EXPERIMENTAL CHEMOTHERAPY OF FILARIASIS

III. Effect of 1-Diethylcarbamyl-4-Methylpiperazine Hydrochloride Against Naturally Acquired Filarial Infections in Cotton Rats and Dogs

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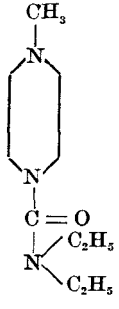
IT HAS been demonstrated in a preceding paper[1] that the intraperitoneal or oral administration of various piperazine derivatives to cotton rats naturally infected with *Litomosoides carinii* causes a rapid and precipitous reduction of microfilariae in the peripheral blood. Of more than one hundred derivatives made available, 1-diethylcarbamyl-4-methylpiperazine hydrochloride (compound 84-L) was selected as the most promising because of its repeated marked effects against microfilariae in both cotton rats and dogs and because of its relatively low toxicity in these animals. Subsequent studies showed that proper dosage regimes killed a large percentage of the adult worms. The structural formula of this compound is given below.



Two hundred twelve cotton rats and twenty-five dogs have been used to test the effectiveness of this compound against both microfilariae and macrofilariae, and the results obtained are reported herein. The methods used for counting microfilariae and for evaluating the effect upon adult worms have been discussed in a preceding paper.[2]

Effect of 1-Diethylcarbamyl-4-Methylpiperazine Hydrochloride Against Microfilariae.—After oral or intraperitoneal treatment with this compound in filaria-infected cotton rats, at doses ranging from 3 to 100 mg. per kilogram, the microfilarial count drops precipitously within twenty-four hours (Tables I and II). It then becomes negative or remains at a very low level for as long as treatment is continued.

There may or may not be a recurrence of microfilariae in the peripheral blood upon cessation of treatment (Tables III, V, IX, and X). The extent of

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TABLE I. EFFECT OF 84-L ON THE MICROFILARIAE OF COTTON RATS (INTRAPERITONEAL DOSAGE)

RAT	DOSE (MG./KG., B.I.D.)	MICROFILARIAE PER 100 LOW-POWER FIELDS — DAYS DURING TREATMENT							
		1	2	3	4	5	6	7	14
566	5	92	10	2	2	4	4	6	4
579	5	36	14	1	1	1	0	0	4
571	5	76	8	14	24	22	8	1	1
584	5	48	12	0	0	0	1	8	0
565	10	112	10	6	2	0	0	0	0
583	10	100	52	8	5	22	10	8	6
588	10	232	56	2	6	1	0	0	0
562	10	548	28	8	4	4	4	1	0
567	25	364	30	1	3	1	2	2	1
572	25	692	20	0	0	4	4	0	1
587	25	452	8	1	2	2	0	1	1
548	25	184	36	6	1	1	2	2	0
568	50	22	14	0	0	4	1	0	0
574	50	408	22	2	0	4	0	0	0
542	50	44	26	8	3	6	4	6	1
586	50	52	2	6	0	1	1	0	0
552	100	36	22	8	4	4	4	6	0
576	100	48	6	0	1	2	3	1	0
580	100	52	30	8	12	44	26	6	0
582	100	284	24	4	1	0	1	1	4

TABLE II. EFFECT OF 84-L ON THE MICROFILARIAE OF COTTON RATS (ORAL DOSAGE)

RAT	DOSE (MG./KG., B.I.D.)	MICROFILARIAE PER 100 LOW-POWER FIELDS — DAYS DURING TREATMENT							
		1	2	3	4	5	6	7	14
900	5	48	12	4	5	1	0	1	0
934	5	228	14	0	10	3	2	4	2
897	5	248	26	22	4	8	3	8	8
912	5	152	36	12	20	8	4	2	6
724	5	68	24	6	6	4	3	0	8
926	5	140	10	6	10	0	3	22	4
898	5	56	16	10	4	1	4	0	0
938	5	92	10	4	4	2	0	2	1
791	5	570	12	8	2	2	2	1	3
927	10	44	20	14	6	6	0	2	2
908	10	408	10	6	10	3	2	1	0
909	10	56	14	8	6	1	0	1	1
911	10	400	2	6	6	14	6	4	1
915	10	12	1	4	6	3	0	0	0
709	10	10	10	3	4	6	2	2	1
917	10	1,260	24	4	6	4	4	0	1
919	10	12	2	0	0	1	1	0	0
920	10	156	6	6	0	4	1	0	1
933	10	68	18	3	2	4	2	2	6
895	25	72	3	0	0	0	0	0	0
940	25	168	8	0	0	0	0	0	0
913	25	40	1	2	0	0	1	0	0
925	25	36	1	0	0	0	0	0	0
931	25	160	3	4	1	2	1	0	0
637	25	156	2	1	0	1	0	0	0
924	25	292	12	1	0	0	0	0	0
930	25	72	6	0	0	0	1	0	0
936	25	288	3	0	0	0	2	6	1
921	25	96	3	0	1	0	0	1	0
775	25	224	6	10	6	0	0	1	0
904	25	18	10	0	0	0	0	0	0
905	25	116	1	0	0	0	1	0	1

