starting material, and no dehydrated compound XIX. No other substance could be detected.

Acknowledgment.—The authors extend their gratitude to Dr. E. Schlittler for his interest in this project. We also wish to thank the members of our Microanalytical and Spectral Sections for their coöperation, and Dr. A. J. Plummer and Dr. L. Witkin for the biological data.

An Empirical Relationship between Anthelmintic Activity and Chemical Structure

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The anthelmintic activity against the mouse pinworm *Syphacia obvelata* is reported for several different types of compound, including N,N-dialkylnaphthamidine salts, some highly substituted dihydropyridines with quaternary salt groupings, and some stilbazole and other quaternary salts. Based on these results it was postulated that an association of a ring moiety with a center which is cationic at physiological pH values might confer anthelmintic properties on molecules. Random selection of four groups of compounds with such structures gave three groups which had active members.

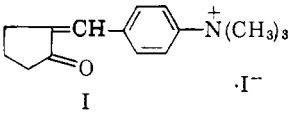
In previous publications[1,2] we have mentioned that one structural feature found in a high proportion of our compounds with anthelmintic activity against the mouse pinworm *Syphacia obvelata* was the existence of a center of positive charge near or in a cyclic system. Some of the compounds tested, which were not previously reported, and the results which led to this empirical observation are given below. This is followed by the results of arbitrary selection of some compounds fitting the same criterion of structure, which previously had been prepared for other purposes, and a description of their activity against *S. obvelata* in the mouse.

Methods

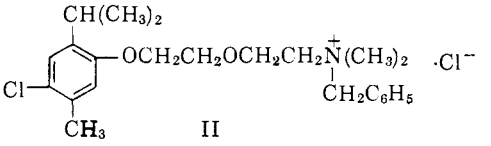
The compounds were prepared by methods reported in the literature unless otherwise annotated. All alkyl groups are normal unless stated otherwise. Those compounds tested initially were selected as representative substances with a high numerical ratio of oral to intraperitoneal LD_{50} in the mouse as determined by Mr. R. V. Fanelli of these Laboratories. Testing for pinworm activity was done essentially as described in a previous publication[3] using mice infected by exposure to infected "source mice" for 8 days, and dosed by gavage once a day for 2 days thereafter, unless otherwise stated. Results are reported as per cent clearance of worms by autopsy in comparison to the worm count in simultaneously infected but untreated "control" mice. Each result and each "control" result are averages of 3 mice.

Experimental Data

Anthelmintic activities are given in Table I for representative examples of the 2 types of compounds, 4-methoxynaphthamidines[4] and quaternary salts of highly substituted 4-(aminophenyl)-1,4-dihydropyridines,[5] of which several members were found to be reasonably active against *S. obvelata* in the mouse, under our test conditions. In addition to these, a few miscellaneous quaternary salts either of substituted anilines (*e.g.*, I) or benzylamines (*e.g.*, II) had appreciable activity.



I

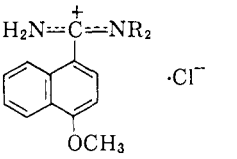


II

A number of other mono- and bis-quaternary salts were prepared or purchased. Those with purely aliphatic substituents were inactive or had only slight activity at a readily attainable dosage. One alicyclic candidate, methyldodecylpiperidinium bromide, had slight activity. As had been reported previously, however, various quaternary piperazinium salts had substantial activity.[1-3]

TABLE I

NAPHTHAMIDINES ACTIVE AGAINST *Syphacia obvelata*



R	Approx. oral LD_{50}	Dose, mg./kg./day	% worm count reduction
i-C_5H_{11}	170	50	51
C_6H_{13}	240	80	93
C_7H_{15}	2000	200	98
C_8H_{17}	3000	200	95

A small group of cinnamamidine salts[6] (III) were found to contain one member, 2-chloro-N,N-dibutylcinnamamidine, R = Cl, R′ = C_4H_9, which cleared an

(1) M. Harfenist, *J. Am. Chem. Soc.*, **79**, 2211 (1957).

