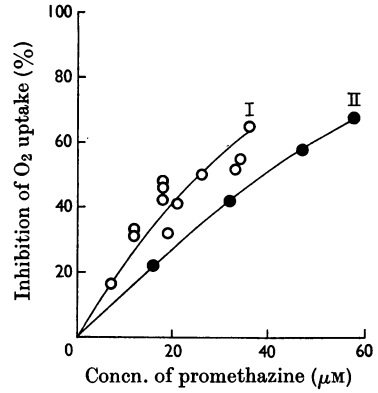

Fig. 2. Effect of promethazine on oxygen uptake by the microsomal $NADPH_2$–ADP–Fe^{2+} system. The experiments were performed at 22° and promethazine was added as a freshly prepared aqueous solution to the reaction mixture when about half the dissolved oxygen had been utilized. The ratio of the rate of oxygen uptake after promethazine addition to that obtained before addition was used to calculate the percentage inhibition produced by the drug. Freshly prepared microsomes were used for the experiments included in curve I, whereas the points on curve II were obtained after the microsomes had been aged overnight at 4°.

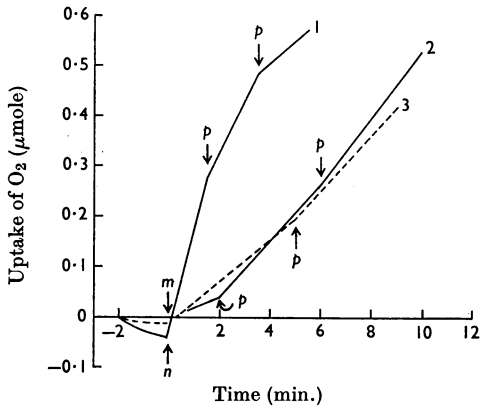

Fig. 3. Effects of promethazine on three systems involving oxygen uptake. Oxygen utilization was monitored with an oxygen electrode assembly. The experimental conditions for (1) orcinol oxidation, (2) sulphite oxidation and (3) succinate oxidation are given in the Methods section. Freshly prepared microsomes (0·2ml., equivalent to approx. 200mg. wet wt. of liver) were used in (2) and freshly prepared mitochondria (0·1ml., equivalent to approx. 50mg. wet wt. of liver) were used in (3). Promethazine (0·1ml.) was added at the times indicated by the arrows (p): for (1) and (3) the initial concentration of promethazine was 3·5mM, for (2) it was 35·3mM. The initial volumes of the incubation mixtures before the addition of promethazine were (1) 3·4ml.; (2) 3·6ml.; (3) 2·7ml. m, Addition of mitochondria; n, addition of aq. ammonia; p, addition of promethazine.

final concentrations of approx. 50 μM (Fig. 2). This effect was also demonstrable when oxygen uptake was measured in a Warburg apparatus, and after the microsomes had been aged overnight at 4°. Promethazine in similar concentrations had no inhibitory effect on two other systems involving oxygen uptake measured with the platinum-electrode assembly: mitochondrial succinate oxidase and sulphite oxidation (Fig. 3). Promethazine had an appreciable effect, however, on the oxidation of ammoniacal orcinol, a process known to be dependent on trace metals (Slater, 1961).

The mean activity of the $NADPH_2$–ADP–Fe^{2+} system in microsomal suspensions prepared from rats that had been dosed 1 hr. previously with 2·5 mg. of promethazine/100 g. body wt. was decreased considerably compared with controls injected with water alone. The results, with numbers of rats in parentheses, were: promethazine group, 35 ± 12 mμmoles of oxygen/min./mg. of protein (8); control group, 62 ± 9 (11). Attempts to determine the amount of promethazine in liver microsomes 1 hr. after intraperitoneal injection of 2·5 mg./100 g. body wt. were unsuccessful. The readings obtained by adding known amounts of promethazine to microsomal extracts before spectrofluorimetry indicated that the concentration of promethazine in the extracts from dosed rats was less than 0·5 μg. in the microsomal equivalent of 1 g. wet wt. of liver.