TABLE III. EFFECT OF 84-L AGAINST FILARIASIS IN COTTON RATS (ORAL TREATMENT EVERY TWO HOURS FOR FORTY-EIGHT HOURS)

RAT	DOSE (MG./KG.)	MICROFILARIAE PER 100 FIELDS: BEFORE TREATMENT	HOURS AFTER TREATMENT: 2	12	24	DAYS AFTER TREATMENT: 4	11	AUTOPSY RECORD
1284	25	148	3	0	0	-	-	Killed at 48 hr.; large mass of live worms
1286	25	340	4	0	0	-	-	Killed at 48 hr.; large mass of live worms
1293	25	48	4	1	0	-	-	Killed at 48 hr.; large mass of live worms
1268	25	220	34	1	0	-	-	Killed at 48 hr.; large mass of live worms
1278	25	156	3	0	0	4	16	Killed on 12th day; mass of live worms; several dead worms; small clump of caseous material
1292	25	248	22	2	0	2	6	Killed on 12th day; one large clump of worms on ventral side of heart, most of these dead; small mass of worms under lungs, some of these living, others dead
1253	25	320	22	0	0	0	16	Killed on 12th day; small clump of live worms, some encased in caseous material
1297	25	964	24	0	0	0	18	Killed on 12th day; many dead and degenerating worms; some living worms
1259	25	664	40	3	1	6	30	Killed on 12th day; mass of live worms; several dead worms
1256	10	148	10	0	0	Dead	-	Dead at 36 hr.; no autopsy
1266	10	676	8	8	2	4	12	Killed on 12th day; small mass of worms, some dead
1226	10	16	0	0	0	0	-	Killed on 8th day; small mass of live worms; 3 dead worms; some caseous material present
1246	10	400	30	8	22	-	-	Killed at 32 hr.; mass of live worms; two clumps of dead and disintegrating worms
1255	10	532	8	0	0	0	-	Killed on 8th day; masses of live worms; 1 dead worm
1264	10	248	8	0	0	0	16	Killed on 12th day; large mass of worms; many dead
1265	10	604	22	16	4	9	92	Killed on 12th day; large mass of worms; many living and some dead
1274	10	592	104	44	9	-	-	Dead at 42 hr.; large mass of live worms; several small clumps of dead worms
1267	10	748	52	14	1	2	48	Killed on 12th day; small mass of live worms; large clump of dead worms; caseous material present
1279	5	176	16	2	0	1	46	Mass of live worms
1281	5	192	8	0	0	0	6	Mass of live worms
1271	5	88	48	6	0	6	26	Mass of live worms
1269	5	148	4	0	0	-	-	Dead on 4th day; no autopsy
1258	5	380	204	14	6	4	38	Small mass of live worms
1299	5	324	52	3	0	3	36	Mass of live worms
1275	5	348	18	0	0	-	-	Dead on 4th day; no autopsy
1291	5	276	4	20	0	6	84	Mass of live worms
1300	5	260	80	8	4	-	-	Dead on 4th day; no autopsy

recurrence is related somewhat to the amount and frequency of dosage, although no predictions can be made in this respect. No definite relationship exists between the recurrence of microfilariae and the height of the initial count

(Table V), nor does the height of the initial count affect the rapidity with which the microfilariae disappear (Tables I and II).

The rapidity of the reduction of microfilariae is well illustrated in Table III which gives the results obtained when treatments were administered every two hours. Within two hours after the first oral dose of 25 mg. per kilogram had been administered all animals showed a reduction of over 90 per cent in circulating microfilariae. At the end of twelve hours of treatment (at 5, 10, and 25 mg. per kilogram) many of the rats exhibited no microfilariae in the peripheral blood.

Microfilariae from cotton rats and frogs undergo contortion and spasmodic contractions when placed in solutions of the drug. The microfilariae of *Folyella dolichoptera* from southern frogs[3] demonstrate this effect very well when placed in dilutions of 1:100, 1:1,000, and 1:10,000 of 1-diethylcarbamyl-4-methylpiperazine hydrochloride. These microfilariae are very large embryos with a narrow whiplike anterior and a thicker posterior end. When placed in contact with solutions of the drug, the anterior end of the embryos contracts immediately into a tight coil, and violent, jerky movements occur. They then straighten out and become completely motionless within five to fifteen minutes. The same effect can be observed upon frog microfilariae in vivo during treatment. Similar effects occur with the microfilariae of *Litomosoides carinii*, although to a lesser extent.

These observations, plus the fact that adult filariae removed from cotton rats in early stages of treatment show no demonstrable damage to the embryos in utero, lead us to believe that this compound acts directly upon the microfilariae in the peripheral blood.

