Brit. J. Pharmacol. (1955), **10**, 191.

THE SCHISTOSOMICIDAL ACTIVITY OF SYMMETRICAL DIAMINODIPHENOXYALKANES

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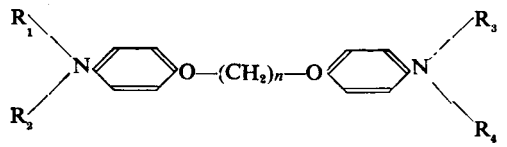
(RECEIVED JANUARY 3, 1955)

After 1917, when tartar emetic was first used in the treatment of schistosomiasis (Christopherson, 1918), little advance was made in the development of schistosomicides until the lucanthone (miracil) series was synthesized by Mauss (1948). These were the first and only non-antimonial compounds shown to possess significant activity against *Schistosoma mansoni* in the mouse (Kikuth, Gönnert, and Mauss, 1946; Gönnert, 1947; Kikuth and Gönnert, 1948, 1949). They were inactive against *S. japonicum* in experimental animals (Vogel and Minning, 1948).

In November, 1952, we discovered schistosomicidal activity in a chemical type not previously known to possess this property. The compound 1:5-bis(*p*-methylaminophenoxy)pentane dihydrochloride (8C51) was shown to possess marked activity against *S. mansoni* in experimentally infected mice. This discovery stimulated the synthesis of many related compounds with the general formula:



where *n* and R were subject to considerable variation. Of these, nearly 300 were found to possess some activity, many being much more active than the original compound. This paper, the first of a series, describes compounds in which $R_1R_2=R_3R_4$. A preliminary announcement of the discovery of schistosomicidal activity in the series has already been made (Raison and Standen, 1954; Wellcome Foundation, 1954a, b; Burroughs Wellcome & Co., Australia, 1954).

MATERIALS AND METHODS

Drugs were tested mainly against an Egyptian strain of *S. mansoni* that had been passaged for several years through *Australorbis glabratus*. A Brazilian strain of *S. mansoni* was employed as a check against possible hyper-susceptibility of this long-established strain. *S. japonicum*, passaged through *Oncomelania hupensis*, was used to check against drug-specificity to *S. mansoni*. No tests were made against *S. haematobium*. The white mouse was selected as the most suitable animal for routine tests, but small-scale experiments were also made on guinea-pigs and rabbits.

All animals were infected by the percutaneous route. Each mouse was exposed to 130 cercariae as described by Standen (1949, 1953). Guinea-pigs, under pentobarbitone anaesthesia, were exposed to *S. japonicum* by smearing the shaven belly with an aqueous suspension of 300 cercariae. Rabbits, under pentobarbitone anaesthesia, were infected with *S. mansoni* by exposure to approximately 500 cercariae by insertion of one ear into a cercarial suspension contained in a boiling-tube. Determination of infection in mice was made by identification of viable eggs in the faeces 56 days after exposure to cercariae. Since guinea-pigs do not pass *S. japonicum* eggs in the faeces the presence of infection was established by observation of eggs in mucosa taken at rectal biopsy. In rabbits, which also do not pass eggs in the faeces, the presence of schistosomes was assumed and confirmation made at autopsy.

All drugs were given orally twice daily for 5 days. Any deviation from this standard dosage is specifically indicated, as in single-dose experiments. Drugs were given, in the first instance, at the maximum tolerated dose level or at 200 mg./kg., whichever was the smaller. Where such dosage killed all or some of the schistosomes the experiment was repeated at reduced doses to determine the limits of activity. The animals were killed and examined 7 days after completion of treatment. Schistosomicidal activity was assessed by recording the shift of the worms from the mesenteric veins to the liver, as first described by Schubert (1948) and modified by Standen (1953). In addition it was possible to provide a more precise comparison of activity by noting the proportion of worms *killed* in mice treated with different but related compounds. Whilst the change in worm distribution contributed much to the estimation of the progress of drug action the final assessment of activity was always made on

the proportion of worms killed. Ten mice were used in each test and the activity of a drug was estimated in terms of the total number of worms found in the group. Worms were considered dead only when completely ensheathed with inflammatory tissue and in process of disintegration.

The preparation of the compounds mentioned in this paper followed established chemical procedures (Wellcome Foundation, 1954a, b; Burroughs Wellcome & Co., Australia, 1954).

Results

Routine Examination of Compounds

The detailed results of the routine examination of compounds for schistosomicidal activity are given in Table I. The term "unit dose" refers to the dose given orally twice daily for 5 days and is therefore one-tenth of the total dose. This table is concerned only with the variation of schistosomicidal activity by symmetrical substitution at both ends of the molecule and by altering the length of the methylene chain; unsymmetrically substituted diaminodiphenoxyalkanes will be dealt with in a further communication. The compounds tested have been grouped according to the variations, listed below, in the general formula shown in Table I.

(i) *Primary amines*: $R_1=R_2=H$ (Table I, A).