Promethazine also inhibited the microsomal-catalysed demethylation of aminopyrine, but the concentration required (0·3 mM gave a 30% depression) was much higher than that found to depress the $NADPH_2$–ADP–Fe^{2+} system strongly.

Fig. 4 gives comparative data for the action of a variety of agents on the microsomal $NADPH_2$–ADP–Fe^{2+} system. Many of these agents are known to inhibit the onset of necrosis after administration of carbon tetrachloride to rats or to prevent the fall in liver $NADPH_2$ content that occurs 1 hr. after dosing with the hepatotoxin. Most of the agents that protect the liver *in vivo* (see legend to Fig. 4) also inhibit the microsomal $NADPH_2$–ADP–Fe^{2+} system. There are, however, important exceptions. For example, Nupercaine and Cetab are effective protectors *in vivo* but have little action on the microsomal system *in vitro*.

Fig. 4 also shows that two other phenothiazine drugs tested (chlorpromazine and Stelazine) were also active in inhibiting the microsomal $NADPH_2$–ADP–Fe^{2+} system, but that higher concentrations were required to produce a given inhibition than was found for promethazine. The tranquillizing drug Librium, which is not a phenothiazine derivative, was much less active in inhibiting the $NADPH_2$–ADP–Fe^{2+} system than was Stelazine, the least active phenothiazine tested.

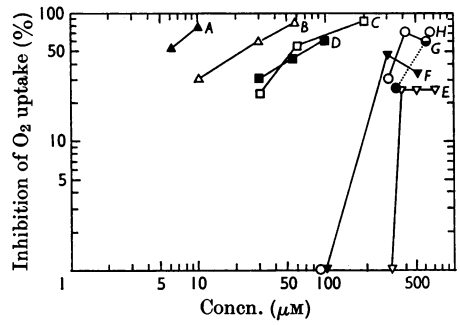

Fig. 4. Inhibitory action of various drugs on the microsomal $NADPH_2$–ADP–Fe^{2+} system. The assays were carried out at 22–24°. All solutions were made up freshly immediately before use. The inhibitory agents were added to the reaction mixture when about half the dissolved oxygen had been utilized. The rates of oxygen uptake before and after the addition of the drug were measured and the percentage inhibition calculated. The results are plotted on a log–log scale. The drugs used were: *A*, propyl gallate; *B*, promethazine; *C*, aminoacetonitrile hydrogen sulphate; *D*, chlorpromazine; *E*, phenobarbitone sodium; *F*, Nupercaine; *G*, Librium; *H*, Cetab. Drugs *B*, *D*, *F* and *H* have been reported to protect rats against liver necrosis assessed histologically 24 hr. after the administration of a single dose of CCl_4 (see Bangham *et al.* 1962). The results shown represent in each case the average inhibitory effects found in a series of experiments with different preparations of microsomes. Individual points have been omitted to aid clarity of representation. However, full details for one drug (promethazine) are shown for example in Fig. 2.

Table 2. *Effect of various agents on the formation of material giving a positive thiobarbituric acid reaction in suspensions of cell sap and microsomes*

The amount of material produced during incubation and giving a positive reaction with thiobarbituric acid in the absence of any drug or CCl_4 is taken as the control blank value of 100%. The effects of added drugs on this 'blank production' are expressed as percentage inhibitions. The stimulatory effect of CCl_4 on the production of thiobarbituric acid-positive material compared with that produced in the blanks is arbitrarily taken to be a 100% stimulation. The effects of drugs on this CCl_4-induced stimulation are again expressed as percentage inhibitions.

Drug	Final concn. (μM)	Blank (% of control blank)	Inhibition of stimulation produced by CCl_4 (%)
Promethazine	0·001	100	40
	0·01	100	100
	2	27	100
Vitamin E	1	100	100
Phenobarbitone sodium	0·1	100	10
	5	140	100
p-Chloromercuribenzoate	90	121	−80*

* *p*-Chloromercuribenzoate increased the stimulatory action of CCl_4 on lipid peroxidation by 80%.