TABLE IV. EFFECT OF 84-L ON THE MICROFILARIAE OF DIROFILARIA IMMITIS

DOG	DOSE (MG./KG.)	MICROFILARIAE PER 0.05 C.C. BLOOD — DAYS DURING TREATMENT								
		1	4	7	14	21	28	35	42	49
100	25 I.P., b.i.d.	896	41	30	1	6	3	-	-	-
221	25 Orally, t.i.d.	378	-	19	1	2	1	0	4	2
222	25 Orally, t.i.d.	735	-	29	0	0	2	1	1	2
260	25 Orally, t.i.d.	734	316	39	50	12	27	20	18	-
262	25 Orally, t.i.d.	235	265	-	32	14	4	-	-	-

In dogs infected with *Dirofilaria immitis*, 1-diethylcarbamyl-4-methylpiperazine hydrochloride produces marked reductions in the number of microfilariae during treatment. This effect is most striking in animals with high initial counts (Table IV). The rate of disappearance in dogs is not so rapid as in cotton rats, but within one or two weeks, more than a 90 per cent reduction generally occurs.

Effect of 1-Diethylcarbamyl-4-Methylpiperazine Hydrochloride Against Adult Filariae in Cotton Rats.—The effects of treatment with this drug on adult filariae in the cotton rat are not demonstrable so quickly as in the case of the microfilariae. It was apparent from the beginning of this work that demonstrable lethal effects on the adult worms involved a number of variables. The

most important of these have been found to be (1) the amount of drug given, (2) the frequency of dosage, and (3) the number of days elapsing from cessation of treatment to autopsy.

Autopsies performed on untreated rats revealed a small number of dead worms in nine of sixty-five (13.9 per cent). No evidence of massive deaths, adhesions, or large deposits of exudate were found in any of these animals, however, and the number of dead worms formed but a small percentage of the total number present.

TABLE V. COMPARISON OF TREATMENT THREE TIMES DAILY FOR FOURTEEN DAYS AND FOR THIRTY DAYS (84-L., 25 MG./KG.; COTTON RATS)

RAT	MICROFILARIAE PER 100 FIELDS, DAYS 1	7	14	21	28	35	42	51	AUTOPSY RECORD (42ND AND 52ND DAYS)
Treatment Three Times Daily for Fourteen Days									
904	18	0	0	6	1	14	18		Small mass of live worms; 4 dead worms
925	36	0	0	6	20	0	1		15 live worms; small clump of dead and disintegrating worms
913	40	0	0	6	34	56	112		Large mass of live worms; 3 dead worms
895	72	0	0	0	0	0	0		Approximately 30 worms, half of them dead and disintegrating
930	72	0	0	8	8	16	64		Small mass of live worms; 7 dead worms
921	96	1	0	2	0	2	10		Mass of live worms; none dead
905	116	0	1	6	12	42	56		Mass of live worms; none dead
637	156	0	0	1	0	0	0		Mass of live worms; small clump of dead worms
931	160	0	0	4	8	60	10		Large mass of live worms; approximately 20 dead worms
940	168	0	0	14	8	0	0		12 to 15 live worms; 5 to 8 dead worms
775	224	1	0	1	4	0	4		Small mass of live worms; none dead
936	288	6	1	30	38	48	112		Mass of live worms; several dead worms
924	292	0	0	40	140	172	60		Mass of live worms; none dead
Treatment Three Times Daily for Thirty Days									
1079	52	0	0	2	3	12		76	Mass of live worms; several clumps of dead worms
1050	72	0	0	0	0	0		6	Large mass of worms; majority dead
1051	96	1	0	0	0	0		0	Mass of live worms; small clump of dead worms
1089	112	2	2	1	1	0		48	Mass of live worms; small clump of dead worms
1074	124	6	0	0	0	0		0	Extensive adhesions; 1 live worm; 1 dead worm
1057	140	16	3	0	3	37		308	Large mass of live worms; none dead
1069	160	2	0	1	0	8		40	Mass of live worms; none dead
1054	208	0	1	1	3	10		44	Extensive adhesions; clump of dead worms; several living worms
1078	260	3	0	0	0	3		110	Mass of worms; approximately half of them dead
1093	264	2	2	0	0	4		24	Extensive adhesions; several live worms
1070	324	0	0	0	1	1		60	Several live and several dead worms
1094	440	0	0	0	0	4		96	Mass of live worms; one small clump of dead worms

Of 150 rats treated with 1-diethylcarbamyl-4-methylpiperazine hydrochloride, with doses of 10 mg. per kilogram or more, twice or more daily, 72 per cent showed either some or all adults dead at autopsy or no adult worms.

Table VIII presents in summary form the results of autopsies on 212 rats treated with doses of from 3.13 to 100 mg. kilogram twice or more daily for from two to four weeks. Both oral and intraperitoneal treatments have been included since there is no measurable difference in the effect produced by either of these routes of administration. In general, animals treated with less than 10 mg. per kilogram did not exhibit as many dead worms at autopsy as did those treated with 10 mg. or more per kilogram.

The frequency of dosage also influenced the rapidity with which adult worms were killed. In Table III, for example, dead worms were found on the twelfth day in many rats after dosage was administered every two hours for two days. Dosage three times daily has given more consistent results than dosage twice daily.

