THE CHEMOTHERAPY OF FILARIAL INFECTIONS

FRANK HAWKING

National Institute for Medical Research, London

In this review an attempt is made to give a general account of the drugs which are chemotherapeutically active against filarial infections. As far as possible reference will be given to sources from which more detailed information may be obtained, but this detail will not be reproduced in the review itself. The attached bibliography is believed to contain most of the significant papers on the subject but it is possible that some have been missed. Further references to other papers on this subject (especially as regards the clinical aspect) can be obtained from Tropical Diseases Bulletin from 1947 onwards. A review up to 1945 has been compiled by Temkin (114); and another useful review to the end of 1949 has been given by Findlay (36). An exhaustive bibliography of papers before 1945 on all aspects of onchocerciasis has been published by the Pan American Sanitary Bureau (89). Valuable contributions on the metabolism of filarial worms (which forms the background to their susceptibility to chemotherapeutic action) have been written by Bueding (21, 22).

BIOLOGY OF FILARIAL INFECTIONS

Most filarial worms are long threadlike creatures, the adult female worm often measuring about 10 cm. long by 0.2 cm. wide; the male worm is often half this size. The adult worms live in the connective tissues, vessels, or cavities of some vertebrate host. The female worm produces thousands of larvae or microfilariae which are typically 100–200 μ long by 5–10 μ wide. These circulate in the blood or move about under the skin of the host. Eventually some of them are ingested by a blood sucking arthropod, in which they undergo further development for about 10–20 days. After that, they penetrate the skin of a new host, while the arthropod vector is sucking blood, and develop into adult worms, either male or female. After conjugation of these the cycle begins anew. From the point of view of chemotherapy there are four different phases at which drugs may act, *viz.*—on the adult worms, on the microfilariae, on the forms developing in the insect vector, and on the infective larvae developing in the vertebrate host. The reactions of these phases are different one from another. The criteria for judging the action of drugs also vary according to the phase. In the case of the microfilariae, it is easy to count the numbers present in the blood or skin before and after treatment. For the forms developing in the arthropod vector, it is similarly easy to dissect the hosts after exposure to the drug (this stage is relatively unimportant for chemotherapy and has been studied but little). In the case of the adult worms it is usual in experimental animals (especially cotton rats infected with *Litomosoides*) to kill the animal at an appropriate time after treatment and to examine the adult worms directly. In some human infections, *e.g.*, *Onchocerca*, which occurs in nodules under the skin, it is possible to inspect the adult

worms directly; but in most human infections it is necessary to rely upon indirect evidence. The most usual criterion is to follow the number of microfilariae in the blood over a long period (as introduced by Culbertson (30, 31)). If after 6 or 12 months the number of microfilariae in the blood or skin has dwindled to zero, it is concluded that the adult female worms have been killed, or at least permanently sterilized. In the case of the infective larvae developing in the vertebrate host, the treatment is administered during the incubation period (between exposure to infection and appearance of microfilariae in the blood) and the effect is judged by seeing whether the appearance of microfilariae is prevented or (with experimental animals) by a postmortem search for adult worms.

There are certain characteristics common to these filarial infections which are important for therapeutics. The parasites are very well adapted to their hosts, and pathological lesions occur in only a small proportion of cases. Thus in many parts of the tropics almost all the adult population may contain, or have contained, the worms; but only one to ten per cent show any ill effects. When lesions occur, they consist most commonly of inflammation (possibly allergic) around the adult worms. This inflammation soon subsides but tends to recur after several weeks or months. Repeated attacks of such inflammation may cause blockage of lymphatics by fibrosis etc. and thus produce lymphstasis in semi-isolated parts of the body, *e.g.*, legs and scrotum; this lymphstasis leads on to the well-known elephantiasis of these parts of the body. Once the lymphatics have been blocked by scar tissue, killing the worms by chemotherapy can not unblock them, so that antifilarial drugs cannot cure elephantiasis although they may prevent recurrence of the attacks of inflammation. On the other hand the administration of suitable drugs should be able to kill the worms and so prevent the development of elephantiasis and similar lesions at a later date. Still better from a public health point of view, it should be possible to treat with appropriate drugs the whole population in a specified area and to kill all the worms; then the filariae would be completely eradicated from the area in question and no further infections or lesions would occur in future. Thus the greatest promise held out by antifilarial drugs is not cure but prevention, especially prevention conducted on a mass-population basis. Technically this might now be feasible in some instances. Unfortunately such prophylactic treatment often produces discomfort in a certain proportion of cases; and while a man may be prepared to undergo some discomfort in order to be cured of a disease which he already has, it is usually difficult to persuade him to undergo such discomfort in order that his neighbour may be prevented from getting the disease at a future date.

Most effective antifilarial drugs tend to produce general febrile reactions, accompanied by inflammation of the tissues around the adult worms or the microfilariae, in a varying proportion of the infected persons treated. These reactions seem to be allergic in nature, excited by the disintegration products of the microfilariae or worms. Since they may be a characteristic of the infection rather than of the drug, it is unlikely that active drugs can be discovered which would be completely free from such effects. Where the fear of allergic effects is an obstacle to therapy, *e.g.*, reactions in the eyes during the treatment of oncho-

cerciasis, it may be possible to prevent or reduce them by antihistamine drugs, or by cortisone.

In experimental infections with *Litomosoides*, female worms are much more susceptible to chemotherapeutic action than male worms. Probably the same is true of the other filarial worms although there is little evidence on the subject. Since few of the lesions are due to the presence of the worms themselves, and since the males cannot produce offspring, the continued survival of male worms after the destruction of the females would be of little importance for the individual patient and of no importance for the public health of the community.

The filarial worms which are important for chemotherapy are as follows:

1) *Wuchereria bancrofti* and *W. malayi*. The adults live in the lymphatics of man; the microfilariae circulate in the blood and are transmitted by mosquitoes; the acute lesions caused are acute lymphangitis of the genitals and limbs, and the chronic lesions are elephantiasis, hydrocoele, chyluria, etc.

2) *Loa loa*, which is limited to the rain-forest areas of Africa. The adults move about in the connective tissues of man, and the microfilariae (which circulate in the blood) are transmitted by the mangrove-fly, *Chrysops*. The resultant lesions include "Calabar swellings", pruritus and other skin troubles, and disturbances due to the adult worm migrating across the front of the eyeball.

3) *Onchocerca volvulus*. The adults live in subcutaneous nodules and the microfilariae migrate under the skin, whence they are picked up by the insect vector, *Simulium*. The lesions are due mostly to the microfilariae, which cause intense itching and later thickening of the skin. Moreover, microfilariae in the head may migrate into the conjunctivae and eyeball and cause eye troubles and sometimes blindness.

4) *Dipetalonema perstans*, *Mansonella* etc., which are common parasites in certain parts of the world; they are non-pathogenic and possess no clinical significance.

All the above cause infections of man.

5) *Dirofilaria immitis* and *D. repens* of dogs. They have sometimes been used for chemotherapeutic and other experimental studies and they are occasionally of veterinary importance, especially in racing greyhounds.

6) *Litomosoides carinii*, a parasite of the cotton rat *Sigmodon hispidus*. This worm has been of fundamental importance to modern chemotherapy, for it has provided the experimental infection for all modern drug-testing. Its use was introduced by Culbertson and Rose (32) and its transmission was worked out by Williams and Brown (126, 126a) and by Scott and Cross (105). Maintenance in the laboratory and the technique for chemotherapeutic tests are described by Hawking and Sewell (50) and by Hewitt (52). The adult worms live in the pleural cavity, and the microfilariae, which circulate in the blood, are transmitted by the mite *Bdellonyssus* (*Liponyssus*) *bacoti*. Unless the adult worms are so numerous as to cause mechanical obstruction inside the thorax, they are not pathogenic.

The response to drugs of these different worms differs one from another, so that each has to be considered separately.

7) *Dracunculus medinensis*, the guinea worm, is sometimes included under

filarial infections, although it differs from those described above in many respects. The adult female worm, which measures about 120 cm. long by 0.15 cm. wide, occurs in the connective tissue of the leg. The larvae are liberated from an ulcer near the ankle; they develop in a fresh water crustacean, *Cyclops*. Transmission to a new host occurs when the crustacean is swallowed in drinking water. *Dracunculus* is not usually considered to respond to the compounds effective for other filarial infections, but Rousset (100) reports that it is sensitive to diethylcarbamazine.

HISTORY OF ANTIFILARIAL CHEMOTHERAPY

Before 1944 there was no convenient experimental animal, and the criteria for judging antifilarial action were not properly understood. In retrospect the investigations carried out in 1920 by Rogers (98) on the action of tartar emetic upon bancroftian filariasis are seen to be well planned and significant. But in general drugs were administered to patients for several weeks, microfilariae in the blood were counted at the end of this time and no significant change was observed, and it was concluded that no antifilarial effect had been produced. It was known from experiments in dogs that large doses of antimonials might kill the adult worms of *Dirofilaria* in dogs but no action could be observed on microfilariae of *W. bancrofti* in man during short term investigations. As already mentioned a comprehensive review of the knowledge at this time is given by Temkin (114).

