Schistosomicidal Activity of Alkylaminooctanethiosulfuric Acids

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The schistosomicidal activity of a new series of alkylaminooctanethiosulfuric acids was studied in white Swiss mice infected with the L.E. strain of Schistosoma mansoni *(Belo Horizonte, MG, Brazil). In a preliminary screening of six compounds, two derivatives - 2-[(1-methylpropyl)amino]-1-octanethiosulfuric acid and 2-[(1-methylethyl)-amino]-1-octanethiosulfuric acid - given orally in doses of 300 mg/kg/day for five consecutive days, caused interruption of the oviposition and the hepatic shift of more than 90% of the worms. Both compounds caused a significant reduction in worm burden and, interestingly, the female schistosomes were more susceptible. With the therapeutic schedule of two doses of 800 mg/kg over a 20 day interval, the death of almost all the females and about 50% of the males was observed. Female worms recovered from treated mice showed scattered vitteline glands. Results of* in vitro *experiments against different developmental stages of the parasite revealed the induction of paralysis and damage to the tegument membrane. The drugs presented no toxic effects on the animals.*

Key words: alkylaminoalkanethiosulfuric acids - Bunte salts - schistosomicides - *Schistosoma mansoni* - schistosomiasis

The synthesis of primary Bunte salts (Formula *1a-c*) was described by Klayman and Gilmore (1964) in an integrated study of potential anti-radiation agents (Jacobus et al. 1966). Similar alkylaminoalkanethiosulfuric acids, supplied by the Walter Reed Army Research Institute, were first tested against *Schistosoma mansoni* by Nelson and Pellegrino (1976) in a preliminary study using *S. mansoni* infected mice or hamsters as experimental models. These authors tested 70 Bunte salts of general formula:

$$RNH_2CH_2CH_2SSO_3^-$$

1

where R equals small or medium sized, apolar alkyl groups. The compounds 2-(butylamino)-1-ethanethiosulfuric acid (*1a*), 2-(octylamino)-1-ethanethiosulfuric acid (*1b*) and 2-(cyclopentylamino)-1-ethanethiosulfuric acid (*1c*) exhibited some activity, as demonstrated by inhibition of oviposition and induction of a hepatic shift. In a previous report, Penido et al. (1990) described the synthesis of similar alkylaminooctanethiosulfuric acid derivatives:

$$RNH_2CH(CH_2)_5CH_3$$
$$|$$
$$CH_2SSO_3^-$$

2

where R = n-butyl (*2a*), 1-methylpropyl (*2b*), 1-methylethyl (*2c*), cyclohexyl (*2d*), 2-methylpropyl (*2e*), n-propyl (*2f*).

The results of preliminary screenings for activity against *S. mansoni* showed that the products 2-[(1-methylpropyl)amino]-1-octanethiosulfuric acid (*2b*) and 2-[(1-methylethyl)amino]-1-octanethiosulfuric acid (*2c*) were active when administered orally to mice at a dose of 300 mg/kg/day for five consecutive days (Penido et al. 1990). Further investigation of the activity of the previously synthesized compounds (*2a - 2d*) and of two newly obtained derivatives (*2e - 2f*) were performed in mice infected with *S. mansoni* at different thera-

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peutic schedules and are reported in the present paper. *In vitro* experiments were also performed using schistosomula, lung stage larvae and adult worms.

MATERIALS AND METHODS

Drugs - The following alkylaminooctanethiosulfuric acids were synthesized according to Penido et al. (1990): 2-(butylamino)-1-octanethiosulfuric acid (*2a*), 2-[(1-methylpropyl)amino]-1-octanethiosulfuric acid (*2b*), 2-[(1-methylethyl)-amino]-1-octanethiosulfuric acid (*2c*), 2-(cyclohexylamino)-1-octanethiosulfuric acid (*2d*), 2-[(2-methylpropyl)amino]-1-octanethiosulfuric acid (*2e*), 2-(propylamino)-1-octanethiosulfuric acid (*2f*) (Formula *2*). All the structures were in accord with infrared, proton nuclear magnetic resonance (^{1}HNMR) and mass spectra and elemental analyses. All the compounds were insoluble in aqueous medium and were administered to mice as a suspension in 10% Cremophor-El (an emulsifying agent - Sigma) after ultrasonication to reduce particle size and produce a uniform suspension. A 0.3 ml volume of the suspension containing the amount of drug stated in each experiment was used in the treatments. The control groups were treated with 10% Cremophor-El solution only.

