EXPERIMENTAL CHEMOTHERAPY OF FILARIASIS

II. Effect of Piperazine Derivatives Against Naturally Acquired Filarial Infections in Cotton Rats and Dogs

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INTRODUCTION

IN THE preceding paper[1] it was mentioned that compound 180-C (1-carbethoxy-4-methylpiperazine hydrochloride) produced a reduction in microfilariae when administered orally or intraperitoneally to filaria-infected cotton rats, and that suggestive action was produced against the adult worms. The effect of this compound, on the microfilariae at least, was so much more rapid than had been reported for any other known compound that an investigation of other derivatives was indicated. The organic chemistry departments of the Lederle Laboratories Division and the Calco Chemical Division of American Cyanamid Company therefore prepared a number of piperazine compounds for testing in the cotton rat. The results of these tests are given in this paper, and the synthetic part of the program is reported elsewhere.[2, 3]

EXPERIMENTAL METHODS

In addition to the general procedures reported in a previous paper[1] for evaluating the effects of new compounds against filariasis in the cotton rat, supplementary methods were used in the case of the piperazines. Several members of this group of compounds were found to produce precipitous reductions in the microfilarial count following various oral or intraperitoneal doses. For comparative evaluations it was necessary therefore to select what appeared to be the most active derivative and to use this compound as a standard, particularly since no other known filaricidal compounds produced comparable effects. During the first few months of this study, 1-carbethoxy-4-methylpiperazine hydrochloride (compound 180-C) produced the most consistent effect against microfilariae and was used as a standard. Data from eighty-one cotton rats treated with various doses of 180-C are given in Table IV.

Although compound 180-C was markedly effective against microfilariae in well-tolerated doses in the cotton rat, the results obtained against adult worms (Table V), although suggestive, were not promising. Moreover, doses which reduced the microfilariae of *Dirofilaria immitis* in the dog frequently caused nausea, muscular weakness, profuse salivation, and prostration. Another compound was subsequently chosen as a standard. This was 1-diethylcarbamyl-4-methylpiperazine hydrochloride (84-L). Marked reductions in microfilariae were produced by this compound in doses as low as 3 mg. per kilogram in cotton rats; no serious toxic symptoms occurred in dogs in therapeutic doses; and by frequent administration a large proportion of the adult worms were killed in cotton rats. A detailed report on the effects produced by 84-L in cotton rats and dogs is given in another paper in this series,[4] but for comparative purposes some data regarding its activity are included herein.

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Received for publication, Aug. 7, 1947.

In testing new piperazine derivatives three rats were used for a preliminary assay. If the compound was soluble, intraperitoneal doses of 50, 25, and 12.5 mg. per kilogram were administered twice daily for from two to four weeks. Insoluble derivatives were administered orally in a 2.5 per cent starch suspension at 200, 100, and 50 mg. per kilogram. If preliminary assays proved the compounds to be toxic in the doses used, second or third trials were made, halving the doses each time. Autopsies were performed within one to four weeks after cessation of treatment.

In the case of piperazines which showed activity against microfilariae several dosage regimes were used and an attempt was made to determine an approximate chemotherapeutic index (Table II).

In several cases, due to a difficult synthesis, relatively small amounts of compounds were available. If these showed no activity in doses which were two or three times greater than the minimum effective dose of 84-L no further tests were performed. It is quite possible, therefore, that some of the compounds listed as inactive in Table I might have shown activity at higher doses.

Types of Piperazines Tested.—In Tables I and II are listed most of the piperazines and related compounds which have been tested thus far in cotton rats. Several other piperazines, some of which were active, will be reported in a later publication. The piperazine nucleus itself showed no activity nor did any of its components. The addition of the carbethoxy radical in position 1, with various substitutions in position 4, produced a number of compounds with high microfilaricidal activity. Alkyl radicals in position 4 retained high activity as far as the propyl and isopropyl derivatives, but butyl substitutions were much less active. Toxicity was increased as the alkyl chain was lengthened.

Retaining the carbethoxy group in position 1, two other substitutions in position 4 retained activity, namely guanyl and carbethoxy.