(2) H. W. Brown, K. L. Hussey, K. F. Chan, M. Harfenist, R. V. Fanelli, and E. Magnien, *Toxicol. Appl. Pharmacol.*, **1**, 350 (1959).

(3) (a) K. F. Chan, *Am. J. Hygiene*, **56**, 22 (1952); (b) M. Harfenist, R. V. Fanelli, R. Baltzly, H. W. Brown, K. L. Hussey, and K. F. Chan, *J. Pharmacol. Exptl. Therap.*, **121**, 347 (1957). Unless otherwise stated the tests reported here were done by Drs. Brown, Hussey, and Chan.

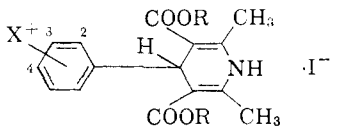
(4) Prepared originally by Mr. E. Lorz as potential local anesthetics. See E. Lorz and R. Baltzly, *J. Am. Chem. Soc.*, **70**, 1904 (1948). These are shown in their symmetrical protonated form, as is reasonable in view of the pK_a values reported by E. Lorz and R. Baltzly, *ibid.*, **71**, 3992 (1949), for related compounds.

(5) Prepared originally as potential curareform substances by Dr. A. P. Phillips. See A. P. Phillips, *ibid.*, **71**, 4003 (1949).

(6) M. Harfenist and A. P. Phillips, *ibid.*, **80**, 6261 (1958).

TABLE II

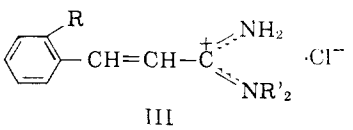
DIHYDROPYRIDINES



X	R	Approx. oral LD_{50}, mg./kg.	Dose, mg./kg. (1 day)	% worm count reduction
3-$N(CH_3)_3$	CH_3	1700	300	0
4-$N(CH_3)_3$	CH_3	1200	500	96
			300	83
4-$N(CH_3)_2C_5H_7$	CH_3	"	300	46
4-$N(CH_3)_2C_7H_9$	CH_3	3500	300	100
4-$N(CH_3)_2C_8H_{11}$	CH_3	910	300	96
4-$N(CH_3)_2CH_2C_6H_5$	CH_3	1700	550	46
3-$N(CH_3)_2$	C_2H_5	1800	250	86
4-$N(CH_3)_2$	C_2H_5	1400	330	94
			200	62
3-$N(C_2H_5)_2$	C_2H_5	3000	300	27
4-$N(CH_3)_2C_5H_7$	C_2H_5	"	300	33
3-$N(C_2H_5)_2$	C_3H_7	"	300	79

[a] Not determined due to shortage of compound.

average of 90% of the worms when given to mice at 200 mg./kg. per day for 3 days.[7]

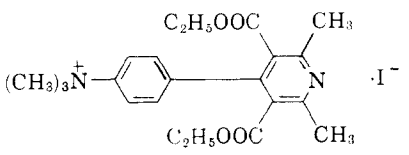


III

However, the analogous 2-methyl-N,N-dibutylcinnamamidine, R = CH_3, R' = C_4H_9, and also N,N-dihexylcinnamamidine, R = H, R' = C_6H_{13}, were essentially inactive against *S. obrelata*.

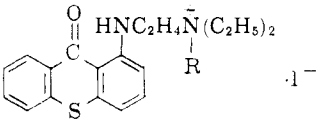
Amidines found to be inactive, or only slightly active, or toxic to the host included those with a variety of other aromatic ring systems holding N,N-dibutylamidine groups, or holding a few other amidine groups of comparable effective size.

