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CHEMOTHERAPY OF EXPERIMENTAL FILARIASIS

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This paper describes an investigation of compounds for their chemotherapeutic action on experimental filariasis due to *Litomosoides carinii* in the cotton rat, *Sigmodon hispidus*. Attention was drawn by Culbertson and Rose (1944) to the value of rats naturally infected with *Litomosoides* for experiments on filariasis, and the transmission by the tropical rat mite, *Liponyssus bacoti*, was demonstrated by Williams and Brown (1945) and by Scott (1946). The method used in the Lederle Laboratories for testing drugs against naturally acquired filarial infections in cotton rats have been described by Hewitt, Wallace, White, and SubbaRow (1947). Early in 1946, a colony of rats infected with *Litomosoides* was established in this Institute, and this has supplied the animals for the present work, which is believed to be the first large scale search for antifilarial remedies which has been made with cotton rats infected in the laboratory.

METHODS

Infection and maintenance of rats.—The procedures for propagating the *L. carinii* infection in cotton rats have already been described in a previous paper (Hawking and Sewell, 1948). Briefly, tanks were set up with entomologically pure colonies of *Liponyssus bacoti*, on a sub-stratum of plaster-of-Paris and sawdust. The mites were infected with the larval stages of *Litomosoides carinii* by allowing them to feed on several cotton rats which had numerous microfilariae in their peripheral blood. The infected rats were then removed and recently weaned rats were placed in the tanks for a period of 14 days. The rats were then freed of mites and removed to a storage room for the incubation period of about fifty days. Smears of tail blood were made weekly during the sixth to ninth weeks of storage. Those rats which failed to show microfilariae in their blood at the fourth examination were exposed to infection a second time. The following notes describe our further experience since the publication of the previous paper.

In the last two and a half years approximately 2,500 cotton rats have been exposed in infection tanks, and of these roughly 80 per cent have been found to have microfilariae in their peripheral blood after the first exposure. A more detailed analysis can be made for the period between January, 1948, and March, 1949, during which time 781 rats were exposed: of these, 121 (15 per cent) died before the termination of the incubation period; 576 (74 per cent) were subsequently found to be infected, while 84 (11 per cent) did not show microfilariae for three months after removal from the tanks. If the number of deaths be subtracted from the number exposed, the net percentage infected was 87. Excluding deaths, the percentage of rats infected was not related to the season of the year. Neither the percentage of rats infected nor the average worm-load per rat in each batch was related to the number of rats in that batch, but this

may have been influenced by the fact that the number of rats in each batch was regulated by appraisal of the number of mites present in the tank which was to contain it. Where the percentage of infected rats in a batch was lower than 80, the average number of female worms per rat was four or less. Higher average counts of worms were not invariably associated with infection of all the rats in a batch, however. Most batches of rats showed averages of five to six female worms per rat.

The earliest appearances of microfilariae in the blood occurred between fifty-six to fifty-nine days after the rats had been *first* put into the infection tanks. The longest period for which an infected rat failed to show microfilariae was sixty-four days, *after* its removal from the tank. Allowance having been made for the spread of fourteen days during which the rats were exposed to infection, it would appear that an interval of fifty-six to sixty-four days must elapse between the day a rat is infected and the first day when microfilariae can be detected in its blood.

Spontaneous disappearance of microfilariae from the blood has been observed in only four rats, and it did not occur before 100 days of patent infection. In two of these rats, no microfilariae were found in the pleural exudate, although there were two and six female worms respectively ; in the other two, living microfilariae were abundant in the pleural exudate and there were three and eight female worms. No accurate figures have been obtained as to the number of male worms present in most of the rats examined ; where complete counts have been made the males have roughly equalled the females in number.

Dead *adult* worms have occasionally been found in otherwise normal rats with ageing infections, but except where very few worms were present, these formed only a small proportion of the total. On the other hand evidence of the spontaneous death of a small number of *immature* worms was found in most infected rats. Usually a small, pale-yellow mass 1–3 mm. in diameter was discovered free in the pleural cavity ; usually there was only one in each of the pleural cavities and a third in the mediastinum. When such a mass was squashed under a cover-slip and examined microscopically the remains of the cuticle of some very small worms were revealed. These are assumed to have been portions of *immature* worms which died in the first weeks after infection of the rat, possibly at the time of their fourth moult. Where the infection had been of short duration the mass was soft, being composed largely of phagocytes. In older infections there was a progressive hardening of this mass, so that in the oldest it was a smooth, spherical nodule with no recognizable contents.

Mortality of cotton rats.—The deaths among our rats were commoner in the cold than in the warm months of the year. Many of them were probably due to a virus infection. On one occasion transport of some rats by rail in cold weather precipitated an outbreak of latent infection. Many rats died after exposure to mites, and signs of an infection were found in most of them at post-mortem examination. In the acute form this intercurrent infection was characterized by general wasting, nasal discharge, diarrhoea, and loss of appetite. At autopsy the intestines were usually flaccid, almost empty of food but filled with mucus and frequently tinted with bile pigments. In some animals the intestinal wall was inflamed. The spleen was always extremely small, perhaps one-fifth of its normal size ; the liver was pale or patchy in colour, and presented a rough surface. At other times, what is thought to have been a milder form of this disease occurred sporadically. The gross pathological findings were similar to those of the acute form, but they were less pronounced, and death was usually attributable to pneumonia or left auricular thrombosis ; in neither was the causative organism found by histological or bacteriological examination. It has been possible to prevent the spread of this disease among rats stored in the laboratory by rigorous attention to cleanliness and by destruction of all insects, particularly houseflies, cockroaches, and bed-bugs. It has frequently

been noticed that the presence of houseflies in moderate numbers has been attended by an outbreak of this disease.

Pugnacity among the rats has also been the cause of many deaths. When males were present in a batch it was not unusual to find one or two of the rats badly mutilated. Trichobenzoar (hairball) frequently occurs in cotton rats fed on a synthetic diet (as noted by Howell *et al.*, 1948), and it is often fatal. When some of the rats in this laboratory died of it, the others were given greenstuff regularly and no further deaths from this cause were observed.

The only gross pathological change shown by infected cotton rats and attributable to the filarial infection was enlargement of the spleen. In those few rats, which harboured over a hundred female worms, the pleura was thickened so as to appear milky, and it was much rougher than in normal rats. Tumours attributable to the presence of the worms were never observed. A small amount of exudate was found in the pleural spaces of most infected rats. This fluid was usually opalescent, being a suspension of microfilariae and large spherical cells resembling the phagocytes which are occasionally found attached to worms.

The testing of chemical compounds.—The drugs used for this study were obtained from the following sources: Drs. J. Walker, D. F. Elliott, W. Ormerod, and H. King, of the National Institute for Medical Research (index numbers F, E, O and A and K); Prof. C. Browning, of Glasgow University (B); Dr. W. O. Kermack, of the Royal College of Physicians, Edinburgh (JEM); Dr. F. E. King, of the Dyson Perrins Laboratory, Oxford (FK); Dr. A. H. Cooke and Sir Ian Heilbron, of Imperial College, London (CH); Boots Pure Drug Co., Ltd., Burroughs Wellcome, Ltd., and May and Baker, Ltd.; Drs. H. Mauss and H. Schmidt, of I. G. Farbenindustrie, Germany (Ms and Sdt); Dr. A. D. Welch, of Western Reserve University, U.S.A.; and miscellaneous other sources.