The only compounds lacking the carbethoxy group which showed marked activity against microfilariae were 1-ethylcarbamyl-4-methylpiperazine hydrochloride (182-L); 1-diisopropylcarbamyl-4-methylpiperazine hydrochloride (177-L); 1-dimethylcarbamyl-4-methylpiperazine hydrochloride (152-L); and 1-Diethylcarbamyl-4-methylpiperazine hydrochloride (84-L). The latter two compounds are considered as the most active of the piperazines yet tested.

Effect of Piperazines on Microfilariae in the Cotton Rat.—The piperazines listed in Tables II and III produced rapid reductions in microfilariae in the peripheral blood of cotton rats. In some cases the amount of drug necessary to produce this effect was very close to the lethal dose, whereas in others it was far beneath the lethal dose. In general active piperazines in proper doses produced a measurable reduction in microfilariae within twenty-four hours (Table III) after the first dose was administered, and this reduction was sustained for as long as treatment was continued. Recurrences of microfilariae frequently occurred after cessation of treatment.

An exhaustive study of all active piperazines has not been made thus far, since the number of animals and amount of drug necessary to carry out such a study would have been prohibitive, from the standpoint of both time and economy. The comparison given in Tables II and III, however, illustrate the effects of eighteen active piperazines, and Table IV illustrates the effects of different dosage upon microfilariae, using 1-carbethoxy-4-methylpiperazine hydrochloride.

TABLE I. PIPERAZINE AND RELATED COMPOUNDS SHOWING NO FILARICIDAL ACTIVITY IN COTTON RATS IN THE DOSES USED

COMPOUND	NAME	HIGHEST* DOSAGE USED (MG./KG.)
148-L	Ethyl carbamate	100 I.P.
150-L	Ethyl N-methylcarbamate	100 I.P.
149-L	Ethyl N-ethylcarbamate	100 I.P.
146-L	Ethyl N,N-diethylcarbamate	100 I.P.
144-L	N-carbethoxyethylenediamine	100 I.P.
220-C	Ethyl N-ethyl-N-(2-hydroxyethyl)-carbamate	100 I.P.
U-930	Piperazine hexahydrate	800 I.P.
63-L	1-Phenylpiperazine hydrochloride	100 I.P.
56-L	1-Ethylpiperazine hydrochloride	100 I.P.
U-910	1,4-Diaminopiperazine hydrochloride	100 I.P.
222-C	1,4-Dimethylpiperazine dihydrochloride	100 I.P.
225-C	1-Carbamyl-4-methylpiperazine	100 I.P.
228-C	1-Methyl-4-thiocarbamylpiperazine hydrochloride	100 I.P.
309-C	Omega-[1-(4-methylpiperazyl)]-propiophenone dihydrochloride	12.5 I.P.
240-C	2,3-Diphenylpiperazine dihydrochloride	100 I.P.
77-L	1,4-Diguanylpiperazine sulfate	100 I.P.
214-C	4-Carbethoxymorpholine	200 Orally
184-L	1-Carbomethoxy-4-methylpiperazine hydrochloride	6.25 I.P.
U-994	1-Carbethoxy-4-nitrosopiperazine hydrochloride	50 Orally
145-L	1-Carbethoxy-4-isobutylpiperazine	100 Orally
224-C	1-Carbethoxy-4-(n-heptyl)-piperazine	200 Orally
193-C	1-Carbethoxy-4-phenylpiperazine	25 I.P.
209-C	1-Carbethoxy-4-benzylpiperazine hydrochloride	200 Orally
60-L	1-Carbethoxy-4-aminopiperazine hydrochloride	100 I.P.
219-C	1-Carbamyl-4-carbethoxypiperazine	100 I.P.
211-C	1-Carbethoxy-4-allylpiperazine	100 I.P.
192-L	4-(β-sulfanylethyl)-1-carbethoxypiperazine	200 Orally
195-L	1-Diethylcarbamyl-4-carbethoxypiperazine	12.5 I.P.
217-C	1,2-Bis-(1′carbethoxy-4′-piperazine) ethane	200 Orally
196-C	1-Ethyl 4[2-(diphenylacetoxy)ethyl]-piperazine	100 I.P.
153-L	1,1′-Carbonyl-bis-(4-methylpiperazine) dihydrochloride	100 I.P.
168-C	1,4-Bis-(α-phenyl-β-benzoylethyl)-piperazine	100 Orally
U-683	Ethyl (1-carbethoxy-4-piperazine)-acetate	100 I.P.
U-682	1-Carbethoxy-4-(2-hydroxyethyl)-piperazine	100 I.P.
162-L	1-Carbomethoxypiperazine	6.25 I.P.
164-L	1-Carbobutoxypiperazine	6.25 I.P.
165-L	1-Carboisobutoxypiperazine	6.25 I.P.
161-L	1,4-Dicarbomethoxypiperazine	6.25 I.P.
163-L	1,4-Dicarbobutoxypiperazine	25 Orally
166-L	1,4-Dicarboisobutoxypiperazine	6.25 I.P.
159-L	1-Diethylcarbamylpiperazine	6.25 I.P.
197-L	1-Diisopropylcarbamylpiperazine	25 I.P.
198-L	1-Di-n-butylcarbamylpiperazine	25 I.P.
199-L	1-Diisoamylcarbamylpiperazine	25 I.P.
176-L	1-Dibutylcarbamyl-4-methylpiperazine hydrochloride	6.25 I.P.
203-L	1-Phenylcarbamyl-4-methylpiperazine	25 I.P.
293-L	1-Benzylcarbamyl-4-methylpiperazine	25 I.P.
158-L	1-Diethylcarbamyl-4-isopropylpiperazine hydrochloride	6.25 I.P.
160-L	1,4-Bis-diethylcarbamylpiperazine	6.25 I.P.

