prepared from the 4- (5-) nitroimidazole and the appropriate alkyl *p*-toluenesulfonate.[17,18]

General Procedure.—2-Isobutyl-4- (5-) nitroimidazole (2.9 g, 0.017 mole) and the β-cyano-*p*-toluenesulfonate (7.72 g, 0.034 mole) were combined and heated to 135° for 12 hr. The cooled mixture was extracted with water, and the aqueous extract was washed once with a small volume of $CHCl_3$ which was discarded. Addition of alkali to pH 9.0 gave a precipitate of the 1-β-cyanoethyl-2-isobutyl-5-nitroimidazole which was extracted out with $CHCl_3$, washed once with water, dried (Na_2SO_4), and evaporated to yield 1.65 g (43%) of the desired compound which was then recrystallized as the *p*-toluenesulfonate salt (mp 211°) from 2-propanol.

1-Alkyl-4-nitroimidazoles were obtained by essentially the same procedure described by Cosar, *et al.*[2]

1-β-Hydroxyethyl-2-methyl-4-nitroimidazole (**68**) is best obtained by heating an ethanolic solution of 2-methyl-4- (5-) nitroimidazole with an excess (2 *M*) of ethylene oxide in the presence of a catalytic amount of NaOH. The desired product is obtained in quantitative yield by evaporation of the solvent, followed by recrystallization from ethyl acetate.

Acknowledgment.—We are greatly indebted to Dr. R. L. Wagner and his group for the microanalyses.

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Anthelmintic Quaternary Salts. Thiacyanines and Hemithiacyanines

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Comparison of the activities of analogs of dithiazinine (III) against gastrointestinal nematodes of sheep showed that the pentamethine chain is essential. Activity may be retained when the 6 position is substituted with alkyl or alkoxy groups, but substitution with electron-withdrawing groups produces inactive compounds. Analogs with activity comparable to dithiazinine tended to be more toxic to mice than dithiazine itself. Two members of a series of hemithiacyanines containing the 4-dimethylamino-1,3-butadienyl group were very active in inhibiting *Ascaris suum* larval migration in mice and in swine and were more effective than dithiazinine in preventing liver pathology due to migratory ascariasis.

Since the initial discovery of anthelmintic activity in cyanine dyes,[1] a number of compounds containing the conjugated amidinium ion characteristic of the cyanines have found use as both veterinary and clinical anthelmintics. Dithiazinine (III), the most potent member of this class of anthelmintics, is effective against a wide range of gastrointestinal nematodes,[2,3] but its use is severely limited by its toxicity. Because of its gastrointestinal side effects, the clinical application of dithiazinine is now restricted to cases of strongyloidiasis and severe trichuriasis.

The structure–activity–toxicity relationships of some structural analogs of dithiazinine were examined with the purpose of discovering compounds with comparable anthelmintic potency but with reduced mammalian toxicity. The structural features which were varied included the length of the polymethine chain, the replacement of the 3-methyl group by ethyl, and the introduction of substituents on the 6 position of the benzothiazole ring. In addition, a group of compounds in which one of the benzothiazole residues was replaced by a dimethylamino group (hemithiacyanines) was also prepared.

The most convenient method for the preparation of the thiadicarbocyanines (pentamethinethiacyanines) was the treatment of 2-methyl-3-alkylbenzothiazolium iodides with 1-methyl-1,2-dihydro-2-iminopyrimidine hydroiodide[4] in the presence of triethylamine.

The 2-methylbenzothiazolium salts were prepared by alkylation of 2-methylbenzothiazoles, which were themselves obtained by the oxidation of substituted thioacetanilides.[5,6]

The monomethine,[7] trimethine,[8] and heptamethine[9] analogs of dithiazinine were prepared by known methods. The hemithiacyanines, which included compounds with 2-dimethylaminovinyl and 4-dimethylamino-1,3-butadienyl substituents, were prepared by procedures which have been described by Brooker and his collaborators.[10]

Biological Properties.—Comparison of the first four compounds in Table I shows that acute toxicity in mice increases sharply with increasing length of the polymethine chain joining the benzothiazole residues. Thus the heptamethine analog IV is 2000 times more toxic than the monomethine analog I. However, only the pentamethine III (dithiazinine) showed appreciable anthelmintic activity. The trimethine analog II was totally inactive while the other two were only weakly active.

