recently been challenged by Pieri *et al.* [22], who indicated that the non-hallucinogenic ergot derivative, lisuride, produced similar neurochemical changes. The hallucinogen phencyclidine, which is structurally related to LSD in possessing the phenylethylamine moiety and elicits cataleptic responses, has been demonstrated to interact with the muscarinic receptors [23] and to exhibit cross-tolerance with cholinomimetic agents [24]. The extent to which the hypothesized interaction with the muscarinic receptor contributes to the psychotomimetic activity of LSD remains to be determined. Accordingly, the effects of LSD and its analogues on ACh turnover and choline uptake, as well as its interaction with the cholinergic receptors *in vitro* and *in vivo*, merit further investigation.

In conclusion, we have demonstrated that, whereas a low dose of LSD (50 $\mu g\ kg^{-1}$, s.c.) significantly increased striatal ChAT activity at 2.5 hr, higher doses exerted no appreciable effects. The intensity of LSD-induced cataleptic responses appears to be dissociated from changes occurring in the ChAT activity.

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Effect of selenite on drug-induced methemoglobinemia in rats

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We reported previously that selenite stimulates the reduction of methemoglobin (metHb) in nitrite-treated erythrocytes *in vitro* [1]. We also demonstrated that the reduction of metHb by thiol compounds, such as reduced glutathione (GSH), is increased by the presence of some selenocompounds, suggesting that this catalytic effect of selenocompounds may participate in the stimulation of metHb reduction observed in nitrite-treated erythrocytes [2]. In fact, selenite had no stimulatory effect in erythrocytes depleted of GSH by *N*-ethylmaleimide treatment [3]. This paper reports that selenite suppresses drug-induced methemoglobinemia (metHbemia) in rats, and its mode of action is discussed.

Male Sprague–Dawley rats (7-weeks-old) were used. Blood samples were obtained by cardiac puncture in heparinized syringes. The techniques used for *in vitro* experiments with washed rat erythrocytes were as described previously [1]; experimental details are described in the legend of Fig. 1. MetHb was determined by the procedure of Evelyn and Malloy [4] and hemoglobin (Hb) by the cyanmethemoglobin technique. Glutathione peroxidase (GSH-Px) activity was measured by a coupled reaction with glutathione reductase using cumene hydroperoxide as substrate, according to the method of Prohaska and Ganther [5]. NADH-metHb reductase was measured by the method of Hegesh *et al.* [6], selenium (Se) by the method of Watkinson [7], and protein by the method of Lowry *et al.* [8]. All chemicals used were of reagent grade.

Figure 1 shows the suppressive effect of selenite on metHbemia induced by aniline or phenylhydrazine in rats. The metHbemia produced by aniline (100 mg/kg, i.p.) increased to a peak level within 30–60 min after aniline injection and then gradually decreased. Selenite, when injected simultane-

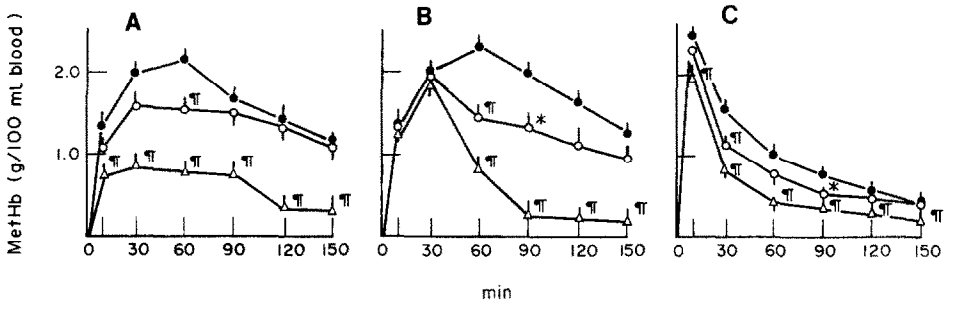

Fig. 1. Effect of selenite on methemoglobinemia induced by aniline and phenylhydrazine in rats. Selenite (0.5 mg/kg and 2.0 mg/kg) was injected with (A), or 30 min after (B), aniline–HCl (100 mg/kg, i.p.). Selenite (0.5 mg/kg and 2.0 mg/kg) was injected with (C) phenylhydrazine–HCl (50 mg/kg, i.p.). Points and bars are means ± S.E. of values in four to six rats. Key: (*) $P < 0.05$; (¶) $P < 0.01$; (●—●) control; (○—○) Na_2SeO_3, 0.5 mg/kg, s.c.; and (△—△) Na_2SeO_3, 2.0 mg/kg, s.c.

ously with aniline, reduced the level of blood metHb dose-dependently (Fig. 1, panel A). Its effect was greatest when it was injected 30 min after aniline (panel B), and it had no effect when injected 30 min before aniline (data not shown).