Low concentrations of carbon tetrachloride are known to increase the spontaneous lipid peroxidation that occurs on incubating mixtures of microsomes and cell sap (Ghoshal & Recknagel, 1965; Comporti, Saccocci & Dianzani, 1965). Promethazine is very active in suppressing the spontaneous peroxidation that occurs on incubating tissue fractions at 37° and, at lower concentrations, inhibits the stimulatory action of carbon tetrachloride. Table 2 gives some relative data on this effect.

DISCUSSION

Many of the drugs used on this study are surface-active and will lower the surface tension of Ringer's solution (Seeman & Bialy, 1963) and the interfacial tension of thin films (Bangham, Rees & Shotlander, 1962). It was thought possible that the powerful inhibition of oxygen uptake via the $NADPH_2$–ADP–Fe^{2+} system produced, for example, by promethazine, might be the result of its surface activity affecting the electrode membrane in a similar way to the action of surface-active agents on reduction potentials at the mercury electrode (Mancy & Okun, 1960). However, this possibility is ruled out for three reasons. First, a similar inhibitory effect by promethazine was found when oxygen uptake was measured in a conventional Warburg assembly. Secondly, promethazine had no similar inhibitory action on oxygen uptake via the succinate oxidase and sulphite oxidase routes monitored by the oxygen electrode. The lack of inhibition on sulphite oxidation at concentrations of approx. 100 μM rules out the possibility that promethazine is inhibiting the $NADPH_2$–ADP–Fe^{2+} system at lower concentrations by forming a complex with oxygen, a reaction known to occur at high phenothiazine concentrations (Martin, Price & Gudzinowicz, 1963). Thirdly, the relative activities of the four tranquillizers tested are not in the same sequential order as found for their action in decreasing surface tension (Seeman & Bialy, 1963). Thus it is probable that promethazine reacts directly with some component of the $NADPH_2$–ADP–Fe^{2+} system, thereby producing a diminished rate of oxygen uptake.

It has been suggested that the drug-metabolizing system and the $NADPH_2$–ADP–Fe^{2+} system involve several common steps (Orrenius, Dallner & Ernster, 1964). If this is true then promethazine must inhibit the $NADPH_2$–ADP–Fe^{2+} system *in vitro* after the point where the two systems diverge, since the drug-detoxication system is only inhibited *in vitro* by promethazine at relatively high (and substrate-level) concentrations compared with its effect on the $NADPH_2$–ADP–Fe^{2+} system.

It is possible that promethazine exerts its inhibitory action through a complex formed with Fe^{2+}, a component known to be necessary for rapid oxygen uptake by the microsomal $NADPH_2$–ADP–Fe^{2+} system and which is added to the system *in vitro* to give a final concentration of 8 μg.atoms/l. Promethazine and other phenothiazines react with a variety of metal ions (Borg & Cotzias, 1962), including Fe^{2+}, and chelating agents such as EDTA (Hochstein & Ernster, 1963) and desferral (T. F. Slater, unpublished work) are known to inhibit the $NADPH_2$–ADP–Fe^{2+} system at very low concentrations. However, although such a reaction of promethazine with exogenous Fe^{2+} may account for part of the inhibition observed *in vitro*, some additional mechanism presumably operates to produce the marked inhibition observed in the $NADPH_2$–ADP–Fe^{2+} system in liver microsomes isolated from rats that had been treated 1 hr. previously with promethazine (Slater, 1965). In such microsomal fractions the concentration of the drug is very low, and yet considerable inhibition of oxygen uptake was found, compared with microsomal fractions isolated from untreated rats, despite the presence of added Fe^{2+}.