Inactive, slightly active, or toxic compounds related to those in Table II which were tested included the tertiary (dimethyl) amines corresponding to certain of these quaternary salts, *i.e.*, dihydropyridines resembling the prototype heading Table II, but with $X^- = N(CH_3)_2$. One true pyridine (IV), corresponding to one of the dihydropyridines (Table II, line 8) with a high apparent therapeutic index, had moderate activity (76% clearance) at 350 mg./kg., which is approximately one-third of its oral LD_{50} in the mouse.



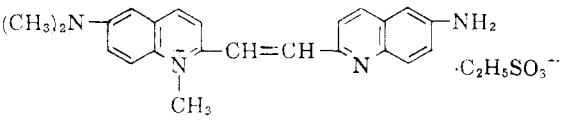
IV

It seemed desirable on the basis of these results and the known activity of gentian violet and of piperazine, to study other compounds with a cyclic structure associated with a quaternary grouping. A small random group of such compounds was obtained through the cooperation of Dr. W. Mark Duffin of the Wellcome Research Laboratories at Beckenham, England. The few such compounds available in the required amounts were selected arbitrarily from files listing compounds made for other purposes at The Wellcome Research Laboratories in England. Among them were representatives of three types of quaternary salts and one tertiary aminopyrrocoline which we quaternized with several alkyl halides.[8] It is of interest that of these four groups, three had members with substantial activity against *S. obrelata* in the mouse. Thus benzthone methiodide[9] (Va) given at 800 mg./kg. for 2 days cleared worms from the infected mice completely, while the corresponding ethiodide[9] (Vb) cleared 96% of the parasites at the same rather high dosage.

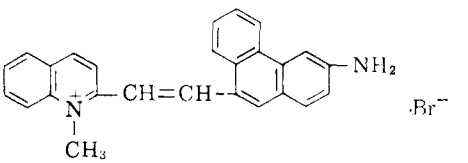


Va, R = CH_3
Vb, R = C_2H_5

Analogously, each of the two rather complex stilbazole quaternary salts VI and VII[10] had appreciable activity in the test, VI clearing all of the worms at a rather high dosage (about 1500 mg./kg.) and VII removing 65% of the worms at 2600 mg./kg. The dosage in each case was less than 1/3 of the LD_{50} but was the most that could conveniently be given.



VI



VII

Examples of the aminoalkylpyrrocoline quaternary salts[8] which were found to be active against *S. obrelata* are tabulated in Table III. These were given to naturally infected mice for only a single day because of a revision in the testing procedure. Compounds in which R was CH_3, C_2H_5, or C_3H_7, and A simultaneously was CH_3, were essentially inactive.

Discussion

The results reported here were responsible for a generalization that one type of structure associated with anthelmintic activity would have a group bearing a positive charge (at physiological pH values) associated with a cyclic moiety. As has been shown, this empirical correlation was used in a successful attempt to select compounds on grounds only of chemical structure, which were found to have a far greater proportion of active compounds than would have been anticipated on statistical grounds. Admittedly these

(7) These results were determined by Dr. R. B. Barrows of these Laboratories.

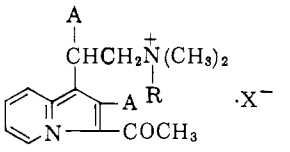
(8) Syntheses of these and related compounds will be described in a Note to be submitted to *J. Org. Chem.*

(9) Prepared by Dr. T. M. Sharp. See *J. Chem. Soc.*, 2962 (1951).

(10) Prepared by Dr. A. G. Caldwell of The Wellcome Research Laboratories, England.