(ii) *Secondary amines*: $R_1=H$; $R_2=CH_3$, C_2H_5, *n*-C_3H_7, *iso*-C_3H_7, C_3H_5 (allyl), *n*-C_4H_9, *iso*-C_4H_9, *n*-C_5H_{11} (Table I, B).

(iii) *Tertiary amines*: $R_1=R_2=CH_3$, C_2H_5, *n*-C_3H_7, C_3H_5, *n*-C_4H_9; $R_1=CH_3$, $R_2=C_2H_5$; $R_1=CH_3$, $R_2=$*n*-C_3H_7, (Table I, C).

(iv) Where necessary the range of values of *n* from 1 to 11 has been covered.

All these compounds have the amino substituents in the *para* positions. Several representatives of the corresponding *meta* and *ortho* series were inactive. The quaternary ammonium ($\dot{N}Me_3$) salts of all three series were also inactive.

Comparative Activity of a Diaminodiphenoxyalkane, Lucanthone and Tartar Emetic

Because of the obviously high activity of the diaminodiphenoxyalkanes, comparative figures were sought for the activity of lucanthone and tartar emetic following single and multiple oral doses. Since many of the diaminodiphenoxyalkanes have a similar order of activity (Table I), the bis-(methylaminophenoxy)heptane (413C52) was selected for these comparative tests. All tests were carried out with mice in groups of 10/dose; most

Table I

STRUCTURES OF DIPHENOXYALKANES AND THEIR SCHISTOSOMICIDAL ACTIVITIES

M.V., mesenteric veins; P.V., portal vein; L., intrahepatic veins. For explanation of "unit dose" see Results.

Compound No.	*n*	R_1	R_2	Unit Dose (mg./kg.)	Worm Distribution % M.V.	P.V.	L.	Worms Killed %
A. Primary Amines								
76T52	2	H	H	200	69	28	3	2
333C50	3	H	H	200	0	0	100	100
				50	47	17	36	36
				25	64	32	4	0
				15	65	35	0	0
334C50	4	H	H	200	0	1	99	99
				50	16	5	79	66
				25	47	28	25	17
				15	74	23	3	0
373C50	5	H	H	200	0	0	100	100
				50	11	0	89	78
				25	44	50	6	3
				15	73	25	2	0
335C50	6	H	H	200	0	0	100	100
				50	1	0	99	99
				25	13	12	75	75
				15	76	19	5	3
153C51	7	H	H	50	0	0	100	100
				25	0	3	97	96
				15	36	20	44	42
154C51	8	H	H	200	0	0	100	100
				50	0	0	100	100
				25	0	6	94	94
				15	56	9	35	32
25C53	9	H	H	50	0	4	96	96
				25	15	13	72	72
				15	49	19	32	29
384C51	10	H	H	200	65	27	8	0
228C53	11	H	H	200	—	—	—	Toxic at this dose
				50	78	22	0	0
B. Secondary Amines								
580C54	1	H	CH_3	200	66	22	12	0
196C54	2	H	CH_3	200	38	11	51	51
				50	70	30	0	0
410C52	3	H	CH_3	200	0	0	100	100
				50	17	21	62	57
				25	63	32	5	0
411C52	4	H	CH_3	200	0	0	100	100
				50	0	2	98	98
				25	40	15	45	36
8C51	5	H	CH_3	200	0	1	99	99
				50	0	1	99	99
				25	31	12	57	55
412C52	6	H	CH_3	50	0	0	100	100
				25	7	3	90	90
413C52	7	H	CH_3	50	0	0	100	100
				25	3	0	97	97
				15	46	13	41	41
414C52	8	H	CH_3	50	0	0	100	100
				25	6	3	91	91
				15	26	17	57	56