Under our conditions, the Se content in erythrocytes reached a maximum 10 min after selenite injection and decreased thereafter, in correspondence with the increase in the Se content of the plasma. According to reports by other investigators [9, 10], when selenite was administered it was taken up rapidly by the erythrocytes and metabolized by the GSH-dependent system, after which a reduced form of Se was expelled into the plasma. From these observations, our results suggest that the expelled Se may be inactive in suppression of the metHbemia. Furthermore, the effect of selenite in increasing the reduction of metHb *in vitro* [1] probably occurred in the erythrocytes. The above experiments do not exclude the possibility that selenite may affect drug-metabolizing systems in the liver, since *p*-aminophenol, a metabolite of aniline, is reported to be the main cause of metHbemia induced by aniline [11, 12]. To examine this possibility, we tested the effect of selenite on phenylhydrazine-induced metHbemia, since phenylhydrazine itself is known to oxidize Hb to metHb. The metHbemia induced by phenylhydrazine (50 mg/kg, i.p.) was maximal after 10 min and decreased more quickly than that induced by aniline. Our results showed that selenite, when injected with phenylhydrazine, suppressed the metHbemia induced by phenylhydrazine (Fig. 1, panel C). These findings indicate that selenite probably acts on the erythrocytes themselves.

The site of action of selenite in erythrocytes was examined by *in vitro* experiments on erythrocyte suspensions. A high concentration of selenite (10^{-3} M) enhanced the oxidation of Hb produced by phenylhydrazine, but a lower concentration of selenite (10^{-5} M) did not. It has been shown that damage of Hb by oxidants *in vitro* is prevented by GSH-Px in the presence of GSH [13], and that Se functions as an integral part of GSH-Px [14, 15], the activity of which is proportional to the amount of dietary Se [16]. Therefore, a possibility remains that GSH-Px might be involved in the effect of selenite observed in the present study. However, under our conditions, the GSH-Px activity of erythrocytes was not increased by treatment with selenite (2.0 mg/kg, s.c.), indicating that this enzyme activity is not involved in the effect of selenite. Furthermore, the fact that the time is too short to expect any increase in GSH-Px and that mature erythrocytes do not incorporate Se into GSH-Px also supports this suggestion.

On the other hand, Beutler *et al.* [17] have demonstrated that small amounts of selenite result in GSH-Px activity which catalyzes the reduction of peroxides by GSH. Therefore GSH-Px activity produced by selenite may be involved in the suppression of metHbemia by selenite. However, since the oxidation of Hb by phenylhydrazine *in vitro* was not protected by the addition of selenite, GSH-Px activity produced by selenite itself did not play a major role in the selenite effect in the present study. Thus, the suppresive effect of selenite on the metHbemia does not seem to be due to decreased oxidation of Hb, but rather to increased reduction of metHb. Reduction of metHb is thought to be mainly due to NADH-metHb reductase [18], and NADH-metHb reductase activity was not changed 30 min after injection of selenite (0.5 and 2.0 mg/kg, s.c.).

The dose (2.0 mg/kg) of selenite used in this study corresponds to about 450 times the daily requirement and 1/3 of the LD_{50} for acute toxicity respectively. Since these amounts of selenite would surely exert some toxic effect, the administration of selenite as a clinical antidote is of doubtful value.

The present findings on the effect of selenite are consistent with the idea that selenite catalyzes the reduction of metHb by GSH, as reported previously [1–3]. In summary, selenite was found to suppress drug-induced metHbemia in rats, and its effect was concluded to be due to increased reduction of metHb, not to decreased oxidation of Hb.

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Some adjuncts to oxime–atropine therapy for organophosphate intoxication—Their effects on acetylcholinesterase

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The standard therapy in cases of poisoning by organophosphorus anticholinesterase compounds is administration of atropine, to alleviate the effects of excess acetylcholine, and an oxime, such as pyridine-2-aldoxime methochloride (2-PAM), to reactivate the inhibited acetylcholinesterase (AChE, acetylcholine acetyl-hydrolase; EC 3.1.1.7)[1]. Various other drugs have been tested as adjuncts to this therapy, in order to control the convulsions which are a side-effect of the intoxication, to assist in countering respiratory failure, which is the primary cause of death [1], or because of their antimuscarinic properties. Some of these drugs have been found to enhance protection against the organophosphorus agents, including benactyzine, chlorpromazine, diazepam, mecamylamine and representatives of the Veratrum alkaloids [2–5]. The anticonvulsant, meprobamate [5], and the alkaloid, galanthamine, might well come into this category also. Galanthamine is a potent reversible cholinesterase inhibitor and as such has been recommended as a prophylactic against nerve agents in combination with atropine and an oxime [6]. The proposed mode of action of each of the other supplementary drugs above is unrelated to any effect on AChE. Nevertheless, since the fate of AChE is central to the problem of organophosphorus poisoning, it is surprising that work on the interaction of the above drugs with this enzyme has been limited to semi-quantitative studies of the inhibition of hydrolysis of acetylcholine or its analogues [7–9]. Even recently reported values of the inhibition constant K_i for chlorpromazine [10] and galanthamine [11] neglect the uncompetitive component of inhibition. A more detailed and comprehensive study is reported in this paper.

