ANTHELMINTICS[1]

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I. INTRODUCTION

In the classical treatise entitled "This Wormy World", Stoll (191) presented a careful estimate of the amount of parasitism of man by helminths based upon survey data obtained throughout the world. His analysis of the global epidemiology of human helminthiases delineated the great parasitic rôle which worms play in the life of man. To cite a few examples, the calculated numbers of human

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helminthic infections *in millions* were 644 for *Ascaris lumbricoides*, 456 for hookworm, 355 for *Trichuris trichiura* and 208 for *Enterobius vermicularis*. The estimated numbers of infections were 114 million for schistosomes and 304 for filariae. Over 2250 million helminthic infections were harbored by a world population of 2166 millions. The frequent occurrence of infection of a single host by multiple species of parasitic worms accounts for the fact that the number of worm infections exceeds the population census of the world. Over 800 million human subjects are infected with worms.

Many of the anthelmintics in use today leave something to be desired in therapeutic effectiveness, lack of side-effects, suitability for mass treatment, low cost or other desirable features. In the light of Stoll's (191) estimates of the enormous number of helminthiases, any improvement in the chemotherapy of these infections and in our knowledge of the mechanisms involved in the action of anthelmintics assumes increased significance. This paper attempts to review some recent studies concerned with the treatment of certain helminthiases and with the possible mode of action of anthelmintics. The chemotherapy of filarial infections is not included because a review of this subject by Hawking (101) was published recently in this journal.

Since this review is concerned primarily with advances in the chemotherapy of helminthiases during recent years, the reader is referred to an earlier review (40) for information on this subject published prior to 1953. A brief summary of salient clinical and biological information about some of these helminthiases is presented to provide the reader with material which has a bearing on the objective of treatment and the proper evaluation of therapeutic results as they are influenced by the bionomics of the parasite.

1. *Mode of action of anthelmintic drugs.* Elucidation of the mechanism of action of anthelmintic drugs requires an understanding of the physiology of helminths. Aside from its intrinsically basic nature, this information is of potential value for the rational development of more effective anthelmintic agents. Such compounds would act by interference with essential metabolic reactions of the parasite without affecting the host in a similar manner. Opportunities would be available if biochemical differences did exist between the mammalian host and the invading organism. This possibility is in apparent conflict with the widely held view about the uniformity of biochemical reactions in all forms of life. Because of the occurrence of many common metabolic patterns, there has been a tendency to overlook differences within and outside of this framework. However, many examples of such variations have been pointed out by Cohen (66). In the case of a number of helminths, their products of carbohydrate metabolism differ from those of the host (31, 35, 40). But even if the reaction products were the same, the nature of the enzymes catalyzing these reactions is not necessarily identical in the parasite and in the host. For example, it has been demonstrated that differences between homologous glycolytic enzymes of *Schistosoma mansoni* and of rabbit muscle exist at various levels. Phosphoglucose isomerase of schistosomes can be distinguished from that of rabbit muscle only by its behavior towards specific antibodies (43). Other glycolytic enzymes of the same parasite

differ from the homologous enzymes of the mammalian host by their respective affinities and specificities towards several substrates, by the effect of the hydrogen ion concentration on their activities, by their coenzyme requirements and by their sensitivities to inhibitors (36, 41, 42, 45, 102, 134, 135). Such differences suggest possibilities for interfering with the functional integrity of the enzyme of the parasite without affecting the enzyme which has the same catalytic function in the host.

It has been demonstrated also that the biochemical characteristics of helminths vary greatly from one species to another. These differences may explain why many drugs which are highly effective against one helminth are devoid of activity against others.

Since a discussion of the comparative physiology and biochemistry of helminths exceeds the scope of this article, the interested reader is referred to other reviews of this subject (31, 40, 207). In this article, consideration of these aspects will be limited to those concerned with the mechanism of action of anthelmintic drugs.

2. Clinical evaluation of anthelmintic drugs. In the assessment of the anthelmintic efficacy of drugs, it is desirable to adhere as closely as practicable to certain criteria. When the nature of infections permits, quantitation of the worm burden by a series of egg counts should be made before and after treatment. The number of diagnostic examinations prior to and following therapy and the length of the post-treatment follow-up period required to provide valid data will vary with the species of helminth. The length of the prepatent period and the possibility of reinfection will influence the selection of a schedule for evaluation after treatment. The use of complete removal of helminths as the criterion for evaluating anthelmintic efficacy of the compound does not provide accurate data on its activity, since it does not indicate the degree of reduction of the worm burden in the host from which the parasites have not been eliminated entirely. In some intestinal helminthiases the use of stool examinations alone is inadequate for measuring the therapeutic effect of drugs and other procedures adjusted to the nature of the infections must be employed. The collection and examination of all intestinal worms passed during and after therapy is desirable when the efficacy of an anthelmintic is being evaluated. The gross effect of the drug on the structure, viability, and the motility of the worms should be ascertained. An untreated control series of patients is desirable to measure the rate of spontaneous cure. It is realized that the human element, when treating groups of patients, and the limitations imposed by field studies often militate against the adherence to ideal criteria for anthelmintic evaluation.

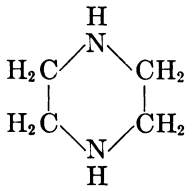
II. ASCARIASIS

1. Life cycle and clinical manifestations. The normal habitat of the adult worms is in the lumen of the small intestine. The worms are not attached to the mucosa. Ascarids apparently may exert pressure against the intestinal mucosa or against other worms by means of loops or coils in order to maintain their position or to migrate against the peristaltic current. The presence of *Ascaris lumbricoides*

in some persons may be related etiologically to abdominal pain or to other complaints. Of more serious import are the complications of ascariasis, such as partial or complete intestinal obstruction, perforation of the intestine and migration of adult worms to extra-intestinal sites. Female ascarids have tremendous egg-laying capacity. Very resistant immature eggs are passed in the stool. Under favorable environmental conditions, the eggs become embryonated and infective in the soil within two weeks. Children frequently acquire the infection through ingestion of the eggs by geophagia. Uncooked vegetables which have been fertilized with human excreta also provide a source of infection. The larvae hatch from the eggs in the small intestine. They enter the portal circulation and are carried to the liver, thence to the heart and lungs, where they grow and moult. If larvae are present in the sufficient numbers, they may produce a clinically evident pneumonitis; however, due to difficulty in proof of etiology, this condition is rarely diagnosed specifically as Ascaris pneumonitis. The parasites ascend the respiratory tract, pass down the esophagus and through the stomach for the second time, once as embryonated eggs and now as free larvae. The adults mature in the small bowel. Female ascarids ordinarily reach a length of 20 to 35 or more cm and are 3 to 6 mm in diameter. Male worms measure about 15 to 30 cm in length and 2 to 4 mm in diameter. The prepatent period, from time of inoculation to the appearance of the diagnostic eggs in the stools, is approximately 2 to 2½ months. Spontaneous loss of infection occurs. The longevity of a single infection is about eight months to one year. Adult worms frequently are passed *per anum* and occasionally *per os* and *nares* in untreated patients. The magnitude of the global prevalence of ascariasis is attested by Stoll's estimate (191) of 644 million infections, which represents more than one-fourth of all of man's helminthiases.

2. Piperazine. One of the most significant recent advances in the field of anthelmintics has been the introduction of piperazine hexahydrate and its salts, such as citrate, adipate and phosphate, for the treatment of ascariasis and enterobiasis. The low toxicity, high anthelmintic efficiency and moderate cost of these compounds has resulted already in their widespread use against these nematode infections. The citrate salt has been employed extensively and its efficacy evaluated precisely for the treatment of patients infected with ascariasis and enterobiasis.

Piperazine (diethylenediamine) is available as a hexahydrate which contains about 44% of the base, as well as in form of various salts.



Piperazine

a. Pharmacological and biochemical aspects. The evidence available to date indicates that piperazine produces a paralysis of Ascaris muscle which results in

the expulsion of the worm through the peristalsis of the host's intestines. In its early stages, this paralysis is reversible. This is borne out by the following observations: a. Incubation of Ascaris in a buffered salt medium containing piperazine results in a paralysis of the worms. If, subsequently, the parasites are transferred into piperazine-free medium, motility is resumed (187). b. When Ascaris, expelled by patients who had received piperazine, are incubated in a salt medium at 37°C they gradually recover from the paralyzing action of the drug and eventually their muscular activity is indistinguishable from that of worms which had not been exposed to piperazine (27, 196).

Recently, Norton and de Beer (152) have investigated the mechanism of action of piperazine on Ascaris muscle. They have found that low concentrations of acetylcholine (2–10 μg/ml) produce a contraction of this tissue. Piperazine blocks this response of Ascaris muscle to acetylcholine. Significantly, piperazine has a much weaker blocking effect on mammalian skeletal muscle (152). Therefore, it appears that piperazine has the properties of a myoneural blocking agent selective for Ascaris muscle. Similar blocking actions were observed with phenergan (152), a phenothiazine derivative. It should be noted that the presence of acetylcholine (141) and of acetylcholinesterase has been demonstrated in Ascaris (38).

The biochemical effects of piperazine on Ascaris have been the subject of another recent study. Ascaris produces large quantities of succinic acid under both aerobic and anaerobic conditions (44, 49). During incubation of Ascaris with paralyzing concentrations of piperazine, production of succinate is reduced markedly. Following transfer into a piperazine-free medium, succinate production is resumed at a normal rate; therefore, this metabolic effect of piperazine is reversible. By contrast, inhibition of succinate production by phenergan is irreversible. There is a close parallelism between the concentrations of piperazine which exert a paralyzing effect on the worm, those which block the stimulatory effects of acetylcholine on Ascaris muscle and those which produce a reduction in succinate formation (49, 152). The inhibitory action of piperazine on succinate production raises the question whether and in what manner this biochemical effect is related to the muscular paralysis produced by this drug. In skeletal muscle of vertebrates the energy produced during the conversion of carbohydrate to lactic acid ultimately is utilized for muscular contraction through the resynthesis of ATP and other energy-rich phosphate compounds. Since lactic acid is not a quantitatively significant endproduct of the anaerobic carbohydrate metabolism of Ascaris (35), metabolic reactions involving the formation or the utilization of succinate may be concerned with the supply of energy required by the parasite for muscular contraction. In such an eventuality reduction or complete suppression of muscular activity produced by piperazine would lower the energy requirement of the muscle and result in a decreased formation of succinate. On the other hand, it is conceivable also that at the myoneural junction chemical reactions associated with the production of succinate, such as the formation of coenzyme A (CoA) or of succinyl CoA, may be required for the response of Ascaris muscle to the stimulatory effects of acetylcholine. While

much more information is needed to provide a correct interpretation of these observations, further studies of the physiological and biochemical effects of piperazine should shed some light not only on the mechanism of the anthelmintic action of this drug, but also on the chemistry of muscular contraction of Ascaris.

b. Clinical effectiveness. Piperazine salts enjoyed long and extensive use several decades ago for the treatment of gout and other conditions. Failing to fulfill its initial promise, piperazine fell into disuse until recently, when it was shown to be an effective agent for the therapy of enterobiasis and ascariasis. The recent literature on the use of piperazine for the treatment of these two infections is so voluminous that it is not practicable to cite all of the contributions which have helped to establish its eminent position and usefulness among the anthelmintics.