The chronic toxicity of each drug was estimated by intraperitoneal injection into mice daily for 4 days. Substances which would not dissolve in water were administered as fine suspensions, agar being added to the suspension fluid if suspensions tended to settle out. The maximum tolerated dose was taken as being the lower of two doses, one twice the other, of which the upper killed but the lower did not. Usually only two mice were treated at each dose-level. When the maximum tolerated dose per kg. had been determined for mice, the drug was injected into infected cotton rats at 0.4 to 0.5 times this dose. Higher doses were occasionally given, but they frequently proved fatal. The dose was repeated daily for six days (see below). Drugs were made up in sterile physiological saline and given intraperitoneally through a patch of skin in the groin which had previously been cleaned with alcoholic iodine. Two rats were treated concurrently; as a rule, only rats which had been infected within the previous three months were used, since the results of tests in which older infections were employed were often anomalous. The intraperitoneal route of administration was chosen as being more convenient, less liable to leakage, and less harmful to the rat than intramuscular or subcutaneous injection, and more reliable than oral administration since insoluble substances might pass through the gut unabsorbed. Drugs were injected only once daily in order to economize labour and diminish trauma to the rat. In order to determine the effect of each drug upon the number of circulating microfilariae, samples of blood were taken from each rat immediately before and after treatment. Five to seven days after the end of treatment the rats were killed with coal-gas and dissected. The contents of the abdomen were quickly inspected to note any lesions caused by the drug. Then the thorax was opened and the worms were removed to a vessel of physiological saline, in which they were studied under a binocular dissecting microscope. If it was doubtful whether or not they were living they were warmed to 37° C. for fifteen minutes and then

exposed to a bright light: this treatment invariably set living worms in violent motion. Particular attention was paid to the numbers of living and dead female worms, since these proved more susceptible to drugs than the males did. The totals of living and dead adult female worms were recorded for each rat, to facilitate the objective assessment of a drug's action. Worms which had lost all structure beyond that of the cuticle were assumed to have died before the beginning of drug treatment. Finally a sample of the pleural exudate was examined for living microfilariae.

Apparently other workers in this field have not noticed that female *L. carinii* are markedly more susceptible to the known filaricides than males are. This observation is important for the study of the physiology of these worms and of the mode of action of the filaricides. It greatly simplified the evaluation of drugs by autopsy findings, since the death of all female worms formed a convenient objective end-point. Doses of active drugs much higher than those which killed all the female worms frequently failed to kill all the males; in fact the males showed a much wider range of response than the females did.

Selection of standard dose schedule.—Preliminary investigations were made on the influence of the arrangement of the doses of the therapeutic effect. In the testing of antimalarial compounds against *P. gallinaceum* the therapeutic effect of a given total amount of drug varies according to whether the drug is given as a large single dose or is divided into eight twice daily ones (Tonkin and Hawking, 1947). The drug chosen for testing the arrangement of antifilarial dose schedules was neostam, since it was readily available and it was one of the original antimonial compounds used by Culbertson and Rose (1944); but the choice proved unfortunate since the compound is apparently not a standardized product and a second batch (which was obtained in order to continue this study) differed from the original batch. Moreover the work was handicapped by the individual variation between animals and by intercurrent infections which made rats more susceptible to toxic effects. For these reasons the final results (which are given in Table I) are incomplete. The animals are grouped into three main groups (according to time of experiment and batch of compound) and the figures of one group are probably not comparable with those of the others. Inside the groups, the doses were chosen to show (1) the relation between size of dose and response and (2) the effect of subdividing the same total dose in different ways. The maximum tolerated intraperitoneal dose (repeated in six days) is probably 250–500 mg./kg. for batch 91155 and 160–250 mg./kg. for batch 6357. Given intraperitoneally to piebald rats for six days, 1,000 mg./kg. killed four out of four, 500 mg. killed three out of six, and 250 mg. killed none out of six. Taking first the figures for batch 6357, it will be seen that 80 mg./kg. daily (as a single dose or subdivided into two) sterilized about half the rats while 130–160 mg./kg. daily always sterilized. There was no difference whether neostam was given as a single daily dose or as two subdivided ones: 80 mg./kg. daily for 6+5 days, or 6+6 days, cured only two out of four rats, just as 80 mg./kg. daily for six days cured one out of two; 780 mg. total given during three days was as effective as 780 mg. spread over six days. Taking the figures for batch 91155, 390–520 mg./kg. given during 3–5 days were effective in four out of eight animals and 260 mg. given as a single dose was effective in one animal. In contrast 720–960 mg./kg. were not effective in any of three animals when spread over 13–20 days; prolongation of the period of treatment does not compensate for the low level of the individual doses. These figures are not conclusive, but they suggest that an almost maximal response of this drug is probably obtained by giving a dose once daily for six days, and that no significant advantage would be obtained by dosing twice daily, or for two weeks, procedures which greatly increase the amount of labour and material required. (With other drugs, the optimum dose-schedule might be different; although with cyanine dyes Peters, Welch, and Higashi

TABLE 1

THE EFFECT OF VARIOUS DOSE SCHEDULES OF NEOSTAM ON THE THERAPEUTIC EFFECT

Doses given intraperitoneally on 6 days in the week. Rats killed about 6 days after last dose. Each line represents one rat.

Total dose, mg./kg.	Total duration, days	Schedule	State of adult worms at post mortem	
			Females	Males
BATCH 6357				
480	6	40 twice daily for 6 days	Dead Most alive	2 alive Most alive
480	6	80 once daily for 6 days	Dead (3 worms) Alive, sluggish (2 worms)	— Alive (2)
960	6	80 twice daily for 6 days	Dead Dead Dead Dead	Dead Dead Dead Dead
960	6	160 once daily for 6 days	Dead Dead	Dead Dead
960	13	40 twice daily for 6+6 days	Alive, sluggish (4 worms) All dead	— Alive (6)
880	12	80 once daily for 6+5 days	All but one dead (many) All dead (many)	Alive All dead
1,430	12	130 once daily for 6+5 days	All dead All dead	All dead Many alive
780	6	65 twice daily for 6 days	Dead Dead	Dead Dead
780	6	130 daily for 6 days	Dead Dead Dead	Dead Dead Dead
780	6	130 daily for 6 days—killed after 3 days	Dead Alive (sluggish)	Dead Alive (sluggish)
780	3	260 daily for 3 successive days	Dead Dead	All but one dead All but one dead
780	5	260 daily for 3 alternate days	Dead Dead	Dead All but one dead
BATCH 91155				
720	20	40 daily 6+6 +6 day	Alive Alive	Alive Alive
960	13	80 daily 6+6	Alive	Alive
390	3	130 daily for 3 successive days	Alive Alive	Alive Alive
390	5	130 daily for 3 alternate days	Dead (many) Alive (many)	One alive, many dead Alive
480	3	160 daily for 3 successive days	Dead (1 worm) Dead (1 worm)	— —
260	1	260 one day	Dead	Dead
520	3	260 twice at 3 day intervals	Alive (many) Dead	Alive (many) Dead
1,500	6	250 daily for 6 days	Dead	Dead
1,500	3	500 daily for 3 days	Alive, sluggish	Alive
240	6	40 daily for 6 days	Most alive (40 worms) Most alive (10 worms)	
480	6	80 daily for 6 days	Most alive (40 worms) Most alive (20 worms)	
900	6	150 daily for 6 days	Dead	All but one dead
1,560	13	130 daily for 6 +6 days	Dead	All dead

(1949) have obtained similar results to our own, viz., that variation of the arrangement of the dose-schedule (within limits) did not greatly affect the therapeutic response and that once daily dosage (for five days) probably yielded optimal results.) The dose schedule adopted for routine use was one dose daily for six days. (The "Therapeutic Index" (see below) for batch 6357 is reckoned as 2, and that of batch 91155 as 2–3).

Two rats (not shown in Table I) were treated with 130 mg. neostam per kg. once daily for six days, and 6+6 days respectively, and watched for three months. The microfilaria count gradually diminished as described by Culbertson and Rose (1944). One rat was killed 13 weeks after treatment, when the microfilarial count of the blood had sunk from 2,900 per 10 cu.mm. to 90 per 10 cu.mm. All the adult worms in the pleural cavity were dead, but a few live microfilariae were still present.