*Treatment administered twice daily for from two to four weeks.

More extensive data concerning the effects of 1-diethylcarbamyl-4-methylpiperazine hydrochloride (84-L) upon microfilariae in vivo and in vitro are presented elsewhere.[4]

Effect of Piperazines on Adult Filariae in Cotton Rats.—In cotton rats treated with several of the piperazines listed in Tables II and III, autopsies performed at various intervals after cessation of treatment revealed no dead

TABLE II. COMPARATIVE MICROFILARICIDAL ACTIVITY AND TOXICITY OF EIGHTEEN PIPERAZINE DERIVATIVES IN COTTON RATS, USING 1-DIETHYLCARBAMYL-4-METHYLPIPERAZINE HYDROCHLORIDE (84-L) AS A STANDARD

COMPOUND	NAME	MINIMUM EFFECTIVE DOSE AGAINST MICROFILARIAE (MG./KG.)	LD_{50} MICE I.P. (MG./KG.)	APPROXIMATE 84-L EQUIVALENT	APPROXIMATE CHEMOTHERAPEUTIC INDEX
163-C	1-Phenylpiperazine hydrochloride	50 I.P.	140	0.06	2.8
162-C	1-Carbethoxypiperazine hydrochloride	25 I.P.	275	0.125	11.0
217-L	1-Methyl-4-(4'-morpholine carbamyl)-piperazine hydrochloride	12.5 I.P.	?	0.25	?
180-C	1-Carbethoxy-4-methylpiperazine hydrochloride	6.25 I.P.	550	0.5	88.0
59-L	1-Carbethoxy-4-ethylpiperazine	50 I.P.	175	0.06	3.5
82-L	1-Carbethoxy-methylpiperazine	100 I.P.	?	0.03	?
U-653	1-Carbethoxy-4-propylpiperazine hydrochloride	25 I.P.	87.5	0.125	3.5
147-L	1-Carbethoxy-4-isopropylpiperazine hydrochloride	25 I.P.	100	0.125	4.0
U-655	1-Carbethoxy-4-butylpiperazine	50 I.P.	125	0.06	2.5
U-801	1-Carbethoxy-4-s. butylpiperazine	100 Orally	175	0.05	1.75*
61-L	1-Guanyl-4-carbethoxy-piperazine sulfate	50 I.P.	285	0.06	5.6
218-C	Bis-(4-carbethoxy-1-piperazine)-methane	25 I.P.	175	0.125	7.0
169-C	1,4-Dicarbethoxypiperazine	100 I.P.	500	0.03	5.0
76-L	1,4-Dicarbethoxy-2-methylpiperazine	200 Orally	375	0.025	1.9*
182-L	1-Ethylcarbamyl-4-methylpiperazine hydrochloride	6.25 I.P.	2,250	0.5	360.0
152-L	1-Dimethylcarbamyl-4-methylpiperazine hydrochloride	6.25 I.P.	310	0.5 to 1.0	48.0
177-L	1-Diisopropylcarbamyl-4-methylpiperazine hydrochloride	6.25 I.P.	160	0.5	25.6
84-L	1-Diethylcarbamyl-4-methylpiperazine hydrochloride	3.13 I.P.	285	1.0	91.0

*Probably not a true value, since the LD_{50} was determined by intraperitoneal injections and the minimum effective dose by oral administration.

adult worms present in the pleural cavity, and there was no macroscopic or microscopic evidence that any damage whatever to the macrofilariae had been effected. In view of the dramatic effects produced against the microfilariae these results were at first disappointing.