Animals and parasites - The LE strain of *S. mansoni* (Belo Horizonte, MG, Brazil), obtained from *Biomphalaria glabrata* snails maintained in the Schistosomiasis Research Unit, Federal University of Minas Gerais, was used in all the experiments. White Swiss mice (22-25 g), obtained from the departmental animal house, were infected by subcutaneous injection of cercariae. The worms were recovered by perfusion following the method of Pellegrino and Siqueira (1956). Mice were provided with water and rodent chow *ad libitum*.

Preliminary screening - Groups of five mice were treated with a daily oral dose of 300 mg/kg of each drug for five consecutive days seven weeks after infection with 100 cercariae. The criteria for assessing drug effects were the hepatic shift of worms (Standen 1953, Buttle & Khayal 1962) and the interruption of egg laying, denoted by the changes in the oogram (Pellegrino & Faria 1965) three days after administration of the last dose. Changes in the oogram are represented by the absence of first to fourth stages of immature eggs in sections of the intestine of the treated animal examined by optical microscopy. These changes signify interruption of egg excretion.

Activity against developmental stages of S. mansoni - Groups of eight mice infected with 30 cercariae were treated daily with 300 mg/kg of compound *2b* for five consecutive days, beginning the treatment one day before and six and twenty-four days after infection. These groups were sacrificed for worm counts 50 days after infection, together with the control group. Reduction of worm burdens of treated groups, compared with the untreated control group, were taken into account for assessment of drug activity.

Dose-response experiments, employing groups of five mice, for testing the efficiency of single doses of 400, 600, 800, 1000 and 1200 mg/kg of *2b* and *2c* showed that these compounds attained a maximum efficacy (50 to 60% reduction of the total worm burdens, including 85 to 90% reduction in the number of female worms) when a single 800 mg/kg dose of each compound was used. Doses of 1000 mg/kg, or more, did not show any improvement in reducing the worm burdens. The toxicity of each compound was evaluated and characteristic symptoms, such as loss of body weight, weakness, behavior or death were not observed. The LD_{50} must be greater than 1.2 g/kg p.o. because no accute toxic effect was observed with this dose. Administration of larger doses was not viable. Based on these results, the following experiments were executed.

Hepatic shift - Five groups of 10 mice infected with 30 cercariae were treated with a single dose of 800 mg/kg of *2b* 70 days after infection. The animals were sacrificed for worm recovery at 1, 2, 6, 12 and 45 days after treatment. After perfusion, male and female worms were counted separately and their distributions between mesenteric veins and liver were determined; a control group was treated only with a 10% Cremophor-El solution.

Therapeutic effect (1) - Four groups of 13 mice infected with 30 cercariae for 70 days were used. Two groups were treated orally with a single 800 mg/kg dose of *2b* and the two remaining groups were treated with the same amount of *2c*. One group of each was sacrificed 20 days after treatment for recovery of worms and the second two groups each received a second 800 mg/kg dose of the respective compounds and were sacrificed 20 days later. (2) - Two groups of 10 mice infected for 70 days with 30 cercariae were treated with *2b*, one with a single 1000 mg/kg dose and the other with five 100 mg/kg doses with intervals of three hours between each administration. (3) - Two groups of 10 mice infected for 70 days with 30 cercariae were treated with five daily doses of 300 mg/kg of *2b* or *2c*. For experiments (2) and (3), recovery of worms was performed 20 days after treatment, and reductions in worm burden were evaluated by comparing with control groups.

The reductions in total worm burden (Total Efficacy) and in male and female populations were calculated according to the formula: R = 100 (C -

W/C), where R is the percent reduction of worms; C is the total worm burden (or male or female worm burden) recovered from control groups; and W is the total worm burden (or male or female worm burden) recovered from treated groups of mice.