TABLE I—*continued*

Compound No.	*n*	R_1	R_2	Unit Dose (mg./kg.)	Worm Distribution % M.V.	P.V.	L.	Worms Killed %
26C53	9	H	CH_3	50	0	0	100	100
				25	10	13	77	77
				15	54	28	18	18
415C52	10	H	CH_3	200	40	19	41	39
				50	53	37	10	0
				25	60	35	5	0
229C53	11	H	CH_3	200	87	13	0	Toxic at this dose
222C54	2	H	C_2H_5	200	25	8	67	67
				50	67	31	2	0
20T53	3	H	C_2H_5	200	0	0	100	100
				50	73	23	4	4
				25	69	29	2	0
90C54	4	H	C_2H_5	50	0	0	100	100
				25	18	4	78	78
201C53	5	H	C_2H_5	200	0	0	100	100
				50	5	2	93	86
				25	50	42	8	0
115C53	6	H	C_2H_5	50	0	0	100	100
				25	34	28	38	38
140C53	7	H	C_2H_5	50	0	0	100	100
				25	1	3	96	96
				15	40	33	27	26
124C53	8	H	C_2H_5	50	0	1	99	99
				25	7	3	90	90
				15	2	0	98	98
215C53	9	H	C_2H_5	50	3	2	95	95
				25	46	13	41	39
223C54	10	H	C_2H_5	200	2	4	94	94
				50	73	26	1	0
411C54	11	H	C_2H_5	200	—	—	—	Toxic at this dose
				50	58	32	10	0
234C54	2	H	n-C_3H_7	200	42	49	9	0
				50	66	31	3	0
235C54	3	H	n-C_3H_7	200	5	0	95	95
				50	69	25	6	0
91C54	4	H	n-C_3H_7	200	0	0	100	100
				50	17	4	79	79
				25	48	51	1	0
263C53	5	H	n-C_3H_7	200	0	0	100	100
				50	70	11	19	15
164C53	6	H	n-C_3H_7	200	25	9	66	66
				50	61	20	19	16
165C53	7	H	n-C_3H_7	50	1	2	97	97
				25	69	22	9	7
166C53	8	H	n-C_3H_7	200	4	0	96	96
				50	7	7	86	86
				25	63	29	8	0
271C53	9	H	n-C_3H_7	200	0	0	100	100
				50	29	29	42	35
266C54	10	H	n-C_3H_7	200	65	31	4	0
				50	56	39	5	0
546C54	3	H	*iso*-C_3H_7	200	70	28	2	0
269C54	4	H	*iso*-C_3H_7	200	12	0	88	88
				50	70	26	4	1
282C54	5	H	*iso*-C_3H_7	200	0	1	99	99
				50	75	23	2	0

TABLE I—*continued*

Compound No.	*n*	R_1	R_2	Unit Dose (mg./kg.)	Worm Distribution % M.V.	P.V.	L.	Worms Killed %
183C53	6	H	*iso*-C_3H_7	200	43	51	6	1
				50	72	27	1	0
221C53	7	H	*iso*-C_3H_7	200	53	30	17	17
				50	77	22	1	0
194C53	8	H	*iso*-C_3H_7	200	56	33	11	11
				50	68	30	2	0
288C54	4	H	C_3H_5	200	0	0	100	100
				50	51	22	27	27
3C54	6	H	C_3H_5	50	25	32	43	43
305C53	8	H	C_3H_5	100	0	0	100	100
				50	6	8	86	86
				25	28	19	53	53
593C54	2	H	n-C_4H_9	200	56	40	4	0
579C54	3	H	n-C_4H_9	200	53	34	13	10
268C54	4	H	n-C_4H_9	200	11	8	81	81
				50	66	26	8	0
296C54	5	H	n-C_4H_9	200	18	18	64	58
				50	71	21	8	0
177C53	6	H	n-C_4H_9	200	74	26	0	0
				50	65	20	15	0
178C53	7	H	n-C_4H_9	200	43	31	26	26
				50	64	33	3	0
179C53	8	H	n-C_4H_9	200	59	30	11	3
				50	67	33	0	0
592C54	3	H	*iso*-C_4H_9	200	79	14	7	6
567C54	4	H	*iso*-C_4H_9	200	49	14	37	37
568C54	5	H	*iso*-C_4H_9	200	68	21	11	11
327C53	6	H	*iso*-C_4H_9	200	45	44	11	0
328C53	7	H	*iso*-C_4H_9	200	39	12	49	49
329C53	8	H	*iso*-C_4H_9	200	55	39	6	0
591C54	4	H	n-C_5H_{11}	200	57	29	14	12
273C53	6	H	n-C_5H_{11}	200	65	32	3	0
				50	63	35	2	0
274C53	7	H	n-C_5H_{11}	200	62	37	1	0
				50	78	19	3	0
275C53	8	H	n-C_5H_{11}	200	58	32	10	0
				50	55	43	2	0
C. Tertiary Amines								
195C54	2	CH_3	CH_3	200	47	35	18	15
416C52	3	CH_3	CH_3	200	0	1	99	99
				50	54	39	7	0
				25	70	23	7	0
417C52	4	CH_3	CH_3	200	8	7	85	85
				50	25	15	60	60
				25	40	28	32	32
70C51	5	CH_3	CH_3	200	0	0	100	100
				50	2	2	96	96
				25	27	27	46	46
418C52	6	CH_3	CH_3	200	7	9	84	84
				50	19	17	64	64
				25	32	26	42	42
27C53	7	CH_3	CH_3	50	0	0	100	100
				25	7	0	93	93
				15	62	25	13	10