TABLE III

ANTHELMINTIC ACTIVITY OF PYRROCOLINE QUATERNARY SALTS

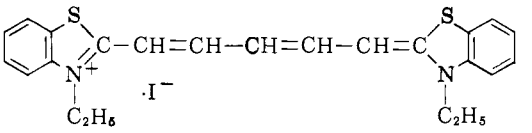


R	X	A	Approx. oral LD_{50}, mg./kg.	Dose, mg./kg. (1 day)	% worm count reduction
C_4H_9	I	CH_3	400	300	77
C_7H_{11}	Br	CH_3	840	300	96
C_8H_{13}	Br	CH_3	2500	300	83
CH_2=CH—CH_2	Br	H	[a]	300	44
CH_2=CH—CH_2	Br	CH_3	2000	300	99
$CH_2C\equiv CH$	Br	CH_3	"	300	7
$CH_2CH_2C\equiv CH$	Tos[b]	CH_3	2000	300	91
$CH_2C_6H_5$	Cl	H	[a]	300	54
$CH_2C_6H_5$	Cl	CH_3	1500	500	100
				400	79
				300	97
$CH_2C_6H_3Cl_2$(2,4)	Cl	CH_3	1700	300	100
				200	45
$CH_2C_6H_4CH_3$(4)	Cl	CH_3	1600	300	100
				200	47

[a] LD_{50} not determined. [b] Tos = *p*-toluenesulfonate.

compounds were selected from those at hand in a laboratory devoted to making potential drugs, and so some selection is implied by the origin of the group from which the selection was made. However, the compounds selected were not synthesized originally for use against intestinal parasites.

This is not to say that all compounds with these structural features are active anthelmintics. Lack of space precludes our listing most of the compounds which were found in our screening tests to have little or no activity against *S. obvelata*, which have these structural features. Also, one cannot say that these structural features are necessary for anthelmintic activity. Examples of antibiotics, phenolic compounds, presumed folic acid antagonists, etc., with activity against *S. obvelata* and against the human pinworm *Enterobius vermicularis* are well known. Several mechanisms may well be operating even in the case of the positively charged compounds known to have anthelmintic activity. For example 3,3′-diethylthiadicarbocyanine iodide (dithiazanine) (VIII), which formally fits our scheme, is believed to affect respiratory enzyme systems



VIII

of nematodes[11] while piperazine apparently affects the neuromuscular junction at the site of action of acetylcholine in the nematode *Ascaris lumbricoides*,[12] and N-carbethoxy-N′-methyl-N′-tetradecylpiperazinium salts and various bisquaternary salts of piperazine cause a rigid paralysis of the same nematode,[12] analogous to that caused by succinyl choline chloride.[13] We may speculate that compounds with a cationic center are likely to have activity at the neuromuscular junction in these nematodes,[14] and that the ring structures may be necessary either to prevent destruction of the compound or to cover a large fixed area on some enzyme onto which the compounds are held by their positive charge. Presumably a quaternary non-cyclic carbon could accomplish both of these objectives as well as a cyclic structure does, but we have not investigated compounds having such groups.

Since useful anthelmintic activity depends on differential effects such as relative non-absorption by the host but absorption by the parasite, action on enzyme systems which differ between host and parasite, or the swamping of the entire parasite by an irritant drug which merely is briefly disturbing to the host, further speculation unsupported by studies of the precise modes of action of our various compounds seems unprofitable.

Acknowledgments.—The author wishes to thank Drs. H. W. Brown, K. F. Chan, and K. L. Hussey of the School of Public Health and Administrative Medicine of Columbia University, and Dr. R. B. Burrows and Mr. R. Fanelli of these Laboratories for permission to use their previously unpublished results, Dr. R. Baltzly of these Laboratories and Dr. S. Norton of the Department of Pharmacology, University of Kansas Medical Center, for their helpful comments and suggestions, and the various chemists who supplied compounds as indicated.

(11) E. Bueding and C. Swartzwelder, *Pharmacol. Rev.*, **9**, 329, especially 337 (1957), *et seq.*

(12) S. Norton, unpublished observations.

(13) S. Norton and E. J. de Beer, *Am. J. Trop. Med. Hyg.*, **6**, 898 (1957).

(14) Compare C. J. Cavallito and A. P. Gray, *Progr. Drug Res.*, **2**, 136 (1960), *et seq.*, in which similar ideas as to the nature of the interactions of quaternary salts and animals are presented and extensively documented.