Histological studies - In order to determine the presence of phenolic compounds in vitteline glands, remaining female worms recovered from mice treated with one 800 mg/kg dose of compounds *2b* or *2c*, as described above for the first therapeutic experiment, were briefly washed in saline, fixed in 70% alcohol for 1 hr, hydrated, stained with 1% Fast Red B (Sigma) for 3 min (Bell & Smith 1958), dehydrated and mounted for examination under a Leitz Ortholux optical microscope and microphotographs were taken with a standard camera attachment using a Kodak Ektachrome Tungsten 169 film.

In vitro *studies* - The Glasgow modification of Eagles Medium (GMEM) (Gibco, Ltd., Paisley, Scotland) containing penicillin (50 UI/ml) and streptomycin (50 µg/ml), buffered with 10 mM Hepes (pH 7.2) and supplemented with 10% (v/v) heat inactivated fetal calf serum (hiFCS; Northumbria Biologicals) was used. To promote solubility, the compounds were first dissolved in fetal calf serum and then added to the incubation medium to achieve the desired concentration. Six pairs of worms obtained by perfusion from mice infected for 10 weeks were washed with warm GMEM three times and then transferred to 5.0 ml of the above-described medium contained in six-well plastic culture dishes (Corning). Mechanically transformed schistosomula (Ramalho-Pinto et al. 1974) were cultured at a concentration of approximately 200 larvae in 1 ml of ready medium in the wells of a 12-well plastic culture plate (Corning). Ten to fifteen lung-stage larvae, obtained from the lungs of mice six days after infection by the method described by Clegg (1965), were cultured as described above for schistosomula. Microphotographs were taken directly from the culture medium with a standard camera attached to the inverted microscope (AO Scientific Instruments Microstar). Control worms were cultured in medium alone. The culture plates were incubated at 37 °C. Alterations, such as loss of the capacity of the adult worm to adhere to the plate, loss of motor activity, morphological alterations in the tegument and death of both adult worms and larvae were observed within 12 to 24 hr of incubation. Death was denoted by absence of any contraction of the worms after being exposed to serotonin (10 µM) (Eshete & Bennet 1990). The results were expressed as minimal concentrations of each compound necessary to induce the above-mentioned alterations.

Statistics - A one-way analysis of variance - Student-Newman-Keuls test (Steel & Torrie 1960) - was carried out, with a significance level of $p < 0.05$.

RESULTS

Preliminary screening - Table I shows that, of all the compounds tested, only the 2-[(1-methylpropyl)amino]-2-octanethiosulfuric (*2b*) and 2-[(1-methylethyl)amino]-2-octanethiosulfuric (*2c*) acids were observed to be active. The five mice in each group, treated with the respective compound, had altered oograms due to interruption of oviposition, since intermediate stages of egg maturation were not found in any of the five mice of each group. The shift of more than 90% of the worms to the liver was evident for both compounds three days after the last day of treatment.

Activity against developmental stages of S. mansoni - A total dose of 1500 mg/kg of *2b* or *2c* given daily in 300 mg/kg doses was not active against the juvenile developmental stages 1 to 10 days after infection. When it was administered within 24 to 29 days after infection, a 50% reduction in the worm burden, including female and male worms, was observed.

Hepatic shift - The administration of compound *2b* in a single 800 mg/kg dose was chosen to illustrate the time course of worm migration from mesenteric veins to the liver of 70 day-infected mice. The distribution of worms between the liver

TABLE I

Preliminary screening. Oogram changes and percentage of worms recovered from livers of white Swiss mice treated with the N-alkylaminooctanethiosulfuric acids *2a* to *2f* in oral doses of 300 mg/kg/day for five consecutive days. Mice were sacrificed three days after the last day of treatment for recovery of worms

Compounds[a]	No. of mice with altered oogram (No. of mice in group)	Worms in the liver (%)
2a	0 (5)	24
2b	5 (5)	90
2c	5 (5)	92
2d	0 (5)	14
2e	0 (5)	21
2f	0 (5)	42
Control	0 (5)	18

a: see Formula *2* and Materials and Methods for structures and names of compounds. Alteration in the oogram means interruption in oviposition, i.e., absence of eggs in the first to fourth developmental stages in sections of intestines of treated mice.

and the mesenteric veins in the control group was 13 and 87%, respectively. In the treated groups, these numbers changed from 77% (mesenteric veins) and 23% (liver) one day after treatment to 16% (mesenteric veins) and 74% (liver) two days after treatment. A maximum shift - 10% (mesenteric veins) and 90% (liver) - was observed six days after treatment. The perfusion of animals 13 and 45 days after treatment showed a 69 and 64% reduction in worm burden, respectively. A greater number of males was able to return to the mesenteric veins (13% after 13 days and 43% after 45 days). Although in reduced number (90% reduction in total number of females both 13 and 45 days after treatment), the females also returned to the mesenteric veins - zero females were recovered from the mesenteric veins 13 days after treatment and three out of five females were perfused from this site 45 days after treatment.