TABLE I—*continued*

Compound No.	*n*	R_1	R_2	Unit Dose (mg./kg.)	Worm Distribution % M.V.	P.V.	L.	Worms Killed %
419C52	8	CH_3	CH_3	200 50 25	28 48 52	25 16 31	47 36 17	46 31 17
50C53	9	CH_3	CH_3	200 50 25	0 0 44	0 8 36	100 92 20	100 92 20
420C52	10	CH_3	CH_3	200 50 25	47 76 72	34 22 23	19 2 5	0 0 0
114C54	11	CH_3	CH_3	200	56	38	6	Toxic at this dose
401C54	2	C_2H_5	C_2H_5	200 50	43 62	22 36	35 2	35 0
116C54	3	C_2H_5	C_2H_5	200 50	0 59	0 38	100 3	100 0
407C53	4	C_2H_5	C_2H_5	200 50	0 0	0 0	100 100	100 100
162C53	5	C_2H_5	C_2H_5	200 50	0 18	2 16	98 66	98 66
82C53	6	C_2H_5	C_2H_5	200 50	0 22	0 11	100 67	100 62
296C53	7	C_2H_5	C_2H_5	200 50 25	0 2 50	0 1 19	100 97 31	100 97 31
306C53	8	C_2H_5	C_2H_5	200 50	0 10	0 8	100 82	87 82
344C53	9	C_2H_5	C_2H_5	200 50	0 50	6 40	94 10	94 8
402C54	10	C_2H_5	C_2H_5	200 50	27 62	9 31	64 7	64 3
598C54	3	CH_3	C_2H_5	50 25	47 56	41 35	12 9	12 4
118C54	4	CH_3	C_2H_5	50 25	3 4	0 10	97 86	97 86
198C53	5	CH_3	C_2H_5	50 25	0 5	3 5	97 90	97 90
123C53	6	CH_3	C_2H_5	50 25	0 24	0 21	100 55	100 54
395C53	7	CH_3	C_2H_5	50 25	0 0	0 5	100 95	100 95
396C53	8	CH_3	C_2H_5	50 25	0 1	0 0	100 99	100 99
397C53	9	CH_3	C_2H_5	50 25	8 56	2 26	90 18	90 15
589C54	3	n-C_3H_7	n-C_3H_7	200	64	21	15	5
291C54	4	n-C_3H_7	n-C_3H_7	200 50	45 55	10 38	45 7	45 0
173C53	5	n-C_3H_7	n-C_3H_7	200 50	60 77	40 23	0 0	0 0
126C53	6	n-C_3H_7	n-C_3H_7	200 50	33 54	27 39	40 7	39 0
297C53	7	n-C_3H_7	n-C_3H_7	200 50	48 49	28 45	24 6	23 0
307C53	8	n-C_3H_7	n-C_3H_7	200 50	56 67	41 31	3 2	0 0
569C54	3	C_3H_5	C_3H_5	200	68	30	2	0
267C54	4	C_3H_5	C_3H_5	200	21	27	52	52

TABLE I—*continued*

Compound No.	*n*	R_1	R_2	Unit Dose (mg./kg.)	Worm Distribution % M.V.	P.V.	L.	Worms Killed %
283C54	5	C_3H_5	C_3H_5	200	19	33	48	45
298C53	6	C_3H_5	C_3H_5	200	64	31	5	0
323C53	7	C_3H_5	C_3H_5	200	66	26	8	7
299C53	8	C_3H_5	C_3H_5	200	63	35	2	0
406C53	7	CH_3	n-C_3H_7	200 100 50	2 32 49	5 20 29	93 48 22	92 47 8
590C54	4	n-C_4H_9	n-C_4H_9	200	74	22	4	0
300C53	7	n-C_4H_9	n-C_4H_9	200	69	31	0	0
308C53	8	n-C_4H_9	n-C_4H_9	200	73	25	2	0

groups were infected with *S. mansoni*, a few with *S. japonicum*. The results are shown in Table II.

Single Oral Dose.—The comparative activity of these three compounds when given as a single oral dose is represented graphically (Fig. 1).

A dose of 160 mg./kg. of 413C52 killed all the worms, whereas 1,000 mg./kg. of lucanthone was required to achieve the same result. With 600 mg./kg. tartar emetic 72% of the worms were killed but only 4/10 mice survived; higher doses killed all the mice and no strict comparison was possible for total worm mortality. At doses which killed all the worms 413C52 was almost 7 times as active as lucanthone. At doses which killed 72% of the worms—the maximum obtainable for tartar emetic—413C52 was 8 times as active as tartar emetic and 9 times as active as lucanthone (Fig. 1). At 160 mg./kg., 413C52

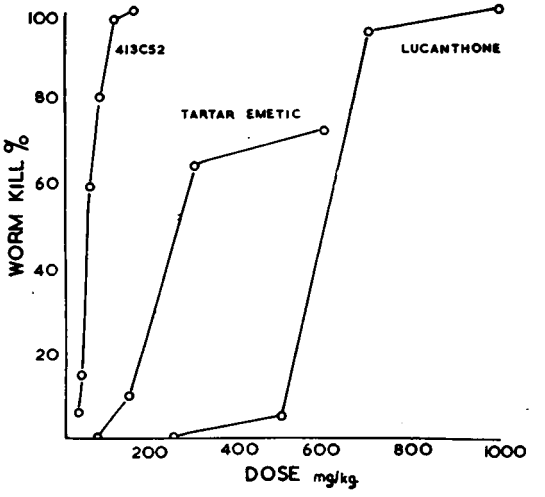


FIG. 1.—Showing the comparative activity in single oral dose of 413C52 [1:7-bis(*p*-methylaminophenoxy)heptane], tartar emetic and lucanthone against *Schistosoma mansoni* in the mouse.

killed all the worms and was 10 times as active as tartar emetic whereas lucanthone failed to kill. However, at 250 mg./kg., lucanthone achieved the appreciable hepatic shift of 69% (Table II), but no worms were killed.