Results

The results of testing a long series of compounds are shown in Table II. The maximum tolerated dose for mice is the maximum dose which failed to kill mice when injected intraperitoneally on four successive days; see above. It is assumed that the maximum tolerated dose for cotton rats was 0.4 of this dose. The number of times the dose was repeated (at daily intervals) is shown by the multiplication sign, e.g., $\times 6$. The letter "D" in the third column indicates that the rat was found dead during treatment, presumably from toxic effects; the results from such rats are unreliable. The letter "K" indicates that the rat was killed, e.g., by trauma, and a post mortem was made soon after death; such results are more significant. "I" indicates that the compound was very irritant to the peritoneum. Therapeutic activity was judged mainly by the proportion of female worms killed. The "Therapeutic Index" was calculated as the ratio of the maximum tolerated dose for cotton rats (derived from the figures for mice) to the minimum dose which killed most (more than half) of the female worms; this index is therefore only a rough approximation.

Discussion of Results

The use of a standardized laboratory infection for chemotherapeutic trials in filariasis is less subject to errors of interpretation than is the use of naturally infected animals, as generally practised in America. In the present work results have been obtained with laboratory-infected rats in which the duration of infection was known. Consequently, it has been unnecessary to perform extensive trials with large numbers of rats for each drug. Two rats per dose-level have usually provided enough evidence to permit a decision as to whether or not a particular compound was active. Where activity has been found, more rats have been expended in order to establish the degree of therapeutic efficiency. The results obtained with active compounds have been mainly consistent, and the numerical conclusions are sound within the limits of accuracy generally reached in biological screening.

In all, 220 compounds have been tested against experimental cotton rat filariasis (Table II). The compounds fall into the following broad groups; antimonials (16), arsenicals (12), arsenic-antimonials (2), amidines, *iso*thioureas, and amidoxines (27), diguanides (14), sulphones (3), sulphonamides (8), hydroxylamine derivatives (4), hydroxamic acids (4), pyridines (4), substances related to hetrazan (8), pyrimidines (4), quinolines, styrylquinolines, and anilquinolines (31), *iso*quinolines (3), cyanines and

TABLE II

THE EFFECT OF VARIOUS COMPOUNDS ON ADULT WORMS IN COTTON RAT FILARIASIS

The names of active compounds are printed in **bold type**; those asterisked have not been used previously against any of the filarial diseases. † Indicates that besides the female worms, for which figures are given, all the male worms as well were dead. "D" in third column indicates that the rat was found dead during treatment; "K" that it was killed, e.g., by trauma, and a post mortem made soon afterwards. "I" indicates that the compound was very irritant to the peritoneum.

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
ANTIMONIALS				
Na 4-acetylamino-2-methylphenylstibonate,* Sdt 626 (500 mg.)	100 × 6	1 D	2/2	
	100 × 6	1	7/7	
	50 × 6	2 D	0/2	
	50 × 6	2	0/9	
	20 × 6	2	1/25	1–2
Na 2-methoxy-4-sulphonamidophenylstibonate,* Sdt 702 (1,000 mg.)	200 × 6	2	All/many	
	200 × 6	1	0/6	
	100 × 6	2	0/14	1–2
Neostibosan, *p*-aminophenylantimonyldiethylamine (750 mg.)	200 × 6	1	10/10†	
	100 × 6	2	All/many	
	100 × 6	2	0/2	
	50 × 6	2	1/38	2–3
Stibosan, Na *m*-chlor-*p*-acetylaminophenylstibonate (100 mg.)	50 × 6	2	40/40	
	20 × 6	3	0/many	1
	10 × 6	2	2/5	
Solustibosan, Na diethylaminoethyl antimony gluconate (2,500 mg.)	500 × 6	2	9/9†	
	200 × 6	4	2/11	
	100 × 6	2	0/8	2
Pentostam, Na stibogluconate (3,750 mg.) ..	1,500 × 6	3 D		
	1,500 × 6	5	All/many†	
	500 × 6	2	All/many	
	200 × 6	2	6/6	
	200 × 6	1	10/20	5–7–15
	200 × 6	2	0/7	
Methylglucamine stibonate* (about 10 g.) ..	1,000 × 6	1	All/many†	
	500 × 6	1	5/5†	
	200 × 6	1	0/8	
	100 × 6	1	1/2	
	50 × 6	1	0/5	
Only limited supplies available	20 × 6	1	0/7	?10
Tartar emetic, K antimony tartrate (10 mg.) ..	20 × 3	1 K	0/20	
	20 × 4	1 K	0/50	
	20 × 6	1	10/20	
	10 × 6	2	5/70	0–1
Anthiomaline, Li antimony thiomalate (100 mg.)	50 × 6	1	All/few†	
	40 × 6	1	All/few	
	20 × 6	2	0/6	1
Fuadin, Na antimony 2-pyrocatechol-3: 5-sodium disulphonate (250 mg.)	50 × 6	2	All/many	
	50 × 6	1	0/5	
	20 × 6	2	8/8†	
	10 × 6	1	0/5	
	10 × 4	1 D	0/4	5

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Na K antimony saccharate,* Sdt 187 (75 mg.) ..	20 × 6 10 × 6 5 × 6	2 2 1	25/25 1/7 0/5	1–2
4-Acetylaminophenyl antimony dipyrocatechinate, tetrasodiumdisulphite,* Sdt 411 (250 mg.)	50 × 6 20 × 6 10 × 6	2 2 2	9/9 11/14 0/7	5
3: 4-Diacetylaminophenyl antimony dipyrocatechinate, tetrasodiumdisulphite,* Sdt 884 (500 mg.)	100 × 6 100 × 6 50 × 6	2 1 2	All/many 0/1 0/4	2
4-Sulphonamidophenyl antimony dipyrocatechinate tetra-sodiumdisulphite,* Sdt 703 (750 mg.)	200 × 6 100 × 6	2 3	8/9 0/many	2
4-Carbamino-3-aminophenyl antimony dipyrocatechinate tetra-sodiumdisulphite,* Sdt 397 (1,750 mg.)	200 × 6 100 × 6 50 × 6	2 2 2	10/10† 11/11† 0/9	7
4-Carbamino-3-(bisdioxypropyl)aminophenyl antimony dipyrocatechinate tetra-sodiumdisulphite,* Sdt 779 (2,500 mg.) Only limited supplies available	100 × 6 50 × 6	1 1	2/2† 6/6	?>20
ARSENICALS				
Na N^1-*p*-arsenophenyl-N^5-*iso*propyl-biguanide (500 mg.)	200 × 6	2	0/many	0
Na N^1-*p*-arsenophenyl-N^5-*p*-chlorophenyl-biguanide (100 mg.)	50 × 6	2	0/many	0
Diphenylarsonic acid, F42 (5 mg.)	2 × 6	2	0/many	0
Butylaminopropylarsonic acid, HCl, F51 (100 mg.)	50 × 1 50 × 2 20 × 1 20 × 2 10 × 2 5 × 4 2 × 6	1 K 1 K 1 K 1 K 1 K 1 K 2	0/3 0/1 0/3 0/2 0/4 0/2 0/9	0
β-Phenylethylaminopropylarsonic acid HCl, F54 (25 mg.)	10 × 2 5 × 4 2 × 5 2 × 6	2 K 2 K 2 K 2	0/7 0/3 0/3 0/6	0
4′-Aminobenzenesulphonyl-4-aminophenylarsinic acid* (1,000 mg.)	500 × 6 500 × 6 400 × 6 200 × 6 100 × 6	1 1 1 1 1	Half/many 8/8 0/many 0/many 0/2	1
Tryparsamide, Na *N*-phenylglycinamide *p*-arsonate (2,500 mg.)	1,000 × 6 1,000 × 6 500 × 6 500 × 6 250 × 0	1 1 K 1 1 K 2	All/many† All/many† All/many Most/many 0/many	1–2
Reduced tryparsamide dithioglycollate, Na *N*-phenylglycinamide-*p*-arsinic dithioglycollate (50 mg.)	30 × 6 10 × 4 10 × 6 6 × 6 5 × 6 3 × 6 3 × 6	2 1 D 1 1 1 1 1	All/many† 5/5 20/20† 20/100 All/many 0/many 4/4	6