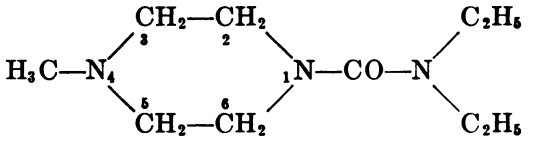
A fundamental advance was made by Culbertson and Rose (32) who studied the action of antimonial compounds on infections of *Litomosoides* in wild cotton rats. They showed that neostibosan and other compounds killed the adult worms, but that the microfilariae persisted in the blood in diminishing numbers for several months. Armed with this knowledge, Culbertson and his colleagues showed (by long follow-up of patients) that repeated high doses of these compounds destroyed (or sterilized) the adult worms of *W. bancrofti* in man. Although of limited value in practice, this was the first effective therapy of bancroftian filariasis. By the use of spontaneous infections of wild cotton rats in the same way, significant antifilarial action was discovered also in the cyanine compounds by Welch *et al.* (122), in the trivalent arsenical compounds by Otto and Maren (86), and in the piperazine series by Hewitt and his colleagues (54, 55, 58). One member of the piperazine series, diethylcarbamazine, or hetrazan, has proved to be much the most effective compound yet known for the treatment of human filariasis. The history of its discovery presents several features of interest for general chemotherapy. Although the work of Culbertson *et al.* (3) had switched attention from microfilariae to adult worms as a criterion of drug action, Hewitt and his colleagues continued to pay much attention to the microfilaricidal action of the compounds which they screened. If they had not done this, the antifilarial activity of the piperazine compounds might well have been missed; for their action on the adult worms of *Litomosoides* is small. (Hewitt was probably fortunate to be using wild cotton rats with old infections, in which the spontaneous deaths of many of the worms increased the apparent antifilarial action of the

drugs.) Although the laboratory findings regarding diethylcarbamazine seemed much less promising than those of other compounds, *e.g.*, cyanines or trivalent arsenicals, it was submitted to clinical trial, and (what is unusual) it was found to be much more effective on human infections than it had been on the experimental one (101, 102). The moral seems to be that every new chemical series, which is found in the laboratory to have specific chemotherapeutic action, ought to be given a small clinical trial in a limited number of cases to see whether the human infection is by chance more responsive than the laboratory one. Unless this is done, many compounds of great practical value will probably be overlooked.

Since the discovery of diethylcarbamazine, no other compounds of practical value have been disclosed in the laboratory although a great number have been screened. The only other compound now known to be useful in human therapy emerged as the result of work in the field. This compound is suramin, which was first produced about 1920 as a powerful drug against trypanosomiasis. During work on trypanosomiasis in volunteers in the Belgian Congo, it was observed by Van Hoof *et al.* (12) that it also acted upon *Onchocerca*; its action does not extend to other filarial infections.

DIETHYLCARBAMAZINE

Diethylcarbamazine, 1-diethylcarbamyl-4-methylpiperazine, is also known



as hetrazan, banocide, notezine, caricide, carbilazine, 3,799 R.P. It was first put out as the chloride, but is now issued as the dihydrogen citrate which contains only half its weight of base. In giving reports it should always be indicated whether the doses refer to a specific salt or to the base.

Relation of structure to activity. The piperazine ring itself appears to be of fundamental importance although piperazine itself showed no activity. Since numerous piperazine derivatives are active against many different round worms in the intestine (see below) it seems probable that this ring has a specific action upon parasitic nematodes in general. A carbethoxy radical in position 1 of the ring with various substitutions in position 4 produced compounds with high antimicrofilarial activity, but as the alkyl chain was increased the toxicity became greater and the activity less. The only compounds lacking the carbethoxy group which showed marked activity against microfilariae possessed an ethyl-, diisopropyl-, dimethyl-, or diethylcarbamyl group in position 1. The alkyl group in position 4 is not essential, as 1-diethylcarbamylpiperazine is still highly active.

Mode of action. The exact mode of action of diethylcarbamazine on microfilariae is unknown. It has no apparent effect on microfilariae *in vitro* at 37°C., nor has the serum from a treated animal (which might be expected to contain activated derivatives of diethylcarbamazine, if such occurred). On the other

hand, if it is administered intravenously to a cotton rat infected with *Litomosoides*, 80 per cent of the microfilariae circulating in the blood disappear within two minutes (5, 51); a similar effect is observed with *Mf. bancrofti* and with *Mf. loa* in man. Such a contrast of apparent inactivity *in vitro* with extremely rapid effect *in vivo* is without parallel among chemotherapeutic agents. When a search, by histological methods, is made for the microfilariae which have disappeared, they are found to be concentrated in the capillaries of the liver of the cotton rat. Within about one hour phagocytes congregate round these trapped microfilariae and within eighteen hours most of them have been destroyed. This collection and destruction of microfilariae in the liver is probably a function of the reticulo-endothelial system which is especially well developed in this organ in rats. Some destruction of microfilariae of *Loa* has been shown to occur in the liver in man also (129); but possibly in this host much of the destruction might be carried out in the bone marrow and spleen. According to these observations it seems that diethylcarbamazine may sensitize the microfilariae in some way so that they become susceptible to the action of fixed macrophages, *i.e.*, it may act something like an opsonin (48, 51).

In order to investigate this action further, the metabolism of the drug labelled with C^{14} has been studied (5). Metabolism is rapid, but none of the four metabolites excreted in the urine has any apparent effect upon microfilariae *in vitro*. Neither microfilariae nor adult worms concentrate the drug or its metabolites significantly above the surrounding fluid or tissue concentration. When microfilariae are examined in an electrophoretic cell, no effect of diethylcarbamazine upon their surface potentials can be observed. It has not been possible to inhibit the action of diethylcarbamazine by compounds of similar chemical structure (except possibly by nicotinic acid (51)).

On adult worms, the action of diethylcarbamazine is less dramatic and it varies according to the species of worm. With *Litomosoides*, Hewitt *et al.* (54) reported the finding of many dead worms in rats which had been treated with the compound and then held for over 70 days before autopsy; they worked with wild rats which had long been infected and the death of the worms may have been due to old age or to immunity of the host. Working with rats freshly infected in the laboratory, Hawking *et al.* (51) found only 9 per cent of 430 adult female worms had been killed by intensive courses. *In vitro* diethylcarbamazine has no effect on adult worms of *Litomosoides*. According to Hewitt *et al.* (54), the adult worms of *Dirofilaria immitis* are not killed by it *in vivo* but good results were reported by Ziegler (131), who gave large doses (20 mg. of the citrate per kg. body weight thrice daily). (See also 25, 76, 93.) In the case of *Loa*, it is generally agreed that diethylcarbamazine causes the death of some adult worms, which form long narrow wheals immediately under the skin; but a single course seldom kills all the worms and repeated courses of treatment may be necessary. With *Onchocerca volvulus*, living adult worms can easily be found (by excision of the subcutaneous nodules) even after repeated courses of treatment. Apparently the compound has only a limited action on these adult worms, or none at all (48, 74, 97). The failure of diethylcarbamazine to kill the adults of *Onchocerca*

can hardly be due to the failure of the drug to penetrate into the fibrous nodules which enclose this worm, since suramin (which is a larger molecule and less diffusible) succeeds in killing them. It must be due to a lack of chemotherapeutic action towards this species of worm. There is evidence that *Onchocerca* differs in its chemotherapeutic reactions from the other filariae, *e.g.*, *Onchocerca* responds to suramin but the other filariae are resistant to it. The action of diethylcarbamazine upon the adult worms of *W. bancrofti* is more difficult to determine as they cannot be observed directly. Since microfilariae do not reaccumulate in the blood of treated patients even during a 10–15 months follow-up period, it seems probable that the compound kills or sterilizes the adult female worms (53, 77, 83). Diethylcarbamazine has no apparent action on the microfilariae of *D. perstans* but it probably has a slight lethal or sterilizing action on the adult worms (77). *Mf. streptocerca* disappears quickly from the skin when diethylcarbamazine is given and there are practically no allergic reactions (121). There is no action on *Mansonella ozzardi* (109). It has an antifilarial action on the frog filaria *Icosiella neglecta*, particularly on the microfilariae (79). The adult worm of *Dracunculus medinensis* (Guinea worm) is said by Rousset (100) to be killed by large doses of diethylcarbamazine. Treatment also destroys the developing stages of the worm if given prophylactically. This most interesting report ought to stimulate further investigation.

As regards other life stages of filarial worms, diethylcarbamazine has no action against the forms of *Dirofilaria repens* which develop in the mosquito; it probably destroys the infective larvae of *Litomosoides*; it is not very effective against the immature female worms of *Litomosoides*, although there may be a selective action on the immature male ones (51). It is said to be effective on "larva migrans", *i.e.*, the infective stage of *Ancylostoma braziliense* which has penetrated into man who is an unnatural host. It is also said not to act on *Ancylostoma caninum* of dogs, but to be effective on *Onchocerca* in mules, on *Toxocara canis* of dogs and cats, and on *Trichinella spiralis* of rats and of man (in two cases) (67). (For action on schistosomes, see p. 295.)