Multiple Oral Doses.—When mice received 10 doses in 5 days, 100% and 92% of the worms were killed with unit doses of 30 and 25 mg./kg. of 413C52, respectively (Table II). Lucanthone killed 96% of the worms at a 100 mg./kg. unit dose, that is 4 times the amount of 413C52 required to elicit a similar dose-response. When 413C52 was given in two doses of 50 mg./kg. in one day 94% of the worms were killed, compared with 96% for lucanthone at 100 mg./kg. twice daily for 5 days. Thus, 10 times as much lucanthone as 413C52 was required to obtain an approximately equal response.

Because of toxicity it was not possible to treat mice with tartar emetic above a unit dose of 40 mg./kg.; at this dose 100% hepatic shift was obtained, but the worms were alive and not ensheathed. In a group of mice similarly treated but autopsied 25 days later, 64% of the worms were in the liver but had not been killed or ensheathed. The remainder were in the mesenteric and portal veins and had, presumably, re-migrated to these vessels.

The small-scale tests with *S. japonicum* (Table II) showed that, when given twice daily for 5 days, a 50 mg./kg. unit dose of 413C52 killed all the worms; a 75 mg./kg. unit dose of lucanthone killed 26% and a 35 mg./kg. unit dose of tartar emetic produced 54% hepatic shift but did not kill.

The multiple-dose tests showed that 413C52 was much more active than either lucanthone or tartar emetic against *S. mansoni* and *S. japonicum* in mice.

Activity Against Other Species and Strains of Schistosome

In addition to the standard tests against *S. mansoni* (Egyptian strain) in mice, three of the most active diaminodiphenoxyheptanes were tested orally against *S. mansoni* (Brazilian strain) in the mouse and against *S. japonicum* in the mouse, guinea-pig, and rabbit. These tests were not extensive and they were performed chiefly to determine whether the activity of the drugs extended beyond the long-established Egyptian strain of

TABLE II

COMPARATIVE ACTIVITY OF 1:7-BIS(*p*-METHYLAMINOPHENOXY)HEPTANE, LUCANTHONE AND TARTAR EMETIC ORALLY AGAINST *S. MANSONI* AND *S. JAPONICUM* IN THE MOUSE

M.V., mesenteric veins; P.V., portal vein; L., intrahepatic veins.

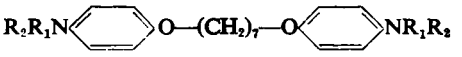
Drug	Schistosome Species	Unit Dose (mg./kg.)	No. of Doses	Total Dose (mg./kg.)	Worm Distribution %			Worms Killed %
					M.V.	P.V.	L.	
413C52 (Table I, B)	*Mansoni*	28	1	28	46	42	12	6
		40	1	40	60	21	19	15
		56	1	56	16	22	62	59
		80	1	80	12	8	80	80
		112	1	112	2	0	98	98
		160	1	160	0	0	100	100
		50	2	100	2	4	94	94
		10	10	100	70	23	7	7
		15	10	150	46	13	41	41
		20	10	200	9	2	89	89
		25	10	250	0	3	97	97
		30	10	300	0	0	100	100
	Japonicum	50	10	500	0	0	100	100
Lucanthone	*Mansoni*	250	1	250	19	12	69	0
		500	1	500	0	17	83	5
		750	1	750	0	0	100	95
		1,000	1	1,000	0	0	100	100
		75	10	750	47	0	53	0
		100	10	1,000	0	0	100	96
	Japonicum	75	10	750	58	16	26	26
Tartar emetic	*Mansoni*	75	1	75	65	33	2	0
		150	1	150	24	65	11	10
		300	1	300	28	7	64	64
		600	1	600	14	14	72	72
		40	10	400	0	0	100	0*
	Japonicum	35	10	350	0	36	54	0

* Examination of a group of mice treated at the same time but autopsied 28 days later than this group showed 64% of the worms still in the liver but not yet dead.

TABLE III

ACTIVITY OF PRIMARY, SECONDARY, AND TERTIARY DIAMINODIPHENOXYHEPTANES AGAINST A RANGE OF SCHISTOSOME SPECIES AND STRAINS IN VARIOUS EXPERIMENTAL HOSTS

All drugs given orally either once or twice daily for 5 days or in single dose. E., Egyptian strain; B., Brazilian strain; C., Chinese strain; M.V., Mesenteric veins; P.V., Portal vein; L., Intrahepatic veins.