Therapeutic effect - The results of therapeutic effect experiments are summarized in Table II. A single 800 mg/kg dose of *2b* or *2c* was effective in reducing the female worm burdens by 90%. A second 800 mg/kg dose, administered 20 days later, increased significantly the reduction in female worms as compared to the control group, no females being recovered from mice treated with *2c* and just one in the group of 13 mice treated with *2b* (Table II, Experiment 1). Other treatment schedules were attempted in order to improve efficacy. Again, reduction of 90% or more in the number of female worms was achieved (Table II, Experiments 2 and 3). Interestingly, administration of five 100 mg/kg doses of *2b* at three-hour intervals resulted in an efficiency (93%) similar to that of a single 1000 mg/kg dose (90%) or five consecutive daily doses of 300 mg/kg of *2b* or *2c* (89% and 92%, respectively; Table II, Experiments 2 and 3).

Morphological alterations observed in worms after in vivo *exposure* - Regression of the vitteline gland of female worms recovered from mice treated with one 800 mg/kg dose of *2b* or *2c* is shown by the lack of staining of phenolic material by Fast Red B in the microphotography of Fig. 1A,B, as compared to the control (Fig. 1C).

In vitro *effect on adult schistosomes* - The first obvious effect noted was the almost immediate loss of the capacity to adhere to the plate surface. Gradually the worms became paralyzed and death occurred within 9 to 12 hr of incubation, when widespread blebbing of the tegument along the worm's body could be noted. No contraction was observed upon incubation of the worms in drug-free medium containing serotonin. Among the compounds tested, *2c* was the most effective in inducing such alterations at a concentration of 0.25 mM (70.7

TABLE II

In vivo antischistosomal activity of 2-[(1-methylpropyl)amino]-1-octanethiosulfuric acid (*2b*) and 2-[(1-methylethyl)-amino]-1-octanethiosulfuric acid (*2c*) in mice

Expt.[a]	Drug dose (mg/kg)	Mean±SD of worms recovered			Worm reduction		Efficacy (%)
		Female	Male	Total	Female (%)	Male (%)	
1a[b]	1 X 800 of *2b*	0.4±0.8	2.7±1.7	3.1±2.4	92	45	68
1b[b]	1 X 800 of *2c*	0.3±0.5	2.5±1.6	2.8±1.9	93	50	71
1c[c]	2 X 800 of *2b*	0.1±0.3[f]	3.1±2.2	3.2±2.5	98	33	65
1d[c]	2 X 800 of *2c*	0.0[f]	2.6±2.2	2.6±2.2	100	47	73
Control	-	4.8±1.2	4.9±1.1	9.7±2.3	-	-	-
2a	1 X 1000 of *2b*	0.2±0.4	1.9±1.4	2.1±1.6	93	49	70
2b[d]	5 X 100 of *2b*	0.3±0.5	1.8±1.5	2.1±1.8	90	51	73
Control	-	3.0±1.0	3.4±2.1	6.4±3.1	-	-	-
3a[e]	5 X 300 of *2b*	0.7±0.7	4.4±1.9[g]	5.1±2.0	89	35	61
3b[e]	5 X 300 of *2c*	0.5±0.9	3.8±1.0	4.2±1.2	92	44	67
Control	-	6.8±1.3	6.2±1.7	13.0±2.8	-	-	-

[a]: the number of the experiment. [b]: groups of 13 mice were sacrificed 20 days after administration of the drug. [c]: the two doses were given within a 20 day interval to groups of 13 mice and the mice were sacrificed 20 days later. [d]: the 5 X 100 mg/kg doses were given in 3 hr intervals; both groups of 10 mice were sacrificed 20 days later. [e]: groups of 10 mice were sacrificed 20 days after administration of the last dose of each drug. [f]: $p < 0.05$ comparing the means of females recovered from groups 1a with 1c and groups 1b with 1d. [g]: except for one group in Experiment 3, all the means presented values of $p < 0.05$ relative to the control groups for each experiment.