Dead worms have been found in animals treated for from two to four weeks, and, as illustrated in Table V, there was no real difference in the condition of the adult worms following these treatment periods. A more striking difference occurred in animals subjected to autopsy at different periods after cessation of treatment. This is illustrated in Table IX and by the comparison of Tables V and X. Of the thirty-eight rats for which data are given in Table X, only three (7.9 per cent) showed all living worms at autopsy. These rats were treated orally three times daily for thirty days with 25 mg. per kilogram, and were then held seventy-seven days before autopsy. All these animals showed relatively high initial microfilarial counts, but in many of them no worms were found at autopsy.

More frequently than not, in animals that showed a rapid and sustained reduction in microfilariae during and after treatment, most of the adult worms were dead or none were found at autopsy. This is illustrated in Tables VI and X. Exceptions did occur, however, as demonstrated in Table VII.

Effect of 1-Diethylcarbamyl-4-Methylpiperazine Hydrochloride Against Adult Filariae in Dogs.—Twenty-five filaria-infected dogs have been treated thus far with this compound or related derivatives. The results are encouraging in that the doses necessary to produce an effect against the microfilariae, and in some cases against the macrofilariae, do not produce signs of severe toxicity. Moreover, oral treatment is effective.

In Table XI, data are given from six dogs treated with intraperitoneal doses. In two of the dogs (Dogs 94 and 108) no adult worms were found in the heart, pulmonary artery, or lung at autopsy. Both of these animals were killed fifty days after the last treatment, and no microfilariae were found in the blood at the time of autopsy. It will be noted, however, that these animals exhibited relatively low initial embryo counts. It has been shown previously[2] that in many untreated dogs with low initial microfilarial counts the adult worms cannot be found at autopsy in any part of the body. A more certain criterion of cure, therefore, is the presence of dead worms in the terminal branches of the pulmonary artery.

TABLE VI. TREATED ANIMALS IN WHICH DEATH OR ABSENCE OF ADULT WORMS AT AUTOPSY WAS ASSOCIATED WITH CONSISTENTLY LOW EMBRYO COUNTS DURING AND AFTER TREATMENT

RAT	84-L (MG./KG.)	NUMBER OF DAYS TREATED	MICROFILIARIAE PER 100 FIELDS — DAYS 1	7	14	21	28	35	42	49	58	107	DAY OF AUTOPSY	AUTOPSY RECORD
481	3 I.P., b.i.d.	16	68	0	2	1	4	4					43	No worms found
488	3 I.P., b.i.d.	16	132	1	1	0	0	1	0				43	2 clumps of dead worms; none living
543	3 I.P., b.i.d.	30	60	0	0	0	0						31	Several dead worms; none living
629	3 I.P., b.i.d.	32	128	3	0	0	0	0	0	0	0		70	No worms found
617	25 I.P., b.i.d.	28	108	0	0	0	1	0	8	1	0		58	2 live worms; 2 dead worms
599	25 I.P., b.i.d.	28	64	0	0	0	0	0	4	1	1		58	4 live worms; 6 clumps of dead worms
593	25 I.P., b.i.d.	30	22	0	0	0	0	2	1				44	3 dead worms; none living
624	25 I.P., b.i.d.	30	88	0	2	1	0	3	4				44	2 dead worms; none living
1050	25 I.P., t.i.d.	30	72	0	0	0	0		6				52	Large clump of dead worms; several living worms
1074	25 I.P., t.i.d.	30	124	0	0	0	0		0				52	Extensive adhesions; 1 live worm; 1 dead
547	25 Orally, b.i.d.	30	26	0	0	0	0						33	Several dead worms; none living
925	25 Orally, t.i.d.	14	36	0	0	6	20	0	1				42	15 live worms; several small clumps of dead worms
895	25 Orally, t.i.d.	14	72	0	0	0	0	0	0				42	25 to 30 worms; half of them dead
940	25 Orally, t.i.d.	14	168	0	0	14	8	0	0				42	12 to 15 live worms; 5 to 8 dead worms
637	25 Orally, t.i.d.	14	156	0	0	1	0	0	0				42	Mass of live worms; several clumps of dead worms
1325	25 Orally, t.i.d.	30	140		0							0	107	2 small clumps of dead worms; none living
1402	25 Orally, t.i.d.	30	330		0							0	107	No worms found
1406	25 Orally, t.i.d.	30	820		0							0	107	No worms found
1409	25 Orally, t.i.d.	30	130		0							0	107	No worms found
1418	25 Orally, t.i.d.	30	124		0							0	107	No worms found
1425	25 Orally, t.i.d.	30	84		0							0	107	No worms found
1511	25 Orally, t.i.d.	30	160		0							0	107	No worms found
1540	25 Orally, t.i.d.	30	145		1							6	107	No worms found
1544	25 Orally, t.i.d.	30	86		0							0	107	No worms found
1553	25 Orally, t.i.d.	30	96		0							0	107	Two small clumps of dead worms; none living
1368	25 Orally, t.i.d.	30	56		0							0	107	No worms found
1403	25 Orally, t.i.d.	30	28		0							0	107	No worms found
1421	25 Orally, t.i.d.	30	210		0							0	107	No worms found
1414	25 Orally, t.i.d.	30	14		0							3	107	2 live worms; small clump of dead worms
1453	25 Orally, t.i.d.	30	36		0								58	1 live worm; clump of dead worms
634	50 Orally, b.i.d.	28	56	0	0	0	0	1	4	8	8		58	No worms found
611	50 Orally, b.i.d.	28	44	0	0	0	0	0	0	0	0		33	No worms found
569	50 Orally, b.i.d.	30	16	0	1	0	0						30	No worms found
623	50 Orally, b.i.d.	28	52	0	0	0	0						33	Several dead worms; none living
552	100 I.P., b.i.d.	30	36	0	0	0	0							

