Effect of An Arsenoso Compound in the Natural Infestation of White Mice With *Syphacia obvelata.* (19525)

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In an earlier paper(1) the marked anthelmintic effect of N-(p-arsenosobenzyl) glycine amide hydrochloride(2), designated Ro 2-1067, in the experimental trichinella infection of mice was described. A single oral administration of a well tolerated dose (500 mg/kg) of this arsenical protected the majority of mice against the fatal outcome of a massive infection with 2000 larvae of *T. spiralis* per mouse. A similarly striking effect was observed in the natural infestation of white mice with the pinworm *Syphacia obvelata.** Preliminary experiments had shown that the worm burden of naturally infected mice was completely eliminated by oral administration of Ro 2-1067. Following the procedure described by Wells(3) who used experimental infections with the mouse pinworm *Aspiculurus tetraptera* and Kam Fai Chan(4) who carried out similar experiments with Syphacia, repeated daily treatment for 5 to 6 successive days was employed in the earlier experiments. The success of this treatment schedule prompted a gradual reduction in frequency of drug administration until it was found that a single dose of the arsenical was sufficient to eliminate pinworms from the intestinal tract. The effect of graded single doses of Ro 2-1067 on the worm burden of the mice is described in this report.

Method. Adult albino mice of 22-26 g body weight were used in the experiments. The presence of Syphacia was ascertained by the demonstration of eggs in perianal smears using the Scotch tape technic. Groups of 20-36 animals received a single oral treatment by gavage with graded doses of the arsenical in aqueous solution and were then kept in cages in groups of 10-12 for a period of 6 days.

TABLE I. Effect of Arsenical Ro 2-1067 in Infestation of Mice with *Syphacia obvelata.*

Ro 2-1067 dose, mg/kg	No. of mice	Avg No. of worms*	Cure rate†
	Exp. #1		
500	20	0	20/20
250	20	0	20/20
125	20	0	20/20
62.5	20	8.6	14/20
50	30	5.4	16/30
25	29§	24.1	6/29
	Exp. #2		
500	36	0	36/36
250	36	.05‡	35/36
125	36	1	31/36
62.5	36	1	27/36

* On 7th day after treatment.
† No. of parasite free mice/No. of treated mice.
‡ One mouse harbored 2 parasites.
§ " " died.

On the 7th day the animals were sacrificed and adult and larval forms of Syphacia present in cecum, colon and rectum counted.

Experimental. During the course of this investigation 418 normal not treated mice were examined which all had shown *Syphacia* eggs in the perianal smears. The autopsy of these animals revealed in 413 of them or 98.8% the presence of adult and larval forms of Syphacia in smaller or larger numbers. The average number was 44 parasites per mouse, but as many as 358 worms were found in one mouse.

The high degree of variation in the number of parasites which seems unavoidable in a natural infection made it advisable to base the evaluation of an anthelmintic effect on the complete disappearance of Syphacia from the intestinal tract. In Table I, in which the observations on the activity of Ro 2-1067 are given, the results of the average worm counts are supplemented by data as to how many treated animals were free of parasites.

It might be seen from the data in Table I that in the 2 experiments recorded a single dose of 500 mg/kg eliminated the parasites in all animals. Following smaller doses such as

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125-250 mg/kg 86-100% of the animals were found free of Syphacia. If the dose was still lower (50-62.5 mg/kg) the number of parasite free animals decreased gradually and at the lowest dose level of 25 mg/kg almost all mice were found to harbor Syphacia. The number of worms recovered from the animals increased with decreasing doses of Ro 2-1067. It might be mentioned that in all treated mice as far as they were not completely free of *Syphacia* the number of larval forms was negligible (4 larvae in 79 mice) and they were, therefore, pooled with the adult forms.

Both the values of the worm counts and the cure rate appeared to be dependent on the dose of the arsenical used and indicate a definite dose-effect relation. The CD_{50} of Ro 2-1067 in the *Syphacia* infection of mice was estimated to be 48 mg/kg. Since the LD_{50} as determined for oral administration was 697 mg/kg the chemotherapeutic ratio LD_{50}/CD_{50} was 14.5.

In the experiments described so far the examination of the intestinal tract of mice was carried out 7 days after therapy. There is, however, evidence that the anthelmintic effect of a single administration of Ro 2-1067 is of longer duration. In one experiment a small group of 6 mice carrying *Syphacia* as shown by preliminary perianal smear was observed for 4 weeks after a single oral dose of Ro 2-1067, 500 mg/kg. The animals were kept without any special precautions in one of the commonly used suspended wire bottom cages. Only one of the mice contained 3 adult forms of *Syphacia* at the time of examination, the others were free of this parasite. Mice treated with Ro 2-1067 once and then kept in one cage together with infected mice were all found to contain *Syphacia* after 4 weeks. Protection against spontaneous infection with pinworms could, however, be achieved if the exposed animal received multiple treatments as in the following experiments:

A group of 14 mice with positive egg findings in the perianal smear were kept together in one cage. Nine of the mice received Ro 2-1067, 500 mg/kg by gavage twice a week, a total of 8 doses. Thirty days after the onset of the experiment and 3 days after the last treatment all treated and untreated mice were sacrificed and examined. In one of the treated mice one adult parasite was found, the other 8 were free of *Syphacia*. All 5 untreated animals harbored still parasites.

Discussion. It has been shown experimentally that a single treatment with the arsenoso compound Ro 2-1067 was able to eliminate *Syphacia obvelata* from infected mice. Ro 2-1067 was the first of our compounds to produce this marked effect which, as a rule, was not observed with most of the many other compounds tested. Known anthelmintics as far as they were active at all required repeated drug administration. The consistently high effect on *Syphacia* which can be achieved with a single administration of the arsenical suggests its usefulness for freeing mice of the parasite preparatory to their experimental infection with *Syphacia*.

Summary. N-(p-arsenosobenzyl)glycine amide hydrochloride possesses marked anthelmintic properties in mice infected with *Syphacia obvelata*. A single oral administration of 125-500 mg/kg eliminated the parasites in 85-100% of the animals. The CD_{50} was approximately 48 mg/kg.

1. Soo-Hoo, G., *Exp. Parasitology,* 1952, in press.
2. Fox, H. H., and Wenner, W., 1949, U. S. Patent No. 2,465,308.
3. Wells, H. S., *J. Infect. Dis.,* 1951, v89, 190.
4. Chan, Kam-Fai, *J. Parasitol.,* 1951, v37, (section 2), 14.

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