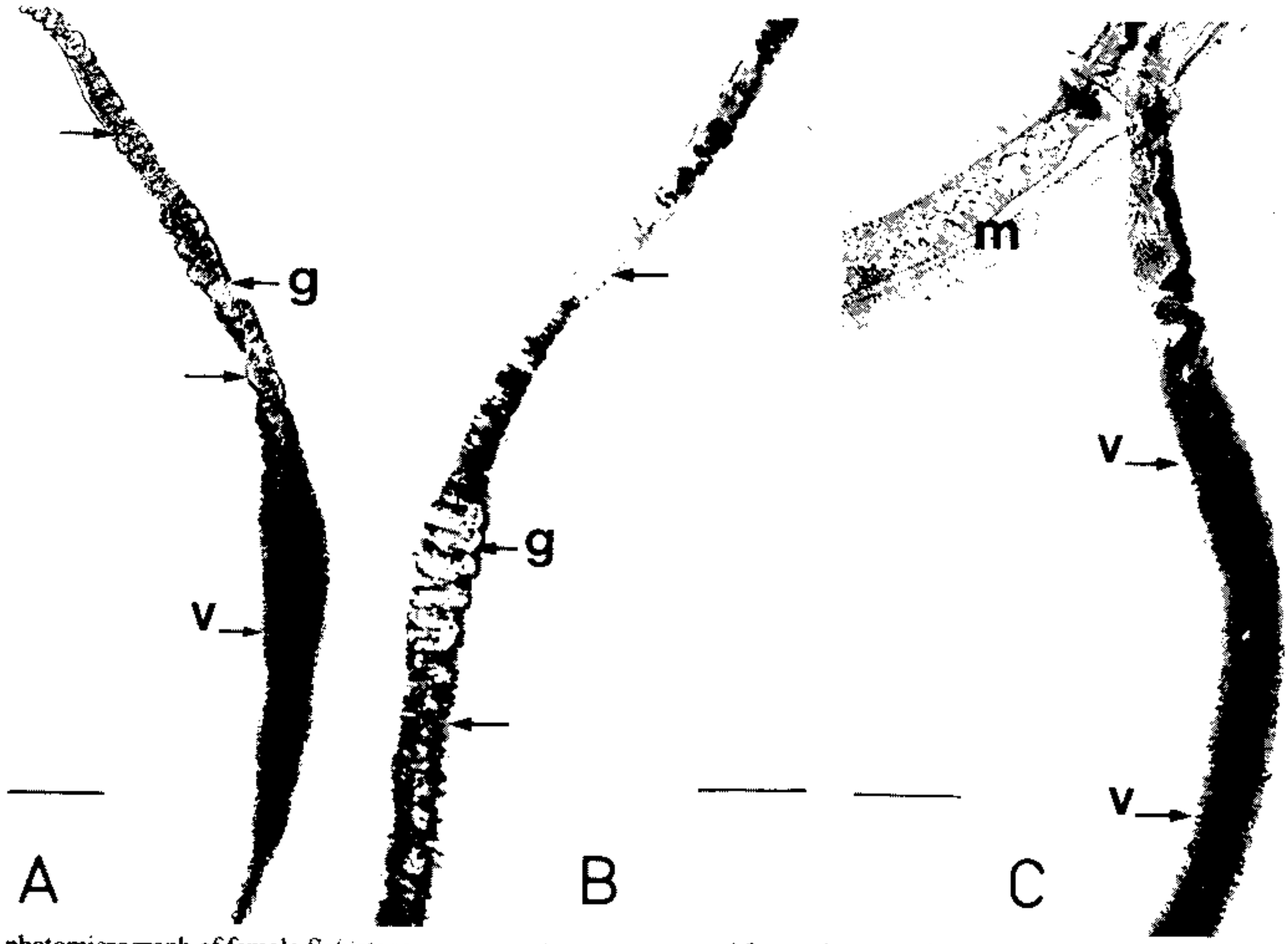

Fig. 1: photomicrograph of female *Schistosoma mansoni* worms recovered from mice treated with compounds *2b* (A) and *2c* (B) and stained with Fast Red B. Arrows indicate regression of vitteline gland (v). (C) Adult female worm recovered from the control group showing fully developed vitteline gland. (g) Gut; (m) male. In (1) bar = 1 mm and, in (2) and (3), bar = 0.7 mm.