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Di(β-carboxy-β-aminoethyl)-4-benzamidethio-arsinite,* K324 (100 mg.)	20 × 5	1 K	Most/many	
	20 × 6	2	6/6†	
	10 × 5	1 D	0/many	
	10 × 6	2	12/12†	
	5 × 6	3	14/14	
	5 × 6	1	2/8	
	2 × 6	4	0/43	
	1 × 6	4	0/23	8
Arsenophenylglycine (200 mg.)	100 × 1	1 K	0/many	
	80 × 6	1	0/many	0
Mapharsen, 3-amino-4-hydroxyphenylarsenious oxide hemialcoholate, HCl (25 mg.)	15 × 3	1 K	0/many	
	10 × 2	1 K	All/many	
	5 × 6	2	0/4	0
Neoarsphenamine, 3: 3′-diamino-4: 4′-dihydroxy-arsenobenzene-*N*-methylene sulphoxylate (200 mg.)	100 × 5	1 K	All/many	
	100 × 6	1	All/many†	
	20 × 6	6	39/41	
	10 × 6	2	20/20	
	10 × 6	2	0/24	
	5 × 6	2	1/16	4
COMPOUNDS OF ARSENIC AND ANTIMONY				
A complex compound containing three atoms of arsenic and two of antimony per molecule, Sdt 386b (100 mg.)	20 × 6	2	All/many	
	10 × 4	1 D	0/4	
	10 × 6	1	0/5	
	10 × 6	1	2/2	
	5 × 6	2	0/15	2
3: 3′-Sodiumdisulphite methylamino-4′-carb-amino-diphenyl arsenostibonate, Sdt 544 (500 mg.)	100 × 2	1 K	All/few†	
	100 × 6	1	All/many†	
	50 × 6	1	1/1	
	20 × 6	2	10/20	5–10
AMIDINES, ISOTHIOUREAS, AND AMIDOXINES				
4: 4′-Bis-dimethylaminostilbene (500 mg.) ..	200 × 1 × 3	2 K	0/22	
	100 × 6	1 K	0/19	
		1	0/17	
	50 × 6	2	0/7	0
4-Nitro-4′-amidinostilbene, HCl (50 mg.) ..	20 × 4	1 K	2/2	
	20 × 6	2	0/many	0
4-Amino-4′-amidinostilbene, di-HCl (50 mg.) ..	20 × 2	1 K	†	
	20 × 6	2 (1 K)	All/many	
	10 × 2	1 K	0/8	
	10 × 6	2	5/5	
	10 × 6	3	0/10	0–1
4:4′-Diamidinodiphenylamine, di-HCl (25 mg.)	10 × 2	1 K	0/1	
	5 × 3	1 K	All/few	
	5 × 6	1	Half/many	
	2 × 6	2	1/14	0–1
4:4′-Diamidino diphenoxyethane, di-HCl (50 mg.)	20 × 6	2	0/many	0
p-Bromobenzamidine, HCl, F53 (100 mg.) ..	100 × 6	1	0/2	
	70 × 6	1	0/3	
	50 × 6	6	2/16	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
3:5-Diiodo-4-hydroxybenzamidine, HCl (250 mg.)	100 × 6	3	1/87	0
p-Tolamidine, HCl, F5 (50 mg.)	50 × 6 20 × 6	1 2	0/many 0/many	0
p-Ethoxybenzamidine, HCl, F14 (100 mg.) ..	100 × 6 50 × 6	1 2	0/1 0/15	0
Phenamidine di-isethionate (75 mg.)	30 × 4 20 × 3 20 × 6 15 × 6	1 1 1 1	All/many 0/many 0/many 0/2	?0
4-β-Hydroxyethoxy benzamidinium chloride, RD408 (500 mg.)	550 × 3 250 × 3 200 × 6	1 K 2 K 1	1/1 0/3-many 0/many	0
4-Aminobenzamidine di-HCl (250 mg.) ..	100 × 6	2	0/many	0
3-Amino-4-hydroxybenzamidinium sulphate, RD353	1,000 × 4 100 × 6	1 K 1	0/2 0/many	0
Tetrahydrogeranamidine HCl, F9 (50 mg.) ..	20 × 6	2	0/many	0
p-2′:5′-Dihydroxybenzenesulphonylbenzamidine, HCl, F50 (500 mg.)	200 × 6 100 × 6	2 2	0/6 0/8	0
4:4′-Diamidinodiphenylsulphone di-HCl, RD421 (100 mg.)	100 × 1 50 × 6	1 K 1	0/many 0/1	0
p-Methylsulphonylbenzamide, HCl, F6 (1,250 mg.)	1,000 × 1 400 × 6 200 × 6	1 K 1 1	0/6 0/few-many 0/many	0
p-Sulphonamidobenzamidine, HCl, F1 (1,000 mg.)	500 × 6 200 × 6	3 1	0/few-many 0/2	0
N-(β-Diethylaminoethyl)benzamidine, RD270 (100 mg.)	500 × 1 100 × 2 100 × 2 50 × 6	1 K 1 K 1 K 2	0/3 0/few Most/many 0/many	0
N-(β-Diethylaminoethyl)-*p*-methylsulphonylbenzamidinium, RD274 (250 mg.)	500 × 2 200 × 6	1 K 1	1/20 0/2	0
2-(4′-Methylsulphonylphenyl)dihydroglyoxalinium chloride, RD302 (100 mg.)	50 × 6 50 × 6 20 × 6	1 1 1	Half/many 0/2 0/many	?0
2-(4′-Aminobenzyl) dihydroglyoxaline monotoluene-*p*-sulphonate, RD304 (100 mg.)	100 × 6 50 × 6	1 1	0/many 0/4	0
S-Methylthiuronium sulphate, F13 (250 mg.) ..	100 × 6	2	0/15	0
p-Anisylthiourea methiodide, F43 (100 mg.) ..	50 × 6	4	0/15	0
S-Methyl-*N*-*p*-sulphonamidophenylthiuronium iodide, F15 (500 mg.)	200 × 6	2	0/many	0
S-2:4-Dichlorophenoxymethyl-thiuronium chloride (50 mg.)	20 × 6	2	0/22	0
p-Sulphonamidobenzamidoxine HCl, F2 (1,500 mg.)	500 × 6 400 × 6	1 1 K	0/many 0/many	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
		DIGUANIDES		
p-Hydroxyphenyldiguanide HCl, F19 (1,000 mg.)	500 × 3 500 × 6	1 K 1	All/many 0/4	 0
p-Chlorophenyldiguanide, HCl, F3 (150 mg.) ..	100 × 6 50 × 6	1 2	0/5 0/many	 0
m-Chlorophenyldiguanide, HCl, F24 (50 mg.) ..	20 × 6	2	0/4	0
p-Bromophenyldiguanide, HCl, F31 (100 mg.) ..	50 × 4 50 × 6 50 × 6	1 K 1 2	0/2 0/many 1/2	 0
N^1-Methyl-N^1-*p*-tolyldiguanide, HCl, F25 (100 mg.)	100 × 6	3	1/many	0
N^1-Methyl-N^1-*p*-anisyldiguanide, HCl, F22 (100 mg.)	50 × 6	2	0/many	0
o-Xylidine-diguanide, HCl, F21 (100 mg.) ..	50 × 6	2	0/many	0
p-Phenetyl-diguanide, HCl, F4 (30 mg.) ..	10 × 2 5 × 6	1 K 2	0/many 0/20	 0
N^1-Ethyl-N^1-*p*-anisyl-diguanide, HCl, F23 (100 mg.)	50 × 6	2	0/many	0
α-Naphthyl-diguanide, HCl, F16 (100 mg.) ..	50 × 4 50 × 6	1 K 2	4/4 0/many	 0
β-Naphthyldiguanide, HCl, F17 (100 mg.) ..	50 × 1 20 × 6	2 K 2	0/few 0/many	 0
Piperonyldiguanide, HCl, F26 (250 mg.) ..	100 × 6	2	0/many	0
Proguanil (paludrine) (25 mg.)	10 × 6	1 1 1	2/30 10/20 19/20	 ?1
N^1-*p*-Chlorophenyl-N^5-methyl*iso*propyl-diguanide acetate, F18 (25 mg.)	10 × 6	2	1/10	0
		SULPHONES		
p-Aminomethylphenyl methylsulphone HCl, F7 (2,500 mg.)	1,000 × 4 800 × 6 200 × 6	1 1 2	0/many 0/many 0/many	 0
p-Aminomethylphenyl ethylsulphone, HCl, F55 (1,000 mg.)	500 × 6	2	0/4	0
2-Chloro-4:4′-diaminodiphenyl sulphone (Insoluble) ..	3,000 × 1 1,900 × 1 s.c.	1 1	0/2 0/few	0
		SULPHONAMIDES		
Sulphaquinoxaline .. .. (Insoluble)	100 × 1 s.c. 100 × 5 Oral	1 2	0/3 0/7	 0
Marfanil, 4-aminomethylbenzene sulphonamide, HCl (>2,000 mg.)	800 × 6	1	0/5	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
8-*p*-Aminobenzene sulphonamido-6-methoxyquinoline (Insoluble)	200 × 1 20 × 1 s.c.	2 2	0/5 0/9	0
1-*p*-Aminobenzene sulphonamidonaphthaline-5-sulphonate (1,000 mg.)	500 × 6	2	0/many	0
3′:5′-Dibromo sulphanilamide	670 × 6	2	0/9	0
Disulphanilamide (1,000 mg.) .. .. .. (Insoluble)	1,000 × 1 s.c.	1	0/6	0
2-*p*-Aminobenzene sulphon(β-diethylaminoethyl)-amide pyridine	5,000 × 10	2	0/many	0
2-Aminopyridine-5-*p*-aminosulphonaniline .. (Insoluble)	2,000 × 1 s.c.	4	1/17	0
DERIVATIVES OF HYDROXYLAMINE				
O-Hexylhydroxylamine, HBr, F44	150 × 6	2	0/3	0
O-Hexylhydroxyguanidine, HNO_3, F45 (10 mg.)	5 × 6	2	0/7	0
Hexane-di-*O*:*O*′-hydroxylamine di-HCl, F47 (250 mg.)	100 × 6	4	1/10	0
1:10-*O*:*O*′Decane hydroxyguanidine di-HNO_3, F46 (50 mg.)	20 × 2 20 × 6	1 D 4	0/5 0/30	0
HYDROXAMIC ACIDS				
Benzhydroxamic acid, F36 (250 mg.)	200 × 6 100 × 6 100 × 6	2 3 1	0/13 0/35 4/5	0
p-Aminobenzhydroxamic acid, F35 (500 mg.) ..	200 × 3 100 × 6	1 K 1	0/3 0/1	0
1:2-Dioxindole, F33 (500 mg.)	200 × 6	1	0/1	0
4-Hydroxy-2-methyl quinazoline-3-oxide, F34	750 × 4	1 K	0/many	0?
PYRIDINES				
α-Aminopyridine sulphate, F52 (50 mg.) ..	20 × 6	2	0/5	0
2-Butoxy-5-aminopyridine di-HCl, F5 (250 mg.)	100 × 6	2	0/many	0
Pyridium. Phenylazo-α,α′-diaminopyridine, HCl, F41 (50 mg.)	20 × 6	2	0/8	0
2:4-Dichlorophenoxymethylpyridinium chloride (100 mg.)	50 × 6	2	0/27	0
PIPERAZINES, MORPHOLINES, AND OTHER SUBSTANCES RELATED TO HETRAZAN				
N-Carbamidomorpholine, F28	2,000 × 6	2	1/many	0
Methyl morpholine, HCl 0/59	200 × 6 50 × 6	1 1	0/1 0/8	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Morpholinomorpholine-*N*-dithiocarboxylate, CH1 (250 mg.)	100 × 6	2	0/13	0
Morpholino-*N*-glycylmorpholine dithiocarboxylate, CH6 (250 mg.)	100 × 6	2	0/many	0
Benzylacetonylpiperidinium bromide, O23 (50 mg.)	30 × 6 20 × 6	2 2	0/17 0/4	0
N:*N*′-Dimethyl-*N*:*N*′-bis-(diethylcarbamyl) ethylene diamine (250 mg.)	100 × 6	2	0/40	0
The above six compounds were not active against microfilariae in vivo				
N:*N*:*N*′-Trimethyl-*N*′-diethylcarbamyl-ethylene diamine (250 mg.)	100 × 6	4	0/49	0
This compound produces a small reduction in the microfilaria count at max. tolerated dose				
4-Dimethylamino-4′-methyldiethylcarbamyl-amidodiphenoxypropane (500 mg.)	200 × 6 100 × 6	2 K 1 2	0/12 0/31 0/14	 0