The mechanism(s) by which promethazine inhibits the $NADPH_2$–ADP–Fe^{2+} system is not yet clear but it may involve interaction of the drug with essential radical species in the electron-transport chain. Phenothiazines are known to enter radical reactions relatively easily (see Murphy, Ravner & Smith, 1950; Piette, Bulow & Yamazaki, 1964). The possible involvement of metabolites of the drug has also to be borne in mind when attempts are made to interpret the results of the experiments *in vivo* (Slater, 1965).

It has been suggested on several occasions that carbon tetrachloride has to undergo an activation stage in the liver to free-radical products before its necrogenic activity can become fully manifest. The details of the activation process, if it occurs, are as yet unknown, but one possibility (Slater, 1966) is that carbon tetrachloride reacts with an endogenous radical associated with the $NADPH_2$–ADP–Fe^{2+} system in the endoplasmic reticulum. Certainly the localization of the enzyme system in the liver microsomes (Hochstein & Ernster, 1963) and the low activity of the system in newborn rats and in chickens (Table 1) are consistent with known features of the hepatotoxicity of carbon tetrachloride (see Van Oettingen, 1965; Dawkins, 1963; Bhattacharya, 1965). Further, the $NADPH_2$–ADP–Fe^{2+} system apparently shares some components with the microsomal detoxication system (Hochstein & Ernster, 1963; Orrenius, 1965), and a correlation between the overall activity of the microsomal detoxication system and the hepatotoxicity of carbon tetrachloride has been provided by the study of McLean & McLean (1966).

The detailed compositions of the microsomal

$NADPH_2$–ADP–Fe^{2+} system and the drug-detoxication system are not known (see Orrenius, 1965), so that it is not possible to identify the component(s) responsible for the activation of carbon tetrachloride. However, the results obtained here show that the activation process in liver microsomes is particularly sensitive to promethazine *in vitro* (as is the liver injury produced by carbon tetrachloride *in vivo*) compared with the action of promethazine on the overall $NADPH_2$–ADP–Fe^{2+} system (Fig. 2) and on the drug-detoxication process (see the text) for the metabolism of aminopyrine. Further, the stimulatory effect of carbon tetrachloride *in vitro* on lipid peroxidation in microsomes is not inhibited by a concentration of *p*-chloromercuribenzoate sufficient to inhibit strongly the $NADPH_2$–ADP–Fe^{2+} system (Table 2, and T. F. Slater, unpublished work). It would therefore appear that the activation of carbon tetrachloride to a free-radical form occurs via an interaction with the early component of the $NADPH_2$-dependent electron-transport chain rather than with the more distal ADP–Fe^{2+} segment involved in the $NADPH_2$–ADP–Fe^{2+} system.

The powerful effect *in vitro* of promethazine on lipid peroxidation implies that the phenothiazines are very active as free-radical 'scavengers' in biological systems. It is possible that such a property is important for the action *in vivo* of these drugs; at least the very low concentrations required to inhibit peroxidation *in vitro* (Table 2) are not greater than those found in plasma and other tissues after clinically effective doses (Seeman & Bialy, 1963; Salzman & Brody, 1956), in contrast with the concentrations *in vitro* required to produce effects on several other enzyme systems: rat liver succinate–neotetrazolium reductase (Slater, Sträuli & Sawyer, 1963); rat liver oxidative phosphorylation (Dawkins, Judah & Rees, 1959); rat liver malate or citrate oxidation (Gallagher, Koch & Mann, 1965); human erythrocyte glucose 6-phosphate dehydrogenase (Carver, Marks & Roesky, 1961); and spontaneous swelling of rat liver mitochondria (Judah, 1960).

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REFERENCES

Bangham, A. D., Rees, K. R. & Shotlander, V. (1962). *Nature, Lond.*, **193**, 754.

Beloff-Chain, A., Catanzaro, R. & Serlupi-Crescenzi, G. (1963). *Nature, Lond.*, **196**, 351.