Action of piperazine derivatives on intestinal nematodes. In all the above cases the worms are embedded in the tissues and it could be imagined that the action of diethylcarbamazine (when present) was exerted by making the adult worms more susceptible to attack by macrophages. A more difficult problem is presented by the action of the compound upon nematodes which occur in the alimentary canal. The best known example of this is that of *Ascaris lumbricoides*, which is often expelled from the intestine by the oral administration of diethylcarbamazine. (From the practical point of view, diethylcarbamazine is probably less effective than the standard remedies, *e.g.*, hexylresorcinol, but when its ease of administration and low toxicity are taken into account, it becomes a means of treatment which is often well worthwhile.) Diethylcarbamazine also has some action in expelling *Enterobius* and similar oxyurids from the large intestine, while *Ancylostoma* is not much affected. But other compounds of piperazine (which are not active against microfilariae) are more potent than diethylcarbamazine in this respect (17, 27, 28, 29, 68, 123, 124). Accordingly this action on

nematodes in the intestine seems to be fundamentally different from the action on microfilariae and to depend on some other property of the piperazine ring.

The action is not a direct lethal one since the worms expelled are usually alive. *In vitro*, diethylcarbamazine has no direct action on Ascaris, except possibly a narcotic one (42a); on isolated muscle preparations from this worm, it may cause slight derangement of the normal rhythmical contractions (78). Apparently the action of diethylcarbamazine and other piperazines in expelling Ascaris is due to some kind of derangement of the normal mechanism by which the worm maintains its position in the intestine.

Toxicity of diethylcarbamazine. The acute toxicity is low, the LD_{50} in mice being 240 mg./kg. given intraperitoneally or 560 mg./kg. given orally. The oral LD_{50} in rats is 395 mg./kg. Chronic toxicity does not appear in laboratory animals even from high dosage, *e.g.*, 170 mg./kg. given intraperitoneally, twice daily, for over 12 doses (cotton rats). All these doses refer to the base. In rats, there seems to be little or no accumulation when repeated doses are given. (See also 45, 46.)

In man the toxic action varies greatly according to whether or not the subject is free from filarial infection and, if he is infected, according to the worm in question. There seem also to be geographical differences. In general, however, diethylcarbamazine is comparatively non-toxic, and its toxic effects although troublesome are never dangerous. In *uninfected* persons, diethylcarbamazine may cause gastro-intestinal disturbances, *e.g.*, anorexia, nausea, and vomiting coming on within one to twenty hours after swallowing the compound in doses of 10–20 mg./kg. Headache and sleepiness have also been noted. All these symptoms are probably due to the direct action of the compound upon the patient. The amount tolerated differs among different native populations. In East Africa, doses of 10–14 mg./kg. were usually tolerated (48) but elsewhere the amounts tolerated have been about 5–8 mg./kg. (All doses refer to base.)

In patients *infected* with some form of filariasis, however, different and more severe untoward effects may occur. These are most marked in patients with *Onchocerca*, less so with *W. malayi* in Malaya, and still less marked in patients with *W. bancrofti* in other parts of the world. In patients with onchocerciasis there is usually a violent reaction which is well marked within 16 hours of the first oral dose. This includes swelling and oedema of the skin, especially the buttocks, thighs, genitals etc. (these being the areas where there are most microfilariae), intense itching, enlargement and tenderness of the inguinal lymph nodes, sometimes a fine papular rash, hyperpyrexia up to 102° F., tachycardia, headache etc. These symptoms persist for 3–7 days and then subside, after which quite high doses (12 mg./kg./day) can be tolerated without further reaction. The severity of the reaction is proportional to the number of microfilariae initially present in the skin and not the size of the initial doses. It is obviously an allergic response to the destruction of many microfilariae in a sensitized subject, and it is so constant that it can be used as a convenient diagnostic test (48, 73). Prophylactic administration of antihistamine compounds diminishes the severity of this reaction but does not abolish it altogether. Similar but milder reactions have

been described in patients in Malaya, with *W. malayi* (128). In patients with *W. bancrofti* treated in West Africa, the chief symptoms were headache, nausea, vomiting, anorexia, cough and pain in the chest, general malaise and pyrexia, and, rarely, a papular rash (77). None of these reactions is so severe as ever to be a danger to life, and they all subside after a few days, even though the administration of diethylcarbamazine is continued. However, they are of great practical importance because they render the mass-administration unpopular and unacceptable, and thus prevent the use of this compound to eradicate filariasis on a community basis.

In addition to the above general symptoms, small focal reactions of pain, tenderness and inflammation sometimes occur in the groins or thighs of persons infected with *W. bancrofti*. They subside in a few days and are probably due to a local reaction round an adult worm which has been killed or damaged.

Pharmacology and distribution. Work on the absorption and metabolism of diethylcarbamazine has been limited because there are no specific sensitive chemical means of estimating piperazine compounds. Lubran (69) has described a colorimetric method for the estimation of blood and urine levels, but this technique has a small 'blank value' in untreated patients and its exact specificity is therefore not known. By means of this method it has been shown that in man diethylcarbamazine is rapidly absorbed from the gut when given by mouth. A single oral dose of 10 mg. base per kg. produces a peak blood level of 4–5 μg./ml. in 3 hours (when toxic symptoms are most prominent); it subsequently falls to zero within about 48 hours. Most of the urinary excretion occurs in the first 24 hours when 10–26 per cent of the dose may be recovered as diethylcarbamazine. When 3 mg. base per kg. is taken twice daily for 4½ days the blood concentration reaches 4–5 μg./ml. by the third day and then falls even though the dosage is continued. The highest concentration observed in the blood was 15 μg./ml. which was seen in an African who had taken 425 mg. base twice daily for 5 doses. Diethylcarbamazine apparently penetrates readily into hydrocoele fluid (and presumably other body fluids) (48).

Using drug labelled with C^{14} in the piperazine ring, Bangham (5) made a more specific analysis of metabolism and distribution in rats and monkeys (5). It has been shown (by chromatographic and carrier techniques) that an oral dose is rapidly absorbed and that about 70 per cent of piperazine metabolites are excreted in the urine in 24 hours. After an intravenous dose it is all excreted in 24 hours, 10–20 per cent appearing as unchanged drug depending on the dose level given. Metabolism is very rapid and the drug is excreted as four different metabolites in all of which the piperazine ring remains intact. Three of these four metabolites, which have been identified chemically, are inactive *in vitro*; the fourth, which is an unstable basic compound, has not been identified, but is known also to be inactive *in vitro*. This compound can be demonstrated in the blood within 2 minutes of an intravenous injection. Additional evidence for this metabolism is found when diethylcarbamazine labelled with C^{14} in the methyl group is injected intravenously. Apparently partial demethylation is an early step in its degradation; further stripping of the side chain then occurs until

finally piperazine and methyl piperazine are excreted as the end products Although metabolism is apparently rapid and extensive, no metabolite so far isolated appears to have a sufficiently profound effect to account for the extraordinary speed of action on the circulating microfilariae. Accordingly, it is probable that this action is due to diethylcarbamazine itself.

Studies of the distribution of the radioactive preparation show that diethylcarbamazine soon equilibrates with all organs, blood cells and tissues (except fat), and that neither the microfilariae nor the adult worms (of *D. immitis*) concentrate it to any significant extent as compared with the surrounding tissues or fluids.

Excretion takes place almost entirely by the urine. The faeces contain only small amounts of piperazine metabolites and there is no significant concentration in the bile. It is possible, however, that the observed effect of diethylcarbamazine in expelling ascaris from the gut may be due to the small amount of piperazine produced which is known to be a potent (and polyvalent) vermifuge.

Clinical uses of diethylcarbamazine. The dose recommended by the manufacturers and widely accepted is 2 mg. (citrate) per kg. thrice daily by mouth for 3 weeks, *i.e.*, for an adult weighing 75 kg., 0.15 g./dose. In primitive populations as in Africa, there is a great gain of simplicity and economy if the whole amount is given as a single dose, *e.g.*, 0.25 g. (the average body weight being lower than in white people) and if the course is restricted to 5–10 days. There is no evidence that the hour of administration or the spacing of doses has any effect upon the therapeutic action, although, possibly, subdivision of the dose might be expected to diminish toxicity (48). The compound is almost always given by mouth, although it has also been prepared for intramuscular injection. The intravenous route is used only during scientific investigations. It has been prepared as a syrup to disguise the taste (which is slightly sweet and unpleasant); and since the usual salt, the citrate, is strongly acid it would seem reasonable to neutralize this acid with the appropriate amount of sodium bicarbonate. By these means, the compound can certainly be made more palatable, but the tendency to produce anorexia and nausea does not seem to be greatly changed.

In infections with *W. bancrofti* or *W. malayi*, short courses of treatment will remove all or most of the microfilariae from the blood and (as indicated above) will probably kill most of the adult worms (26, 40, 43, 60, 61, 71, 99, 101, 102, 116). The main lesions of filariasis, *viz.* elephantiasis and hydrocoele are not affected since they are due to irreversible blockage of the lymphatics and not to the presence of worms. On the other hand, according to experience in East Africa, the repeated attacks of fever, pain and lymphangitis are much diminished in frequency and severity by such treatment (35). With infections with *Loa*, suitable treatment kills the adult worms and microfilariae and prevents further symptoms, *e.g.*, pruritus, Calabar swellings, etc.; several courses of treatment may be needed to destroy all the worms (18, 19, 38, 65, 80, 104, 108, 110, 111, 113, 118). In onchocerciasis treatment with diethylcarbamazine (which provokes an intense, possibly allergic, reaction) kills the microfilariae in the skin and temporarily relieves many of the symptoms; but the adult worms are not killed

so that the microfilariae will reappear and the symptoms recur (13, 41, 48, 48a, 72, 73, 94, 95, 96, 119, 120). It may be possible, however, to hold both in check by periodic short courses of treatment.

