Table 2. Rate constant of ageing of soman-inhibited membrane-bound (GB-) and solubilized (GS-) AChE from rat and human erythrocytes in the absence and presence of various concentrations of atropine at pH 7.4 and room temperature*

Concn of atropine (mM)	Rate constant (10^{-3} min^{-1}) Rat GB-AChE	Rat GS-AChE	Human GB-AChE	Human GS-AChE
0	20.5 ± 2.6 (3)	21.5 ± 2.7 (4)	256 ± 17 (3)	231 ± 16 (3)
0.01	16.9 ± 2.4 (3)	21.0 (1)	259 ± 9 (3)	216 ± 20 (3)
0.1	14.9 ± 2.9 (3)	13.9 ± 3.9 (3)	252 ± 14 (3)	236 ± 9 (3)
1	8.0 ± 0.6 (3)†	10.5 ± 3.2 (3)	184 ± 9 (3)†	161 ± 10 (3)†

* All values represent the mean ±SEM: number of experiments is given in parentheses.
† Significantly different from control (no addition); $P < 0.05$ (Student's t-test, two-tailed).

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Acute effects of oltipraz on adult *Schistosoma mansoni* and its antagonism *in vitro*

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Oltipraz is an effective antischistosomal compound whose mode of action is yet to be determined. Bueding *et al.* [1] observed a depression of parasite glutathione (GSH) content as one of the earliest biochemical changes after administration of oltipraz *in vivo*. To further define the relationship between the antischistosomal activity of oltipraz and parasite GSH levels, the acute effects of oltipraz on various biochemical and physiological parameters of *Schistosoma mansoni in vivo* and *in vitro* are presented.

Methods

Parasite preparation and incubation media. Adult *Schistosoma mansoni*, 45–55 days post-infection, were dissected from Swiss Webster mice by the method of Fetterer *et al.* [2]. Where male parasites were used, worms were dissected into media containing 0.05% sodium pentobarbital, mechanically separated and the males placed into fresh media and maintained at 37° until assay.

For *in vivo* experiments, mice were dosed by gavage at either 125 mg/kg or 250 mg/kg oltipraz in peanut oil and killed at the specified times after dosing.

Glutathione assay. After incubation, worms were filtered, weighed and homogenized (1:10, w/v) in 6% trichloroacetic acid (TCA). Aliquots were removed for protein determination by the method of Albro [3] or centrifuged at 12,000 *g* for 10 min and then derivatized and analyzed for GSH by HPLC as described by Reed *et al.* [4].

Characterization of ^{35}S-labeled metabolites. Male schistosomes were transferred to wells (multi-well tissue culture plates, Flow Laboratories, Inc., McLean, VA) containing

2.5 ml RPMI 1640 and 3×10^5 dpm/well [^{35}S]cysteine or [^{35}S]cystine (Amersham Corp., Arlington Heights, IL). Parasites were maintained at 37° in a dark chamber, for the time periods specified. Parasites were rinsed, weighed and homogenized 1:10 (w/v) in 3.5% perchloric acid. The homogenate was centrifuged at 12,000 *g* for 10 min, 0.5-ml aliquots of acid-soluble supernatant fraction were derivatized [5], and the derivatives were analyzed by HPLC as described by Reed *et al.* [4]. Eluant was monitored at 352 nm and collected in 0.5-ml fractions. Fractions were assayed for radioactivity by liquid scintillation (Beckman Instruments, Inc., Fullerton, CA).

Biophysical measurements. Surface electrical activity was recorded by suction electrodes attached to the parasites as described by Semeyn *et al.* [6]. Recordings of tegumental potentials were obtained with glass microelectrodes as described by Thompson *et al.* [7].

Results and discussion

Oltipraz effect on parasite glutathione levels. In vivo, oltipraz (250 mg/kg) effected a significant reduction in male parasite GSH levels by 1 hr, with maximum depression by 3 hr. *In vitro,* oltipraz (10 μM) significantly depressed schistosome GSH levels by 1 hr, an effect not observed when parasites were coincubated in GSH (1 mM), cysteine (1 mM) or methionine (1 mM). Neither dithiothreitol (DTT) nor 2-mercaptoethanol (1 mM or 100 μM) blocked the oltipraz-induced depression of schistosome GSH *in vitro.*

Oltipraz effect on uptake and incorporation of [^{35}S]cysteine and [^{35}S]cystine. By 1 hr, the uptake of [^{35}S]cysteine or [^{35}S]cystine by male schistosomes was inhibited significantly (41 and 36% of control respectively) during *in vivo* incubations in the presence of 5 μM oltipraz (Fig. 1). [^{35}S]GSH formation occurred in the presence of 5 μM oltipraz and, although less total label was incorporated, the assimilation of labeled precursor into the GSH pool (in terms of relative percent distribution) was comparable to control. There were no significant differences between the cysteine and cystine controls.