Drug	Schistosome Species and Strain	Host Animal	Unit Dose (mg./kg.)	Total Dose (mg./kg.)	Worm Distribution %			Worms Killed %
					M.V.	P.V.	L.	
R_1,R_2 =H	*Mansoni* (E)	Mouse	50	500	0	3	97	97
	Mansoni (B)	,,	50	500	0	4	96	96
	Japonicum (C)	,,	50	500	0	12	88	88
R_1=H R_2 =CH_3	*Mansoni* (E)	,,	100	100	0	4	96	96
	Mansoni (B)	,,	100	100	3	7	90	90
	Japonicum (C)	,,	50	500	0	0	100	100
R_1,R_2 =CH_3	*Mansoni* (E)	,,	100	100	2	13	85	85
	Japonicum (C)	,,	50	500	0	0	100	100
	Japonicum (C)	Guinea-pig	100	1,000	9*	3	88	97
	Japonicum (C)	Rabbit	100	500	7	9	84	84

* Worms ensheathed and dead in mesenteric veins.

S. mansoni used for routine screening. The compounds used—the primary amine and the secondary and tertiary methylamines—were highly active against both strains of *S. mansoni* and against *S. japonicum* in a variety of rodents (Table III).

Discussion

The Amino, Methylamino and Dimethylamino Series

The first of these forms the basis for all our structural variations; the other two may be regarded as the simplest examples of monoalkyl and dialkyl substitution.

In the *primary amine* series (Table I; Fig. 2, A) at the highest unit dose (200 mg./kg.) there is full activity (100% worm mortality) over the range of values of n from 3 to 9 inclusive. Maximum activity lies in the region where $n=7$ and 8, both of which compounds give over 90% mortality at 25 mg./kg. (Fig. 2).

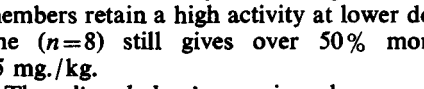
The *methylamino* series follows the same pattern as the primary amines, but there are signs of a slightly greater activity in the methylamines. For example, the compounds where $n=2$ and $n=10$ show definite activity at the highest dose; more members retain a high activity at lower doses; and one ($n=8$) still gives over 50% mortality at 15 mg./kg.

The *dimethylamino* series shows a different range of activity from any of the other series of drugs now described (Table I, C; Fig. 2, D). Whereas at a low dose (25 mg./kg.) the compound with $n=7$ is outstanding with >90% mortality, at the higher doses there is a definite alternation of activity with the number of carbon atoms in the central chain, odd-numbered members being more active than the adjacent even-numbered members (Fig. 2) This type of variation is known in certain physical properties of members of a homologous series, and the present biological results might, at first sight, seem to be related to such a phenomenon. It is true in the present series that there is an alternation of some characteristics (melting-point, solubility) with the value of n, but it is not confined to the dimethylamino derivatives and is found, for example, in the primary amines and monomethylamino derivatives which do not show an alternation in schistosomicidal activity.

The Higher Alkylamino Series

At the two highest doses the *ethylamino* series strongly resembles the methylamino, both in absolute activity and in the variation with n. At 25 mg./kg., however, the results differ; a graph of activity against n shows two peaks, one still at $n=7$–8 with 90–100% mortality and another at $n=4$ with nearly 80% mortality (Fig. 2, B).

The introduction of alkyl groups higher than ethyl progressively reduces the therapeutic effect. The activity of the n-*propylamino* series at 50 mg./kg. bears a strong resemblance both in absolute values and variation with n to that of the ethylamino series at the dose of 25 mg./kg. The iso-*propylamino* compounds only show activity at the highest dose with virtually only the lower peak at $n=4$–5 (Fig. 2, C). At 50 mg./kg. the activity has disappeared. This difference in schistosomicidal activity between *n-* and *iso*-propylamino compounds may reflect the known difference in their chemical reactivities, for the latter are sometimes sterically hindered in reactions at the nitrogen atom. The few compounds made with *allylamino* substituents seem to resemble the *n*-propyl rather than the *iso*-propyl series.

The n-*butylamino* and iso-*butylamino* series are similar and activity is found only at the highest dose. The former shows a peak at $n=4$ and a lower peak at $n=7$, but the latter shows equal peaks at the same points. It is interesting that this branching, which is farther from the nitrogen atom  , has not had so profound an effect on the activity as with the propyl isomers.

The n-*amylamino* compounds appear to show very little activity.

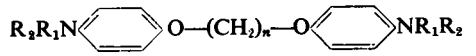
The Higher Dialkylamino Series

The *ethyl-methylamino* series resembles the ethylamino and has a comparable activity. The high activity at 50 mg./kg. over the whole range ($n=4$–9) breaks down at 25 mg./kg. into two peaks, one at $n=7$–8 and the other at $n=4$–5.