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(3) M. C. McCowen, M. E. Callender, and M. C. Brandt, *Am. J. Trop. Med. Hyg.*, **6**, 894 (1957).

(4) D. J. Fry, J. D. Kendall, and A. J. Morgan [British Patent 870,633 (June 14, 1961)] described this compound, which is obtained by treating 2-aminopyrimidine with methyl iodide, as a quaternary salt. The correct structure was established by Brown and his collaborators, who have studied the fission of the pyrimidine ring in compounds of this type: D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1276 (1963), and earlier papers.

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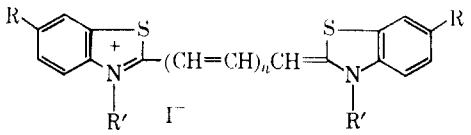
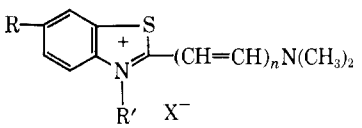
TABLE I
THIACYANINES



No.	R	R′	n	Mp, °C	Yield, %	Formula	N, % Calcd	N, % Found	LD50 (mice), mg/kg ip	Act. vs. nematodes in sheep[a]
I[b]	H	CH_3	0						200	Slight active
II[c]	H	C_2H_5	1						3	Inactive
III[d]	H	C_2H_5	2						3	Very active
IV[e]	H	C_2H_5	3						0.1	Slight active
V	CH_3	CH_3	2	290–292	40	$C_{23}H_{23}IN_2S_2$	5.30	5.40	0.1	Very active
VI	CH_3	C_2H_5	2	259–260	70	$C_{25}H_{27}IN_2S_2$	5.13	5.04	1	Very active
VII	CH_3O	CH_3	2	241	50	$C_{23}H_{23}IN_2O_2S_2$	5.09	4.93	2	Inactive
VIII	CH_3O	C_2H_5	2	240–242	57	$C_{25}H_{27}IN_2O_2S_2$	4.84	4.47	0.05	Active
IX	C_2H_5O	CH_3	2	242–243	69	$C_{25}H_{29}IN_2O_2S_2$	4.84	4.47	100	Slight active
X	Cl	CH_3	2	310–312	49	$C_{21}H_{17}Cl_2IN_2S_2$	5.01	5.27	300	Inactive
XI[f]	Cl	C_2H_5	2						200	Inactive
XII	Br	CH_3	2	294–296	58	$C_{21}H_{17}Br_2IN_2S_2$	4.32	4.44	750	Slight active
XIII	NO_2	CH_3	2	305	84	$C_{21}H_{17}IN_4O_4S_2$	9.65	9.51	300	Inactive

[a] The compounds were tested on sheep which had been experimentally infected with six species of gastrointestinal nematodes: *Haemonchus contortus*, *Cooperia curticei*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Ostertagia circumcincta*, and *Nematodirus spathiger*. The compounds were administered in two equal doses on consecutive days, and the reduction of the worm burden was estimated after determination of the egg count per gram of feces. The activity recorded is the average of the activity against the six helminths tested; no marked selectivity of action was observed with respect to any one of the six species. [b] Reference 7. [c] Reference 8. [d] Reference 4. [e] Reference 9. [f] B. Berlenson and F. M. Hamer, *J. Chem. Soc.*, 1225 (1936).

TABLE II
HEMITHIACYANINES



No.	R	R′	n	X	Mp, °C	Yield, %	Formula	N, % Calcd	N, % Found	Act. vs. nematodes in sheep[a]	Act. vs. *A. suum* in mice[b]
XV[c]	H	C_2H_5	1	I						Inactive	Inactive
XVI	CH_3	CH_3	2	I	233–235	50	$C_{15}H_{19}IN_2S$	7.25	7.29	Slight active	Very active
XVII	CH_3	C_2H_5	2	Cl	225–227	86	$C_{16}H_{21}ClN_2S \cdot 2H_2O$	8.12	8.47	Slight active	Inactive
XVIII	CH_3O	CH_3	2	I	222–223	60	$C_{15}H_{19}IN_2OS$	6.97	6.99	Inactive	Very active
XIX	C_2H_5O	CH_3	2	I	231	66	$C_{16}H_{21}IN_2OS$	6.73	6.60	Slight active	Active

[a] See footnote *a*, Table I. [b] The compounds were administered to groups of three mice in two doses (10, 5, or 2.5 mg/kg) 4 hr apart. An infection of 10,000 embryonated *Ascaris* eggs was administered immediately after the first treatment. The mice were sacrificed 8 days after infection and examined for lung lesions. Larval counts were taken of mice with very light or no lung lesions. The activity of the administered compounds was judged according to the freedom from lung lesions and the reduction of larval count as compared with unmedicated controls. [c] H. Meerwein, W. Florian, N. Schön, and G. Stopp, *Ann.*, **641**, 1 (1961).