Oltipraz effect on schistosome surface electrical potentials. The amplitude and frequency of endogenous electrical transients in *S. mansoni* were depressed profoundly after *in vivo* exposure to oltipraz, even at a dose of 125 mg/kg. The onset of significant depression occurred by 6 hr, with the high amplitude (>40 μV) potentials exhibiting a greater degree of sensitivity to the effects of oltipraz. Schistosomes exposed to 3.5 μM oltipraz *in vitro* exhibited a similar level of depression to the drug, with high amplitude electrical activity being significantly depressed after incubations as brief as 1 hr. The profound depression of surface electrical activity observed after 24-hr incubations in 10 μM oltipraz was prevented when the parasites were coincubated in 1 mM cysteine or 1 mM GSH. The depression was not prevented by either 1 mM or 100 μM 2-mercaptoethanol. The oxy analog of oltipraz (100 μM) did not alter parasite surface electrical activity from control levels.

Oltipraz effect on schistosome tegument potential. Tegument potentials recorded from male schistosomes were depolarized significantly by 5 μM oltipraz following 18 hr of *in vitro* incubation (Fig. 2). This effect was prevented by the addition of 1 mM cysteine to the incubation medium (−54.6 + 2.2 mV). Cysteine did not alter the tegument potential nor did the oxy analog of oltipraz.

Bueding *et al.* [1] observed that *in vivo* treatment of *S. mansoni* with a 250 mg/kg dose of oltipraz resulted in a depression of parasite GSH levels to roughly 40% of control over the first week after treatment. In the present study, depletion of GSH was observed by 1 hr in the parasite at a dose of 250 mg/kg *per os.* The fact that GSH levels in parasite tissue were not rapidly restored to control levels, as was seen in host tissues [1], indicates that there may be important differences in the biochemical regulation of GSH between the parasite and the host. A similar depletion of parasite GSH levels in the presence of oltipraz was observed *in vitro,* allowing the acute actions of oltipraz on the parasite to be studied independently, without the variables of host reaction to the drug.

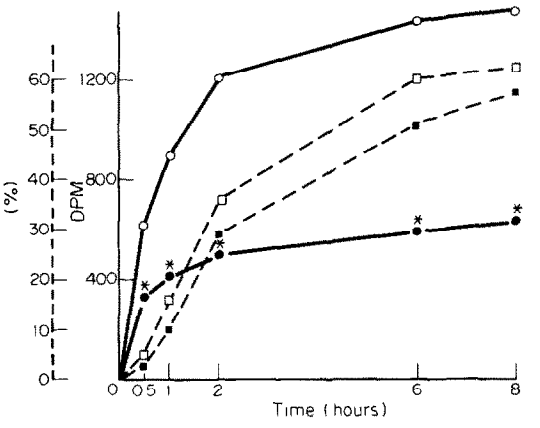

Fig. 1. [^{35}S]Cysteine uptake and incorporation into schistosomes *in vitro.* Male schistosomes were incubated in 2.5 ml RPMI 1640 containing 3×10^5 dpm [^{35}S]cysteine per well at 37°, in the dark, in the presence of 5 μM oltipraz (solid symbols) or vehicle only (open symbols). Key: (● or ○, solid line) total uptake (disintegrations/min/male) of [^{35}S]cysteine; (□ or ■, broken line) the relative percentage of total ^{35}S as [^{35}S]GSH and oxidized [^{35}S]GSH. There were ten males per well; values represent means of four wells per data point. An asterisk denotes statistically different ($P < 0.05$) from control uptake. Relative distribution of label, once assimilated by the parasite, into [^{35}S]GSH and oxidized [^{35}S]GSH was not statistically different from control.

Bueding *et al.* speculated that the chemotherapeutic activity of oltipraz may be due to competition of the drug with γ-glutamyl cysteine, a precursor of GSH synthesis. By this mechanism, the non-stoichiometric depletion of GSH could occur at a rate dependent on the degree of inhibition of synthesis and the turnover rate for parasite intracellular GSH. The results of the present study suggest that there may be an alternative explanation for the effect of oltipraz on GSH levels. First, the inhibition of cysteine uptake by 5 μM oltipraz corroborates results by Bueding *et al.* [1] showing that uptake of both glutathione and cysteine, but not the uptake of other amino acids, is retarded by oltipraz. Second, in the presence of oltipraz, male schistosomes were