TABLE VII. TREATED ANIMALS IN WHICH LOW EMBRYO COUNTS AT AUTOPSY DID NOT SIGNIFY DEATH OF THE MAJORITY OF ADULT WORMS

RAT	84-L (MG./KG.)	NUMBER OF DAYS TREATED	MICROFILARIAE PER 100 FIELDS — DAYS									DAY OF AUTOPSY	AUTOPSY RECORD
			1	7	14	21	28	35	42	49	58		
484	3 I.P., b.i.d.	16	224	8	6	7	0	0				36	Several live worms
486	3 I.P., b.i.d.	16	236	2	0	0	1	0				36	Several live worms
487	3 I.P., b.i.d.	16	204	6	4	16	24	64			0	58	Several live worms
480	6¼ Orally, b.i.d.	15	46	10	1	1	2	0		0		49	Mass of live worms
908	10 Orally, t.i.d.	14	408	1	0	6	24	10	8			42	Mass of live worms; 4 dead worms
917	10 Orally, t.i.d.	14	1,260	0	1	0	6	1	0			42	Mass of live worms; small clump of dead worms
548	25 I.P., b.i.d.	30	184	0	0	0	0	0				35	Mass of live worms
921	25 Orally, t.i.d.	14	96	1	0	2	0	2	10			42	All worms alive
775	25 Orally, t.i.d.	14	224	1	0	1	4	0	4			42	All worms alive
1051	25 Orally, t.i.d.	30	96	1	0	0	0	0		0		52	Large mass of live worms; 1 small clump of dead worms
621	50 Orally, b.i.d.	28	608	0	0	0	0	0	0			42	Large mass of live worms
555	50 Orally, b.i.d.	30	100	0	1	0	0	0				35	Mass of live worms

TABLE VIII. EFFECT OF DIFFERENT DOSES OF 84-L ON ADULT FILARIAE IN COTTON RATS, AS COMPARED WITH NONTREATED CONTROLS

DOSE* (MG./KG.)	NUMBER OF RATS	CONDITION OF ADULT WORMS AT AUTOPSY			
		ALL ALIVE (%)	LESS THAN 50 PER CENT DEAD (%)	MORE THAN 50 PER CENT DEAD (%)	ALL DEAD OR NONE FOUND (%)
3.13	34	63.2	14.7	8.8	13.3
5 and 6¼	28	78.7	14.3	7.0	0.0
10 and 12½	27	40.7	48.1	7.4	3.8
25	104	24.0	42.3	15.3	18.4
50 and 100	19	31.5	15.7	26.3	26.5
Total	212	40.5	33.9	13.2	12.4
Nontreated controls	65	86.1	13.9	0.0	0.0

*Including treatments twice or more daily for from two to four weeks. Autopsies were performed at varying periods of time after cessation of treatment.

TABLE IX. EFFECT OF 84-L AGAINST FILARIASIS IN COTTON RATS (AUTOPSIES AT FOUR-, SIX-, AND EIGHT-WEEK PERIODS; TREATMENT FOR FOUR WEEKS)

RAT	DOSE (MG./KG., B.I.D.)	MICROFILARIAE PER 100 FIELDS, WK.									WEEK OF AUTOPSY	AUTOPSY RECORD
		0	1	2	3	4	5	6	7	8		
623	50 Orally	52	0	0	0	0					4	No worms found
601	25 I.P.	34	1	0	1	0					4	2 live worms; clump of dead and deteriorated worms
600	25 I.P.	64	0	3	1	0					4	Mass of worms, approximately 50% dead
602	25 I.P.	40	2	1	0						3	Several live and several dead worms
606	50 Orally	52	1	0	0	0					4	Several live and several dead worms
607	50 Orally	108	2	0	0	0					4	Several live and several dead worms
615	25 I.P.	22	1	2	1	4					4	Mass of live worms
619	50 Orally	288	1	1	0	0					4	Mass of live worms
593	25 I.P.	22	0	0	0	0	2	1			6	3 dead worms; none living
624	25 I.P.	88	0	2	1	0	3	4			6	2 dead worms; none living
592	25 I.P.	52	0	1	0	0	0	14			6	1 live worm; none dead
620	50 Orally	104	0	1	3	0	4	10			6	6 live worms
621	50 Orally	608	0	0	0	0	0	0			6	Mass of live worms
626	50 Orally	224	0	0	0	1	4	28			6	Mass of live worms
611	50 Orally	44	0	0	0	0	0	0	0	0	8	No worms found
634	50 Orally	56	0	0	0	0	1	4	8	8	8	1 live worm; clump of dead worms
616	25 I.P.	152	1	1	1	0	5	4	3	72	8	2 live worms; mass of dead worms
599	25 I.P.	64	0	0	0	0	0	4	1	1	8	4 live worms; mass of dead worms
618	50 Orally	68	0	0	0	0	6	6	14		8	1 live worm; several dead worms
590	50 Orally	52	1	2	0	0	4	12	28	18	8	Several live worms; several dead worms
617	25 I.P.	108	0	0	0	1	0	8	1	0	8	2 live worms; 2 dead worms
608	50 Orally	100	0	1	1	0	32	22	72	68	8	Several live worms; none dead