Eradication of W. bancrofti. The most promising use of diethylcarbamazine lies in attempts to eradicate filariasis due to *W. bancrofti* and *W. malayi* by mass treatment of all the carriers in a given area. Since filarial worms are narrowly specialized as regards their hosts and there is no reservoir of infection apart from man, sterilization of all the carriers of microfilariae would prevent all further infections and the disease would die out. Even if the number of microfilariae were merely reduced to a low level without complete extinction, this might well be sufficient for the infection to dwindle away; since (in contrast to what occurs in protozoal and bacterial infections) one infecting unit, *i.e.*, one worm, stays one worm until it has met another worm of the opposite sex in the human body, and it is only after this meeting that multiplication can occur. The difficulties of one worm finding another in the body must be very great, so that meetings will take place only if many members of both sexes are present. If the number of worms falls below a certain concentration, conjugation of the sexes and reproduction will become increasingly rare, until ultimately the species will become extinct. Thus the biological conditions are favourable for attempts to eradicate filariasis by a mass chemotherapeutic attack.

Several pilot experiments on these lines have been undertaken (9, 10, 33, 56, 63, 70, 77). Briefly, if diethylcarbamazine is taken by the carriers of microfilariae even in quite short courses, a profound and prolonged diminution in the numbers of microfilariae can be produced. Unfortunately some of the people suffer minor toxic effects, *e.g.*, headache, anorexia, nausea, etc., which render the drug unpopular and often inacceptable to the people concerned. It is only when the full cooperation of the indigenous people can be secured, (by their confidence in the doctor, by education, or by the acute fear of elephantiasis and lymphangitis) that filarial infection can be eradicated in this way. But the benefits of such eradication would be so great and so long lasting, and the means of effecting it are relatively so simple, that all efforts should be made to carry out such a measure wherever lymphangitis, hydrocoele, and elephantiasis are at all common.

ANTIMONIALS

As was said above, reliable evidence concerning the action of antimony compounds upon filarial infections was first given in 1920 by Rogers (98) who showed that if tartar emetic was administered intravenously to patients carrying *Mf. bancrofti*, the number of microfilariae in the blood was greatly reduced, the number sometimes continuing to diminish for several months after the end of treatment. However, this work was overlooked. In 1939 a compound called stibsol (described as "antimonial-3-catechol-thiosalicylic acid sodium") was reported to be effective against *D. immitis* in dogs (15). In 1944–1947 the action of antimonials in filariasis was analysed in detail by Culbertson and his colleagues (30, 31, 32) first on *Litomosoides* in cotton rats, and subsequently on *W. bancrofti* and other infections in man. Briefly, they showed that suitable treatment

with antimony compounds acted primarily on the adult worms, causing their death. (This was proved at autopsy to have occurred in treated cotton rats, and it was inferred to have happened also in man.) The number of microfilariae in the peripheral circulation then gradually died away during a period of 3–19 months from lack of replenishment. In addition there might be a certain amount of action directly upon the microfilariae, as shown by an immediate fall in their number directly after treatment; this action however was much less important than that on the adults. Altogether 96 persons infected with *W. bancrofti* were treated with antimonials. The most satisfactory compound was a pentavalent one called neostibosan, which sterilized 25 out of 35 patients; high doses which approached the toxic level were needed. Somewhat less good results were obtained with neostam, with urea stilbamine, and with stibanose; fuadin and anthiomaline seemed rather less effective; four patients were treated with tartar emetic, but one showed a severe reaction to the drug and this treatment was not pursued. Twenty-five patients with *W. bancrofti* were treated with lithium antimony thiomalate (anthiomaline) by Brown (13, 14), who found that in all except one patient the microfilaria count was reduced by 85–100 per cent, but that toxic symptoms (vomiting, joint pains, slight fever and rash) might occur. Several patients with *Loa* infection were treated with neostibosan by Culbertson and his colleagues (30) with encouraging results. Twenty patients with *Onchocerca volvulus* were also treated, but five showed severe toxic effects and one died; there was no significant permanent effect on the number of microfilariae in the skin. (See also 6.)

This work of Culbertson and others on antimonials was of great importance, since it showed that effective chemotherapy of filariasis was possible and it analysed the criteria for judging in man whether an antifilarial action had been produced. But the antimonials have not been received into general practical use, partly because of the accompanying toxic effects (while filarial infection is often a symtomless condition), and partly because of the introduction of diethylcarbamazine which provided a more effective and less toxic alternative.

Clinical trials have been made in East Africa with pentavalent N-methylglucamine antimoniate (protostib), with neostibosan, and with solustibosan; these have a definite antifilarial effect (exerted mostly on the adult worm) but they are probably less effective than diethylcarbamazine while being more toxic and more expensive (35).

Various experimental investigations on antimony compounds have been made with *Litomosoides*. Thus 16 compounds were examined by Sewell and Hawking (106), most of which were active in killing the adult worms. Another compound, MSb (sodium *p*-melaminylphenylstibonate) has been shown by Kershaw and Williamson (62, 127) to be effective as a prophylactic for six months after a single dose in cotton rats exposed to infective mites. Another compound, antimony α,α'-dimercaptopotassium succinate, has been shown to be active upon *Litomosoides* in cotton rats. It is reported to be well tolerated in man when used for the treatment of schistosomiasis, but tests against human filariasis have not

yet been described (39). When tartar emetic, containing radioactive antimony, was injected intravenously into dogs infected with *D. immitis*, the following concentration of antimony was found: in the liver 10.7 μg./g., in the thyroid 3.8 μg./g., and in the adult worms 1.5 μg./g. (11). Investigations on antimonials in cotton rats and dogs were also carried out by Otto and Maren (87). Following treatment with tartar emetic necrosis has been found in the ovaries of female worms (3, 8).

ARSENICALS

The use of "aminoarsenophenol" for the treatment of bancroftian filariasis was recommended by Noc in 1923 (82), and the marked microfilaricidal action of phenylarsenoxide compound *in vitro* was noticed by Hawking (47) in 1940. Little serious attention, however, was given to these compounds until the work of Otto and Maren (86, 87, 88). They tested a large number of phenyl arsenoxide derivatives *in vitro* and *in vivo* and concluded that the most promising member of the series was benzamide *p*-arsenoxide (OAs  $CONH_2$) which has since been prepared as a soluble thioglycollate derivative named "arsenamide". The action of arsenamide in the treatment of bancroftian filariasis in man has been investigated by various workers (48, 75, 84, 85, 115). Briefly, if the drug is given intravenously in doses of 1 mg./kg. daily for 15 days the microfilariae disappear from the blood at, or soon after, the end of treatment and do not return during follow-up periods of 12–17 months. This indicates a direct lethal action on the microfilariae and a lethal (or sterilizing) action on the adult worms. In most of the patients this treatment is well tolerated, but several cases of jaundice have occurred in the 60 or 70 patients reported in the literature, and in one patient acute fatal necrosis of the liver developed after only 3 daily doses of 2.4 mg./kg. (75). Apparently arsenamide (like most other compounds of arsenic) may exert a profound toxic action upon the liver in a small proportion of patients. Although the likelihood of this toxic effect can probably be diminished by giving a high protein diet before treatment, it is hardly justifiable to use a potentially dangerous drug for the treatment of a nonfatal infection such as filariasis, especially as a safe and effective alternative remedy is available in diethylcarbamazine.

Arsenamide was given to 3 patients with onchocerciasis and some degeneration of the worms occurred (2). The compound has been employed by Drudge (34) for the treatment of *D. immitis* in dogs. Intravenous doses of 0.45 mg. As per kg. daily for 15 days regularly killed the adult worms but smaller doses were ineffective. Similar trials were also carried out by Jackson (59) and Foster (37).

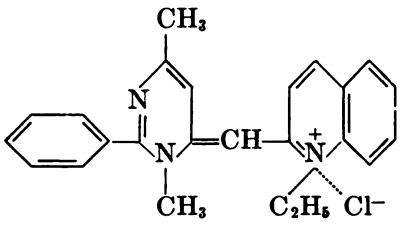
Melarsen oxide has been given to 18 patients with *Mf. bancrofti* present in the blood and 8 became free from microfilariae (30); but this compound is too dangerous for general use in a non-fatal infection such as filariasis.

When radioactive arsenic as sodium arsenite was administered to cotton rats infected with *Litomosoides* a high concentration (12.8 μg. As per g. of wet

tissue) was found in the adult worms; the kidney and liver contained slightly higher concentrations, while the other tissues contained much less (66).