PYRIMIDINES

2:4:5-Triamino-6-hydroxypyrimidine bisulphite, F30 (250 mg.)	100 × 6	2	0/many	
2′-*p*-Sulphonamidophenyl-4′:6′-dimethylpyrimidine, F49	1,000 × 3 850 × 5	1 K 1	0/2 0/3	0
Sulphadiazine, 2-sulphanilamide pyrimidine (250 mg.)	100 × 6	1	0/many	0
2′-*p*-Guanylphenylsulphonamido-4′:5′-dimethylpyrimidine, F48 (250 mg.)	150 × 6 100 × 6	2 4	0/5 2/33	0

QUINOLINES (various); STYRYLQUINOLINES; ANILQUINOLINES

4:7-Dichloro-3-methylquinoline (1,000 mg.) ..	500 × 6 200 × 6 50 × 6	1 D 1 2 2	0/3 0/20 0/17 0/26	 0
4:8-Dichloro-3-methylquinoline (about 1,000 mg.)	500 × 6	2	0/10	0
4:7-Dichloro-3:8-dimethylquinoline (about 1,000 mg.)	500 × 6	1 K 2	 0/12	 0
6-Methoxy-4-δ-diethylamino-α-methylbutylaminoquinaldine di-HBr, F12 (50 mg.)	20 × 6	2	0/many	0
Na 6-methoxyquinaldine-4-sulphonate, F11 (2,500 mg.)	1,000 × 6	2	0/many	0
2-*p*-Aminophenylquinoline, HCl, K515 (10 mg.)	5 × 6 1 × 6	2 2	0/14 0/5	 0
m-Aminophenylquinoline methochloride, HCl, K516 (10 mg.)	5 × 6	2	0/13	0
4-Quinolylalanine di-HCl, E28 (350 mg.) ..	150 × 6	2	0/many	0
6-Methoxy-4-quinolyl alanine di-HCl, E44 (350 mg.)	150 × 6	1	0/many	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Surfen C, bis-2-methyl-4-amino-quinolyl-6-melamine (50 mg.)	20 × 2	1 D	0/4	
	20 × 6	1	0/3	
	10 × 6	1	0/1	0
Sontoquin (150 mg.)	20 × 6	1	0/75	0
Pamaquin (50 mg.)	10 × 4	1 D	0/many	
	10	1	0/50	0
Bis-(2-anilino-4-quinolylamino-ethyl)-methyl-amine, 3HCl, A290 (25 mg.)	10 × 6	2	0/14	0
2(*p*-Aminostyryl)-β-naphthoquinoline methochloride,* B209a (10 mg.)	10 × 1	1 K	All/many†	
	5 × 6	1 I	All/many†	
	4 × 6	1 I	1/4	1
2(*p*-Dimethylaminostyryl)-β-naphthoquinoline dimethochloride, B325 (5 mg.)	4 × 6	1	0/many	
	2 × 6	1	0/2	0
2(*p*-Acetylaminostyryl)-10-β-naphthoquinoline methochloride, B207	50 × 2	1 K	Half/many	
	50 × 3	1 K	0/many	
	20 × 6	1	0/many	0
2(*p*-Acetylaminostyryl)-β-4-aminonaphthoquinoline methoacetate, B230(i)	100 × 1	1 K	0/2	
	100 × 4	1 K	0/many	
	80 × 2	1 K	0/many	
	30 × 6	1	0/many	0
2(*p*-Aminostyryl)-6-acetylaminoquinoline methochloride, B147 (100 mg.)	100 × 6	1 K	All/many	
	40 × 6	1	0/many	0
2(*p*-Dimethylaminostyryl)-6-aminoquinoline methiodide,* B62c (10 mg.)	20 × 1	1	All/many	
	10 × 1	1	All/many	
	4 × 6	1	5/7	
	4 × 6	1	All/many†	
	2 × 6	1	0/6	
	2 × 6	1	0/many	
	1 × 6	1	0/many	1
2(*p*-Dimethylaminostyryl)-6-aminoquinoline dimethiodide, B326 (10 mg.)	10 × 6	1	0/many	
	4 × 6	1	0/many	0
2(*p*-Dimethylaminostyryl)-4-carboxamido quinoline methiodide, B393 (100 mg.)	80 × 6	1	0/many	
	40 × 6	1	0/many	
	40 × 6	1	6/6	?0
2(*p*-Dimethylaminostyryl)-quinoline 4-carboxymethylamide methochloride,* B408 (100 mg.)	50 × 6	1	2/2	1
2(*p*-Dimethylaminostyryl)-4-dimethylaminoquinoline methochloride,* B440 (5 mg.)	4 × 6	1 I	All/many†	
	2 × 6	1	All/many†	
	1 × 6	1	All/many†	
	1 × 6	1	Half/many	
	0.5 × 6	2	1/10	4
2(*p*-Dimethylaminostyryl)-4-acetylaminoquinoline methochloride, B431 (50 mg.)	20 × 6	1 K	0/many	
	20 × 6	3	All/many†	
	10 × 6	1	All/many†	
	10 × 3	1 K	0/4	
	5 × 6	1 K	All/many†	
	5 × 6	2	All/many†	
	2 × 6	2	5/5†	
	1 × 6	1	2/6	
	1 × 6	1	0/many	
	0.5 × 6	1	2/13	
	0.5 × 6	1	0/many	10