The introduction of a 6-methyl group gave a compound (VI) with activity comparable to dithiazinine, but with increased toxicity. The 6-methoxy derivative (VIII) was somewhat less effective and also highly toxic. The replacement of the 3-ethyl group by methyl had no effect on the activity of the 6-methyl analog (V) but greatly reduced the activity of the 6-methoxy derivative (VII). The 3-methyl-6-ethoxy analog IX was weakly active and much less toxic. Substitution with electron-withdrawing groups like halogen and nitro produced compounds which were both inactive as anthelmintics and relatively nontoxic.

The 2-dimethylaminovinyl derivative XV (Table II) was totally inactive. The 4-dimethylamino-1,3-butadienyl derivatives XVI–XIX were of the same order of mammalian toxicity as dithiazinine but showed considerably less activity against the nematodes of sheep. However, several members of this series were found to be effective as prophylactic agents against *Ascaris suum* larvae in mice, and the two most active compounds (XVI and XVIII) were tested against *A. suum* in swine.

Dithiazinine has been reported to inhibit the larval migration of *A. suum* in pigs at a dosage of 0.04% in feed.[11,12] At this dosage, it is effective in reducing the number of larvae found at autopsy and in preventing lung pathology, but it does not provide effective protection against liver damage. In comparison, thiabendazole was stated to be effective against migratory ascariasis at the relatively high dosage of 0.4% in feed, while other common anthelmintics are inactive.[13]

The above observations with respect to dithiazinine are confirmed by the results given in Table III. XVI and XVIII were effective in preventing lung damage at a dosage of 0.01% in feed and were significantly more active than dithiazinine in preventing the development of liver lesions.

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(12) R. F. Shumard and J. C. Hendrix, *ibid.*, **46**, 9 (1960).
(13) G. W. Kelley, *Vet. Med.*, **47**, 891 (1962).

TABLE III
Ascaris suum IN SWINE[a]

No.	Dose, % in feed	Liver lesions	No. of larvae in lungs	Lung pathol
III	0.02	>500	0–600	0–0
	0.04	140–160	0–600	0–0
XVI	0.01	54–125	0–400	0–0
XVIII	0.01	9–86	400–1600	0–1
Unmedicated controls		>500	10,000–15,000	3–4

[a] The test compounds were administered at the stated dosage level in feed for a period of 10 days to two pigs in concrete-floored pens. An infection of 10,000 embryonated *Ascaris suum* eggs was administered 3 days after the start of the inclusion of the test compound in the feed. The animals were sacrificed after 10 days and examined for liver lesions and lung pathology.

Experimental Section[14]

3,3′-Diethyl-6,6′-dimethylthiadicarbocyanine Iodide (VI).—Triethylamine (7.3 ml) was added to a refluxing solution of 2,6-dimethyl-3-ethylbenzothiazolium iodide[15] (16.0 g, 0.05 mole) and 1-methyl-1,2-dihydro-2-iminopyrimidine hydriodide[4] (5.9 g, 0.025 mole) in ethanol (150 ml). The resulting deep blue mixture was refluxed for 30 min, cooled, and filtered, giving VI, mp 259–260°, yield 9.5 g (70%).

The other symmetrical thiadicarbocyanines listed in Table I were prepared in the same way.