One of the first compounds found to produce suggestive activity against the adult worms was 1-carbethoxy-4-isopropylpiperazine hydrochloride (147-L). Dead worms were found in one of the three rats first used to test this compound, and no adult worms were found in another. The third rat revealed all living worms. Attempts to repeat these results in a larger series of rats failed, because this compound was very toxic to the host and prolonged dosage could not be administered.

Thirty rats treated with various doses of 1-carbethoxy-4-methylpiperazine hydrochloride were then brought to autopsy, and, as illustrated in Table V,

TABLE III. EFFECT OF VARIOUS PIPERAZINE DERIVATIVES AGAINST MICROFILARIAE IN COTTON RATS DURING FIRST WEEK OF TREATMENT

COMPOUND	NAME	DOSE (MG./KG.) B.I.D.	MICROFILARIAE PER 100 FIELDS — DAY 1	2	3	4	5	6	7
163-C	1-Phenylpiperazine hydrochloride	25 I.P.	340	284	310	440	144	250	108
		50 I.P.	92	8	0	60	18	24	0
162-C	1-Carbethoxypiperazine hydrochloride	25 I.P.	180	6	4	6	6	8	6
		50 I.P.	652	252	96	18	14	14	12
		100 I.P.	1,360	84	8	10	4	6	6
217-L	1-Methyl-4-(4'-morpholine carbamyl-piperazine hydrochloride	25 I.P.	268			40			2
		50 I.P.	14						1
		100 I.P.	92			6			0
180-C	1-Carbethoxy-4-methylpiperazine hydrochloride	6.25 I.P.	430	190	56	250	52	8	22
		12.5 I.P.	128	72	60	48	22	12	56
		25 I.P.	160	112	28	38	40	6	14
		50 I.P.	384	40	22	12	4	0	4
		100 I.P.	264	26	8	2	6	8	4
		200 I.P.	168	12	2	1	1	4	0
59-L	1-Carbethoxy-4-ethylpiperazine	25 I.P.	1,580	800			430		360
		50 I.P.	1,940	600			188		100
		100 I.P.	136	6			0		1
82-L	1-Carbethoxy-methylpiperazine	25 I.P.	140	144	132	116	248	308	140
		50 I.P.	212	320	124	124	156	136	84
		100 I.P.	76	14	18	14	12	10	6
U-653	1-Carbethoxy-4-propylpiperazine hydrochloride	25 I.P.	64	28	22		32		14
		50 I.P.	248	160	36		18		14
147-L	1-Carbethoxy-4-isopropylpiperazine hydrochloride	25 I.P.	44	3	10		4		1
		50 I.P.	26	6	1		4		8
		100 I.P.	8	0	0		0		0
U-655	1-Carbethoxy-4-butylpiperazine	25 I.P.	284	656	696		208		336
		50 I.P.	160	18	10		6		12
		100 I.P.	116	10	9		10		4
U-801	1-Carbethoxy-4-s. butylpiperazine	50 Orally	388	468	148		568		340
		100 Orally	140	80	26		28		14
		200 Orally	440	44	8		24		0
61-L	1-Guanyl-4-carbethoxypiperazine sulfate	25 I.P.	380	212		436	272	412	304
		50 I.P.	388	68		18	4	24	6
		100 I.P.	104	12		6	8	6	8
218-C	Bis-(4-carbethoxy-1-piperazine)-methane	25 I.P.	272	68	60				22
		50 I.P.	76	32	6				3
169-C	1,4-Dicarbethoxypiperazine	12.5 I.P.	132	196	116	172	40	120	88
		25 I.P.	188	140	140	60	23	48	128
		50 I.P.	440	552	476	676	348	148	188
		100 I.P.	344	88	38	22	56	36	26
		200 I.P.	740	132	30	6	18	6	34
76-L	1,4-Dicarbethoxy-2-methylpiperazine	50 Orally	568	400	248	92	320	400	160
		100 Orally	292	292	412	76	72	268	116
		200 Orally	52	64	38	10	6	2	8
182-L	1-Ethylcarbamyl-4-methylpiperazine hydrochloride	1.5 I.P.	188	332	188	232	176	76	244
		3.13 I.P.	432	1,060	324	472	712	490	612
		6.25 I.P.	36	72	48	28	4	10	6
152-L	1-Dimethylcarbamyl-4-methylpiperazine hydrochloride	25 I.P.	80	20	2	3	6	4	4
		50 I.P.	1,190	2,180	740	870	960	560	310*
		100 I.P.	172	60	8	12	6	4	1
177-L	1-Diisopropylcarbamyl-4-methylpiperazine hydrochloride	1.5 I.P.	152		28	380	10	104	26
		3.13 I.P.	432		104	136	336	244	180
		6.25 I.P.	60		1	20	10	6	2