TABLE X. EFFECT OF 84-L AGAINST FILARIASIS IN COTTON RATS (ORAL TREATMENT, 25 MG. PER KILOGRAM, THREE TIMES DAILY FOR THIRTY DAYS)

RAT	MICROFILARIAE PER 100 FIELDS, DAYS 1	DAYS 15	DAYS 107	AUTOPSY RECORD (DAY 107, 77 DAYS AFTER LAST TREATMENT)
1406	820	0	0	No worms found
1511	160	0	0	No worms found
1540	145	1	0	No worms found
1544	86	0	6	No worms found
1553	96	0	0	No worms found
1403	28	0	0	No worms found
1421	210	0	0	No worms found
1414	14	0	0	No worms found
1402	330	0	0	No live worms found; probable remnants of dead worms
1409	130	0	0	No live worms found; probable remnants of dead worms
1418	124	0	0	No live worms found; probable remnants of dead worms
1425	84	0	0	No live worms found; some adhesions
1325	140	0	0	No live worms found; two small clumps of dead worms
1368	56	0	0	No live worms found; two small clumps of dead worms
1410	170	1	24	1 live worm; 1 dead worm; probable remnants of other dead worms
1453	36	0	3	2 live worms; small clump of dead worms
1539	200	1	36	5 or 6 live worms; small clump of dead worms
1312	500	1	120	Small mass of live worms; two small clumps of dead worms
1419	340	0	260	Small mass of live worms; 2 or 3 dead worms
1434	370	1	48	Mass of live worms; two small clumps of dead worms
1442	280	0	72	Large mass of live worms; two small clumps of dead worms
1457	400	0	310	Several live worms; several dead and disintegrated worms
1444	320	0	440	Large mass of live worms; one small clump of dead worms
1474	410	0	56	Large mass of live worms; small clump of dead worms
1498	260	0	220	Large mass of live worms; small clump of dead worms
1480	200	2	280	Large mass of live worms; three clumps of dead worms
1513	180	0	14	7 live worms; 2 or 3 dead worms
1525	280	0	400	Mass of live worms; two clumps of dead worms
1536	440	1	96	Small mass of live worms; small clump of dead worms
1424	120	0	72	Small mass of live worms; two small clumps of dead worms
1528	900	0	480	Mass of live worms; two large clumps of dead worms
1530	540	0	112	Mass of live worms; small clump of dead worms
1508	740	0	390	Large mass of live worms; none dead
1512	36	0	0	3 live worms; none dead
1388	440	0	92	2 live worms; none dead
1395	96	1	112	3 live worms; 1 dead worm
1408	560	0	68	2 live worms; 2 dead worms
1478	320	0	48	Small mass of live worms; small clump of dead worms

In three dogs (Table XI, Dogs 101, 95, and 71) no worms were found in the heart at autopsy, but living worms were recovered from various portions of the pulmonary artery, some in the terminal branches. The remaining dog (Table XI, Dog. 100) was killed relatively soon after cessation of treatment because of a severe case of distemper. Live adults were found in the heart of this animal, but others occurred in the pulmonary artery at various levels.

Data from ten dogs treated orally with 84-L are given in Table XII. In two of these dogs (Dogs 191 and 193) no adult worms were found at autopsy. Marked reductions in microfilariae occurred in all animals with high initial counts, and in three dogs (Dogs 222, 260, and 261) many dead worms were found in the terminal lobes of the lungs. Extensive areas of infarction surrounded the plugged vessels. Upon section,* worms in various stages of disintegration were found.

Dog 225 died after two doses of the drug had been administered. This animal had a very high initial microfilarial count, was severely emaciated, and coughed persistently before and during treatment. Within a short time after the first dose was given it was noted that the animal breathed with difficulty and was in considerable distress. It was found dead in the cage shortly after the second dose had been given. At autopsy a large mass of worms was found in the pulmonary artery near the heart, and these completely plugged the vessel. Since this is the only instance in which this condition occurred we are not convinced that it was due to the administration of the drug. It is quite possible, however, that a simultaneous migration of this large mass of worms from the right ventricle to the pulmonary artery, caused by the presence of the drug in the circulatory system, might have caused the death of the animal.