2-(4-Dimethylamino-1,3-butadienyl)-3-methyl-6-ethoxybenzothiazolium Iodide (XIX).—A mixture of 2,3-dimethyl-6-ethoxybenzothiazolium iodide[16] (12.0 g, 0.035 mole) and N,N′-1-propen-1-yl-3-ylidenedianiline hydrochloride (9.1 g, 0.035 mole) in acetic anhydride (100 ml) was refluxed for 1 hr. The mixture was cooled and filtered, giving 2-(4-acetanilido-1,3-butadienyl)-3-methyl-6-ethoxybenzothiazolium iodide, mp 230–232°, yield 15.0 g. The acetanilido derivative (14 g) was added to a solution of dimethylamine (10 g) in ethanol (50 ml), and the mixture was refluxed for 30 min. The product, which separated out on cooling, was filtered and recrystallized from methanol to a constant melting point of 231° dec, yield 9.6 g (66%).

The other hemithiacyanines listed in Table II were made in the same way.

2,3-Dimethyl-6-nitrobenzothiazolium Iodide.—2-Methyl-6-nitrobenzothiazole[17] (25 g, 0.13 mole) was heated with methyl iodide (23 g, 0.16 mole) in a pressure bottle at 100° for 24 hr. The solid product was triturated in ether, giving 1.4 g (99%), mp 240° dec after recrystallization from nitromethane.

Anal. Calcd for $C_9H_9IN_2O_2S$: C, 32.15; H, 2.70; I, 37.76; N, 8.33; S, 9.54. Found: C, 32.25; H, 2.96; I, 38.10; N, 8.26; S, 9.53.

(14) Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Elementary analyses were performed by the Microanalytical Laboratory, Abbott Laboratories, North Chicago, Ill.

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Acknowledgment.—The authors are indebted to Dr. A. O. Geiszler for invaluable help in the coordination of the research program.

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Pyrazine Diuretics. IV. N-Amidino-3-amino-6-substituted Pyrazinecarboxamides

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The synthesis of a series of N-amidino-3-amino-6-substituted pyrazinecarboxamides is described. Since more direct synthetic routes were unsuccessful, the intermediate 3-amino-6-substituted pyrazinecarboxylic acids were synthesized by nucleophilic displacement of the chlorine from 6-chloro-4(3H)-pteridinones or their 3-methyl derivatives, followed by alkaline hydrolysis. These were cyclized to the 2-methyl-6-substituted 4H-pyrazino[2,3-*d*][1,3]oxazin-4-ones, which upon reaction with a guanidine followed by hydrolysis, afforded the desired compounds. When the N-amidino-3-amino-6-substituted pyrazinecarboxamides were assayed for saluretic and diuretic activity in normal rats, the 6-methylmercapto and the 6-benzylmercapto derivatives proved to be the most potent, while in the corresponding 3-acetamido series, the outstanding members were the 6-methylmercapto and the 6-methoxy derivatives.

The observation that the diuretic activity of N-amidino-3-aminopyrazinecarboxamide is markedly increased by the introduction of a 6-halo[1] or 6-methyl[2] substituent prompted the extension of the series to include a variety of 6-substituted derivatives (VIII and IX).

Chemistry.—The synthetic route for the compounds of this series is summarized by Scheme I. Since this route affords the intermediate 3-amino-6-substituted pyrazinecarboxylic acids (V), it was found most convenient to prepare the N-amidino-3-amino-6-substituted pyrazinecarboxamides (IX) by the previously described method[1] involving the reaction of a "cyclic iminoanhydride," 2-methyl-6-substituted 4H-pyrazino[2,3-*d*][1,3]-oxazin-4-one (VII), with guanidine followed by hydrolysis of the 3-acetamido intermediate (VIII).

In this way, the reaction was carried out with "iminoanhydrides" (VII) bearing eight different 6-substituents (VIIa–h). The N-amidino-3-acetamido-6-substituted pyrazinecarboxamides (VIII) were readily isolated, and, except in two instances (VIIIb and c), they were characterized. Representative members of the series were hydrolyzed to the corresponding N-amidino-3-amino-6-substituted pyrazinecarboxamides (IX).

Initially, attempts were made to prepare the intermediate 3-amino-6-substituted pyrazinecarboxylic acids

(1) Paper I of this series: J. B. Bicking, J. W. Mason, O. W. Woltersdorf, Jr., J. H. Jones, S. F. Kwong, C. M. Robb, and E. J. Cragoe, Jr., *J. Med. Chem.*, **8**, 638 (1965).

(2) Paper III of this series: J. B. Bicking, C. M. Robb, S. F. Kwong, and E. J. Cragoe, Jr., *ibid.*, **10**, 598 (1967).