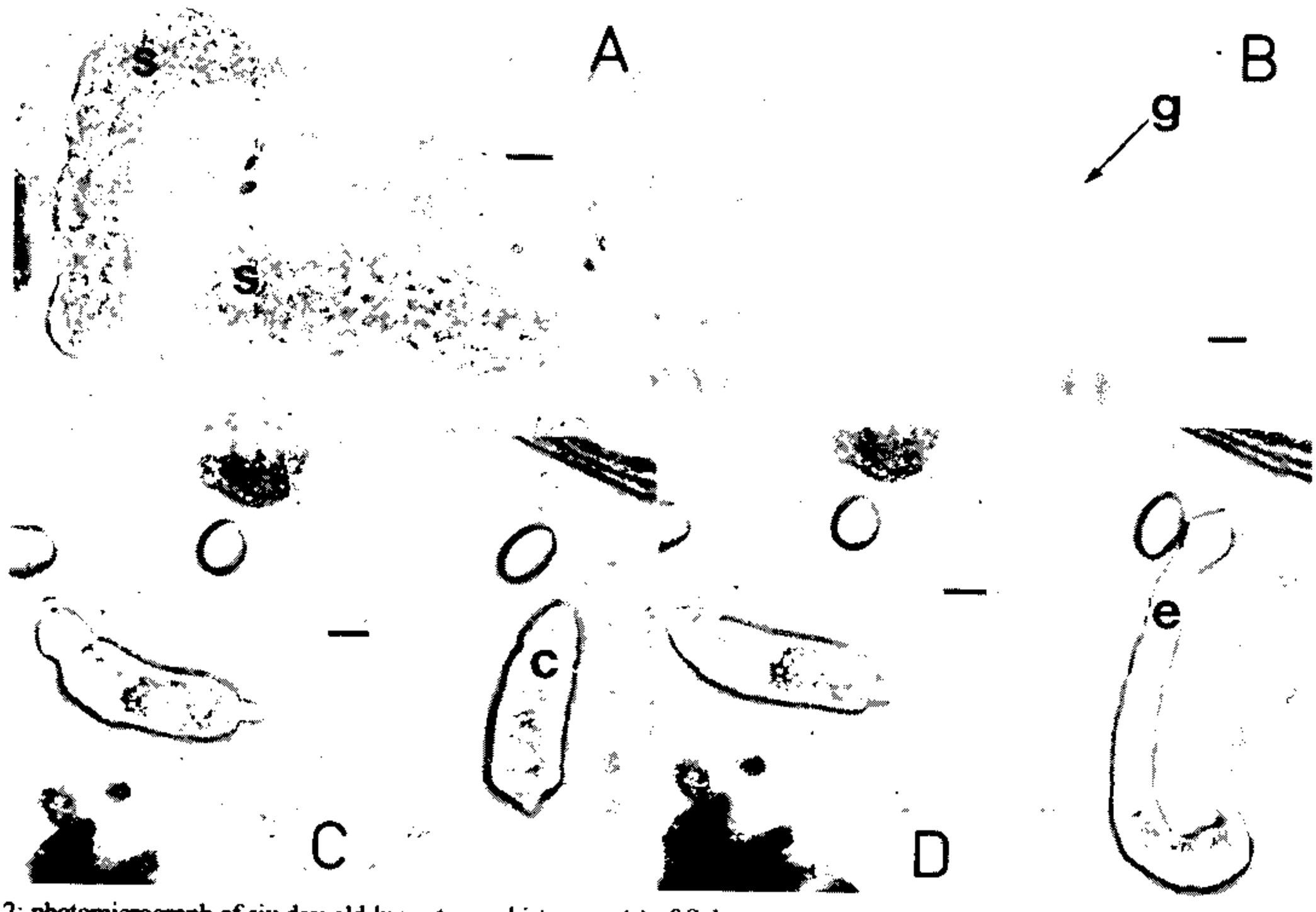

Fig. 2: photomicrograph of six day-old lung stage schistosoma (s) of *Schistosoma mansoni* taken directly from the culture medium after 12 hr of incubation with (A) 0.05 mM (14.1 µg/ml) of *2c*, (B) 0.30 mM (89.1 µg/ml) of *2b*; (g) gut. (C) Contraction (c) and (D) elongation (e) of a larva from the control group incubated for 12 hr. Photomicrographs C and D are of the same field. In (A) and (B), bar = 0.03 mm and, in (C) and (D), bar = 0.05 mm.

μg/ml), followed by *2f* (0.5 mM; 141.5 μg/ml) and *2b* (1.0 mM; 297 μg/ml). Compounds *2a*, *2d* and *2e* were not active at a 0.8 mM concentration after 24 hr of incubation.

In vitro *effect against schistosomula* - The most striking effect observed was the paralysis of the larvae and even of the residual cercariae and cercariae tails which are always present in the schistosomula suspension. The larvae became progressively paralyzed within 6 to 12 hr of incubation when gradual blebbing of the tegument also became apparent. The compounds *2c* and *2f* were the most active, causing paralysis of 100% of larvae within 12 hr at a concentration of 0.40 mM (113,2 μg/ml). Compound *2b*, at a concentration of 0.8 mM (237.6 μg/ml), required 18 to 24 hr to induce paralysis and blebbing of 100% of the lavae, and compounds *2a*, *2d* and *2e* were not active, even at 0.8 mM over a 24-hr incubation period. The immobilization of the schistosomula was considered a sign of death because they did not recover movement upon re-incubation in drug-free medium.

In Vitro *effect against lung-stage schistosomula* - Interestingly, compounds *2b* and *2c* caused damage to the larvae in different manners after 12 hr of incubation. Compound *2c* caused paralysis and general damage to the tegument of 100% of the larvae at a concentration