*No adult worms were found in the pleural cavity at autopsy. Four worms were found in the abdominal cavity.

TABLE III—CONT'D

COMPOUND	NAME	DOSE (MG./KG.) B.I.D.	MICROFILARIAE PER 100 FIELDS DAY 1	2	3	4	5	6	7
84-L	1-Diethylcarbamyl-4-methylpiperazine hydrochloride	1.5 I.P.	480	400	340	270	290	210	184
		3.13 I.P.	1,220	108	40	56	4	8	2
		6.25 I.P.	600	116	44	22	72	40	22
		12.5 I.P.	180	64	8	8	8	1	8
		25 I.P.	396	100	20	8	10	6	1
		50 I.P.	476	76	22	13	12	2	3
		100 I.P.	192	12	6	5	4	10	1
		5 Orally	228	14	0	10	3	2	4
		10 Orally	408	10	6	10	3	2	1
		25 Orally	168	8	0	0	0	0	0
	Nontreated controls	–	1,630	252	590	1560	224	1020	1180
			46	20	28	18	44	40	72
			36	26	56	44	52	58	36
			40	60	88	22	40	24	56
			560	212	208	184	280	244	410
			120	248	348	100	404	628	780
			40	24	24	10	12	42	56

the effects upon the adult worms were extremely variable. Most of the adult worms were alive, however. Similar results were obtained on a smaller series of animals treated with other piperazines effective against microfilariae.

Nine of sixty-five untreated rats which were autopsied showed one or two dead adult worms at autopsy, but in no case were the majority or all of the worms dead. The effects produced by several of the piperazines upon the adult worms in a few rats in each series, therefore, suggested that a wide variety of dosage schedules, with autopsies at different periods after cessation of treatment, might throw some light on the amount of drug and the length of time necessary to kill a larger proportion of the adult worms than had been accomplished in earlier trials. The compound selected for these extensive tests was 1-diethylcarbamyl-4-methylpiperazine hydrochloride, since it produced predictable and rapid reductions in microfilariae in oral or intraperitoneal doses as low as 3 mg. per kilogram, was relatively nontoxic to cotton rats, and was made available in large quantities.

Subsequent data obtained with this compound and reported in a following paper showed that frequent oral dosage for several weeks killed a large proportion of the adult worms. None of the other compounds listed in Tables II and III have been studied so extensively, but it is believed that the best balance between activity and toxicity has been achieved with 1-diethylcarbamyl-4-methylpiperazine hydrochloride. For the reasons described, the activity of the piperazines listed in Tables II and III is based entirely upon their effectiveness against microfilariae. In most cases relatively high toxicity to the host rules out further studies upon their effectiveness against adult worms, and in the few instances where good effects against microfilariae were obtained with relatively nontoxic doses (182-L, 152-L, 180-C) it is felt that further studies would produce little more pertinent information than has been obtained with 84-L.