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
2(*p*-Diethylaminostyryl)-4-dimethylaminoquinoline methochloride,* B441 (2.5 mg.)	1 × 6	1	0/4	
	1 × 6	3	All/many†	
	0.5 × 6	2	2/many	
	0.5 × 6	2	0/many	
	0.2 × 6	1	5/many	
	0.1 × 6	1	0/1	1
2(*p*-Acetylaminostyryl)-6-aminoquinoline methochloride, B154(i) (35 mg.)	30 × 6	1 I	0/many	
	15 × 6	1	0/3	0
2-*p*-Acetaminostyryl-6-methylaminoquinoline methosulphate,* B314 (50 mg.)	20 × 6	1	20/30	
	20 × 12	1	80/100	1
2(-*p*-Dimethylaminoanil)-6-methylquinoline methochloride, B48 (10 mg.)	5 × 6	1 K	0/many	
	5 × 6	1	0/many	0
2(*p*-Dimethylaminoanil)-6-*N*-caproylaminoquinoline methochloride,* B68 (100 mg.)	50 × 3	1 K	6/6†	
	50 × 6	1 K	0/1	
	20 × 6	1 I	4/8	
	20 × 6	1	9/10	
	10 × 6	1 K	0/few	
	10 × 6	1	0/many	1–2
2(*p*-Dimethylaminoanil)-4-acetylaminoquinoline methochloride, B405a (50 mg.)	20 × 6	2	0/many	0
2(*p*-Dimethylaminoanil)-4-carboxyamido quinoline methochloride, B395 (100 mg.)	50 × 6	2	0/3	0
ISOQUINOLINES				
Emetine, HCl (10 mg.)	4 × 6	1	0/many	0
3-Chloro-1-(4′-diethylamino-1′-methylbutyl)-amino*iso*quinoline (25 mg.)	10 × 6	2 D	0/5	
	10 × 6	2	0/10	0
5-Amino-1-(4′-diethylamino-1′-methylbutyl-amino*iso*quinoline, HCl (30 mg.)	50 × 6	3	6/many	
	20 × 6	1	0/1	0–1
CYANINE				
1′-Ethyl-3:6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine, chem. ctr. 863, Kodak 81 (2.5 mg.)	2 × 6	1	All/many†	
	1 × 6	1	All/many†	
	0.5 × 6	1	All/many†	
	0.2 × 6	1	90/100	
	0.2 × 6	1	All/many	
	0.2 × 6	2	6/6	
	0.1 × 6	2	6/many	
	0.1 × 6	4	All/many†	
	0.1 × 12‡	3	All/many†	
	0.1 × 12‡	1	10/12	
	0.05 × 6	3	0/15	20
PHENANTHRIDINIUM SALTS				
7-Amino-9-*p*-aminophenyl-10-methyl phenanthridinium Cl,* K506 (50 mg.)	20 × 6	2	13/15†	
	10 × 6	3	60/63	
	5 × 6	2	0/14	2
Dimidium Br, 1553, 2:7-diamino-9-phenylphenanthridine 10-methobromide* (10 mg.)	40 × 3	1 D	5/15	
	20 × 6	1 D	2/2	
	10 × 6	1	7/8	
	5 × 6	3	3/many	1

‡ Given twice daily for 6 days.