Materials. Most materials were obtained from commercial sources. Galanthamine was obtained as a 1% aqueous solution from "Medexport", U.S.S.R., for whom the Australian agents are the Malco Agency, Sydney. Cevadine (veratrine), from K & K Laboratories, Plainview, NY, U.S.A., probably includes 25–30% of the related alkaloid, veratridine, as indicated by its n.m.r. spectrum in $CDCl_3$. The spectrum was consistent with that of cevadine [12] but included a sharp peak at $\delta 3.91$, attributable to the aromatic methoxyl groups of veratridine [12]. Diazepam was donated by Roche Products, Dee Why, Australia. Bovine erthrocyte AChE, from the Sigma Chemical Co., St. Louis, MO, U.S.A., was the enzyme used except where mentioned otherwise. *Electrophorus* eel AChE, from the Worthington Biochemical Corp., Freehold, NJ, U.S.A., was used in some experiments. Sarin was synthesized in these laboratories by Mr. D. Amos.

General details. All experiments were done in 45 mM phosphate buffer, pH 7.0, at 25° except where indicated otherwise. Diazepam was introduced into aqueous solution in methanol or ethanol of final concentration 1–2%. Enzyme assays were performed at pH 7.0, 25°, by the method of Ellman *et al.* [13] using a Unicam SP 600 or SP 1750 spectrophotometer.

Hydrolysis of acetylthiocholine (ASCh) by AChE. Six concentrations of ASCh (0.025 to 0.50 mM) were generally employed, and these were run in a convoluted sequence designed to minimize the effect of any enzyme denaturation during the set of assays [14]. When a reversible inhibitor was present, corresponding assays in the presence and absence of inhibitor were run simultaneously. The kinetic parameters K_m (Michaelis constant) and V (maximum velocity) were obtained via the computer program "Median" provided by Dr. A. Cornish-Bowden [15–17].

Inactivation of AChE by physostigmine or phospholine (diethoxyphosphinylthiocholine iodide). Some experiments at 1.6 μM physostigmine were done in the presence of substrate [0.5 mM ASCh, with 0.05 mM 5,5′-dithiobis-(2-nitrobenzoic acid), DTNB] by the method of Hart and O'Brien [18]. Preliminary experiments established that there was no interaction between physostigmine and DTNB at the concentrations used [19]. Other experiments were done in the absence of substrate. At selected times (0.5 to 3.5 min) after mixing solutions of AChE and physostigmine (0.23 μM) or phospholine (0.29 μM), 0.1-ml aliquots were assayed with 1 mM ASCh. No further inhibition occurred during the period of the assay. The apparent first-order rate constant was determined by plotting ln (activity) vs time. The half-life in the absence of a reversile inhibitor was approximately 1 min in all cases.

Reactivation of dimethylcarbamyl-AChE and diethylphosphoryl-AChE. The procedure for reactivation of dimethylcarbamyl-AChE has been described [20]. The same procedure was used for reactivation of diethylphosphoryl-AChE except that the activity of uninhibited enzyme was determined by an appropriate control experiment, rather than by accelerated reactivation. Diethylphosphoryl-AChE was produced by incubation of AChE for 30 min with 0.13 μM phospholine, and its reactivation was followed over 6 hr.

Ageing of isopropylmethylphosphonyl-AChE. AChE in 5 mM Tris, pH 8.7, was incubated with 50 nM Sarin for 30 min at 37°, and for 10 min at room temperature. Approximately 97 per cent inhibition of enzyme activity occurred over this period. Excess Sarin was removed by filtration through a Pharmacia PD 10 column. Studies of the rate of ageing of inactivated AChE were done at 37° in 8.2 ml phosphate buffer. At set times (0–6 hr), 1.0-ml aliquots were treated with 0.2 ml of 3 mM 2-PAM, incubated for 30 min at 25° and freed of 2-PAM by filtration through a PD 10 column. The solution of AChE was then assayed with 0.5 mM ASCh. The first-order rate constant was obtained by plotting ln (activity) vs time.

Hydrolysis of acetylthiocholine (ASCh). All drugs under study inhibited the hydrolysis of ASCh by AChE. The inhibition was of the mixed reversible type, i.e. the drugs increased both K_m/V and $1/V$ where K_m and V are the Michaelis constant and maximum velocity of hydrolysis respectively