Bhattacharya, K. (1965). *J. Path. Bact.* **90**, 151.

Bieri, J. G. & Anderson, A. A. (1960). *Arch. Biochem. Biophys.* **90**, 105.

Borg, D. C. & Cotzias, G. C. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 617.

Butler, T. C. (1961). *J. Pharmacol.* **134**, 311.

Cameron, G. R. & Karunaratne, W. A. E. (1936). *J. Path. Bact.* **42**, 1.

Carver, M. J., Marks, J. D. & Roesky, N. (1961). *Experientia*, **17**, 315.

Comporti, M., Saccocci, C. & Dianzani, M. U. (1965). *Enzymologia*, **29**, 185.

Dawkins, M. J. R. (1963). *J. Path. Bact.* **85**, 189.

Dawkins, M. J. R., Judah, J. D. & Rees, K. R. (1959). *Biochem. J.* **72**, 204.

Fridovich, I. & Handler, P. (1961). *J. biol. Chem.* **236**, 1836.

Gallagher, C. H., Koch, J. S. & Mann, D. M. (1965). *Biochem. Pharmacol.* **14**, 789.

Ghoshal, A. K. & Recknagel, R. O. (1965). *Life Sci.* **4**, 1521.

Hochstein, P. & Ernster, L. (1963). *Biochem. biophys. Res. Commun.* **12**, 388.

Hochstein, P., Nordenbrand, K. & Ernster, L. (1964). *Biochem. biophys. Res. Commun.* **14**, 323.

Judah, J. D. (1960). *Exp. Cell Res.* **19**, 404.

McLean, A. E. M. & McLean, E. K. (1966). *Biochem. J.* **100**, 564.

Mancy, K. H. & Okun, D. A. (1960). *Analyt. Chem.* **32**, 108.

Martin, H. F., Price, S. & Gudzinowicz, B. J. (1963). *Arch. Biochem. Biophys.* **103**, 196.

Murphy, C. M., Ravner, H. & Smith, N. L. (1950). *Industr. Engng Chem.* **42**, 2479.

Nash, T. (1953). *Biochem. J.* **55**, 416.

Orrenius, S. (1965). *J. Cell Biol.* **26**, 713.

Orrenius, S., Dallner, G. & Ernster, L. (1964). *Biochem. biophys. Res. Commun.* **14**, 329.

Piette, L. H., Bulow, G. & Yamasaki, I. (1964). *Biochim. biophys. Acta*, **88**, 120.

Ragland, J. B. & Kinross-Wright, V. J. (1964). *Analyt. Chem.* **36**, 1356.

Salzman, N. P. & Brodie, B. B. (1956). *J. Pharmacol.* **118**, 46.

Saslow, L. D., Corwin, K. M. & Waravdekar, V. S. (1966). *Arch. Biochem. Biophys.* **114**, 61.

Schneider, A. K., Smith, E. E. & Hunter, F. E., jun. (1964). *Biochemistry*, **3**, 1470.

Seeman, P. M. & Bialy, H. S. (1963). *Biochem. Pharmacol.* **12**, 1181.

Slater, T. F. (1961). *Nature, Lond.*, **192**, 420.

Slater, T. F. (1965). *Biochem. J.* **97**, 22c.

Slater, T. F. (1966). *Nature, Lond.*, **209**, 36.

Slater, T. F., Sträuli, U. D. & Sawyer, B. C. (1963). *Biochim. biophys. Acta*, **77**, 365.

Swoboda, P. A. T. & Lea, C. H. (1958). *Chem. & Ind.* p. 1090.

Van Oettingen, W. F. (1955). *Publ. Hlth Serv. Publ., Wash., No.* 414: *The Halogenated Hydrocarbons; Toxicity and Potential Dangers.*

Wills, E. D. (1965). *Biochim. biophys. Acta*, **98**, 238.

Wirtschaft, Z. T. & Cronyn, M. W. (1964). *Environm. Hlth*, **9**, 186.