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
		ACRIDINES		
Acriflavine (10 mg.)	4 × 6	1	0/60	0
Rivanol, F37 (10 mg.)	5 × 6	2	0/7	0
Mepacrine	40 × 4 20 × 6	1 D 1	0/many 0/75	0
5-Heptylaminoacridine, HCl, H_2O, F387 ..	50 × 1 50 × 4 20 × 6	1 D 1 D 2	3/3† 0/2 0/20	0
		BENZTHIAZOLES		
2(*p*-Dimethylaminoanil) benzthiazole methochloride, B252a (25 mg.)	10 × 2 10 × 6 10 × 6 10 × 6	1 D 1 K 1 1	6/12 0/1 3/7 0/many	0–1
2(*p*-Dimethylaminoanil) acetylaminobenzthiazole methosulphate, B329 (250 mg.)	100 × 6 50 × 6 50 × 6	2 K 1 1	3/many Quarter/many 0/6	0–1
5-Acetamido-3-methyl-2-styrylbenzthiazolium Cl, JHM41 (100 mg.)	50 × 6	2	0/12	0
2(*p*-Aminostyryl)-6-acetyllactylaminobenzthiazole methochloride, B398 (100 mg.)	80 × 6 40 × 6 20 × 6 20 × 6 10 × 6	1 1 1 1 1	All/many† All/many† All/many Half/many 1/many	4
6-Nitro-2-dimethylaminostyrylbenzthiazole ..	1,500 × 6	2	0/12	0
5-Acetamido-2-(*p*-dimethylaminostyryl)-3-benzthiazolium Cl, JEM40 (10 mg.)	5 × 6 5 × 6	1 3	6/8 0/many	0
2(*p*-Acetylaminostyryl) aminobenzthiazole methochloride, B368 (100 mg.)	100 × 1 40 × 6	1 K 1	0/many 0/many	0
2(*p*-Acetyllactylaminostyryl)-6-aminobenzthiazole methochloride, B392 (30 mg.)	20 × 6 20 × 6	1 1	Half/many 0/6	0–1
2:5-Diaminobenzthiazole, F56 (500 mg.) ..	200 × 4 200 × 6 50 × 6	1 K 1 2	0/4 0/2 0/13	0
5-Acetamido-2:3-dimethylbenzthiazolium Cl, JEM39 (250 mg.)	100 × 6 100 × 6	2 2	3/16 0/8	0
3-Methyl-6-acetamido-2-*p*-dimethylaminostyrylbenzthiazolium I, BD49	100 × 6 50 × 6	2 D 1 1 2	7/20 4/4† 2/8 0/33	0–1
2-Methylbenzthiazole, HCl, BD/A	50 × 6	1 D 3 (1 D)	31/31† 0/23	0
2-Methyl-6-nitrobenzthiazole, BD/B	500 × 6	2	0/21	0
2:3-Dimethylbenzthiazolium Cl, BD38	50 × 6	2	0/14	0
2:3-Dimethyl-6-nitrobenzthiazolium Cl, BD39..	100 × 6	2	0/9	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
2-Methyl-5-diethylaminoethylaminobenzthiazole di-HCl, A135 (200 mg.)	80 × 6	2	0/many	0
2-*p*-Aminobenzene sulphonamido-6-ethoxybenzthiazole (Scarcely soluble)	100 × 1‡ 100 × 6	1 2	0/9 0/5	0
BENZIMINAZOLES				
2-Methyl-5-methoxy-6-chlorobenziminazole, HCl, TJ2b (50 mg.)	20 × 6	1	0/75	0
2-Carboxyethyl-5-chlorobenziminazole, HCl, TJ9 (250 mg.)	100 × 6	1	0/50	0
2-Hydroxymethyl-5-chlorobenziminazole, HCl, TJ10 (250 mg.)	100 × 6	1	0/75	0
1-Methyl-2-diethylamino-*n*-propyl-5:6-dichlorobenziminazole, FK56 (100 mg.)	50 × 6	2	0/4	0
2-Guanidyl-5-chlorobenziminazole di-HCl, F20 (50 mg.)	40 × 3 20 × 6	1 K 3	0/few 0/6	0
2-Guanidyl-5-methoxybenziminazole di-HCl, FK42 (125 mg.)	50 × 2 50 × 4	1 K 1 K	0/many 0/many	0
BENZOXAZOLE				
2(*p*-Dimethylaminostyryl)benzoxazole methochloride, B270 (10 mg.)	10 × 1 4 × 6	1 K 1 I	0/many 0/3	0
TETRAZOLES				
2:3:5-Triphenyltetrazolium Br (5 mg.) ..	2 × 6	2	0/many	0
2:5-Diphenyl-3-*p*-tolyltetrazolium Br (5 mg.) ..	2 × 5 2 × 6	1 K 1	0/many 0/many	0 0
THIAZOLINES				
2-Mercapto-4-*p*-hydroxybenzyl-5-ketothiazoline, CH7	500 × 1 200 × 6 100 × 6	1 K 1 1	0/many 3/7 0/many	0
2-Mercapto-4-*p*-methoxybenzyl-5-ketothiazoline, CH4 (100 mg.)	50 × 6	2	0/6	0
2-Mercapto-4-phenyl-5-ketothiazoline, CH5 (1,000 mg.)	500 × 6	1 K	0/2	0
2-Mercapto-4-benzylidine-5-ketothiazoline, CH9 (250 mg.)	100 × 6	2	0/many	0
2-Mercapto-4-ethoxymethylene-5-ketothiazoline, CH10 (50 mg.)	50 × 6 20 × 6	2 2	0/many 1/many	0
2-Mercapto-5-ketothiazoline, CH8	1,000 × 1 500 × 1	1 K 1	0/1 0/many	0
XANTHONES AND THIOXANTHONES				
Miracil A, 1-methyl-4-diethylaminoethylaminoxanthone HCl (100 mg.)	50 × 6	2	0/many	0

‡ s.c. § oral.

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Miracil B, 1-methyl-4-diethylaminoethylamino-8-chloroxanthone (100 mg.)	50 × 6	2	0/many	0
Miracil C, 1-methyl-4-diethylaminoethylamino-xanthydrol (50 mg.)	20 × 6	2	0/many	0
Miracil D, 1-methyl-4-diethylaminoethylamino-thioxanthone, HCl (250 mg.)	200 × 6 Oral	1	0/many	0
1-Diethylaminoethylamino-4-methylthioxanthone, Ms803 (200 mg.)	100 × 6	2 I	0/many	0
1-Methyl-4-diethylaminoethylamino-8-chloro-thioxanthone, Ms786 (200 mg.)	100 × 5 100 × 6	1 K 1 I	0/many 0/10	0
BENZEPINES				
7-Chloro-2:4-diamino-1:3:5-triazobenzepine hemisulphate, FE59 (100 mg.)	50 × 4 50 × 6	1 D 4	0/5 0/15	0
1-*Iso*propyl-2:4-diamino-8-chloro-1:3:5-triazobenzepine SO_4, FK61 (250 mg.)	100 × 6	2	0/20	0
DYES				
Erythrosin, C.I., No. 773 (250 mg.)	100 × 6	2	0/many	0
Methylene blue (50 mg.)	20 × 6	2	0/82	0
Methylene violet Bernthsen (150 mg.)	100 × 6	2	0/many	0
Methylene green, C.I., No. 924 (75 mg.) ..	50 × 6 50 × 4 20 × 6	1 I 2 D 1 D	0/few 0/7 0/6	0
Kiton fast green, F40 (50 mg.)	20 × 6	2	0/6	0
Xylene fast green B, F39 (500 mg.)	200 × 5 200 × 6	1 K 2	0/4 0/12	0
Neutral red, C.I., No. 825 (250 mg.)	100 × 6	2	1/30	0
Brilliant cresyl blue, C.I., No. 877 (50 mg.) ..	20 × 6	4	0/51	0
Nile blue sulphate, C.I., No. 913 (50 mg.) ..	20 × 6	2	1/9	0
Resorcin blue, C.I., No. 908 (250 mg.) ..	100 × 6	2	1/23	0
Gallocyanin, C.I., No. 883 (250 mg.)	100 × 5 100 × 6 50 × 6	1 D 1 2	0/16 0/13 0/3	0
New methylene blue, C.I., No. 927 (50 mg.) ..	20 × 6	2	0/27	0
Thionin, C.I., No. 920 2.2'diaminothiazine ..	200 × 6 200 × 2 100 × 6 100 × 4	2 (1 D) 1 D 1 1 D	2/25 0/24 0/5 0/6	0
Azocarmine, C.I., No. 828	80 × 6 50 × 6	2 D 3	20/21† 0/12	0
Gallamine blue, C.I., No. 894	500 × 6	4 2 D	0/22 0/21	0

TABLE II—*Continued*

Drug (Maximum tolerated i.p. dose for mice per kg. in parentheses)	Dose mg. per kg.	No. of rats	No. female worms dead/ total No.	Therapeutic index
Indulin (water soluble), C.I., No. 861	100 × 6	2 (1 D)	0/30	
		1 D	7/7	0
	50 × 6	2	0/23	
Toluidine blue, C.I., No. 925 (50 mg.)	20 × 6	2	0/37	0
MISCELLANEOUS COMPOUNDS				
Sanocrysin (100 mg.)	50 × 6	4	0/9	0
Neosolganal (1,000 mg.)	400 × 6	1	0/350	0
p-Aminobenzoic acid (100 mg.)	40 × 6	1	0/15	0
1:4-Bis-amino-*n*-propoxybenzene di-HCl, F10 (100 mg.)	50 × 6	2	0/many	0
Diethyl urea, F32 (1,000 mg.)	500 × 6	2	0/many	0
2:4-Dichlorophenoxymethyl chloride (50 mg.) ..	20 × 6	2	0/20	0
Aminoacetamide thiocarbamate, CH2 (250 mg.)	100 × 6	2	0/many	0
N-Carbethoxypiperidine, F29 (500 mg.) ..	400 × 6	1 D	0/5	
	200 × 6	1	0/many	
N-Carbamidopiperidine, F27 (1,000 mg.) ..	1,000 × 1	1 K	0/many	
	500 × 3	1 K	0/many	
	500 × 5	2	0/2	0
3:5-Dicarbamylmethyltetrahydro-1:3:5:2-thiadiazino-2-thione, CH3 (500 mg.)	200 × 6	2	0/many	0
Suramin (100 mg.)	100 × 6	2	0/10	
	50 × 6	2	0/9	0
Antrycide	16 × 6	4	0/56	0
α-*p*-Methoxyphenylpiperidyl ethanol, HBr (100 mg.)	50 × 6	2	0/13	0
3:3′:5:5′-Tetrabromo-2:2′-dihydroxybenzil .. (Insoluble)	200 × 1	1 K	0/10	
	20 × 1	2 K	1/18	
	20 × 1 s.c.	2	0/7	0
Quinine HCl (100 mg.)	20 × 6	1	0/350	0
Chloromycetin	100 × 6	2	0/82	0

phenanthridiniums (3), acridines (4), benzthiazoles (17), benziminazoles and benzoxazoles (7), tetrazoles (2), thiazolines (6), xanthones and thioxanthones (6), benzepines (2), dyes (17), and miscellania (16). For ease of reference the active compounds are printed in bold type in Table II. Although no new important filaricidal compounds have been discovered in the present work, it has been possible to make comparisons among those already known and to draw attention to some new ones.