as low as 0.05 mM (14.1 μg/ml), and the destruction of the tegument occurred in such a way that the internal structure could not be observed (Fig. 2A). On the other hand, *2b* killed 100% of the larvae at a concentration of 0.30 mM (89.1 μg/ml), but the morphology and internal structure appeared to be preserved (Fig. 2B). Figs. 2C,D show the characteristic movements of contraction (c) and elongation (e) of a lung stage schistosomula from the control group incubated for 12 hr.

DISCUSSION

The control of schistosomiasis is mainly performed by mass chemotherapy (Warren 1982). In Brazil, the high cost of Praziquantel, to date the most efficient drug of choice against schistosomiasis (Archer 1985, Harnett 1988), has made oxamniquine the preferred drug for mass treatment. However, drug resistant strains have been described by several authors (Katz et al. 1973, Dias et al. 1978, Araujo et al. 1980, Dias et al. 1988). There is, therefore, a need for the development of new, less expensive, schistosomicidal compounds and the continued identification and evaluation of new schistosomicidal agents are important.

In this paper, two of the six new compounds presented - 2-[(1-methylpropyl)amino]-1-octanethiosulfuric acid (*2b*) and 2-[(1-methylethyl)-amino]-1-octanethiosulfuric acid (*2c*) - have proved to be effective in reducing adult worm burdens of *S. mansoni* in mice. The lack of activity shown by compounds with very closely related chemical structures, such as the acids 2-[(2-methylpropyl)amino]-1-octanethiosulfuric acid (*2e*) and 2-(propylamino)-1-octanethiosulfuric acid (*2f*) which differ from *2b* and *2c*, respectively, only in the absence of an alpha methyl group on the carbon atom bound to the amino group, suggests that such an alpha methyl group may be important for schistosomicidal activity.