CYANINE COMPOUNDS

Attention was drawn to the antifilarial action of these compounds by Welch *et al.* (122) and Peters *et al.* (91, 92). Working with cotton rats, infected with *Litomosoides*, they found the most active member of the series to be cyanine 863 which has the probable structure

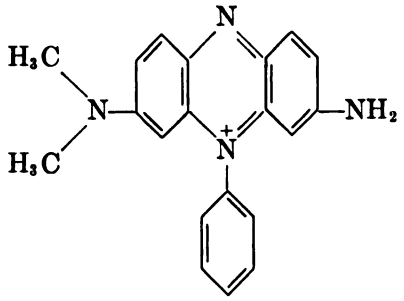


1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride. Many other cyanine and styryl dyes possessed similar (but smaller) filaricidal action, which seemed to be associated with the presence of an amidinium-ion system in which a positively charged quaternary nitrogen is linked to a tertiary nitrogen by a conjugated chain consisting of an uneven number of members

$$> \overset{+}{N}{=}C{-}(C{=}C)_n{-}N < \rightleftharpoons > N{-}(C{=}C)_n{-}C{=}\overset{+}{N} < \text{(12)}.$$

Cyanine 863 killed all the worms in cotton rats in daily doses of 1.0 mg./kg given intravenously on 3–6 days, while toxic effects were seen only with doses of 10 mg. or more per kg. per day. Unfortunately when this compound was tested in 37 patients infected with *W. bancrofti* the results were less favourable (90). The compound was given as 4–8 intravenous infusions of 2.0 mg./kg. either daily or on alternate days. When it was given daily signs of toxicity, including dyspnoea, severe persistent vomiting, headache and marked hypotension with bradycardia, began to appear after the second or third dose, necessitating interruption of treatment. Microfilaria counts showed a marked reduction of the microfilariaemia during and immediately after treatment. However, within a few weeks the number of microfilariae returned to its previous level and stayed there. Accordingly, this compound seems to have only incomplete action against *W. bancrofti*. Its action on other filariae of man has not been examined. Another cyanine dye No. 715 has been investigated as an anthelmintic in dogs (44). Cyanine dyes in concentrations as low as 5×10^{-6}M inhibit the oxidative metabolism of *Litomosoides*, and this inhibition may well explain their lethal action upon the worms (20); concentrations 1,000 times greater than this have no effect upon the oxygen uptake of mammalian tissues.

A somewhat similar compound is methylene violet, which belongs to the safranin series and has the formula:



It was studied by Hawking *et al.* (49). This compound had a maximum tolerated dose in cotton rats of 3.5 mg./kg. given intraperitoneally daily for 6 days and a minimum effective dose of 0.5 mg. Its main action was upon the adult worms. It was administered intravenously in an average total dose of 8–12.6 mg./kg. during 10–14 days to 50 patients infected with *W. bancrofti*. This was well tolerated apart from a curious toxic effect upon the development of the finger nails 3 weeks after the end of treatment. However, it had no action upon the microfilariae or adult worms of *W. bancrofti*, *D. perstans* or *O. volvulus*. This compound has a vinylogous amidinium-ion system similar to that of the cyanine series. For further information about the safranin series of compounds, see 16, 42, 107. Antifilarial action (as seen in cotton rats) has also been reported in various styrylquinoline compounds and also in phenanthridinium compounds (106) which is probably due to the presence of the same structure.

SURAMIN

This compound, which is also known as Bayer 205, antrypol, naphuride sodium, moranyl, germanin, belganin etc. was introduced as an effective remedy against trypanosomiasis in 1921 and it has been widely used for this purpose ever since. Its action against onchocerciasis was discovered in 1945 by Van Hoof, Henrard, Peel and Wanson during experiments with trypanosomiasis on volunteers who were also infected with *Onchocerca* (120); this reference also gives the fullest account of its use. It was given by the Belgian workers in courses of 1 g. intravenously once weekly for 5 to 10 doses; at a meeting of the W.H.O. Expert Committee on Onchocerciasis in 1953 (125) a dosage of 1 g. weekly for 5 weeks was recommended. Such courses of treatment may be expected to produce concentrations of 4–6 mg. per 100 ml. in the blood (47a, 120). This compound acts principally on the adult female worms which die or degenerate by the 4th to 5th week of treatment. The male adults are more resistant and remain alive and motile much longer than the females. With the longer courses of 10 doses, the ova and intra-uterine embryos are destroyed by the 6th to 10th week; and later the free microfilariae are also killed. The dead worms excite the usual inflammatory reactions round them and are then gradually absorbed (2, 120). After about the

5th or 6th dose of suramin there are various reactions, such as pains in the joints and various other places, and erythematous and pruritic eruptions. The fever may last a few days to 2 weeks, and the temperature may rise to 39–40°C. There are often acute pains in the knee and the foot. There may be violent prurigo of the parts of the skin which harbour microfilariae; there may be transient conjunctivitis and photophobia. All these symptoms may represent an allergic reaction to the destruction of microfilariae and to the gradual liberation of their protein in a body which is sensitized to them. The reaction to suramin is similar to that provoked by diethylcarbamazine, but it is much later in appearing, is more prolonged and less severe (in keeping with the more gradual liberation of microfilarial protein). In addition, there may be the usual toxic effects of suramin *e.g.*, albuminuria (common and mild) and exfoliative dermatitis (rare but severe) which are described in standard textbooks dealing with trypanosomiasis. Other accounts of the use of suramin for the treatment of onchocerciasis are given by Sarkis (103), Burch and Ashburn (24) and by Ashburn *et al.* (2).

As regards the place of suramin in the practical treatment of onchocerciasis, it is certainly the only effective method known at present for the destruction of the adult worms (which are not affected by diethylcarbamazine). But since a prolonged course of treatment may be necessary to kill all the worms, the possible toxic effects of this must be weighed against the fact that (unless the eyes are involved) onchocerciasis is usually a benign infection. If suramin is administered it should be given only under careful medical supervision. The value of combined treatment, first with diethylcarbamazine and then with suramin deserves further investigation (58a).

As regards other filarial infections, suramin has no effect on *W. bancrofti*, *L. loa*, *D. perstans* or *D. streptocerca* (120). In the experience of the writer and of Dr. E. Lagrange (quoted by Wanson (120)) it also has no effect on *Litomosoides* in the cotton rat, but the writer has heard of one laboratory in which such an effect was recognised.

MISCELLANEOUS COMPOUNDS

Gentian violet was tried carefully in 13 men carrying *Mf. bancrofti* but was found to be ineffective (4). Slight action against *Litomosoides* has been detected in proguanil (106), but further examination of related compounds failed to reveal anything of promise. In the author's laboratory during the past four years over five hundred compounds have been screened on *Litomosoides* without detecting significant filarial activity of a type different from those already known; the only exception is the finding that sodium vanadyl tartrate kills many of the microfilariae of *Litomosoides*, but apparently it does not destroy the adult worms (unpublished observations).

CONCLUSION

It is interesting to compare the chemotherapeutic reactions of filarial worms with those of other parasites and micro-organisms. The nematodes of the intestine live in an environment too different from the tissues to permit a close

comparison; as mentioned above, many intestinal round worms seem to be expelled (but not killed) by piperazine compounds, many compounds of this type being more active than diethylcarbamazine in this respect. Most of the compounds which are effective on the intestinal nematodes, *e.g.*, hexylresorcinol, carbon tetrachloride, etc., are too toxic for the tissues to permit them having a useful action on filariae. The other important class of worms invading the body itself is that of the trematodes, especially the schistosomes. Both filariae and schistosomes are sensitive to antimonials, although their relative sensitivities to the different compounds are not quite the same. Diethylcarbamazine has a slight therapeutic action in human schistosomiasis which is of theoretical interest but not of practical importance (7, 81). Otherwise there is little resemblance between these two types of parasite. Leucanthone (miracil, nilodin), which acts on schistosomes, has no action upon filariae (*Litomosoides*). The bacteria seem to have no chemotherapeutic resemblances to filariae; sulphonamides, antibiotics, isoniazide etc. all have no action upon filariae. Similarly, malaria parasites show no resemblances; none of the well-known antimalarial compounds has been found to possess antifilarial activity (apart from the slight action exhibited by proguanil).

With the trypanosomes, however, there are many remarkable similarities. Thus filariae are very sensitive to arsenicals and also to antimonials. *Litomosoides* is sensitive to phenanthridinium and styrylquinoline compounds, which destroy *Trypanosoma congolense*; and *Onchocerca* responds to suramin. On the other hand, *Litomosoides* does not respond to diamidine compounds, to antrycide, or to acriflavine, all of which are trypanocidal, while trypanosomes do not respond to diethylcarbamazine, or to cyanine 863.

Finally, consideration may be given to the further requirements of chemotherapeutic research in this field. From a clinical point of view the chief requirement is a drug to kill *Onchocerca volvulus*. At present diethylcarbamazine does not kill the adult worms, and suramin is potentially toxic. In filariasis due to *Wuchereria* or to *Loa*, diethylcarbamazine is so active that it seems unreasonable to ask for greater activity; but it would be valuable if some means could be discovered of avoiding the minor toxic effects which are such a handicap to mass treatments. This might be achieved by discovering a completely new compound, or by modifying the molecule or the pharmaceutical vehicle of diethylcarbamazine so that its effects were exerted more slowly. It would be advantageous to have a preparation of diethylcarbamazine which could be administered on fewer occasions while exerting an equally prolonged action in the body. There is no compound known at present which will kill *Dracunculus* (apart from the doubtful action of diethylcarbamazine), and it would be valuable to find one. The search would be much facilitated by obtaining an analogous helminthic infection in a laboratory animal (similar to *Litomosoides* in cotton rats which made possible the discovery of diethylcarbamazine). All new knowledge of the biology and biochemistry of filarial worms will assist in the provision of a rational chemotherapy, while the discovery of new chemotherapeutic agents will probably throw new light on the metabolism of the worms.

ACKNOWLEDGMENTS

Grateful acknowledgments are due to Dr. D. R. Bangham for allowing reference to be made to unpublished work, and to Dr. J. W. Walker for assistance with chemical nomenclature.