Fayard (78) was the first to report the anthelmintic activity of piperazine hydrate against *Ascaris lumbricoides*. Later, cases were reported in which the use of piperazine hydrate or piperazine diphenylacetate resulted in the passage of ascarids or in complete elimination of the infection (13, 15, 148, 204, 214). Some of these observations were made during the treatment of patients for enterobiasis with these piperazine compounds (48, 204). More thorough evaluations of piperazine citrate for the treatment of ascariasis have been reported recently (22, 24, 25, 79, 142, 193, 196, 198). The use of piperazine citrate has resulted in almost complete removal of ascarids over a wide range of dosage and treatment schedules. Short courses of therapy with this drug have been found to be highly effective against ascariasis (7, 25, 27, 79, 107, 142, 156, 193, 198). Two dose courses of therapy with piperazine citrate ordinarily provide a cure rate above 90% (142, 198). An effective schedule for the treatment of patients with ascariasis is as follows: an initial dose of 150 mg (hexahydrate equivalent) of piperazine citrate (in syrup form) per kg of body weight, with a maximum of 3 g; the same treatment is repeated, with another single dose, one week later (142, 198). Since a single dose therapy for ascariasis would have considerable appeal and practicability for mass treatment, several evaluations of this form of treatment have been conducted but it was less effective than the two dose treatment (7, 25, 79, 92, 107, 142, 193, 198).

No significant difference in the therapeutic efficacy of piperazine citrate, adipate and phosphate against *Ascaris lumbricoides* in humans has been demonstrated (17, 107, 142, 198). Standen (187) tested piperazine citrate, adipate and phosphate *in vitro* against *A. lumbricoides var. suis*. He observed that these piperazine salts were equally active in inducing a state of paralysis in the worms. The drug effect was gradual and did not stimulate violent movements of the helminths. Thus *in vivo* observations on the therapeutic value of these three piperazine salts against ascarids in man correlate with the findings on the *in vitro* effect of these compounds on ascarids from pigs. Also, there is no essential difference in the systemic absorption of these salts in man (86).

The preparation of piperazine citrate in a palatable syrup facilitates the treatment of infants and children who can not swallow tablets. Fasting prior to treatment and purgation after therapy are unnecessary. There is no hazard of local tissue damage from the drug. Since the ascarids usually are alive when

passed during or after treatment, the possibility of absorption of disintegration products of the parasites, if any, is minimized. The sluggish condition of the ascarids reduces the hazard of migration. All of these factors, in addition to the high efficacy of two dose treatments, contribute to the suitability of piperazine citrate for individual or mass treatment for ascariasis.

Jenkins and Beach (111) reported results of a conservative treatment for partial intestinal obstruction due to *Ascaris lumbricoides*. Prompt use of an anthelmintic was an integral part of their therapy. The measures consisted of Wangensteen drainage, parenteral fluids, saline enemas and small doses of hexylresorcinol (crystoids anthelmintic) on alternate days without a laxative, or diethylcarbamazine three times a day for one week. When the effectiveness of piperazine for the elimination of worms in cases of uncomplicated intestinal ascariasis was observed, one of the authors and his associates (179, 195, 198), employed this compound with considerable success in patents in whom there was clinical evidence of partial obstruction. Basneuevo *et al.* (16) reported cure of a case of partial intestinal obstruction by Ascaris with piperazine hexahydrate. Piperazine citrate syrup has several qualities which render it suitable for use in patients with this complication of ascariasis. The drug is in liquid form and can be delivered easily through the drainage tube. There is a high concentration of the active ingredient in the syrup; therefore, effective doses can be introduced into the intestine in small volumes of fluid. The drug immobilizes the worms without producing their disintegration within the bowel. Conservative measures for the management of partial intestinal obstruction and the administration of piperazine citrate syrup through the drainage tube have obviated the necessity of surgery in some patients with this complication of ascariasis (179, 198).

Peña *et al.* treated 36 typhoid patients harboring *Ascaris lumbricoides* with piperazine citrate without untoward reactions. The flaccid condition of the worms appeared to minimize the possibility of intestinal perforation by the ascarids in patients with typhoid fever (159a).

Piperazine citrate causes the elimination of both mature and immature ascarids from the intestine (92, 196). The worms are eliminated in greatest numbers within one to three days after the initiation of treatment (159, 196). The presence of food in the digestive tract has little, if any, effect on the activity of piperazine citrate against *A. lumbricoides* in man (25).

The vast experience with treatment of patients with ascariasis and enterobiasis with a variety of dosages and regimes of piperazine citrate for periods as long as 15 days indicate that this anthelmintic is safe and that side-effects are infrequent (25, 74, 77, 79, 105, 107, 193, 196, 198). Apparently there is a wide range between effective therapeutic and toxic dosages of piperazine citrate (25, 196). The oral LD_{50} of piperazine citrate for mice is 11 g/kg. Laboratory studies, including BUN, cephalin flocculation, thymol turbidity, urinalysis and complete blood counts, were made on patients who received 150 mg (hexahydrate equivalent) of piperazine citrate per kg of body weight daily for five days. No abnormal findings were obtained (196). Attempts to sensitize guinea pigs to

syrup of piperazine citrate ('antepar') have failed. In view of the absence of sensitizing properties when tested in the guinea pig, the drug has been regarded as nonallergenic. It also has been judged nonanaphylactogenic in this animal (170).

Some of the occasional or rare reactions to either piperazine hydrate or citrate have been nausea, vomiting, headache, abdominal cramps, urticaria, vertigo, tremor, incoordination, muscular weakness, difficulty in focusing vision, a sense of detachment, memory defects, dropping of articles, erythema multiforme, mild diarrhea and lethargy (7, 73, 109, 167, 186, 198, 213). Transient neurological side-effects have been recorded mostly in adults. Some of these may have resulted from excessive dosage due to the viscosity of the syrup and from variations in content of tablespoons (186, 213). Piperazine citrate or hydrate have been employed without ill effect during pregnancy (12, 167). The only toxic manifestations resulting from the accidental ingestion of approximately 9 g of piperazine by a three year old girl (weighing 10.5 kg) consisted in the transitory development of hypoactive patellar reflexes and of an apparent inability to sit erect (196). Less extensive experience with piperazine adipate and phosphate suggests that these salts also are well tolerated (74, 100, 107, 171, 194, 198).

3. Other drugs. According to Hoekenga (104, 107), none of the therapeutic results observed with diethylcarbamazine ('hetrazan'), sodium santoninate, nematolyt (activated papain), hexylresorcinol monoacetate, hexylresorcinol or a chenopodium-chloroform mixture was as favorable as those obtained with piperazine. Of the various single dose treatments with the above drugs, hexylresorcinol gave the best results with only 42% cures; the chenopodium-chloroform mixture ranked second in effectiveness with a 40% rate of cure. A two dose regime of hexylresorcinol produced a cure rate of 86% and a four day course of diethylcarbamazine eliminated the infection in 80% of the patients treated for ascariasis (107).

Other anthelmintics which contain proteolytic enzymes, such as papain (activated by cysteine) have some therapeutic effect on *Ascaris lumbricoides* infections in man (58, 116). Since the papain-containing anthelmintics often require dietary preparation and post-treatment purgation and have a relatively low therapeutic index, they do not compare favorably with the piperazine salts in efficacy or simplicity of administration.

Diethylcarbamazine gave unsatisfactory results in the mass therapy for ascariasis in school children (87). Promethazine hydrochloride [N-(2'-dimethylamino-2'-methyl)ethylphenothiazine hydrochloride], failed to show evidence of significant therapeutic value for ascariasis when given in large single doses of 125 mg (201).

Swartzwelder *et al.* (200a) obtained complete elimination of *Ascaris lumbricoides* during therapy with dithiazanine (see below) for multiple infections with trichuriasis and ascariasis. The observation that this compound is active against both *A. lumbricoides* and *Trichuris trichiura* is significant since these two helminths frequently coexist in the same host. Both immature and mature ascarids were passed during the course of therapy with this anthelmintic. The worms were

stained a light greenish-blue color. The discoloration involved the cuticle and the muscle cells; the anterior portion of the intestinal tract of some ascarids was stained deeply. The discolored adult worms were extremely soft, flaccid and non-motile when expelled from the intestine. Upon rinsing these worms with distilled water and incubation at 37°C, the limp state disappeared and motility returned. Thus the effect of the drug on the motility of ascarids is reversible (200, 200a).

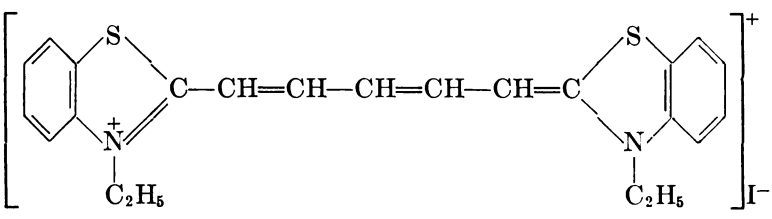
III. TRICHURIASIS

1. Life cycle and clinical manifestations. The adults of the roundworm *Trichuris trichiura*, derive their common name, whipworm, from the long thread-like anterior portion and the wider posterior end. Their size range is between 30 and 50 mm in length. The posterior portion of the male characteristically is coiled like a watchspring. The anterior end of the worm usually is embedded in the mucosa of the cecum, appendix and colon. In heavy infections, the nematodes may be found throughout the colon and in the rectum.