Effect of Piperazines Upon Dirofilaria Immitis in the Dog.—Several piperazines showing high activity in the cotton rat were tested for effectiveness against

TABLE IV. EFFECT OF 1-CARBETHOXY-4-METHYLPIPERAZINE HYDROCHLORIDE (180-C) AGAINST MICROFILARIAE IN COTTON RATS

RAT	SERIES	DOSE (MG./KG.)	TREATMENT	PER CENT REDUCTION OF MICROFILARIAE	
				7 DAYS	LAST DAY OF TREATMENT
370	RF-32	12½	I.P., b.i.d., 14 days	56.3	59.4
44	RF-9	25	I.P., b.i.d., 14 days	97.5	100.0
38	RF-9	25	I.P., b.i.d., 14 days	70.0	90.0
75	RF-9	25	I.P., b.i.d., 14 days	100.0	100.0
109	RF-9	25	I.P., b.i.d., 14 days	92.3	94.3
70	RF-9	25	I.P., b.i.d., 14 days	25.0	75.0
76	RF-9	25	I.P., b.i.d., 14 days	95.6	94.0
82	RF-9	25	I.P., b.i.d., 14 days	97.8	77.5
133	RF-10	25	I.P., b.i.d., 27 days	100.0	91.7
134	RF-10	25	I.P., b.i.d., 27 days	94.5	77.8
135	RF-10	25	I.P., b.i.d., 27 days	100.0	D.
137	RF-10	25	I.P., b.i.d., 27 days	100.0	14.3
138	RF-10	25	I.P., b.i.d., 27 days	50.0	62.5
372	RF-32	25	I.P., b.i.d., 14 days	91.3	93.8
373	RF-32	25	I.P., b.i.d., 14 days	74.0	88.1
205	RF-16	25	Orally, b.i.d., 28 days	99.6	99.1
210	RF-16	25	Orally, b.i.d., 28 days	99.4	D.
215	RF-16	25	Orally, b.i.d., 28 days	99.4	D.
218	RF-16	25	Orally, b.i.d., 28 days	98.5	99.4
222	RF-16	25	Orally, b.i.d., 28 days	98.2	D.
139	RF-10	50	I.P., b.i.d., 27 days	100.0	100.0
140	RF-10	50	I.P., b.i.d., 27 days	50.0	87.5
141	RF-10	50	I.P., b.i.d., 27 days	83.4	100.0
142	RF-10	50	I.P., b.i.d., 27 days	87.5	100.0
143	RF-10	50	I.P., b.i.d., 27 days	100.0	D.
159	RF-10	50	I.P., b.i.d., 27 days	100.0	100.0
160	RF-10	50	I.P., b.i.d., 27 days	99.4	100.0
374	RF-32	50	I.P., b.i.d., 14 days	100.0	D.
375	RF-32	50	I.P., b.i.d., 14 days	99.0	99.0
207	RF-16	50	Orally, b.i.d., 28 days	100.0	99.3
209	RF-16	50	Orally, b.i.d., 28 days	99.4	D.
216	RF-16	50	Orally, b.i.d., 28 days	95.0	98.8
219	RF-16	50	Orally, b.i.d., 28 days	97.7	98.5
183	RF-15	50	I.P., O.D., 28 days	96.3	100.0
189	RF-15	50	I.P., O.D., 28 days	92.9	100.0
190	RF-15	50	I.P., O.D., 28 days	100.0	96.6
184	RF-15	50	I.P., A.D., 28 days	94.7	D.
187	RF-15	50	I.P., A.D., 28 days	93.4	96.7
197	RF-15	50	I.P., A.D., 28 days	20.0	0
144	RF-10	100	I.P., b.i.d., 14 days	95.0	80.0
145	RF-10	100	I.P., b.i.d., 14 days	99.0	99.0
146	RF-10	100	I.P., b.i.d., 14 days	100.0	D.
148	RF-10	100	I.P., b.i.d., 14 days	100.0	D.
149	RF-10	100	I.P., b.i.d., 14 days	100.0	100.0
376	RF-32	100	I.P., b.i.d., 14 days	97.6	100.0
385	RF-32	100	I.P., b.i.d., 14 days	98.4	100.0
56	RF-11	100	I.P., b.i.d., 29 days	100.0	98.8
131	RF-11	100	I.P., b.i.d., 29 days	100.0	99.6
90	RF-11	100	I.P., b.i.d., 29 days	100.0	100.0
206	RF-16	100	Orally, b.i.d., 28 days	97.3	100.0
208	RF-16	100	Orally, b.i.d., 28 days	98.9	D.
213	RF-16	100	Orally, b.i.d., 28 days	98.8	98.8
221	RF-16	100	Orally, b.i.d., 28 days	98.4	D.
186	RF-15	100	I.P., O.D., 28 days	87.0	78.3
196	RF-15	100	I.P., O.D., 28 days	98.1	94.3
212	RF-15	100	I.P., O.D., 28 days	99.0	100.0
194	RF-15	100	I.P., A.D., 28 days	92.3	69.3
203	RF-15	100	I.P., A.D., 28 days	90.5	54.1
204	RF-15	100	I.P., A.D., 28 days	87.0	98.2
155	RF-19	100	*	D.	D.