BIBLIOGRAPHY

1. Adams, J. C. L. and Woodruff, A. W.: Diethyl-carbamazine in the treatment of onchocerciasis. Tr. Roy. Soc. Trop. Med. & Hyg., 47: 66–69, 1953.
2. Ashburn, L. L., Burch, T. A. and Brady, F. J.: Pathologic effects of suramin, hetrazan and arsenamide on adult *Onchocerca volvulus*. Bol. Ofic. san. panam., 28: 1107–1117, 1949.
3. Ashburn, L. L., Perrin, T. L., Brady, F. J. and Lawton, A. H.: Histological changes in ovary and uterus of live *Dirofilaria immitis* recovered from dogs treated with trivalent antimony compounds. Arch. Path., 40: 334–339, 1945.
4. Ashford, B. K. and Snyder, H. McC.: Gentian violet in filariasis. Puerto Rico J. Pub. Health, 8: 375–384, 1933.
5. Bangham, D. R.: Unpublished observations. Submitted to Brit. J. Pharmacol.
6. Bartter, F. C., Burch, T. A., Cowie, D. B., Ashburn, L. L. and Brady, F. J.: Experimental therapy of onchocerciasis with trivalent antimonials. Ann. New York Acad. Sc., 50: 89–96, 1948.
7. Belhommet, F.: Essai de thérapeutique de la bilharsiose par la carbilasine. Ann. Soc. belge Méd. trop., 33: 3–11, 1953.
8. Bettini, S. and Lagrange, E.: Controllo istologico sulle filarie dell'asione antifilarica di medicamenti. Riv. Parassit., 8: 191–196, 1947.
9. Beye, H. K., Edgar, S. A., Mille, R., Kessel, J. F. and Bambridge, B.: Preliminary observations on the prevalence, clinical manifestations and control of filariasis in the Society Islands. Am. J. Trop. Med. & Hyg., 1: 637–661, 1952.
10. Beye, H. K., Kessel, J. F., Heuls, J., Thooris, G. and Bambridge, B.: Nouvelles recherches sur l'importance, les manifestations cliniques, et la lutte contre la filariose à Tahiti, Océanie Francaise. Bull. Soc. path. exot., 137: 144–163, 1953.
11. Brady, F. J., Lawton, A. H., Cowie, D. B., Andrews, H. L., Nees, A. T. and Ogden, G. E.: Localization of trivalent radioactive antimony following intravenous administration to dogs infected with *D. immitis*. Am. J. Trop. Med., 25: 103–107, 1945.
12. Brooker, L. G. S.: Chemistry of the cyanine dyes. Ann. New York Acad. Sc., 50: 108, 1948.
13. Brown, H. W.: Treatment of filariasis with antiomaline (lithium antimony thiomalate). Ann. New York Acad. Sc., 50: 51–72, 1948.
14. Brown, H. W.: The treatment of filariasis (*Wuchereria bancrofti*). J. A. M. A., 125: 953–958, 1944.
15. Brown, H. W. and Austin, J. A.: Treatment of heartworm in dogs with stibsol—a new drug. J. Am. Vet. Med. A., 95: 566–569, 1939.
16. Brown, H. W. and Hussey, K. L.: Experimental therapy of paragonimiasis in dogs. J. Parasitol., 33: 33–35, 1947.
17. Brown, H. W. and Sternman, M. M.: Treatment of *Ascaris lumbricoides* infections with piperasine citrate. Am. J. Trop. Med. & Hyg., 3: 750–754, 1954.
18. Brumpt, L. C.: Le mode d'action de la diéthylcarbamazine sur les filaires. Compt. rend. Soc. biol., 146: 209–211, 1952.
19. Brygoo, E.: Essai de traitement de la filariose (*F. loa* et *F. perstans*) par le 3.799 R.P. Bull. Soc. path. exot., 42: 313–323, 1949.
20. Bueding, E.: Effect of cyanine dyes on the metabolism of *Litomosoides carinii*. Ann. New York Acad. Sc., 50: 115–116, 1948.
21. Bueding, E.: Studies on the metabolism of the filarial worm, *Litomosoides carinii*. J. Exper. Med., 89: 107–130, 1949.
22. Bueding, E.: Metabolism of parasitic helminths. Physiol. Rev., 29: 195–218, 1949.
23. Burch, T. A.: Experimental therapy of onchocerciasis with suramin and hetrazan. Bol. Ofic. san. panam., 28: 232–245, 1949.
24. Burch, T. A. and Ashburn, L. L.: Experimental therapy of onchocerciasis with suramin and hetrazan. Am. J. Trop. Med., 31: 617–633, 1951.
25. Canet, J. and Jahan, P.: Treatment of filariasis due to *W. bancrofti* in Indo-China by a new filaricide: 1-diethyl carbamyl 4-methyl-piperasine, 3.799 R.P. or hetrazan. Bull. Soc. path. exot., 42: 408–414, 1949.
26. Casile, M. and Saccharin, H.: Sur un cas de lésions génitales lymphatico-veineuses dans la filariose de Bancroft. Bull. Soc. path. exot., 45: 56–60, 1952.
27. Cavier, R.: Les anthelmintiques: (1) Médicaments destiné à lutter contre les nématodes intestinaux. Prod. pharm., 8: 239–248, 1953.
28. Colbourne, M. J.: Unsuccessful treatment of ascariasis with hetrazan. Lancet, 1: 996, 1950.
29. Corcos, C., Dupoux, R. and Abitbol, S.: Traitement des parasitoses intestinales à vers ronds par le diéthyl carbamyl 4 méthyl pipérasine (notésine). Bull. Soc. path. exot., 44: 209–215, 1951.
30. Culbertson, J. T.: Experimental chemotherapy of filariasis. Tr. Roy. Soc. Trop. Med. & Hyg., 41: 18–54, 1947.
31. Culbertson, J. T.: Treatment of filariasis with neostibosan and some other compounds. Ann. New York Acad. Sc., 50: 73–88, 1948.