Very light whipworm infections usually produce no symptomatology. The presence of a large worm burden may be associated with diarrhea of long duration, dysentery, mucoid stools, abdominal pain and tenderness, dehydration, severe anemia, weight loss and weakness. Occasionally prolapse of the rectum occurs. Adult worms may be observed on the prolapsed bowel or by sigmoidoscopy. Some patients with heavy infections are acutely ill. As in hookworm infection, egg counts frequently are desirable in order to determine the size and significance of the whipworm infection.

Trichuriasis is acquired in a manner similar to ascariasis and frequently the two helminthiases coexist in the same individual. Immature eggs are passed in the stool. They embryonate in the soil under favorable conditions and become infective within a few weeks. Upon ingestion of the eggs, by geophagia, or in food or drink contaminated by infested soil, the parasites mature in the intestine. Unlike *Ascaris lumbricoides* the whipworm requires no migration through the lung of its host. The adult female whipworm lays several thousand eggs daily. Diagnosis is made by demonstration of typical eggs in the stool. The adult whipworms are not commonly observed in the stool except after treatment or in the exudate from patients with severe clinical infections.

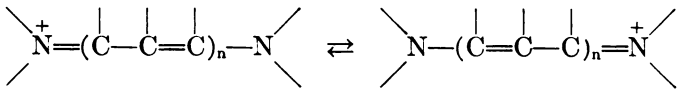
2. Cyanine dyes. a. Effects of cyanine dyes on the metabolism of helminths. A very promising development in the chemotherapy of whipworm infection is the demonstration of the therapeutic effectiveness of 3,3′-diethylthiadicarbocyanine iodide (dithiazanine) (84, 200, 200a), a cyanine dye.



Dithiazanine

Earlier studies demonstrated the anthelmintic activity of cyanine dyes against the filarial worm of the cotton rat, *Litomosoides carinii* (163, 210). In extremely low concentrations (5×10^{-8}M) the cyanines produce an inhibition of the oxygen uptake of adult *Litomosoides* (30, 32). This respiratory inhibition is associated with a compensatory increase in aerobic glycolysis. The oxygen uptake of filariae of cotton rats to whom subcurative doses of cyanines had been administered is decreased and their aerobic glycolysis is increased. Therefore, it is probable that these compounds exert their chemotherapeutic effect through inhibition of enzyme systems concerned with oxidative metabolism. Concentrations of cyanine dyes a thousand times higher than those exerting an inhibitory effect on the respiration of Litomosoides have no effect on the oxygen uptake of slices or homogenates of mammalian tissues or on the activities of cytochrome c or of cytochrome oxidase (32). Neither of the latter two respiratory enzymes could be detected in Litomosoides (37). Therefore, it appears that cyanines interfere in these worms with respiratory enzyme systems which play no role or only a minor one in mammalian tissues.

The cyanine dyes contain the *amidinium* ion system in which a quaternary nitrogen is separated from a tertiary nitrogen by a resonating or "conjugated' carbon chain of alternating double and single bonds:



If both nitrogens are incorporated in two heterocyclic rings, such compounds are known as *cyanines*: in *styryl* dyes only one of the nitrogens is a member of a ring. For a detailed discussion of the chemistry of these compounds the reader is referred to reviews by Brooker (19, 20, 21). Inhibition of the oxygen uptake of *Litomosoides in vitro* and antifilarial activity *in vivo* are exerted by a great variety of amidinium compounds. Activities *in vitro* and *in vivo* are not restricted to any particular ring and are maintained despite wide variations in structure. However, any modification which abolishes the possibility of amidinium ion resonance results in a disappearance of metabolic and chemotherapeutic activities against Litomosoides (163). Administration of a cyanine dye to hamsters infected with *Schistosoma mansoni* resulted in a marked reduction of the oxygen uptake of the parasite (39); yet, cyanines had no observable chemotherapeutic activity in experimental schistosomiasis (39). Therefore, in contrast to Litomosoides, oxidative metabolism does not supply a major portion of the energy required for the survival of *Schistosoma mansoni*. This is also borne out by the fact that the rate of glycolysis of schistosomes is not affected by cyanine dyes (33). If this parasite were dependent on aerobic metabolism, inhibition of oxidative reactions by cyanine dyes should result in a compensatory increase in the rate of glycolysis. The complete lack of chemotherapeutic activity of cyanines against *Wuchereria bancrofti* (99, 177) suggests that this filarial worm, in contrast to Litomosoides does not require oxidative metabolism for survival. Therefore, despite the close morphological relationship between *Litomosoides carinii* and *Wuchereria ban*

crofti, biochemical differences exist between these two filarial worms. The chemotherapeutic actions of cyanines against the former and their lack of activity against the latter demonstrate that the sensitivity of a parasitic worm to anthelmintic agents is determined to a much greater degree by biochemical than by morphological or taxonomic characteristics.

Recent studies have revealed that in another helminth, *Trichuris vulpis*, the whipworm of dogs, anaerobic metabolic reactions, essential for the survival of the parasite, are inhibited by cyanine dyes. While these compounds do not affect the rate of anaerobic glucose utilization of *Litomosoides carinii* (32), of *Schistosoma mansoni* (33, 39) or of the nematode, *Dracunculus insignis* (34), incubation of *Trichuris vulpis* for short periods of time with low concentrations (2×10^{-6} M or 50 μg/ml) of cyanine dyes resulted in an irreversible inhibition of the anaerobic carbohydrate metabolism of this worm (51). In contrast to the other helminths mentioned above, lactic acid production accounts for less than 50% of the carbohydrate utilized anaerobically by *Trichuris vulpis* (51). Since lactic acid formation by this organism was not inhibited by cyanines, these dyes must interfere with another metabolic reaction involved in the anaerobic utilization of glucose by *Trichuris vulpis*. The dependence of this helminth on anaerobic rather than on aerobic metabolism is suggested not only by the low oxygen tension of its habitat, the colon of the dog, but also by the fact that the motility of the parasite is not reduced during incubation for 24 hours in an atmosphere of nitrogen (51). The biochemical and chemotherapeutic actions of cyanines on Trichuris indicate that the effects of these dyes on the metabolism of helminths are not limited to oxidative processes, but may involve anaerobic reactions also. Interference with one of the latter may account for the anthelmintic effects of the cyanines in trichuriasis and in other intestinal helminthiases (see below).

b. Therapeutic actions. Frye *et al.*, in preliminary studies (84), obtained high cure rates in persons infected with *Trichuris trichiura* who were treated with dithiazanine. The infection was completely eliminated in 14 of 16 patients who received 200 mg of the drug in CAP-coated tablets by mouth three times daily for 5 days. Twelve of 17 patients (71%) were cured with a lower dosage of 200 mg twice daily for 5 days. Patients whose weight was less than 40 kg received half the above dosages. There were no side reactions in any of the 33 patients who received the coated tablets. When the drug was administered in gelatin capsules, nausea and vomiting frequently occurred. The worms were dead and partially stained by the blue dye when passed in the stool during therapy.

Studies by Swartzwelder *et al.* (200a) on the therapy of severe whipworm infections with diarrhea, dysentery and prolapse and large worm burdens quantitated by egg counts, indicate that clinical cure of such cases can be achieved by oral administration of dithiazanine. Within a few days after the start of treatment the dysentery terminated. Higher dosage and/or longer treatment schedules may be required to attain complete parasitic cure of patients with heavy infections. The above workers also observed that the adult trichurids usually were flaccid and appeared to be non-viable upon passage in the stool. Rinsing in distilled water and incubation at 37°C failed to induce a return of motility in worms

expelled during therapy. Thus, the effect of the drug on trichurids was not reversible in contrast to the findings on ascarids. Mature whipworms were passed in large numbers after only one day of treatment and continued to be expelled in the stool during the course of therapy. Many of the worms appeared visibly stained blue at the anterior whiplike and/or the posterior portion of the parasite. The long tubular intestine and the reproductive system of the worms apparently contained the dye also. Eggs of *Trichuris trichiura* and of *Ascaris lumbricoides* in the stools of patients under therapy with this anthelmintic were intensely stained.

Pérez-Santiago *et al.* (161) reported the recovery of adult whipworms following oral administration of another cyanine dye, pyrvinium, in six patients with hookworm disease. Since this observation was made during a comparative evaluation of the effect of the cyanine dye and tetrachlorethylene, based upon recovery of adult hookworms in the stools, the cure rate of the compound on *T. trichiura* infections was not determined. Nausea, vomiting and epigastric pain were frequent complaints during therapy, but these reactions were not sufficient to warrant discontinuation of treatment.

The broad anthelmintic range of the cyanine dyes is evident from their activity against filariasis in the cotton rat (210), several species of intestinal nematodes of dogs (99, 138), pinworms of rats and mice (199, 211), *Strongyloides ratti* (199, 211), and intestinal nematodes of man (84, 149, 161, 175, 180, 199, 200, 200a) as well as from their ability to interfere in low concentrations with metabolic reactions in helminths.

3. Other drugs. Retention enemas with hexylresorcinol are beneficial for the treatment of patients with severe clinical infections and heavy worm burdens (9, 10, 31, 115, 118). However, this method of therapy usually requires hospitalization and is attended by some hazard. It is not very practicable for general use in areas where trichuriasis is heavily endemic nor desirable for the treatment of persons with light whipworm infections.

In view of the activity of certain piperazine salts against *Ascaris lumbricoides* and *Enterobius vermicularis*, an evaluation of their effect on *Trichuris trichiura* was inevitable. Most of the reports have indicated that piperazine citrate failed, with the many dosages and regimes employed, to eliminate whipworm infections in a significant percentage of the patients treated (7, 27, 105, 106, 194, 196). However, Guill (95a) reported a high cure rate of trichuriasis in adults when 6 g of piperazine citrate were given "in two divided doses in the morning and afternoon followed the first time in three to four hours by a good saline laxative". This observation could not be confirmed by Swartzwelder *et al.* (201a).

There is disagreement on the therapeutic value of piperazine adipate against trichuriasis. Dunn (74) has reported that the stools of 28 of 31 children were free of Trichuris eggs after a three day course of therapy with piperazine adipate In those cases in which the infection was not completely eliminated, the eggs became extremely scanty. Hoekenga (106) obtained a 50% cure rate in 22 cases treated with a single dose of 3.5 g of piperazine adipate. In contrast, other workers (194) failed to observe any therapeutic effect of piperazine adipate on trichuriasis in 32 patients treated with 2 g of the drug daily for 20 days.

Preparations such as "nematolyt" and "vermizym", which contain papain, failed to cure whipworm infection in the majority of cases (104, 158). The administration of enteric-coated capsules containing papain, however, was reported to be effective for the treatment of trichuriasis (131).