In addition to the effects produced by the drug on the filariae in dogs, it is of interest that most dogs placed under treatment improved markedly in physical appearance. Most of the dogs when received were emaciated, sickly, and unkempt. Good care and adequate food probably accounted for some of their subsequent improvement, but even in dogs with high initial microfilarial counts, a marked gain in weight and disappearance of symptoms which might be attributed to the presence of Dirofilaria took place during and after treatment.

As yet, too few dogs have been used to determine an optimum treatment schedule, but it seems apparent that frequent administration of the drug for several weeks is desirable. Toxicity in dogs with therapeutic doses has thus far been limited to occasional transient nausea.

DISCUSSION

The effect produced against microfilariae in cotton rats and dogs by 1-diethylcarbamyl-4-methylpiperazine hydrochloride is certainly more rapid than that reported for any other known compound. Moreover, to our knowledge, this is the first compound shown to produce this effect in experimental animals when administered by the oral route. No data have been obtained thus far with regard to the rate of absorption and excretion of this com-

*Sections prepared and studied by Dr. Frederick Dessau.

pound, but indirect evidence denotes that it is absorbed rapidly when given either orally or parenterally. The better effects produced by frequent dosage indicate that it either is excreted rapidly or is degraded in the body.

Studies are now in progress regarding the fate of the embryos once they are removed from the peripheral circulation.

The failure of this compound to kill all adult worms in all animals treated may be due in part to individual variations in the absorption of the drug. Furthermore, the state of maturity of adult worms, or the number of worms present in the pleural cavity, may influence the effectiveness of treatment. There is no question concerning the more rapid lethal effect of parenteral doses of several antimony derivatives and cyanine dyes[4-7] against adult worms in cotton rats. We believe it important, however, that for the first time an organic, nonmetallic compound has been found which produces a very marked effect against filariasis in experimental animals when given by mouth.

It should be pointed out that many different dosage regimes have been included in the 212 treated rats for which summary data are given in Table VIII. The best results, as shown in Table X, have been obtained by treating three times daily for thirty days, and then holding the animals for two months before autopsy. In many of the rats treated by this method, no adult worms were found at autopsy, in spite of the fact that high microfilarial counts were present before treatment was initiated. In other rats in this series, varying numbers of dead adult worms were found at autopsy, and in three rats all adult worms were alive. These data, plus the fact that microfilariae sometimes do and sometimes do not recur in the peripheral blood after cessation of treatment demonstrate that a number of unknown variables probably influence the effectiveness of treatment.

The mode of action of this compound against micro- or macrofilariae in the cotton rat is not known. Worms have been found in various stages of decay and disintegration within the pleural cavity after treatment. Worms recovered shortly after death occurred showed no movement when placed in physiologic saline, but no external or internal changes in the appearance of these worms could be noted. Living or dead embryos occurred within the uteri. In other cases, dead worms were found enveloped in caseous, purulent material such as has been described by Culbertson and Rose[4] following treatment with Neostibosan and Neostam. In very late stages of decay the worms occurred in tight clumps, were usually embedded in a firm, yellowish mass, and were fragmented. In the many cases where no worms were found, the chest cavity was clean and no evidence of fragments of dead worms was found. We have assumed that the dead worm tissue is eventually absorbed.

In some cases where large masses of dead worms were found, extensive adhesions of the heart and lung to the walls of the pleural cavity occurred.

The data obtained thus far from filaria-infected dogs treated with 1-diethylcarbamyl-4-methylpiperazine hydrochloride suggest that this drug may prove useful in the treatment of this infection. As in the case of the cotton rats, individual variations occurred in the susceptibility of the worms to treatment. Emphasis has not been placed upon treated cases in which no adult worms

TABLE XI. RESULTS OF TREATMENT OF DIROFILARIA-INFECTED DOGS WITH INTRAPERITONEAL INJECTIONS OF 84-L

DOG	DOSE (MG./KG.)	MICROFILARIAE PER 0.05 C.C. WEEK 0	1	2	3	4	5	6	7	8	9	10	MICROFILARIAE (LAST DAY BEFORE AUTOPSY)	AUTOPSY (DAYS AFTER LAST TREATMENT)	AUTOPSY RECORD
100	25 I.P., b.i.d., for 14 days	896	30	1	6	3							3	16	Killed because of severe distemper; 36 live worms; several in heart, others in pulmonary artery at various levels
101*	50 I.P., b.i.d., for 13 days	354	16	1	13	11	21		62	67			67	41	No worms in heart; 15 live worms in pulmonary artery near heart; 10 live worms in pulmonary vessels in center of lung
95*	50 I.P., b.i.d., for 13 days	130	12	63									63	2	3 live worms in pulmonary artery; 1 live worm in lung, nearly at terminus of blood vessel
94	50 I.P., b.i.d., for 9 days 100 I.P., b.i.d., for 2 days	8	0	2	0	0	3	1			0		0	50	No worms found
108	50 I.P., b.i.d., for 9 days 100 I.P., b.i.d., for 2 days	28	10	18	1	5					0		0	50	No worms found
71	50 I.P., b.i.d., for 13 days	22	5	19	7	11	7	18			38		38	50	No worms in heart; small clump of live worms (3 or 4) in terminal pulmonary vessel

*Treated several weeks previously with 25 mg. per kilogram b.i.d. for fifteen days. Microfilariae count reduced, but relapsed upon cessation of treatment.