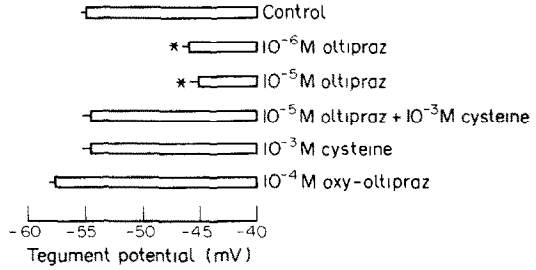

Fig. 2. Tegument resting membrane potential of male schistosomes *in vitro*: Effect of oltipraz and antagonism of effect by cysteine. Male schistosomes were incubated in HS/RPMI containing oltipraz or oltipraz plus cysteine as indicated, or in the presence of 100 μM oxy-oltipraz, for 18 hr at 37°, in the dark. Values are the means recorded from five parasites; vertical lines are one SE. An asterisk denotes statistically different ($P < 0.05$) from control value.

able to incorporate [^{35}S]cysteine label into the glutathione pool, as GSH and oxidized GSH, at a relative distribution percentage that was not significantly different from control. Therefore, under the conditions of acute exposure, it is unlikely that GSH synthesis is being directly inhibited by oltipraz. Further, the fact that the oxy derivative of oltipraz did not affect the uptake of [^{35}S]cysteine into the parasites, even at 50 μM, suggests that the thione group of the drug molecule is a necessary participant in the oltipraz-induced effects on transport mechanisms.

In the present study, GSH (1 mM), cysteine (1 mM) and methionine (1 mM), but neither DTT (1 mM or 100 μM) nor 2-mercaptoethanol (1 mM or 100 μM), were able to block the *in vitro* effect of oltipraz on GSH levels occurring during incubation. The antagonism of oltipraz effects by GSH or GSH precursors may be mediated through a process of mass action on the replenishment of GSH stores. DTT and 2-mercaptoethanol, unlike the other compounds tested, are incapable of participating in the production of GSH, which may explain their inability to antagonize the effects of oltipraz. Alternatively, the inactivity of the oxy derivative of oltipraz and the rather stringent structural requirements for the maintenance of antischistosomal activity of a series of oltipraz congeners [1] suggest that oltipraz may influence a membrane receptor which possibly functions as a dipeptidase or transport carrier molecule for amino acids.

Tegumental morphology is not affected by *in vitro* treatment of the parasites with 10 μM oltipraz for 12 hr [8], indicating that structural damage to the tegument is not a contributary factor in the induction of acute oltipraz effects on membrane transport. While the structural integrity of the outer tegumental membrane was maintained, microelectrode recordings which showed depolarization of the tegument suggest that a significant redistribution of ions across the tegumental membrane had occurred. The effect(s) of a drug-induced ionic imbalance on the Na^+-driven carrier-mediated cotransport of glucose [9] may have contributed to a decrease in glucose uptake which was also observed (unpublished results). Perturbations of ionic equilibria across the tegument may also contribute to the inhibition by oltipraz of the uptake of GSH precursors. Consistent with the effects on other parameters measured, tegument potentials of worms incubated in 1 mM cysteine or 10 μM oltipraz plus 1 mM cysteine were not significantly different from control values. These results indicate that cysteine is able to prevent oltipraz-induced ionic imbalances *in vitro* without directly affecting ionic fluxes across the membrane. The oxy derivative of oltipraz did not affect the tegumental membrane potential, suggesting that the disruption of membrane conductance may contribute to the antischistosomal efficacy of oltipraz.

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Intestinal first-pass metabolism of phenacetin, acetaminophen, ethenzamide and salicylamide in rabbits pretreated with 3,4-benzo[*a*]pyrene

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The liver is usually considered to be the major drug-eliminating organ. However, all drugs that are administered orally must traverse the gastrointestinal wall before reaching the systemic circulation. The loss of drug as it passes through the gastrointestinal wall and the liver during the absorption process is known as the first-pass effect.

Phenacetin (PHT), used as an analgesic and an antipyretic, is metabolized extensively, the major metabolic route being O-de-ethylation to acetaminophen (NAPA). NAPA is subsequently conjugated to form a glucuronide (NAPA glucuronide, NAPAG) and a sulfate (NAPA sulfate, NAPAS). Similarly, the primary pathway for ethenzamide (ETB) metabolism is via O-de-ethylation to salicylamide (SAM), and SAM is further metabolized to SAM glucuronide (SAMG) and SAM sulfate (SAMS).

It has been reported that there is in rats an enzyme system in the mucosa of the small intestine that is capable of metabolizing PHT and that the activity of this enzyme system is increased in rats pretreated with 3,4-benzo[*a*]pyrene [1, 2] and 3-methylcholanthrene [3]. Recently, Klippert *et al.* [4] found that 3-methylcholanthrene pretreatment results in enhanced PHT disposition as was shown from decreased plasma half-life time, decreased oral availability, increased clearance, and an increase in metabolite levels. In 3-methylcholanthrene-pretreated rats the intestine contributes significantly, and predominantly over the liver, to PHT first-pass metabolism. In contrast, gut wall metabolism in control rats could not be demonstrated [4].

To provide more information on the role of intestinal