A trichuricidal drug of particular interest is 3-methyl-1-pentyn-3-yl acid phthalate ('phthalofyne', 'whipcide'). This compound was found to be effective and safe against the canine whipworm, *Trichuris vulpis*, in a single oral dose (75). It was moderately effective in eliminating trichurids of man in single or multiple oral doses, but was not curative when administered in a retention enema (106). At the higher dosages which were found effective in the dog, a type of ocular toxicity appeared in humans which was not present in other species studied, including monkeys. It did occur, however, in chimpanzees. The toxic manifestations were conjunctivitis and/or keratitis, ocular pain and photophobia. The duration of these disturbances was one to three days. No residual eye injury remained in patients in whom these manifestations occurred (56, 106).

The maximal percentages of cures obtained with a variety of anthelmintics employed by Hoekenga (106) in patients with trichuriasis were as follows: a combination of tetrachlorethylene and oil of chenopodium in a single dose, 50; leche de higueron, 18.5 (with an average reduction in egg count of 62.8%); hexylresorcinol (crystoids), 36; sodium santoninate, 37.5; methylbenzene (toluene), 33.3; and methylbenzene and diphenthane ('vermiplex'), 18.5. Twenty-two patients who received up to 4 g of puromycin during four to ten days still had *Trichuris trichiura* infection (218).

IV. HOOKWORM INFECTIONS

1. Life cycle. The site of predilection of adult hookworms (*Ancylostoma duodenale* and *Nector americanus*) is the small intestine where they are attached to the mucosa. Blood from the villi is pumped into and through the intestine of the hookworms. The blood loss by the parasitized host may, under certain conditions, cause a microcytic hypochromic anemia. The clinical manifestations and physical findings in cases of severe hookworm disease originate in a large measure from anemia. Hookworm eggs appear in stools of infected persons. Under favorable environmental conditions, infective larvae may develop in soil contaminated with excreta from such individuals. The infection is acquired by contact with soil. The larvae penetrate the skin, enter the circulatory system, reach the lungs, erupt into the alveoli, ascend the respiratory tract and ultimately reach the small intestine where they attach and mature into adult worms. These nematodes are cylindrical in shape and measure about 10 mm in length. Eggs appear in the stool five or more weeks after invasion of the host by the larvae.

2. Clinical approaches to the treatment of hookworm infections. Tetrachlorethylene has been used for many years for the removal of hookworms. It is still considered the drug of choice for the treatment of persons with hookworm infection, provided that the patients do not also have ascariasis. In the past, purgation with sodium sulfate or magnesium sulfate within a few hours after administration of the anthelmintic has been recommended. The significant

change in the therapy of patients infected with hookworms has been in the improvement of the method of usage of an established anthelmintic rather than in the introduction of new drugs. Carr *et al.* (59) observed that when tetrachlorethylene was administered *without* subsequent purgation, there was less shock to the patient and more effective removal of worms than when the anthelmintic was followed by a saline purge. These workers observed that patients with severe anemia due to hookworm infection can be treated with a full dose of tetrachlorethylene, without serious unfavorable reactions from the treatment, if purgation is not employed. Dosage schedules of 0.1 or 0.12 ml of tetrachlorethylene per kg. of body weight, with maximal total doses of 4 or 5 ml in a single dose, were highly effective for removal of hookworms and well tolerated by the patients. These two dosages were employed effectively and safely in mass therapy. Precise evaluations by Carr and his associates of the therapeutic efficacy of the above schedules indicated that a single treatment usually expelled most of the worms. However, two or more treatments at four day intervals were required to obtain complete removal of the worm burden. Single doses of either 4 or 5 ml of tetrachlorethylene removed a higher percentage of hookworms than 3 ml which generally has been recommended in the past. Saenz *et al.*, based upon extensive experience in Costa Rica, prefer to use tetrachlorethylene without subsequent purgation (176a).

In Africa, Pinto *et al.* (166) employed tetrachlorethylene, in a dosage of 0.12 ml/kg, with a maximum single dose of 5 ml every four days until the infection was eliminated completely. In 165 patients with hemoglobin values ranging from 25 to 85% of normal, complete removal of worms was obtained in 95% of the cases with four treatments or less. The above dosage of tetrachlorethylene was tolerated well, even in the cases with severe anemia. Pinto and his associates (166) concurred in the observations of Carr *et al.* (59) that the omission of the saline purge after treatment of hookworm infection with tetrachlorethylene increases the effectiveness of the drug, shortens the time of treatment, lowers its toxicity and cost and facilitates the administration of mass treatment. Precisely, it is better for the patients and worse for the worms.

Following the use of tetrachlorethylene, vertigo, headache, a burning sensation in the stomach, abdominal cramps, nausea and vomiting occur some times. Patients with severe anemia may collapse during therapy, especially if purgation is employed. The pharmacology of tetrachlorethylene has been studied extensively over two decades ago and will not be reviewed here. However, despite claims that tetrachlorethylene is not readily absorbed from the gastrointestinal tract, vertigo frequently occurs in patients treated with this drug.

The precise mode of action of tetrachlorethylene on adult hookworms apparently has not been elucidated. When purgation is employed a few hours after the administration of tetrachlorethylene, motile worms may be recovered from the stool. Although the worms appear viable after exposure to the anthelmintic within the intestine, they have been unable to maintain their attachment to the mucosa of the small intestine. It has been *assumed* in the past that the hookworms have been paralyzed sufficiently by the drug to release their attach-

ment to the intestinal wall and that the worms should be expelled by purgation before they may reattach themselves to the intestine. However, as discussed above, it appears that purgation is not required for removal of worms from the intestine (59, 166).

When ascariasis and hookworm infections occur in the same patient, it is generally considered that tetrachlorethylene is contraindicated. However, this view is not concurred in by many physicians with wide experience in the tropics. In such cases, it is a common practice to employ hexylresorcinol. This anthelmintic is active against both helminthiases, but is less effective than tetrachlorethylene against hookworms. After the ascarids have been removed, the patient may be treated with tetrachlorethylene in order to eliminate or further reduce the hookworm burden. An alternate method of treating patients with mixed infections with hookworm and *Ascaris lumbricoides* consists in the elimination of the ascariasis with a short intensive regime of piperazine citrate and then proceed with the treatment of the patient with tetrachlorethylene without purgation.

It should be borne in mind that an essential part of the treatment of hookworm disease, *i. e.*, hookworm infection and anemia, is therapy directed toward the restoration of normal hemoglobin values. Administration of ferrous sulfate, and in cases of extreme anemia, transfusion with whole blood or packed red corpuscles may be indicated. In view of the frequent association of anemia, hypoproteinemia and malnutrition with hookworm disease, improvement or correction of diet also is often indicated.

Evidence of the activity of cyanine dyes against *Necator americanus* has been obtained (161,200a.) One compound was less effective for the removal of hookworms in Puerto Rican patients than tetrachlorethylene. Nausea, vomiting, epigastric pain, anorexia, diarrhea and vertigo were frequent complaints during administration of the cyanine dye. The recovered worms were dead and had been stained by the dye (161). Dithiazanine produced marked reduction of egg counts and passage of adult hookworms in Costa Rican patients (200a).

The anthelmintic activity of the piperazine salts against hookworms in man has not been measured adequately in infections quantitated by egg counts (90, 92, 105, 106, 196). From the limited data available now, it appears that piperazine and its derivatives are inferior to tetrachlorethylene for the treatment of hookworm infection.

Clinical trials of 1-bromo-β-naphthol have indicated that it has significant activity against *Ancylostoma duodenale*. It is claimed that this compound can be used in large dosage because of its low toxicity. No purgation is required when the drug is given. It produced only occasional minor reactions in patients who were treated for hookworm infection (144).

In view of the high effectiveness of N-(2,4-dichlorobenzyl)-N-(2-hydroxyethyl) dichloroacetamide (Win 5047 or mantomide®), reported by Sheth *et al.* (183) in India, further evaluation of the efficacy of this compound against hookworm seems highly desirable.

A comparison of the therapeutic efficacy of a variety of anthelmintics against *Necator americanus* infections by Hoekenga (106) gave the following maximum

percentages of complete worm removal: methylbenzene, 50; a combination of methylbenzene and diphenanthene (vermiplex®), 31; activated papain (nematolyt), 60; and hexylresorcinol (crystoids anthelmintic), 25. A mixture of 2.7 ml of tetrachlorethylene and 0.3 ml of oil of chenopodium in a single dose, followed by a purge, resulted in elimination of all worms in 50% of the patients treated. Puromycin failed to eliminate hookworm infection in 16 persons who received from 0.8–4 g of the drug (218).

V. STRONGYLOIDIASIS

1. Clinical manifestations. The minute parasitic adult female *Strongyloides stercoralis* may be imbedded in the mucosa of the small intestine, especially in the duodenum. The presence of the infection in some individuals may elicit a tissue reaction associated with clinical, and sometimes radiographic, evidence of a duodenitis. Frequent symptoms and findings in clinical cases of strongyloidiasis are pain in the epigastrium, nausea, vomiting, anorexia, weight loss, weakness, diarrhea and urticaria. The manifestations may recur frequently. These recurrences of symptoms possibly are associated with hyperinfection or internal autoinfection. Strongyloidiasis initially is acquired by the invasion of the skin by infective filariform larvae from infested soil. The prepatent period for human strongyloidiasis is about one month. In this helminth infection, non-infective rhabditiform larvae usually are passed in the stool. At times these larvae may develop into the filariform stage in the large intestine or in the perianal region and reinvade the same host. Thus, reinfection may occur repeatedly and frequently. Strongyloidiasis, through these mechanisms, may persist for many years in the same host.

2. Clinical evaluation of drugs. Dithiazanine has significant activity against *Strongyloides stercoralis* and, in the writer's experience, is the first effective therapeutic for strongyloidiasis. A cure rate of 89% was obtained in a series of 18 cases treated with 200 mg of this compound three times daily for from 5 to 21 days. The criteria for cure were the absence of diagnostic forms in a series of examinations of both stools and duodenal fluids after therapy. This evidence of anthelmintic activity of dithiazanine against Strongyloides was supplemented by complete elimination of *S. ratti* infection, the experimental murine counterpart of human strongyloidiasis, in 100% of rats given this compound. Both the tablet and syrup forms of the drug were tolerated well by adult patients who received the medication (199).