The size of a single oral dose of *2b* or *2c* required to reduce the parasite burden by 60%, with a reduction in female worms of at least 90%, was 800 mg/kg. However, when a total dose of 500 mg/kg was divided into five 100 mg/kg doses given orally in intervals of 3 hr, an efficacy comparable to that of a single 1000 mg/kg dose, or five daily doses of 300 mg/kg (Table II, Experiments 2 and 3), was observed. This fact suggests that, similar to Praziquantel (Gonnert & Andrews 1977), the influence of the length of exposure to the drug itself, or to any active metabolite, on the schistosomicidal activity is more important than that of the maximum plasma concentration. Gonnert and Andrews found that five doses of 50 mg/kg of praziquantel administered at three hour intervals eliminated all the worms, whereas the treatment with a single 1000 mg/kg dose reduced the worm burden by 95%.

Like oxamniquine, both *2b* and *2c* have a delayed effect on parasites. For oxamniquine, the hepatic shift of the majority of the worms occurs about six days after treatment (Foster & Cheetham, 1973), and for *2b*, after two days. The maximum reduction in worm burden could be noted 13 days after treatment, almost the same number of worms being recovered 45 days after treatment. Some of the worms were able to return to the mesenteric veins within this interval. Therefore, a second treatment with 800 mg/kg within a 20 day interval was attempted in order to achieve total elimination of the female worms. In fact, no females were recovered from the group of 13 mice treated with *2c* (47% reduction in males), and just one from the group treated with *2b* (33% reduction in population of males) (Table II, Experiment 1).

The most interesting and intriguing aspect concerning the *in vivo* action of these compounds was their selective action against the female worms. In the surviving females recovered from the group of mice treated with a single 800 mg/kg dose, optical microscopic observations revealed a regression of the vitelline gland (Fig. 1). It is not known if this effect could be directly linked to drug action or if it originated from a reversible effect due to

cessation of male stimulation of the female because of non-pairing. In *S. mansoni*, the female does not complete sexual maturation and shows suboptimal growth and development in the absence of the male (Erasmus 1973).

A metabolite of *2b*, recovered from worms after *in vitro* and *in vivo* experiments, was identified by gas cromatography and mass spectral analysis (GC-MS) as being the disulfide derivative of the parent alkylaminooctanethiosulfuric acid (manuscript in preparation). A probable mechanism of action of these compounds should be based on the occurrence of the disulfide derivative of the parent alkylaminooctanethiosulfuric acid in the worm. This fact raises the possibility of the occurrence of disulfide interchange reactions (Kelley et al. 1967). These reactions would involve free thiol groups of proteins, among other compounds. The disulfide interchange reactions have been described as an interfering factor in thiol enzyme activity, as reviewed by Kitson (1988). One site may be the parasite enzymes, whose activities depend on essential sulfhydryl groups. For example, the hemoglobinases are responsible for the digestion of hemoglobin (Chappell & Dresden 1987), which is an important nutritional factor in worm mantainance (Clegg & Smithers 1972, Zussman et al. 1970); its impairment could be related to the lack of the vittelinic protein material shown above (Fig. 1).

The alkylaminooctanethiosulfuric acids have been demonstrated to be inactive *in vivo* against developmental stages of the parasites, only 24 to 29 day-old parasites being affected. Meanwhile, the *in vitro* experiments revealed an activity characterized by paralysis and bebbling of the tegument of the adult worm and schistosomula after 12 or 24 hr of incubation. In the lung stage, blebbing was not observed and quite different effects were observed between compounds *2b* and *2c* (Fig. 2A,B). These results may reflect differences in exposure time or in tegument compositions among the developmental stages of the worms (McLaren 1980).

The single 800 mg/kg dose required to eliminate 50 to 60% of the adult worms, including a 90% reduction of females, could be reduced in animals with a body weight greater than that of the mouse. For example, a single 1000 mg/kg dose of praziquantel was necessary to achieve 95% elimination of worms in the mouse. However, 249 mg/kg and 100 mg/kg doses were effective in hamsters and primates, respectively (Gonnert & Andrews 1977), due to the lower rate of metabolism observed in larger animals (Andrews 1976, Steiner et al. 1976). Therefore, there is good reason to believe that smaller doses would be efficient in larger animals. This fact encourages further work on this class of substances, mainly because the chemical scheme for their synthesis is versatile, the raw materials are inexpensive and more active compounds could be readily obtained. Also, the elucidation of the mechanism(s) of action of these compounds, considering the greater specificity against females than males, would be valuable for achieving a better comprehension of the differences in the metabolic events in both adult parasites.

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