Among the antimonials tested, only one, tartar emetic, failed to show significant activity against the adult worms. All the remaining sixteen were active, although in

differing degrees. The most active, Sdt 779, with a therapeutic index of at least 20, is five to ten times better in this respect than neostibosan, hitherto regarded as the best antimonial for the treatment of filariasis; next is methylglucamine stibonate, with an index of 10; but with both compounds results are incomplete owing to scantiness of supplies. Both are markedly non-toxic to mice, the latter being tolerated at 10 g./kg. Anthiomaline, solustibosan, neostibosan, neostam, and fuadin, in that order, are shown to have therapeutic indices ranging from one to five. With the exception of solustibosan they have been considered by American workers to have lower therapeutic indices than these (possibly because the American workers rated as cured only those rats in which all the parasites were dead, and not only the females). Fuadin, with a therapeutic index in cotton rats of five, has been administered to patients with filariasis at relatively lower doses than any other of the compounds mentioned above because of the severe reactions for which it can be responsible. For this reason it is thought that the fuadin-type drugs Sdt 379 and Sdt 779 may not be as useful in man as their high therapeutic indices in cotton rats suggest. Solustibosan, although not very effective in man at comparatively high doses, was the only compound used by Culbertson and his co-workers which did not produce unpleasant reactions in patients with filariasis; as it has only a low therapeutic index for cotton rats it is not surprising that few cures resulted from its use. Two similar compounds, pentostam and methyl-glucamine stibonate, are shown in the present work to have high therapeutic indices in rats, and there is hope that they might be given to man in doses sufficiently high to achieve radical cure but not so high as to produce serious side-effects. These drugs are excreted rapidly, and doses would have to be given frequently.

Several of the arsenic compounds have been found to have quite high therapeutic indices, although seven were without action. K 324 and neoarsphenamine showed activity comparable to that of the better antimonials, as did Sdt 544, which contains both arsenic and antimony. K 324 is a compound which was developed by Strangeways (1935) against trypanosomiasis, and administered to patients with sleeping sickness by Murgatroyd (1937). It is practically identical with arsenamide, which has been put forward by Thetford, Otto, Brown, and Maren (1948) for the treatment of human filariasis. One of us had the opportunity of using it in the treatment of four patients in East Africa, and the preliminary results were encouraging (Hawking, 1950). Further consideration should be given to the possible use of arsenicals for killing adult filarial worms.

Activity was found among eight members of the cyanine-quinoline group and in two phenanthridinium compounds, and it was confirmed in the cyanine 863. The best of the new compounds had a therapeutic index of 10. The high degree of activity found among the styryl- and anil- -quinolines and -benzthiazoles is not surprising in view of the extremely good results reported for the related cyanine dyes (Peters, Bueding, Valk, Higashi, and Welch, 1949). One of these (863) has been found by us to be more active than any of the other cyanine-quinolines we have examined. Unfortunately this compound has proved disappointing when tried in man (Peters, Welch, and Higashi, 1949), and as the antifilarial action of all this group seems to be essentially the same it did not seem worth while to continue its exploration. One of the styryl-quinolines (B440) has been reported to induce the

formation of tumours when injected subcutaneously into mice (Browning *et al.*, 1936).

The only group which has not been explored by other workers and in which activity has been found is the diguanide group. Unfortunately the (low) antifilarial activity discovered with paludrine was not encountered in any other member of the series, so that this clue was disappointing. Suramin was studied because of its action in onchocerciasis, and miracil because of its action in schistosomiasis ; both proved inactive both on adult worms and on microfilariae.

Action on microfilariae.—While the main object of this work has been to study the action of drugs on the adult filariae, a watch has been kept on the microfilariae both in the blood stream and in the pleural cavities of the treated rats. Microfilariae were frequently found to be absent from the pleural cavities after all adult worms had been killed, although still present in the blood. With two exceptions (hetrazan and *N* : *N* : *N'*-trimethyl-*N'*-diethylcarbamylethylene diamine, $Me_2N.CH_2CH_2NMeCO.NEt_2$) none of the drugs tested appeared to have a direct action on the circulating microfilariae ; slight reductions may have occurred in some cases, but they were not studied, since they are difficult to distinguish from

TABLE III

The effect of *N*: *N*: *N'*-trimethyl-*N'*-diethylcarbamylethylene diamine on circulating microfilariae when given as 6 daily doses of 100 mg. per kg.

Rat	Microfilariae per cu.mm. of tail blood					
	Before first dose	Days after first dose				
		1	2	3	4	5
2377 ..	46					4
2378 ..	30					10
2426 ..	54	76	12	6	16	4
2429 ..	50	24	42	56	72	2

the spontaneous fluctuations of the microfilaria count and since our main attention was given to compounds which kill the adult worms. Of particular interest are the morpholines ; *N*-carbamido-morpholine (F28), morpholinomorpholine-*N*-dithiocarboxylate (CH1), and morpholino-*N*-glycylmorpholine dithiocarboxylate (CH6) ; the piperidine derivative, benzylacetonylpiperidinium chloride ; and the diamine derivatives: *N* : *N'*-dimethyl-*N* : *N'*-bis-(diethylcarbamyl)ethylene diamine and 4-dimethylamino-4'-methyldiethylcarbamylamido-$\alpha\gamma$-diphenoxypropane, all of which bear some structural resemblance to hetrazan, but were without activity. Hetrazan itself was remarkably active against microfilariae, but had little action on adult worms ; the analysis of its action has been described in a separate paper by Hawking, Sewell, and Thurston (1950). *N* : *N* : *N'*-trimethyl-*N'*-diethylcarbamylethylene diamine was only feebly active against microfilariae, as Table III shows.

SUMMARY

1. Two hundred and twenty substances have been examined for *in vivo* activity against the adults and microfilariae of *Litomosoides carinii*.

2. No activity against the adult worms has been discovered in 183 substances belonging to the following chemical groups: amidines, *iso*thioureas, and amidoxines; sulphones; sulphonamides; derivatives of hydroxylamine; hydroxamic acids; pyridines; morpholines, a piperazine, a piperidinium, and an ethylene diamine derivative; pyrimidines; simple quinolines; *iso*quinolines; simple benzthiazoles; benziminazoles and a benzoxazole; tetrazoles; thiazolines; xanthones and thioxanthones; benzepines; miscellaneous dyes, and other miscellaneous compounds.

3. Some activity against adult worms has been found in all of the 18 compounds of antimony studied. Three of these—pentostam, methyl glucamine stibonate, and Sdt 779—are outstanding, having therapeutic indices of 6, 10, and ?20 respectively.

4. Some activity against adult worms has been found in four of the twelve compounds of arsenic studied. Three of them—a derivative of *p*-benzamide arsenoxide (D 324), neoarsphenamine, and a trivalent derivative of tryparsamide—have therapeutic indices of 4–8. Activity against adult worms has also been found in two compounds containing both antimony and arsenic.

5. Some activity against adult worms has been found in 23 of 35 anil- and styryl--quinolines and -benzthiazoles and in two phenanthridinium salts and a cyanine dye. However, clinical trials on this type of compound by Peters, Welch, and their colleagues have been discouraging.

6. Only one compound in the diguanide series, viz., proguanil, was found to be slightly active.

7. Of all the substances tested, only hetrazan and $N : N : N'$-trimethyl-N'-diethylcarbamylethylene diamine affected the microfilariae directly.

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