Several drugs, such as intravenous gentian violet, miracil D and mantomide, have been recommended as therapeutic agents for strongyloidiasis (91, 139, 157). However, limited attempts to repeat these studies, with rigid criteria for cure, have failed to provide evidence of therapeutic effectiveness of these drugs (114). It is well known that stool examination is not consistently reliable for the demonstration of *S. stercoralis* larvae. Jones and Abadie (113) recently confirmed this observation and precisely defined the comparative efficiency of diagnosis of strongyloidiasis by examination of feces and duodenal fluid. From their data, it is obvious that evaluation of the results of therapy, based upon examination of

stools alone, is likely to be misleading. Final evaluation of the efficacy of drugs against strongyloidiasis should be based on a series of examinations of both stools and duodenal fluid.

Browne (29) encountered thrombosis of the veins, pain and discomfort from intravenous gentian violet therapy. In addition, this form of treatment was almost uniformly unsuccessful when evaluated by examination of both stools and duodenal aspirates (29). The lack of effectiveness and the difficulties encountered in the administration of gentian violet solution intravenously contraindicated its use (29). Jung and Faust (119) expressed a similar view.

The therapy of strongyloidiasis by oral administration of gentian violet, from the standpoint of complete elimination of the infection, is almost uniformly unsatisfactory. The diagnostic stages of the parasite may be recovered from either the stool and/or duodenal contents regardless of the dosage schedule used orally or intraduodenally. However, in many cases, treatment with gentian violet may be attended by amelioration of symptoms, decrease in the total leukocyte and eosinophil counts and temporary disappearance of larvae from the stools. For these reasons, at present, gentian violet is still used widely for the treatment of patients with strongyloidiasis. It should be emphasized that gentian violet tablets specially coated to release the dye 1½ hours after administration should be employed. These tablets are taken one hour before meals. Gentian violet tablets with longer release times may liberate the drug below the site of the infection.

For small children who cannot swallow tablets, Basnuevo and Borbolla (11) prepared an alkalinized granular mixture containing gentian violet which can be dissolved in milk, fruit juice or in water sweetened with sugar. A suspension of the phenolphthalein salt of gentian violet was well tolerated by children who were treated for pinworm infection (55). It is possible that this gentian violet suspension may be of some use in the treatment for strongyloidiasis in view of the reported absence of side reactions which frequently ensue after administration of gentian violet tablets.

Evidence of activity of gentian violet on *S. stercoralis* is indicated by the disappearance of larvae from the stools for long periods in some patients treated with this methylrosaniline dye. It has long been recognized that the diagnostic rhabditiform larvae may be actively motile, and seemingly unaffected, in stools which are deeply stained with gentian violet during a course of therapy. It should be noted that gentian violet contains the amidinium ion system (see above); therefore, the mode of the anthelmintic action of this compound may be identical with that of cyanine dyes.

Miracil D, a thioxanthone derivative, apparently is also the subject of conflicting observations on its efficacy for the treatment of strongyloidiasis (91, 114). Gillet *et al.* (91) reported high rates of cure when this drug was employed for 2 to 6 days. Jones *et al.* (114) used approximately the same dosage as Gillet *et al.* (91) and observed that the stools and/or the duodenal drainage fluid still contained larvae after completion of therapy. McHardy and his associates (139) reported the elimination of *S. stercoralis* larvae from stools in 7 of a series of 27 patients

treated with mantomide. Since these patients had previously shown no therapeutic response to gentian violet and since mantomide was tolerated better than the dye, McHardy *et al.* suggested that mantomide may be useful as a therapeutic agent. The efficacy of mantomide against *S. stercoralis* is probably very low, since larvae were recovered in duodenal aspirate of five consecutive patients who were treated with 5 g of the drug daily for 20 days (114).

An array of compounds, such as phenergan, stibophen (administered intramuscularly), hexylresorcinol (by duodenal intubation), quinacrine, tetracyclines, and fumagillin uniformly failed to eradicate strongyloidiasis (114). Puromycin was not curative of this infection (218). Diethylcarbamazine (63, 114, 202), piperazine citrate and piperazine adipate (7, 42, 92, 114, 194, 195, 196) also augment the long list of drugs which are not significantly effective against strongyloidiasis.

VI. ENTEROBIASIS (OXYURIASIS)

1. Life cycle and clinical aspects. Despite the fact that *Enterobius vermicularis*, the pinworm, seldom is the cause of serious illness, enterobiasis frequently provides clinical, epidemiologic and therapeutic problems. The macroscopic female worms measure from 8 to 12 mm in length. The male is only 2 to 5 mm long and is seldom observed. The adult worms reside in the cecum, appendix and adjacent portions of the ileum and the large intestine. They are essentially lumen dwellers; only occasionally are they observed in the intestinal wall. The gravid females migrate from the intestine to the perianal and perineal areas where their eggs may be deposited or liberated by drying or rupture of the worms during scratching to relieve the associated pruritus. The eggs become infective within a few hours. Worm migration occurs from two to four weeks after intake of infective eggs. The larvae emerge from the eggs and develop to maturity within the bowel without the necessity of a circulatory phase in their life cycle. Important sources of infection or reinfection may be night clothing, sheets and bathroom fixtures. The infection is frequently familial and treatment of the entire family may be indicated. Since thousands of eggs may be liberated in the anal and perianal regions, a common source of reinfection is direct from anus to mouth by finger contamination. Airborne eggs, dislodged from shaken sheets or night clothes, may be inhaled and cause infection or reinfection.

The most frequent manifestation of pinworm infection is anal pruritus. Excoriation and infection of the perianal area may result from scratching. The pruritus may cause disturbed sleep and resultant irritability. Since the adult female worms at times migrate to the vagina, not infrequently a vulvovaginitis may be associated with enterobiasis in young females. Contrary to commonly expressed views, pinworms are not a common cause of appendicitis, nor are these and other common intestinal nematodes, related etiologically to nervous or emotional disturbances in children.

Diagnosis of enterobiasis is made primarily by microscopic demonstration of the typical eggs in cellulose adhesive tape preparations obtained by applying the tape to the anal and perianal areas in the morning before bathing. For practical

purposes, examination of three such scotch tape preparations made on consecutive or alternate mornings is sufficient to demonstrate a large percentage of infections. For evaluation of therapy, seven scotch swabs, taken after completion of treatment, should be examined to provide desirable criteria for cure. Since the prepatent period for pinworm infection is from 15 to 28 days and reinfection is common, the post-treatment diagnostic schedule should be completed at least within two weeks after the termination of therapy. The rate of natural loss of pinworm infection within a period of three weeks, observed during studies on the evaluation of chemotherapeutics, has been about 20% in untreated control series of patients. Owing to the length of the life cycle of *Enterobius vermicularis*, the possibilities of reinfection, autoinfection and retrofection, and the periods required for diagnosis, treatment and follow-up, properly controlled clinical trials against pinworm infection are extremely complicated.

2. Therapeutic agents. The introduction of piperazine salts has greatly improved the treatment for enterobiasis and represents one of the most significant recent advances in chemotherapy in the field of anthelmintics. The simple piperazine compounds, such as piperazine citrate or phosphate, are highly efficacious in the elimination of pinworm infection and are much better tolerated by patients than is gentian violet. The latter drug in some cases is more troublesome than pinworm infection itself. The palatability of the piperazine products, the ease of administration of the drug to children and its low toxicity have provided physicians and patients alike, for the first time, with an effective, pleasant, safe and reasonably economical solution to the previous vexious problem of therapy for enterobiasis.

In 1951, Mouriquand *et al.* (147, 148) recorded the successful use of piperazine hydrate in the treatment for enterobiasis. Later, Turpin *et al.* (204) reported that piperazine diphenylacetate produced a very high cure rate when it was employed against *E. vermicularis* infection. Clinical trials by White and Standen (212) with piperazine hydrate syrup, with adequate post-treatment examinations, demonstrated that a cure rate of 97% was obtained when the drug was administered daily in divided doses for two alternate weeks if the daily dose exceeded 50 mg/kg of body weight. The observations of the above investigators on the efficacy of piperazine salts for the elimination of pinworms have been confirmed or extended in many other reports (3, 14, 26, 27, 54, 109, 167, 178, 195, 197, 198)

Shorter treatment schedules for enterobiasis with piperazine citrate for 6, 7 and 10 days, which would be more practicable and economical, have been evaluated recently (26, 27, 109, 176 178). Studies by Swartzwelder, Miller and Sappenfield (178, 197, 198) have shown that a 6-day course of therapy with a *single daily dose* of piperazine citrate (antepar®) was equally effective in eliminating pinworm infections as 14-day treatment schedules with equivalent total daily dosage given in divided doses. The reduction in duration and cost of medication, without loss of therapeutic efficiency, are significant factors in the treatment for this common and frequently familial infection. A cure rate of 95% was obtained with a dosage schedule of 65 mg of piperazine citrate (hexahydrate equivalent) per kg, with a maximal daily total dose of 2 g (20 ml of piperazine citrate syrup)

in a single daily dose for six consecutive days. Brown *et al.* obtained equally high cure rates with either a 7-day regime with single daily doses of piperazine citrate (27) or a 14-day treatment schedule with divided doses (26).

Although piperazine citrate syrup has been most widely used for the treatment of enterobiasis, piperazine adipate (entacyl®) (17, 154, 184) and piperazine phosphate (antepar®, piperazate®) are reportedly effective therapeutics for pinworm infection.

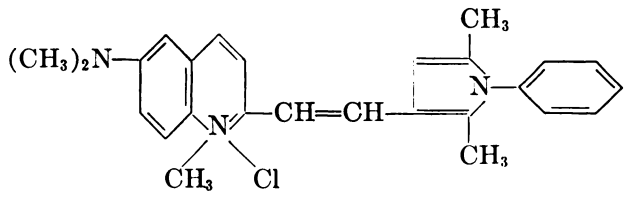
The infrequent reactions which have been reported during therapy with the simple piperazine salts are listed and discussed in this paper in the section on ascariasis. Since a longer course of treatment is employed for enterobiasis than for ascariasis and since the drug has been used more extensively for pinworm infection, most of the reactions to therapy which have been described have occurred in patients who were under therapy for this infection.

The advent of anthelmintic products containing piperazine salts, which are easy to administer, efficacious and relatively free of toxic or other objectionable side reactions, permits large scale treatment measures previously not considered feasible. Small scale trials of community treatment for pinworms with piperazine citrate and piperazine adipate were conducted in two localities by Siemens (184). More adequate and repeated post-treatment examinations are necessary to assess the value of such therapeutic projects.