The *diethylamino* series appears to be less active than the previous one, for already at 50 mg./kg. the graph of activity against n produces two peaks, one at $n=7$–8 and the other at $n=4$.

Both the *di*-n-*propylamino* and the *diallylamino* series show a low level of activity, and the *di*-n-*butylamino* compounds are inactive.

Comparing the monoalkyl with the dialkyl series, it is apparent that, for a given *total* of carbon atoms present as alkyl substituents, a dialkyl is in general more active than a monoalkyl compound. For, although dimethyl is approximately equal to ethyl, the following inequalities have been observed: ethylmethyl>*n*-propyl; diethyl>*n*-butyl or *iso*-butyl; and, in a single comparison, methyl-*n*-propyl>*n*-butyl.



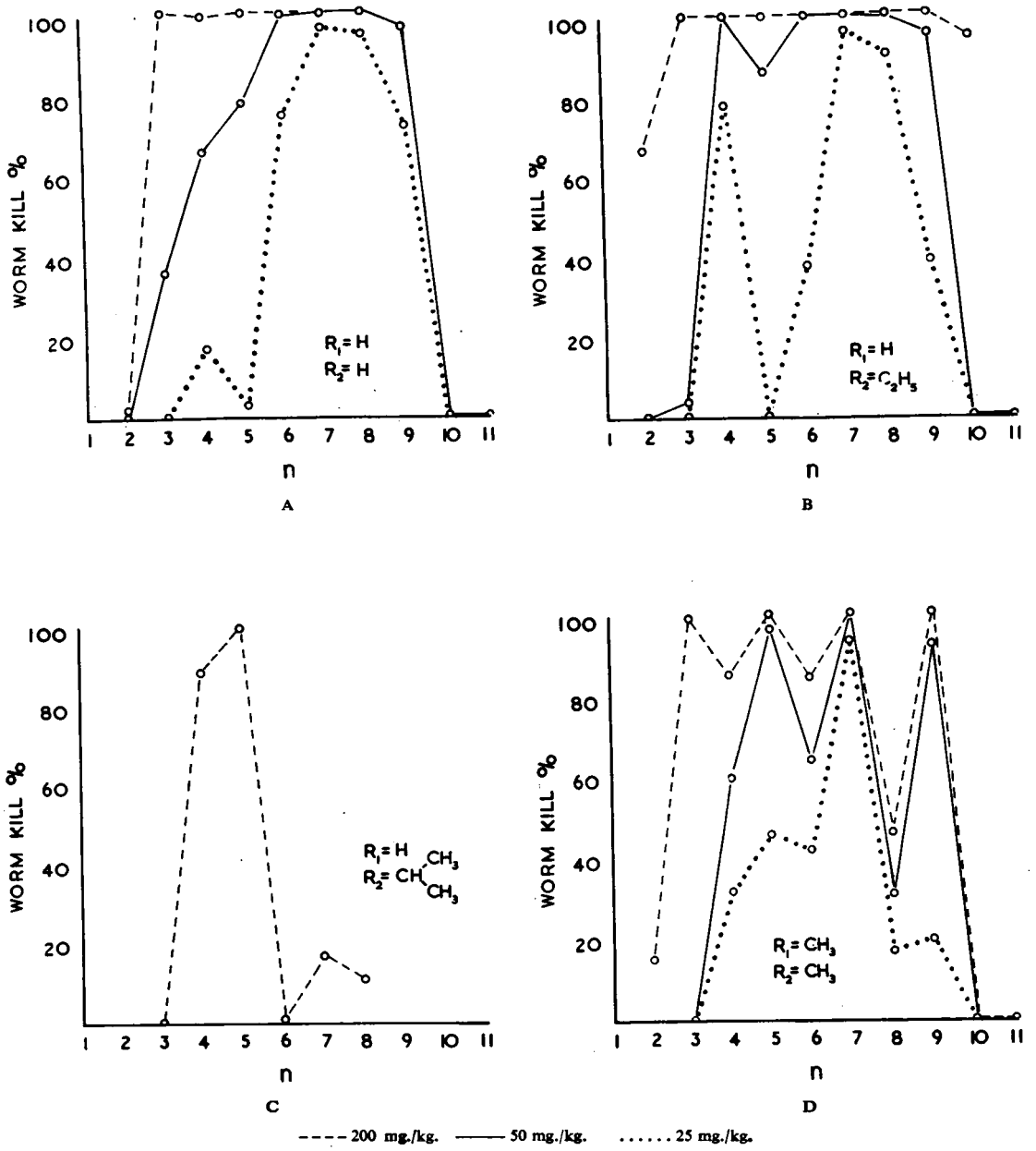


FIG. 2.—Showing the four types of relationship between structure and schistosomicidal activity of diaminodiphenoxyalkanes.

Fig. 2 illustrates the four types of relationship between structure and schistosomicidal activity which have been observed in these compounds.