32. CULBERTSON, J. T. AND ROSE, H. M.: Chemotherapy of filariasis in the cotton rat by administration of neostam and of neostibosan. J. Pharmacol. & Exper. Therap., 81: 189–196, 1944.
33. DASSANAYAKE, W. L. P.: A follow-up of 230 cases of bancroftian filariasis treated with hetrazan at the filariasis clinic at Dehiwala, Ceylon. Ann. Trop. Med., 48: 123–126, 1954.
34. DRUDGE, J. H.: Arsenamide in the treatment of canine filariasis. Am. J. Vet. Res., 8: 220–235, 1952.
35. EAST AFRICA HIGH COMMISSION: Filariasis Research Ann. Rep., 1949, 1950, 1951, 1952, 1953.
36. FINDLAY, C. M.: Recent advances in chemotherapy, 1: 95–98. Churchill Ltd., London, 3rd ed., 1950.
37. FOSTER, A. O.: Notes on veterinary parasitology. Vet. Med., 44: 460, 1949.
38. FREMONT, O.: Traitement de la filariose humaine par un nouveau dérivé de la pipérazine, le 1 diéthyl carbamyl 4 méthyl pipérazine. Librairie Arnette, Paris 1949.
39. FRIEDHEIM, E. A. H., DE SILVA, J. R. AND MARTINS, A. V.: Treatment of schistosomiasis mansoni with antimony—a,a'-dimercapto-potassium succinate (TWSb). Am. J. Trop. Med. & Hyg., 3: 714–727, 1954.
40. GALLIARD, H. AND MILLE, R.: Essais de traitement de la filariose à *Wuchereria bancrofti* var. pacifica par le 1-diéthyl carbamyl, 4-méthyl pipérazine, à Tahiti. Bull. Soc. path. exot., 42: 304–313, 1949.
41. GIBSON, C. L. AND BURCH, T. A.: Estudios preliminares sobre la infectividad de microfilarias de *Onchocerca volvulus* que reaparacen despues del tratamiento con hetrazan. Rev. Col. Med. Guatemala, 2: 63–66, 1951.
42. GINELER, A. M.: Experimental tubular nephritis produced by safranin O. Proc. Soc. Exper. Biol. & Med., 61: 231–234, 1946.
42a. GOODWIN, L. G. AND STANDEN, O. D.: Treatment of roundworm with piperazine citrate ("antepar"). Brit. Med. J., 2: 1332–1333, 1954.
43. HALAWANI, A., BAZ, I. AND DAWOOD, M.: Preliminary report on treatment of ambulant cases of bancroftian filariasis with hetrazan in Egypt. J. Roy. Egyptian Med. A., 32: 395–403, 1949.
44. HALES, D. R. AND WELCH, A. D.: A preliminary study of the anthelmintic activity of cyanine dye 715 in dogs. J. Pharmacol. & Exper. Therap., 107: 310–314, 1953.
45. HARNED, B. K., CUNNINGHAM, R. W., HALLIDAY, S., VESSEY, R. E., YUDA, N. N., CLARK, M. C. AND SUBBAROW, Y.: Some toxicological and pharmacological properties of 1-diethyl-4-methylpiperazine hydrochloride, hetrazan. Ann. New York Acad. Sc., 50: 141–160, 1948.
46. HARNED, B. K., CUNNINGHAM, R. W., HALLIDAY, S., VESSEY, R. E., YUDA, N. N., CLARK, M. C., HINE, C. H., COSGROVE, R. AND SUBBAROW, Y.: Studies on the chemotherapy of filariasis. VI. Some pharmacodynamic properties of 1-diethylcarbamyl-4-methylpiperazine hydrochloride, hetrazan. J. Lab. & Clin. Med., 33: 216–235, 1948.
47. HAWKING, F.: Chemotherapy of filariasis *in vivo* and *in vitro*. J. Trop Med., 43: 204–207, 1940.
47a. HAWKING, F.: Concentration of Bayer 205 (germanin) in human blood and cerebrospinal fluid after treatment. Tr. Roy. Soc. Trop. Med. & Hyg., 34: 37–52, 1940.
48. HAWKING, F.: Some recent work on filariasis. Tr. Roy. Soc. Trop. Med. & Hyg., 44: 153–192, 1950.
48a. HAWKING, F.: A histological study of onchocerciasis treated with hetrazan. Brit. Med. J., 1: 992–994, 1952.
49. HAWKING, F., ORMEROD, W. E., THURSTON, J. P. AND WEBBER, W. A. F.: The antifilarial action and toxicity of methylene violet and of other compounds of the phenosafranine series. Brit. J. Pharmacol., 7: 494–508, 1952.
50. HAWKING, F. AND SEWELL, P.: The maintenance of a filarial infection (*Litomosoides carinii*) for chemotherapeutic investigations. Brit. J. Pharmacol., 3: 285–296, 1948.
51. HAWKING, F., SEWELL, P. AND THURSTON, J. P.: The mode of action of hetrazan on filarial worms. Brit. J. Pharmacol., 5: 217–238, 1950.
52. HEWITT, R.: General experimental methods used in studying filaricides. Ann. New York Acad. Sc., 50: 27–38, 1948.
53. HEWITT, R., KENNEY, M., CHAN, A. AND MOHAMED, H.: Follow-up observations on the treatment of bancroftian filariasis with hetrazan in British Guiana. Am. J. Trop. Med., 30: 217–237, 1950.
54. HEWITT, R. I., KUSHNER, S., STEWART, H. W., WHITE, E., WALLACE, W. S. AND SUBBAROW, Y.: Experimental chemotherapy of filariasis. III. Effect of 1-diethylcarbamyl-4-methylpiperazine hydrochloride against naturally acquired filarial infections in cotton rats and dogs. J. Lab. & Clin. Med., 23: 1314–1329, 1947.
55. HEWITT, R. I., WALLACE, W. S., WHITE, E. AND SUBBAROW, Y.: Experimental chemotherapy of filariasis. I. Experimental methods for testing drugs against naturally acquired filarial infections in cotton rats and dogs. J. Lab. & Clin. Med., 32: 1293–1303, 1947.
56. HEWITT, R. I., WHITE, E., HEWITT, D. B., HARDY, S. M., WALLACE, W. S. AND ANDUZE, R.: The first year's results of a mass treatment program with hetrazan for the control of bancroftian filariasis on St. Croix, American Virgin Islands. Am. J. Trop. Med., 30: 443–452, 1950.
57. HEWITT, R. I., WHITE, D. E., KUSHNER, S., WALLACE, W. S., STEWART, H. W. AND SUBBAROW, Y.: Parasitology of piperazines in the treatment of filariasis. Ann. New York Acad. Sc., 50: 128–140, 1948.
58. HEWITT, R. I., WHITE, E., WALLACE, W. S., STEWART, H. W., KUSHNER, S. AND SUBBAROW, Y.: Experimental chemotherapy of filariasis. II. Effect of piperazine derivatives against naturally acquired filarial infections in cotton rats and dogs. J. Lab. & Clin. Med., 32: 1304–1313, 1947.
58a. HUTTON, P. W.: Severe onchocercal dermatosis responding to suramin and diethylcarbamasine: an illustrative case. Tr. Roy. Soc. Trop. Med. & Hyg., 48: 522–525, 1954.
59. JACKSON, R. F.: The treatment of heart-worm infected dogs with arsenamide, a phenylarsenoxide. J. Am. Vet. Med. A., 115: 17–22, 1949.
60. KENNEY, M. AND HEWITT, R. I.: Psychoneurotic disturbances in filariasis, and their relief by removal of adult worms or treatment with hetrazan. Am. J. Trop. Med., 30: 895–899, 1950.

61. KENNEY, M. AND HEWITT, R. I.: Treatment of bancroftian filariasis with hetrazan in British Guiana. Am. J. Trop. Med., 29: 89–114, 1949.
62. KERSHAW, W. E. AND WILLIAMSON, J.: Studies on the chemoprophylaxis of experimental filariasis with MSb (Friedheim). 1. The prophylactic activity during an interval of six months. Ann. Trop. Med., 46: 268–275, 1952.
63. KESSEL, J. F., THOORIS, G. C. AND BAMBRIDGE, B.: The use of diethylcarbamazine (hetrazan or notezine) in Tahiti as an aid in the control of filariasis. Am. J. Trop. Med. & Hyg., 2: 1050–1061, 1953.
64. KUSHNER, S., BRANCONE, L. M., HEWITT, R. I., MCEWEN, W. L., SUBBAROW, Y., STEWART, H. W., TURNER, R. J. AND DENTON, J. J.: The chemistry of piperazine compounds in the chemotherapy of filariasis. Ann. New York Acad. Sc., 50: 120–127, 1948.
65. LAGRANGE, E.: Essais de traitement des filarioses à *Loa loa* et *O. volvulus* par le diéthylcarbamazine Chl. Ann. Soc. belge Méd. trop., 29: 19–22, 1949.
66. LAWTON, A. H., NESS, A. T., BRADY, F. J. AND DEAN, B. C.: Distribution of radioactive arsenic following intraperitoneal injection of sodium arsenite into cotton rats infected with *Litomosoides carinii*. Science, 102: 120–122, 1945.
67. LEDERLE LABORATORIES DIVISION: Hetrazan diethylcarbamazine for the treatment of bancroftian filariasis and onchocerciasis. Am. Cyanamid Co., New York 1948.
68. LOUGHLIN, E. H., JOSEPH, A. A., RAPPAPORT, I. AND MULLIN, W. G.: Treatment of human ascariasis with hetrazan. Lancet, 2: 1197–1200, 1951.
69. LUBRAN, M.: Determination of hetrazan in biological fluids. Brit. J. Pharmacol., 5: 210–216, 1950.
70. MALDONADO, J. F., HERNANDEZ-MORALES, F., VELEZ-HERRERA, F. AND THILLET, C. J.: The role of hetrazan in the control of filariasis bancrofti. Puerto Rico J. Pub. Health, 25: 291–299, 1950.
71. MANSON BAHR, P.: The action of hetrazan in Pacific filariasis. J. Trop. Med., 55: 169–173, 1953.
72. MAZZOTTI, L.: Observations on use of hetrazan in onchocerciasis in Mexico. Am. J. trop. Med., 31: 628–632, 1951.
73. MAZZOTTI, L.: Posibilidad de utilizar domo medio diagnostico auxiliar en la oncocercosis, las reacciones alergicas consecutivas a la administracion del "hetrazan". Rev. Inst. salub. y enferm. trop., 9: 235–237, 1948.
74. MAZZOTTI, L. AND HEWITT, R.: Tratamiente de la onchocercosis par el clerure de l-dietilcarbamil-4-metilpiperasine (hetrazan). Medicina, Mexico, 28: 39–42, 1948.
75. MCFADZEAN, J. A. AND HAWKING, F.: Arsenamide treatment of filariasis due to *W. bancrofti* and *A. perstans*. Brit. Med. J., 1: 956–959, 1954.
76. MCGAUGHEY, C. A.: Filariasis of dogs in Ceylon: treatment with diethylcarbamazine. Vet. Rec., 64: 66–68, 1952.
77. MCGREGOR, I. A., HAWKING, F. AND SMITH, D. A.: The control of filariasis with hetrazan. A field trial in a rural village (Keneba) in the Gambia. Brit. Med. J., 2: 908–911, 1952.
78. MELLANBY, H.: Unpublished observations, 1953.
79. MINNING, W. AND DING, P.-C.: Hetrazan-Wirkung bei Frosch-Filariasis. Ztschr. Tropenmed. u. Parasitol., 2: 535–543, 1951.
80. MURGATROYD, F. AND WOODRUFF, A. W.: Loiasis treated with hetrazan (banocide). Lancet, 2: 147–149, 1949.
81. NEWSOME, J.: Hetrazan and stibophen in schistosomiasis. Tr. Roy. Soc. Trop. Med. & Hyg., 48: 445–446, 1954.
82. NOC, F.: Chimiothérapie des filarioses. Action de l'aminoarséno-phénol (132) sur *Mf. bancrofti*. Bull. Soc. path. exot., 16: 126–132, 1923.
83. OLIVER-GONZALEZ, J., SANTIAGO-STEVENSON, D. AND MALONADO, J. F.: Treatment of filariasis bancrofti with hetrazan. Follow-up observations 15 months after treatment. J. A. M. A., 139: 308–309, 1949.
84. OTTO, G. F., BROWN, H. W., BELL, S. D. JR. AND THETFORD, N. D.: Arsenamide in the treatment of infections with the periodic form of the filaria, *Wuchereria bancrofti*. Am. J. Trop. Med. & Hyg., 1: 470–473, 1952.
85. OTTO, G. F., JACHOWSKI, L. A. JR. AND WHARTON, J. D.: Filariasis in American Samoa. III. Studies on chemotherapy against the nonperiodic form of *Wuchereria bancrofti*. Am. J. Trop. Med. & Hyg., 2: 495–516, 1953.
86. OTTO, G. F. AND MAREN, T. H.: Filaricidal activity of substituted phenyl arsenoxides. Science, 106: 105–107, 1947.
87. OTTO, G. F. AND MAREN, T. H.: Studies on the chemotherapy of filariasis. Am. J. Hyg., 50: 92–141, 1949.
88. OTTO, G. F. AND MAREN, T. H.: Use of arsenicals in filariasis. Ann. New York Acad. Sc., 50: 39–50, 1948.
89. PAN AMERICAN SANITARY BUREAU: Bibliography of onchocercosis. Oficina Sanitaria Panamericana, Washington 1950.
90. PETERS, L.: Antifilarial action, toxicology, and clinical trial of cyanine dyes in filariasis. Ann. New York Acad. Sc., 50: 117–119, 1948.
91. PETERS, L., BUEDING, E., VALK, A. D. JR., HIGASHI, A. AND WELCH, A. D.: The antifilarial action of cyanine dyes. (1) The relative antifilarial activity of a series of cyanine dyes against *Litomosoides carinii, in vitro* and in the cotton rat. J. Pharmacol. & Exper. Therap., 95: 213–239, 1949.
92. PETERS, L., WELCH, A. D. AND HIGASHI, A.: The antifilarial action of cyanine dyes. (2) Selection of 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride (863) for further study as a potential antifilarial agent. J. Pharmacol. & Exper. Therap., 96: 460–471, 1949.
93. PURCHASE, H. S.: Hetrazan in the treatment of *Dirofilaria immitis* (Leidy 1856). Vet. Rec., 62: 34, 1950.
94. PUYUELO, R.: Le traitement de l'onchocercose en Haute-Volta. Premiers essais chimiothérapiques par la 1-diéthylcarbamyl 4-méthylpipérasine. Bull. Soc. path. exot., 43: 462–470, 1950.
95. PUYUELO, R.: Note préliminaire sur l'épidémiologie et le traitement de l'onchocercose humaine à *O. volvulus* en pays Mossi. Le 3799 R.P. (notézine). Bull. Soc. path. exot., 42: 558–561, 1949.
96. PUYUELO, R. AND HOLSTEIN, M. M.: L'onchocercose humaine en Afrique Noire Française. Méd. trop., 10: 397–510, 1950.