Pinworm infection frequently is a problem in custodial and other types of institutions. Chemoprophylaxis for enterobiasis with piperazine citrate has been employed in institutions with some success (60, 120).

Studies on the effect of piperazine on *Syphacia obvelata*, a pinworm of mice, demonstrated great efficacy of the drug against the adult worms but only slight activity against the immature stages (23). This observation suggested that in trials against human enterobiasis an interrupted treatment schedule spread over several weeks might be especially effective, since it would allow the immature forms to develop into mature worms which might be more susceptible to the action of the drug. However, the high cure rates achieved with short courses of therapy suggest that the piperazine salts are active against both immature and mature forms of *Enterobius vermicularis*.

Since the introduction of piperazine for the treatment for enterobiasis, cyanine dyes also have been found to be effective for the elimination of pinworms. One of these dyes is pyrvinium chloride (vanquin®, poquil®), (6-dimethylamino-2-[2-(2,5-dimethyl-1-phenyl-3-pyrryl)vinyl]-1-methylquinolinium chloride) dihydrate.



Pyrvinium chloride

It is not appreciably absorbed from the gastrointestinal tract (99). Sawitz and Karpinski (180) and Royer (175) found that this compound was very effective

against enterobiasis when taken for either six or eight days. No significant changes were observed in the urine, blood and bone marrow of treated patients. No untoward effects of clinical importance were noted with the dosages employed (175, 180).

A five day course of therapy with another cyanine dye, dithiazanine, with a dosage of 100 mg three times daily, also was very efficacious for the elimination of pinworm infection (200). Urine analyses, hemograms, blood urea nitrogen, and liver function studies on several patients who received a similar dosage of dithiazanine during therapy for strongyloidiasis (149) revealed no significant changes during or after therapy.

The intravenous administration of some cyanines is followed by the rapid accumulation of high concentrations of the dyes in the renal tubular cells (172) resulting in generalized renal functional damage and histologically demonstrable degenerative changes (162). Intravenous injection of certain cyanines in small doses which do not produce any morphological damage or general depression of kidney function inhibit the renal tubular secretion of certain organic bases (165, 172). It does not appear likely that the oral administration of pyrvinum chloride or dithiazanine would produce a toxic action on the kidney because cyanines as a group are not absorbed readily from the gastrointestinal tract (163, 164). Furthermore, observations of Hales and Welch (99) indicate that the systemic absorption of pyrvinium chloride must be of a low order (99). Because of the selective accumulation of cyanines in the kidney and because of the availability of a sensitive analytical method (172), the question whether or not a particular cyanine is absorbed could be studied readily by measuring its concentration in the kidney.

Single large doses of 125 mg of promethazine hydrochloride (phenergan®) were reported to be curative for enterobiasis (8). Further evaluation of this antihistaminic failed to corroborate the claim for its effectiveness against pinworm infection (28, 143, 176). Only two (20 %) of ten patients treated with single doses of 75 to 125 mg of the drug were free of infection on subsequent examinations (143). The natural rate of loss of pinworm infections in control series is about 20 %. The drug usually produced drowsiness. Restlessness and nightmares occurred on the night of medication in some patients and were sufficiently disturbing to countraindicate the use of the drug in such high dosage as a treatment for enterobiasis even if the drug showed significant anthelmintic efficacy (28, 143).

A large number of other compounds have been evaluated or reevaluated in recent years for their effectiveness against enterobiasis. Various degrees of therapeutic success have been obtained by the use of a suspension of a phenolphthalein salt of gentian violet (55), a combined treatment of gentian violet orally and gentian violet suppositories (1), a combination of bacitracin and succinyl sulfathiazole (61), oxytetracycline (52), puromycin (218), and phthalyl sulfathiazole (cremothalidine®) (6, 176). Most of these forms of therapy suffer by comparison with the piperazine salts or the cyanine dyes either on the basis of efficacy, ease of administration, palatability, side reactions, cost or of other

factors. Tetrachlorethylene, phenothiazine, benzylphenyl carbamate (diphenan®, butolan®) (52, 117), hexylresorcinol, thymyl-N-isoamylcarbamate (egressin®) (52, 53, 117), pyrathiazine hydrochloride (28), and garlic (54) are not ideal for the treatment of enterobiasis either because of their relative ineffectiveness, toxicity or impracticability.

There is need for additional studies on therapy and drug prophylaxis for enterobiasis, particularly with reference to prevention of reinfection from the household environment. Precise studies should be conducted to determine, if, with present anthelmintics, economical and practicable regimes of treatment can be designed which will not only be therapeutic but also prevent immediate reinfection within the home. These chemotherapeutic studies should be coordinated with epidemiologic studies regarding the duration of infectivity of the environment among the treated families. The development of an effective regime consisting of short treatment schedules or single doses of an acceptable chemotherapeutic agent repeated at intervals over a limited period of time, beyond the infectious period of the environment, would be of considerable value.

VII. TRICHINOSIS

No chemotherapeutic agent is available which affects the larvae of *Trichinella spiralis*. As demonstrated by Davis and Most (68) and by Luovar *et al.* (129), administration of ACTH to patients with trichinosis produced a marked improvement of the severe clinical manifestations of the invasive phase of the infection and resulted in eventual recovery. Similar favorable effects in this condition have been reported with cortisone (174, 185). While these two hormones do not affect the larvae (68), they produce an alteration of the host's reaction to the invading parasites and in this manner they may prove highly beneficial. By contrast, ACTH or cortisone have no favorable effect on the course of experimental trichinosis in guinea pigs (129) nor in mice (192). Chan and Brown (62) have found that massive doses of piperazine administered to mice infected with Trichinella markedly reduces the number of the adult worms. No attempt was made to ascertain the effect of piperazine on the larvae. These authors point out that most Trichinella infections are not diagnosed before the invasion of the muscle by the larvae produces characteristic clinical manifestations. However, since the adult females deposit larvae for several weeks, effective antiadult therapy during this period would reduce production of larvae. For these reasons trials of large daily doses of piperazine are recommended in human trichinosis (62).

VIII. TAENIASIS

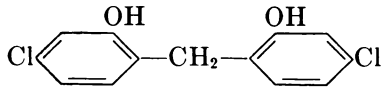
1. Life cycles. Tapeworms are hermaphroditic, segmented flatworms consisting of a small scolex, ("head"), a "neck", and a variable number of proglottids ("segments") which carry out the nutritional, excretory and reproductive functions. The proglottids are regenerated from the scolex and neck; therefore, effective anthelmintic therapy must result in the elimination of the scolex. The cestodes which parasitize the intestine of man vary in size from about 40 mm

[*e.g.*, *Hymenolepis nana*] to over 20 m [*e.g.*, *Diphyllobothrium latum*]. *Taenia saginata*, the beef tapeworm, infects man when undercooked beef, containing the larval forms, is ingested. The scolex is attached to the small intestine and the chain of segments may have a length of over 10 m. Eggs deposited in the soil infect cattle and the larvae [cysticerci] develop in the muscles and other tissues of the intermediate host. Infections with *Taenia solium* are acquired by eating undercooked pork containing the larvae of the parasite. Taeniasis may be associated with abdominal pain, weight loss, diarrhea and weakness. Eggs of *T. solium* may reach the upper intestinal tract either by ingestion or regurgitation. After digestion of the eggs, the liberated larvae may be carried *via* the circulation to various tissues, such as the muscles, brain or eye; thus, producing cysticercosis. The latter is not susceptible to chemotherapy by any known anthelmintic agent. *Diphyllobothrium latum* is introduced by the ingestion of poorly cooked fish containing the larvae. It has been known for a long time that some infections by *D. latum* are associated with pernicious anemia. Elimination of the parasite by anthelmintic therapy results in a rapid return of the blood picture to normal and a permanent remission. The investigations of von Bonsdorff (205, 206) have demonstrated that, in contrast to other cestodes, *D. latum* assimilates large amounts of vitamin B_{12} and that the concentration of this vitamin in *D. latum* is 50 times higher than that in *Taenia saginata* (153). *Hymenolepis nana*, or dwarf tapeworm, can develop entirely in man and does not require an intermediate host. It affects children much more frequently than adults. The adult worm is attached to the mucosa of the small intestine. Abdominal pain and diarrhea have been ascribed to this infection.

2. Chemotherapy. Several studies have supplied confirmatory evidence that quinacrine is better tolerated and is more effective than oleoresin of aspidium in the treatment of infections caused by *T. saginata* and by *D. latum* (76, 108, 127, 168). Therefore, quinacrine is considered as the drug of choice in these two infections. The potential danger of producing cysticercosis by the use of quinacrine against *T. solium* has been pointed out previously (40). In contrast to its high activity against other cestodes, quinacrine is not very effective in the treatment of infections produced by *H. nana* (160). Because of the lack of safe and effective anthelmintics against *T. solium* and *H. nana* and because of the need for a drug producing less side-effects (vomiting) than quinacrine, the search for chemotherapeutic agents against cestodes is continuing. Wigand and Warnecke (215) have reported that intraduodenal administration of a low boiling fraction [50 to 70°C] of petroleum ether, containing mainly hexane and heptane, results in lysis and destruction of *T. saginata*. Nausea and vomiting are frequently observed side-effects of this method of treatment. The systemic absorption and toxicity of the large dose of petroleum ether (60 ml) used in this study have not been investigated. Since the habitat of cestodes, the intestinal tract, is essentially anaerobic, Wigand and Warnecke (216) have postulated that oxygen should be harmful to these worms. Based on this consideration, they insufflated large amounts of air intraduodenally during a period of 8 days. They state that this procedure enhanced the effect of drugs active against *T. saginata* (216). However,

there is no evidence of a deleterious effect of oxygen or of aerobic metabolism on cestodes. According to Anttonen (5), flavaspidic acid is the active anthelmintic principle of oleoresin of aspidium and is more effective and less toxic than the crude resin. The claim that diiodophenylpropionic acid has taeniacidal properties (88) has not been confirmed (155).