TABLE XII. RESULTS OF TREATMENT OF DIROFILARIA INFECTED DOGS WITH ORAL DOSES OF SN L

DOG	DOSE (MG./KG.)	MICROFILARIAE PER 0.05 C.C. WEEK 0	1	2	3	4	5	6	7	8	9	10	MICROFILARIAE (LAST DAY BEFORE AUTOPSY)	AUTOPSY (DAYS AFTER LAST TREATMENT)	AUTOPSY RECORD
191	25 Orally, t.i.d., for 23 days	1	0	0	3	0	0	0	1	0		0	0	49	No worms found
193	25 Orally, t.i.d., for 23 days	18	2	0	0	1	1	1	2	1		1	1	49	No worms found
194	25 Orally, t.i.d., for 23 days	7	22	5	2	0	2	9	3	2		0	0	49	No worms in heart; one pair of live worms in pulmonary artery
221	25 Orally, t.i.d., for 64 days	378	19	1	2	1	0	4	2	1	0		0	1	No worms in heart; 15 living worms in pulmonary artery and in lung
222	25 Orally, t.i.d., for 64 days	735	29	0	0	2	1	1	2	2	1	1	1	17	No worms in heart; 2 live worms in pulmonary artery; 4 "cysts" in terminal lobe of lung; upon section these were found to contain worms, with various stages of organization of the thrombus; small hemorrhagic infarcts present
225	25 Orally, 2 doses	1,613											-	-	Animal died in afternoon of first treatment day; large mass of live worms in heart and plugged in pulmonary artery; labored breathing before and during treatment
260	25 Orally, t.i.d., for 60 days	734	39	50	12	27	20	18	20	11	2	8	16	30	10 live worms in heart; 10 live worms in pulmonary artery; 2 live worms in lung; lungs show extensive infarction; many worms found in terminal arteries with thrombus formation
262	25 Orally, t.i.d., for 58 days	235	265	32	14	4	26	16	5	11	134	50	172	30	No worms in heart; 9 live worms in pulmonary artery; no macroscopic lesions in lungs
259*	35 Orally, t.i.d., for 27 days	232	17	14	7	3	3						3	4	8 live worms in heart; none found in lung; no lesions found in lung
261*	50 Orally, b.i.d., for 27 days	209	58	124	88	75	43						43	4	3 worms in heart; 1 live worm in pulmonary artery; massive hemorrhage and infarction in lower quadrant of lower right lobe of lung; several hard cystlike structures containing remnants of dead worms; living and dead embryos within these remnants

*Treated several weeks previously with 30 mg. per kilogram of compound 180-C, orally, t.i.d. for forty-seven days. No sharp reduction in microfilariae occurred.

were found at autopsy, since it is well known[2] that adult worms cannot always be found in untreated dogs which show microfilariae in the blood stream, particularly when the microfilariae are few in number. It is believed important, however, that in three dogs with relatively high initial microfilaria counts large number of dead adult worms were found in the terminal blood vessels of the lung following treatment. These dogs showed marked physical improvement during the course of their treatment; all gained weight and the microfilariae had been reduced rapidly in number. A large series of cases has now been put under observation in order to determine an optimum treatment schedule. It is emphasized again that no disturbing toxic symptoms have occurred during treatment, even though the drug was administered in some cases three times daily for two months. The one death which occurred after two doses had been administered was that of a dog with a very heavy worm burden, and this dog had been in obvious distress several days before treatment was started.

The use of piperazines therapeutically in man is not new, although the use to which they have been put previously has largely been abandoned. Hanzlik[8] gives a summary of the literature on the use of piperazine hexahydrate and several similar compounds as urate solvents. Doses as high as 6 Gm. of piperazine hexahydrate have been administered to human subjects without undesirable effects. Other investigators mentioned by Hanzlik, however, report that large doses produce headache, clonic spasms of the extremities, muscular prostration, incoordination, tremor, malaise, and nausea. Piperazine hexahydrate has been reported by several investigators[8] to be rapidly absorbed and excreted by human patients.

SUMMARY

Data are presented which show the effects produced by 1-diethylcarbamyl-4-methylpiperazine hydrochloride on natural infections with filariae in cotton rats and dogs. An immediate and sustained reduction in microfilariae is produced in cotton rats following oral or parenteral administration of the drug. The effect produced against microfilariae in dogs is less rapid but is substantially the same.

Various factors which influence the effects produced on the adult worms in cotton rats are discussed. The maximum number of adult worms are killed when treatment is administered at frequent daily intervals for a period of three weeks or longer, with doses of 10 mg. or more per kilogram.

It is demonstrated that in some Dirofilaria-infected dogs treated with the compound the worms were not present in the heart; but were located in terminal branches of the pulmonary artery; and in three dogs, many worms were dead and surrounded by areas of hemorrhage and infarction.

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