TABLE IV—CONT'D

RAT	SERIES	DOSE (MG./KG.)	TREATMENT	PER CENT REDUCTION OF MICROFILARIAE	
				7 DAYS	LAST DAY OF TREATMENT
153	RF-19	100	*	98.3	98.3
157	RF-19	100	*	100.0	100.0
158	RF-19	100	*	D.	D.
98	RF-11	200	I.P., b.i.d., 29 days	D.	D.
124	RF-11	200	I.P., b.i.d., 29 days	100.0	100.0
102	RF-11	200	I.P., b.i.d., 29 days	100.0	100.0
386	RF-32	200	I.P., b.i.d., 14 days	98.0	100.0
387	RF-32	200	I.P., b.i.d., 14 days	100.0	99.3
18	RF-11	200	Orally, b.i.d., 29 days	99.3	100.0
33	RF-11	200	Orally, b.i.d., 29 days	99.2	D.
40	RF-11	200	Orally, b.i.d., 29 days	100.0	D.
248	RF-19	200	*	97.8	97.8
250	RF-19	200	*	100.0	100.0
251	RF-19	200	*	100.0	100.0
252	RF-19	200	*	100.0	100.0
50	RF-11	400	Orally, b.i.d., 29 days	100.0	D.
55	RF-11	400	Orally, b.i.d., 29 days	100.0	100.0
127	RF-11	400	Orally, b.i.d., 29 days	100.0	D.
88	RF-12	Diet 0.5%	15 days	97.5	98.4
92	RF-12	Diet 0.5%	15 days	100.0	98.9
96	RF-12	Diet 0.5%	15 days	91.6	D.

I.P., Intraperitoneal b.i.d., twice daily; O.D., once daily; A.D., once on alternate days; D., death.

*Series RF-19 treated every two hours for forty-eight hours, then twice daily for six more days.

Dirofilaria immitis in the dog. The only two compounds which produced measurable reductions in microfilariae in tolerated doses were 1-carbethoxy-4-methylpiperazine hydrochloride (180-C) and 1-diethylcarbamyl-4-methylpiperazine hydrochloride (84-L). The administration of oral or intraperitoneal doses of compound 180-C which produced reductions of microfilariae (25 to 100 mg. per kilogram twice daily) invariably produced nausea, profuse salivation, and muscular weakness. Compound 84-L was much better tolerated, and subsequent experiments in dogs were confined to this derivative. The results obtained with 84-L in dogs, against both micro- and macrofilariae, are presented in another paper in this series.

DISCUSSION

The effectiveness of several piperazine derivatives (administered orally or intraperitoneally) in rapidly reducing the microfilarial count in filaria-infected cotton rats is unique in the experimental chemotherapy of this infection. If the effectiveness of the piperazines was confined to the microfilariae, it would probably indicate that their potential usefulness for treating filarial diseases in other animals and man would be limited. The fact that several of the piperazines tested showed suggestive action against the adult worms, and that one in particular killed a large proportion of the adult worms when administered frequently in tolerated doses for several weeks, certainly removes this group of compounds from the class of experimental curiosities.

The value of the cotton rat bio-assay method for predicting potential filaricidal activity against related diseases in human beings has not been fully tested.