Perhaps a more promising approach to this problem may be found in the use of tin compounds. The reports of Le Gac (126) and of Hirte (103) about the effectiveness of tin preparations in cestode infections have been confirmed by Kuhls (124) and by Gras (94). According to Kuhls (124), administration of a mixture of tin oxide, metallic tin and zinc chloride to 197 patients infected with *T. saginata* resulted in cures in 183 cases. The same preparation was effective against *D. latum* (two cases) and against *T. solium* (three cases). With the exception of occasional minor gastrointestinal disturbances, no undesirable side-effects were observed. Unfortunately, the exact composition and dosage of the tin preparation used in these trials are not given. In view of the reported high effectiveness and low toxicity of this material and in view of the activity *in vivo* of organic tin compounds against *Rallietina cesticillus* (121), a tapeworm of chickens, further investigation of the action of tin preparations on cestodes appears highly desirable. Another veterinary taenicidal agent, 2,2′-methylene-bis-(5-chlorophenol) (Di-phenthane 70®)



has been reported recently to be effective in human subjects infected with *T. saginata* and with *T. solium* (91a, 137a). The drug was used at an oral dose level of 200 mg/kg in divided doses of 1 g, administered every four hours.

IX. BILHARZIASIS

1. Life cycle and incidence

Bilharziasis (schistosomiasis) is a trematode infection caused by one of three species of schistosomes or blood flukes, *Schistosoma haematobium, S. mansoni and S. japonicum.* They differ in their geographical distribution and in the location of the adult worms in the mesenteric, vesical and pelvic veins. Infections with *S. haematobium* are distributed widely throughout Africa, the Near East and the Middle East. *S. mansoni* is encountered in the same areas, but also in parts of the Western Hemisphere, *i. e.*, Puerto Rico, Venezuela, Dutch Guiana, and in northeastern Brazil, while *S. japonicum* is confined to Eastern Asia (China, Japan) and the Southwest Pacific (Philippines). Man becomes infected by exposure of the skin (through bathing, working, washing or wading) to water containing schistosome larvae, the cercariae, which have emerged from molluscan intermediate hosts. The adult worms establish themselves in the portal and mesenteric veins. In contrast to the other two species of schistosomes, *S. haematobium* to a large extent migrates to the pelvic veins and the venules of the urinary bladder. Therefore, the eggs of *S. haematobium* are excreted mainly in

the urine, while those of *S. mansoni* and of *S. japonicum* are eliminated by the feces.

If an egg reaches water a ciliated miracidium emerges and may penetrate a suitable snail host. Within the snail one miracidium ultimately can give rise to thousands of cercariae. The pathological manifestations of schistosomiasis are caused by eggs extruded into the intestines or the urinary bladder or deposited in the liver resulting in inflammatory and hemorrhagic lesions and abscesses of the intestinal mucosa, liver cirrhosis and, in the case of *S. haematobium*, cystitis and inflammatory reactions and abscesses of the urinary bladder, the ureter and the urethra.

Next to malaria, schistosomiasis is considered the parasitic disease which causes the most disability and death of man. Since remarkable progress was made during and following World War II in the chemotherapy and control of malaria, the relative importance of schistosomiasis as a world health problem has increased. According to Stoll (191), 114 million human subjects, or about 5% of the population of this globe, were infected with schistosomes in 1947. Since then Meleney (140) has pointed out that the endemic areas of all three species of schistosomes have increased.

2. Some pharmacological and biochemical aspects of the chemotherapy of bilharziasis

Up to the present, organic antimonials have remained the most effective available schistosomacidal agents (95, 130, 140, 182, 217). Generally, it has been assumed that the parasiticidal action of trivalent antimonials is brought about by mechanisms similar to those of arsenicals, *i. e.*, by interaction with and inactivation of sulfhydryl groups of enzymes (80). However, antimonials do not affect glucokinase of *Schistosoma mansoni* while the activity of this enzyme is inhibited by arsenicals (135) and other sulfydryl reagents, *e. g.*, p-chloromercuric benzoate (33). Therefore, it appears that different mechanisms are involved in the inhibition of enzymatic activity by arsenicals and by antimonials. Studies concerned with the mode of action of trivalent organic antimonials revealed that these compounds reduce the utilization of carbohydrate by schistosomes (33). By the use of cell-free extracts of these organisms, it was found that potassium antimony tartrate and stibophen interfere with a single step in the glycolysis of *S. mansoni*, the phosphorylation of fructose-6-phosphate (F-6-P) by adenosine triphosphate (ATP) to fructose-1,6-diphosphate (FDP) (135) according to the following reaction:

$$\text{F-6-P} + \text{ATP} \rightarrow \text{FDP} + \text{ADP}$$

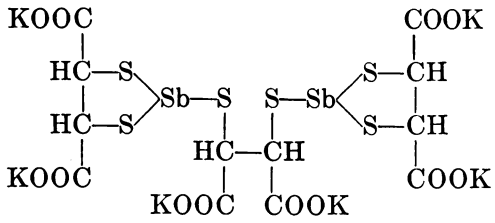
This reaction is catalyzed by phosphofructokinase. Inhibition of this reaction accounts for the reduced rate of glycolysis produced by trivalent antimonials (46). The relationship between the selective action of these compounds on schistosome phosphofructokinase and their schistosomacidal action has been the subject of a recent investigation (48). The effect of a drug on an enzyme does

not imply necessarily that this drug-enzyme interaction is responsible for the pharmacological or chemotherapeutic actions of the same compound. In several reviews dealing with this problem, attention has been directed to requirements which must be met before proof can be accepted that a drug acts by inhibiting a particular enzyme (110, 209). In the following, application of these criteria to the inhibition of schistosome phosphofructokinase activity by trivalent antimonials will be discussed. 1. The enzyme should be inhibited in the intact cell: exposure of the parasites to low concentrations of potassium antimony tartrate or administration of subcurative doses of stibophen to the host resulted in accumulation of F-6-P and a decrease in the concentration of FDP in the worms. The increase in the substrate of the enzyme and the decrease in its product indicates that the antimonials inhibit phosphofructokinase activity within the intact schistosomes (48). 2. Enzyme inhibition should explain quantitatively the effects of the drug on the intact organism: inhibition of phosphofructokinase is responsible for the reduction in the rate of glycolysis of schistosomes (46). Since glycolysis supplies the major, if not the exclusive source of energy for schistosomes (33, 36), inhibition of glycolysis could result in the death of the worms. 3. Enzyme inhibition must occur with an amount of drug no greater than that necessary to produce the drug action: survival of the parasite *in vitro* is reduced very markedly (from 30 days to 8 hours) by exposure to antimonials in concentrations which produce an inhibition of phosphofructokinase to an extent of 50% (48); thus, there is a satisfactory agreement between the concentration required to inhibit the enzyme and that exerting a schistosomacidal effect. 4. Other cell constituents must not bind or inactivate a substantial fraction of the drug: given concentrations of potassium antimony tartrate or of stibophen interfered with the phosphofructokinase activity of crude schistosome homogenates to the same degree as with that of purified preparations of the enzyme (50); these findings indicate the absence of binding of antimonials by other constituents of schistosome cells. The observations discussed above indicate that inhibition of phosphofructokinase activity may be the mechanism of the chemotherapeutic action of trivalent organic antimonials in schistosomiasis.

It should be pointed out that mammalian phosphofructokinase is much less sensitive to the inhibitory effects of antimonials than the enzyme catalyzing the same reaction in schistosomes (135). Therefore, it appears that differences in the nature of these two homologous enzymes form the basis for the chemotherapeutic action of this group of drugs and that their toxicity for the host can not be ascribed to an interference with phosphofructokinase activity of mammalian tissues.

In contrast to trivalent antimony derivatives, pentavalent antimonials have no schistosomicidal activity *in vitro* (125) nor do they reduce the rates of glycolysis or of phosphofructokinase activity (135). Since these compounds have chemotherapeutic activity in schistosomiasis, it appears that, as with arsenicals, reduction of pentavalent antimonials occurs in the tissues of the host.

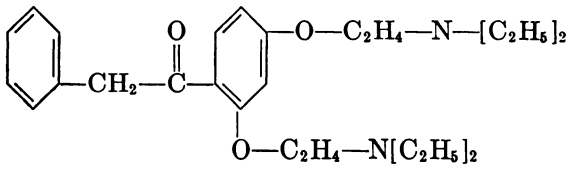
Friedheim *et al.* (81, 82, 83) have reported that antimony α,α-dimercapto potassium succinate (TWSb)



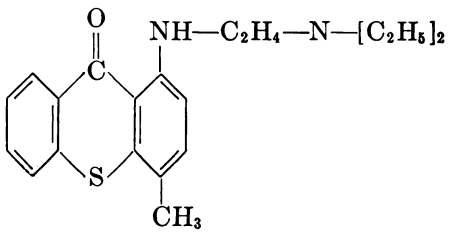
is equally effective as stibophen in the treatment of human infections caused by *S. mansoni* and by *S. haematobium*, and that the former antimonial causes less severe toxic reactions, particularly with regards to cardiovascular and gastro-intestinal manifestations. TWSb was administered intramuscularly or intravenously at doses, varying between 0.2 to 0.5 g daily for five to seven consecutive days, with the total dosage varying from 1.8 to 2.5 g. Luttermoser (130) has observed that in mice infected with *S. mansoni* maximally tolerated doses of TWSb are significantly less effective than are those of stibophen.

The necessity of repeated parenteral administrations of organic antimonials is one of their most serious disadvantages as chemotherapeutic agents against bilharziasis (2). There is general agreement that successful mass treatment in areas where this infection is prevalent requires an orally effective schistosomacidal agent. Up to the present, thioxanthones have been the only types of compounds available clinically for this purpose. Reports dealing with Miracil D and other thioxanthones confirm earlier observations and conclusions that they are more effective against *Schistosoma haematobium* than against *S. mansoni* and they have no activity against *S. japonicum* (69, 95, 97, 98, 122, 128, 130, 132, 140, 151, 173, 181). But even in the treatment of infections caused by *S. haematobium* failures occur not infrequently (4, 122, 140, 151, 182, 217). Also, a high incidence of toxic side-effects (nausea, vomiting, abdominal colic, dizziness, insomnia and mental disorders) is encountered (96, 128, 140, 145, 151). It has been claimed that their severity is reduced by the administration of atropine or of antihistaminics (203).

Deschiens *et al.* (71, 72) have reported that bis-(diethylaminoethoxy)-2,3-desoxybenzoine has schistosomacidal activity *in vivo*. In this compound the distance between one of the diethylamino groups and the carbonyl group is the same as in Miracil D:



Bis-(diethylaminoethoxy)-2,3-desoxybenzoine



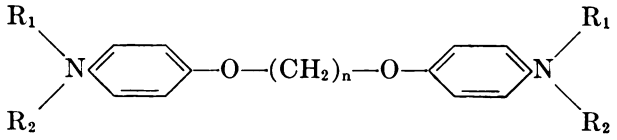
Miracil D

Following oral administration of the desoxybenzoine (400 mg daily for eight days) to 33 patients infected with *S. haematobium* disappearance of the eggs

from the urine was observed in 22 cases. In schistosomiasis mansoni the results were less favorable.