97. RODHAIN, J.: Considérations sur le rôle des microfilaires dans la pathogénie des filarioses. Ann. Soc. belge Méd. trop., 29: 177–190, 1949.
98. ROGERS, L.: Further work on antimony intravenously in filariasis. Brit. Med. J., 1: 596–598, 1920.
99. ROSSI, A. L. B. AND HEWITT, R. I.: Treatment of bancroftian filariasis with hetrazan in Puerto Cabello, Venezuela. South. Med. J., 42: 978–981, 1949.
100. ROUSSET, P.: Essai de prophylaxie et de traitement de la dracunculose par la notézine en Adrar. Bull. méd. A.O.F., 9: 351–568, 1952.
101. SANTIAGO-STEVENSON, D., OLIVER-GONZALEZ, J. AND HEWITT, R. I.: Treatment of human filariasis with hetrazan. J.A.M.A., 135: 708–712, 1947.
102. SANTIAGO-STEVENSON, D., OLIVER-GONZALEZ, J. AND HEWITT, R. I.: The treatment of filariasis bancrofti with 1-diethyl-carbamyl-4-methylpiperazine hydrochloride (hetrazan). Ann. New York Acad. Sc., 50: 161–171, 1948.
103. SARKIES, J. W. R.: Antrypol in the treatment of onchocerciasis. Tr. Roy. Soc. Trop. Med. & Hyg., 46: 435–436, 1952.
104. SCHNEIDER, J.: Traitement de la filariose à *F. loa* par la 1-diéthyl-carbamyl 4-méthylpipérazine. Bull. Soc. path. exot., 43: 270–275, 1950.
105. SCOTT, J. A. AND CROSS, J. B.: A laboratory infection of the rat with filarial worms. Am. J. Trop Med., 26: 849–855, 1946.
106. SEWELL, P. AND HAWKING, F.: Chemotherapy of experimental filariasis. Brit. J. Pharmacol., 5: 239–260, 1950.
107. SHEEHAN, H. L.: The deposition of dyes in the mammalian kidney. J. Physiol., 72: 201–246, 1931.
108. SHOOKHOFF, H. B. AND DWORK, K. G.: Treatment of *Loa loa* infections with hetrazan. Am. J. Trop. Med., 29: 589–593, 1949.
109. SNAPPER, I. AND MERLISS, R.: Observations on two cases of human filariasis (*Wuchereria bancrofti*, and *Mansonella ozzardi*). J. Mt. Sinai Hosp., 12: 1032–1038, 1946.
110. STEFANOPOULO, G. J.: The symptomatology, diagnosis and treatment of filariasis due to *Loa loa*. Brit. Med. A. Proc. Ann. Meet., 1949, pp. 283–287.
111. STEFANOPOULO, G. J. AND SCHNEIDER, J.: Essais de traitement de la filariose à *F. loa* par la 1-diéthyl-carbamyl 4-méthylpipérazine. Compt. rend. Soc. biol., 142: 930–931, 1948.
112. STRANGEWAY, W. E. AND STRANGEWAY, A. K.: *Onchocerca volvulus* in Angola, Africa. Canad. Med. A. J., 64: 427–429, 1951.
113. TASQUE, M.: Essais de traitement de 17 cas de filariose à *Loa loa* par le 3.799 R.P. Bull Soc. path. exot. 42: 556–557, 1949.
114. TEMKIN, O.: A report on the medicinal treatment of filariasis bancrofti. N. R. C., Washington 1945.
115. THETFORD, N. D., OTTO, G. F., BROWN, H. W. AND MAREN, T. H.: The use of a phenyl arsenoxide in the treatment of *Wuchereria bancrofti* infections. Am. J. Trop. Med., 28: 577–583, 1948.
116. TUBANGUI, M. AND CABRERA, B. D.: Studies on filariasis in Philippines; treatment of bancroftian filariasis with hetrazan. Acta med. Philippina, 6: 1–7, 1949.
117. VANBREUSEGHEM, R.: Difficultés du diagnostic de la filariose à *Loa loa* et son traitement par le diéthylcarbamasine. Ann. Soc. belge Méd. trop., 30: 71–77, 1950.
118. WANSON, M.: Essai de traitement curatif de la filariose à *Loa loa* et de la filariose apériodique par les dérivés de la pipérazine. Ann. Soc. belge Méd. trop., 29: 73–80, 1949.
119. WANSON, M.: L'hetrazan dans la période d'invasion de l'onchocercose. Ann. Soc. belge Méd. trop., 29: 85–89, 1949.
120. WANSON, M.: Contribution à l'étude de l'onchocercose africaine humaine (problèmes de prophylaxie à Leopoldville). Ann. Soc. belge Méd. trop., 30: 671–863, 1950.
121. WANSON, M., BORGERS, C. AND PANNIER, L.: Activité de l'hetrazan sur *Dipetalonema streptocerca*. Ann. Soc. belge Méd. trop., 30: 91–95, 1950.
122. WELCH, A. D., PETERS, L., BUEDING, E., VALK, A. JR. AND HIGASHI, A.: A new class of antifilarial compounds. Science, 105: 486–488, 1947.
123. WHITE, R. H. R.: Ascariasis treated with piperazine hydrate. Lancet, 2: 315–316, 1954.
124. WHITE, R. H. R. AND STANDEN, O. D.: Piperazine in the treatment of threadworms in children. Brit. Med. J., 2: 755–757, 1953.
125. W. H. O. EXPERT COMMITTEE ON ONCHOCERCIASIS: First Report, Jan. 30, 1954.
126. WILLIAMS, R. W. AND BROWN, H. W.: The development of *Litomosoides carinii*, filariid parasite of the cotton rat, in the tropical rat mite. Science, 102: 482–483, 1945.
126a. WILLIAMS, R. W. AND BROWN, H. W.: The transmission of *Litomosoides carinii*, filariid parasite of the cotton rat, by the tropical rat mite, Liponyssus bacoti. Science, 103: 224, 1946.
127. WILLIAMSON, J. AND KERSHAW, W. E.: Studies on the chemoprophylaxis of experimental filariasis with MSb (Friedheim). 2. The retention and distribution of the drug. Ann. Trop. Med., 46: 330–330, 1952.
128. WILSON, T.: Hetrazan in the treatment of filariasis due to *Wuchereria malayi*. Tr. Roy. Soc. Trop. Med. & Hyg., 44: 49–66, 1950.
129. WOODRUFF, A. W.: Destruction of microfilariae of *Loa loa* in the liver in loiasis treated with banocide (hetrazan). Tr. Roy. Soc. Trop. Med. & Hyg., 44: 479–480, 1951.
130. WRIGHT, H. N., LITCHFIELD, J. T. JR., BERY, T., CRANSTON, E. M., CUCKLER, A. C. AND BIETER, R. N.: Chemotherapeutic activity of cyanines and related compounds in filariasis in the cotton rat. Ann. New York Acad. Sc., 50: 109–114, 1948.
131. ZIEGLER, C. G.: Treatment of canine filariasis with caricide, diethylcarbamasine. J. Am. Vet. Med. A., 116: 209–210, 1950.