It is too early yet to attempt a detailed explanation of our results, but the variations of *in vivo* activity with substitution on the nitrogen atom and with variation in the methylene bridge suggest a superposition of effects. In elaborating the primary amines in the manner described, the picture obtained by plotting activity against n changes its form at the point (NHC_2H_5 in monosubstitution and $N(CH_3)_2$ in disubstitution) where two carbon atoms have been added to each nitrogen atom. Apart from the unique dimethylamino series, the change is from a single peak at $n=7$–8 to double peaks at $n=4$–5 and $n=7$–8, followed then by a relative accentuation of the lower peak as the substitution on the nitrogen atom increases. It is possible at this stage to envisage these *in vivo* results as representing a summation of intrinsic schistosomicidal activity and some property which determines the absorption of the compounds in the host. It is hoped that further detailed studies will help to elucidate these points.

The Effect of Drug Treatment

All active schistosomicides when administered to infected animals affect the worms in more or less the same way. The worms in mesenteric and portal veins are rendered unable to maintain their position in the vessels; they are swept back passively by the portal blood, and accumulate in the liver, where they are surrounded by inflammatory tissue and then destroyed by phagocytosis. This sequence of events has been followed in detail in mice treated with lucanthone and trivalent antimonials (Standen, 1953): unless treatment is prolonged, or unless dosage is sufficiently high to produce immobility of the worms for sufficient time, the worms recover and return to the mesenteric veins. By comparison, the diaminodiphenoxyalkanes produce a novel effect. The degree of hepatic shift is proportional to the amount of drug given and is also related to the absolute activity of any given compound. At any dose producing some degree of hepatic shift, even with compounds of relatively low activity, all worms which drift back to the liver become ensheathed in inflammatory tissue within the liver and are subsequently destroyed by phagocytosis. With lucanthone and the trivalent antimonials a low degree of hepatic shift is invariably followed by remigration to the mesenteric veins. It appears that the effect of the new compounds is irreversible. Therefore, although the mode of action of the diaminodiphenoxyalkanes conforms in most ways to that of schistosomicides in general, this fundamental and irreversible effect constitutes an outstanding difference. Because of this it has been possible to obtain controlled dose-response in terms of worm mortality rather than hepatic shift, and thus to provide a more accurate measure of drug activity.

A detailed account of the process of worm destruction in the liver will appear elsewhere, but it can be recorded here that these compounds are not themselves specifically schistosomicidal but that they prepare the parasites for invasion by phagocytes. The onset of phagocytosis is gradual, invasion commencing where the cuticle of the worm is in contact with the wall of the intrahepatic vein and then proceeding to affect the worm throughout its length. In one experiment ensheathed and phagocytosed worms were observed in the mesenteric veins of a treated guinea-pig infected with *S. japonicum* (Table III). This is atypical and is alone amongst many thousand observations where phagocytosis was seen to occur only in the liver.

Conclusions

Symmetrical diaminodiphenoxyalkanes having the amino-groups in the *para* positions are highly active schistosomicides. Altering the position of the amino-groups or quaternization destroys this activity. Within the active series, considerable variation of alkyl substitution on the nitrogen and of the methylene bridge is possible while still retaining activity. A detailed investigation of the influence of these changes on biological activity has been made, but at present any attempt to account for these findings can be only speculative. It appears likely that a final appraisement must take into account the dual need for absorption by both host and parasite.

Against *S. mansoni* in mice the outstanding differences between these compounds and lucanthone or the antimonials are the very high activity at low dosage and the more potent effect upon the parasites. Also, although the experiments performed with *S. japonicum* have not been extensive, sufficient evidence of activity against this parasite is available to justify the conclusion that the schistosomicidal action of the diaminodiphenoxyalkanes is not species specific.

Summary

1. High schistosomicidal activity has been discovered in symmetrical $p:p'$-diaminodiphenoxyalkanes when tested orally against *S. mansoni* and *S. japonicum* in mice, guinea-pigs, and rabbits.

2. The influence on activity of alkyl substitution in the amino-groups and of variation in the methylene bridge has been investigated in detail.

3. The primary amines and mono-methylamines show a peak of activity at a chain length (n) of 7–8. The dimethylamines are unique in showing an alternation of activity with n, but still show a peak where $n=7$. All the remaining mono- and di-alkylamines (C_2–C_5) show peaks at $n=4$ and $n=7$–8 and in general show decreasing activity as the size of alkyl group increases.

4. The most active of the diaminodiphenoxyalkanes are several times as active as lucanthone or tartar emetic when given orally to mice infected with *S. mansoni* or *S. japonicum*.

We are indebted to Dr. A. G. Caldwell, Wellcome Research Laboratories, and to Mr. T. M. Sharp, Chief Chemist, Wellcome Laboratories of Tropical Medicine, for the preparation of certain compounds mentioned in this paper. We wish to record that the work described would have been impossible but for the valuable and loyal assistance of our technical staffs, and we extend to them our most grateful appreciation.

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