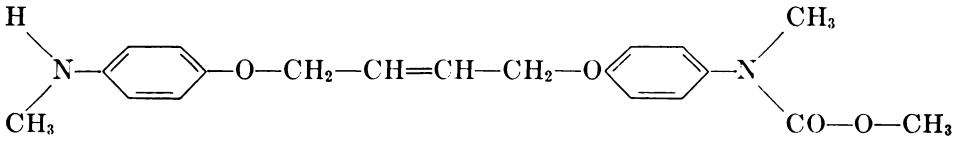
During the past few years several papers have appeared in which it is stated that oral administration of stannous oxide (4 g daily for 8 days) has a curative effect in schistosomiasis (*S. mansoni*). Following one or several courses Deschiens (70) found that in the majority of cases eggs of *S. mansoni* had disappeared from the stools and that the latter remained negative for a period of at least 22 weeks. There were no severe side-effects; however, quite often, nausea, vomiting and headaches were encountered. These observations were confirmed by Mause and Arnaud (137) and by Bellon (18). Unfortunately, no rectal biopsies were performed in any of these studies. As shown by Newsome (151), in many instances in which examination of the stools failed to reveal the presence of schistosome eggs, the latter can be found on rectal biopsy. In view of the ease of administration and the low toxicity of stannous oxide, it would appear very desirable to test the schistosomacidal activity of this compound by the use of more stringent and carefully established methods of evaluation.

A new series of compounds orally effective in schistosome infections (*S. mansoni* and *S. japonicum*) of experimental animals (mice, hamsters, guinea pigs, rabbits), discovered by Raison and Standen (169), are symmetrical diaminodiphenoxyalkanes of the general structure:

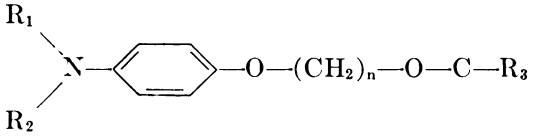


In an extremely well conducted and carefully documented study, Standen *et al.* (57, 93, 169, 189) have investigated the relationship between chemical structure and schistosomacidal activity. Their most important findings are summarized in the following. Regardless of whether or not alkyl groups were introduced into the nitrogens, optimal activity was obtained when the number of carbons (n) in the central chain amounted to seven. Under these conditions, schistosomacidal activity was approximately the same with no, one (R_1) or two (R_1, R_2) methyl or ethyl substituents. N-alkyl groups higher than ethyl progressively reduced chemotherapeutic activity. On the other hand, activity was retained with hydroxy alkyl, with carboxy alkyl and with aldehyde bisulfite substituents (93). Quaternization of the nitrogens or alteration of the nitrogen from the para to the ortho or meta position resulted in a complete loss of activity. Substitution by methyl, methoxy, chlorine, hydroxyl or amino groups in the meta or ortho positions of the p-amino phenol rings of these compounds resulted in a very marked reduction or a complete loss of activity (189). Omission or replacement of the ether oxygens by a wide variety of alkyl or aryl groups abolished activity while some of the latter was retained when the oxygens were replaced by sulfurs (93). Compounds with branched central carbon chains invariably had lower activity than their unbranched analogs. Replacement of one or more methylene groups of the central chain by an oxygen generally decreased activity; however, the latter was retained with an —O·$(CH_2)_4$·O·$(CH_2)_4$·O— configura-

tion. Unsaturated compounds with a triple bond in the central chain proved less active than the corresponding saturated derivatives. On the other hand, the presence of a double bond frequently did not reduce the activity and a compound of this type with one terminal basic and one terminal urethane group,



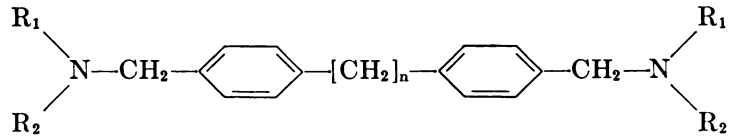
proved to one of the most active substances in the entire series. Replacement of the urethane by an acetamido group resulted in an equally active compound (93). In an attempt to uncover the minimal structural requirements for schistosomacidal activity *in vivo*, Caldwell and Standen (57) have tested a considerable number of asymmetrical diaminodiphenoxylalkane and mono-p-amino phenoxylalkane derivatives. This study revealed that the following basic structure is essential for activity:



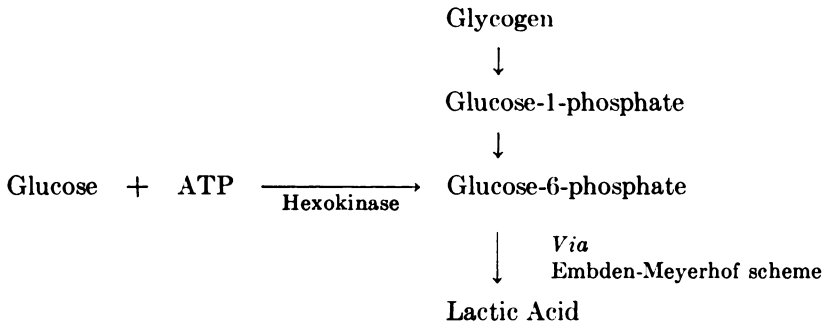
Highest activity was observed when R_1 and R_2 were either H, CH_3 or C_2H_5, when n equaled 7, and when R_3 represented a wide variety of alkyl or substituted phenyl groups. The possibility that the activity of these compounds is due to their metabolic alteration by the host to p-aminophenol has been ruled out because the latter compound had no activity when administered orally or parenterally.

Observations of Standen (188) indicate that the active compounds of this series exert their schistosomacidal activity through a combined effect on the parasite and on the host. Shortly after the oral administration of 1:7-bis(p-dimethylaminophenoxy) heptane, the worms lose their attachment in the mesenteric veins, probably because of a decrease or loss of muscle tone, and are carried to the liver where they are ensheathed and phagocytosed. Since schistosomes of untreated animals fail to elicit such a host defense mechanism, it appears that the aminophenoxyalkane derivatives either bring about changes in the worms which in turn stimulate an inflammatory reaction of the host or they exert this effect by a direct action on the host. The involvement of the host in the schistosomacidal action of these compounds is indicated also by their low schistosomacidal activity *in vitro* (50).

Higher *in vitro* activity against *Schistosoma mansoni* has been observed with a series of symmetrical diaminodibenzylalkanes (47):



The basic structure of these compounds differs from that of the aminophenoxyalkanes by the fact that they are benzylamine rather than aniline derivatives. Maximal activity was obtained with a central chain of six carbons. An increase or decrease above or below this number progressively reduced the activity. Quaternization of the amino group abolished the activity. In contrast to the diaminophenoxyalkanes, secondary amines (R_1: alkyl; R_2:H) were considerably more potent *in vitro* than their tertiary amine analogs (R_1: alkyl; R_2: alkyl). Activity was essentially the same with methyl- and isopropyl amines. Incubation of *Schistosoma mansoni* with these compounds produced an irreversible paralysis of the worms; this was preceded by marked muscular hyperactivity. These effects were antagonized by two amphoteric quaternary ammonium compounds, betaine and carnitine, but not by choline, acetylcholine or other cholinergic agents (47). Diaminodibenzylalkanes also had high activity *in vitro* against two other trematodes, *Fasciola hepatica* and *Clonorchis sinensis* (136). When schistosomes were incubated in the presence of low concentrations of dibenzylalkylamines, changes in the motility of the worms were preceded by a decrease in the uptake of glucose (50). Lactic acid production was reduced to a much lesser degree than glucose utilization (50), indicating that under these conditions lactic acid was produced from endogenous glycogen. This was confirmed by the observation that glycogenolysis of intact schistosomes was increased by these compounds (50). Following the formation of glucose-6-phosphate, the pathways and enzymes concerned with the production of lactic acid by schistosomes are identical for glucose and for glycogen:



Diaminodibenzylalkanes did not inhibit the activity of schistosome hexokinase (50), the enzyme catalyzing the production of glucose-6-phosphate from glucose and ATP. Furthermore, the diamines had no inhibitory effect on the rate of glycolysis of glucose by schistosome homogenates nor did they stimulate the activity of phosphorylase, or phosphoglucomutase or of the ATPases of the parasite (50). Because of the absence of any direct effect of diaminobenzylalkanes on enzymes involved in carbohydrate metabolism of the parasite it is concluded that they interfere with the uptake by, or the active transport into, the worms of exogenous glucose. It remains to be determined whether the paralyzing action of these compounds on schistosomes is based on their inhibitory effect on the

uptake of glucose. Since this organism is dependent on a high rate of carbohydrate metabolism, it is conceivable that the parasite may prove vulnerable to inhibition of glucose uptake and that an alteration of this process may offer an opportunity for the development of potential schistosomacidal agents.

X. OTHER TREMATODE INFECTIONS

Two trematode infections, which so far have proved resistant to chemotherapy and which are characterized by a parasitic invasion of the bile ducts or the lungs are caused by *Clonorchis sinensis* of *Paragonimus westermani*, respectively. Results obtained with antimonials or with emetine combined with sulfonamides have proved unsatisfactory (64, 67, 89, 123, 146, 208). Also, Miracil D is ineffective in these infections (208). It has been stated that administration of chloroquine over long periods in either infection results in marked clinical improvement and in a reduction or even the disappearance of eggs from the bile, the gastric juice, the stools or the sputum (64, 65, 67, 85). Should these reports be confirmed, a significant advance in the chemotherapy of clonorchiasis and of paragonimiasis would have been achieved.

XI. CONCLUSIONS

The introduction of piperazine as an agent for the treatment of ascariasis and enterobiasis and the demonstration of the broad spectrum anthelmintic activity of cyanine dyes have been the most significant developments in the field of anthelmintics during the past few years. Also, some progress has been made towards an understanding of the mechanism of action of these and of other anthelmintic agents. However, a great deal more investigative effort will be required before the wide gap is bridged between our knowledge of the morphology and taxonomy of parasitic worms on one hand, and that of their physiology and biochemistry on the other. Such information should supply a rational basis for the development of drugs for the treatment of infections produced by those helminths against which safe and effective chemotherapeutic agents as yet are not available.

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