TABLE V. EFFECT OF 1-CARBETHOXY-4-METHYLPIPERAZINE HYDROCHLORIDE AGAINST ADULT WORMS IN COTTON RATS

RAT	DOSE (MG./KG.)	TREATMENT	MICROFILARIAE PER 100 FIELDS			AUTOPSY (DAYS AFTER LAST TREATMENT)	AUTOPSY RECORD
			DAY 1	DAY 7	DAY OF AUTOPSY		
117	25 I.P.	b.i.d., 7 days	24	4	84	15	Mass of live worms
119	25 I.P.	b.i.d., 7 days	34	0	104	15	Mass of live worms
38	25 I.P.	b.i.d., 14 days	40	6	4	1	Several dead worms; none alive
75	25 I.P.	b.i.d., 14 days	30	0	0	1	Several live and several dead worms
109	25 I.P.	b.i.d., 14 days	52	4	2	1	Several live and several dead worms
70	25 I.P.	b.i.d., 14 days	56	42	8	1	Several live and several dead worms
76	25 I.P.	b.i.d., 14 days	68	3	4	1	Several live and several dead worms
82	25 I.P.	b.i.d., 14 days	132	3	4	1	Mass of live worms
135	25 I.P.	b.i.d., 10 days	18	0	0	1	Several live and several dead worms
143	50 I.P.	b.i.d., 15 days	4	0	0	1	Mass of live worms
146	100 I.P.	b.i.d., 19 days	8	0	0	1	Mass of live worms
141	50 I.P.	b.i.d., 26 days	6	1	0	13	Several live worms
40	200 Orally	b.i.d., 11 days	200	0	4	3	Mass of live worms
90	100 I.P.	b.i.d., 28 days	64	0	424	55	Mass of dead worms; none alive
18	200 Orally	b.i.d., 28 days	132	1	0	8	Several dead worms; none alive
55	400 Orally	b.i.d., 28 days	356	0	40	8	Mass of live worms
215	25 I.P.	b.i.d., 12 days	146	3	2	1	Mass of live worms
209	50 Orally	b.i.d., 13 days	160	1	0	1	Mass of live worms
208	100 Orally	b.i.d., 11 days	348	4	0	1	Mass of live worms
195	1.5 I.P.	b.i.d., 14 days	740	76	428	56	Mass of live worms
198	3.13 I.P.	b.i.d., 14 days	1,840	248	192	56	Mass of live worms
41	6.25 I.P.	b.i.d., 14 days	430	22	52	56	Mass of live worms
104	12.5 I.P.	b.i.d., 14 days	104	16	184	56	Mass of live worms
539	5 Orally	b.i.d., 10 days	1,700	180	528	1	Mass of live worms
527	5 I.P.	b.i.d., 15 days	72	172	2	112	Mass of live worms
522	5 I.P.	b.i.d., 15 days	252	80	380	112	Mass of live worms
532	5 I.P.	b.i.d., 15 days	26	6	84	112	Mass of live worms
530	5 Orally	b.i.d., 15 days	1,080	370	630	112	Mass of live worms
529	5 Orally	b.i.d., 15 days	204	40	24	112	Mass of live worms
540	5 Orally	b.i.d., 15 days	600	112	212	112	Mass of live worms

Culbertson, Rose, and Olive-Gonzalez,[5-7] however, have demonstrated that a correlation exists between the effects produced by Neostibosan against *Litomosoides carinii* in cotton rats and *Wuchereria bancrofti* infections in man.

We believe it to be important, also, that several of the most active piperazines are effective when administered orally. With the exception of malaria and the intestinal helminth diseases, the chemotherapy of parasitic diseases in man is largely confined to the parenteral administration of various drugs, usually compounds containing antimony or arsenic. The value of some of the heavy metals in the treatment of several of the most important parasitic diseases is not disputed, but the advantages of an effective oral treatment for any disease in human beings or domestic animals are obvious, particularly if no toxic effects are produced.

There is a measurable correlation between structure and microfilaricidal activity in the piperazines, as demonstrated in Table II. We cannot be sure that the most active member of the group has been found, and there is room for further investigation in this respect.

In comparison with several antimony derivatives, the piperazines produce a much more rapid effect upon the microfilariae of cotton rats in the peripheral blood.[8, 9] The effect produced against adult worms by piperazines is very slow in comparison with that of antimony salts. An entirely different mode of action must therefore be involved. No studies have been made thus far in attempts to determine how the piperazines produce their effects upon either microfilariae or macrofilariae.

SUMMARY

The effects of various piperazine derivatives against naturally acquired filaria infections in cotton rats and dogs are discussed. Several compounds in this group produce rapid reductions in microfilariae following oral or intraperitoneal dosage, and one in particular produces a lethal effect against adult filariae when administered frequently for a period of several weeks.

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