(2) Highly purified lysine-vasopressin causes higher plasma 17-OHCS levels than highly purified arginine-vasopressin when administered by means of an intravenous injection. (3) Increase in plasma concentration of free 17-OHCS observed when Pitressin is administered in man can be completely accounted for by the vasopressin content of Pitressin.

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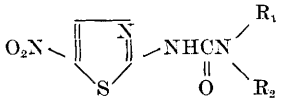
Nithiazide I. Chemical and Biological Studies on 1-ethyl-3-(5-nitro-2-thiazolyl) urea and Related Compounds. (22519)

ASHTON C. CUCKLER, CHRISTINE M. MALANGA, ARTHUR J. BASSO, ROBERT C. O'NEILL AND KARL PFISTER, 3rd. (Introduced by H. J. Robinson.)
Merck Institute for Therapeutic Research and Merck, Sharp and Dohme Research Laboratories, Rahway, N. J.

Enterohepatitis (blackhead disease) may be an important cause of morbidity and mortality in turkey flocks. Although a number of substituted 8-hydroxyquinolines and arsenical compounds(1,2) were known to have some value in enterohepatitis, the discovery of the effectiveness of 2-amino-5-nitrothiazole by Waletzky *et al.*(3) revealed an important new chemical means of controlling this disease. During the course of screening various substituted ureas(4), we found that 1-ethyl-3-(5-nitro-2-thiazolyl) urea was effective in controlling the morbidity and mortality of enterohepatitis. This compound has been assigned the generic name of nithiazide. The present paper reports the results of the chemical and biological studies on nithiazide and related compounds.

Materials and methods. Chemical. The 1-mono-substituted nitrothiazolylureas employed in this study were prepared by the addition of various isocyanates to 2-amino-5-nitrothiazole. The preparation of 1-ethyl-3-(5-nitro-2-thiazolyl) urea (Example 1) is representative of this general method. Synthesis of 1,1-disubstituted nitrothiazolylureas was conveniently effected by heating 2-amino-5-nitrothiazole and a disubstituted carbamyl chloride in an inert solvent (Example 2). The basicity of 2-amino-5-nitrothiazole is sufficiently feeble that the hydrogen chloride produced in this reaction is readily expelled by the refluxing solvent. *Example 1. 1-Ethyl-3-(5-nitro-2-thiazolyl) urea.* To a solution of 69 g (0.97 mole) of ethyl isocyanate and 1200 ml of dry toluene was added 94 g (0.648 mole) of 2-amino-5-nitrothiazole. The stirred suspension was heated 16 hours at vigorous reflux under scrupulously anhydrous conditions. The warm slurry (80°) was filtered, and the pale yellow crystalline cake of 1-ethyl-3-(5-nitro-2-thiazolyl) urea was washed well with benzene and ether, and then dried to constant weight. Yield: 137.7 g (98.5%); m.p. 228° (dec.) Anal. Calcd. for $C_6H_8O_3N_4S$: C, 33.33; H, 3.73; N, 25.91. Found: C, 33.70; H, 3.58; N, 25.44. Infrared spectrum (Nujol mull): Intense carbonyl band at 5.95 μ; broad N-H absorption at 3.2 μ. Ultraviolet spectrum (ethanolic hydrochloric acid): $\lambda_{max.}$ 350 mμ $\epsilon_{max.}$ 14910; $\lambda_{max.}$ 234 mμ, $\epsilon_{max.}$ 7890. *Example 2. 1,1-*

TABLE I. Comparative Antihistomonad Activity of 1-Substituted-3-(5-nitro-2-thiazolyl) Ureas.



1-substituted-3-(5-nitro-2-thiazolyl) ureas

Substituent	R_1	R_2	Melting point	Relative antihistomonad activity (%) compared to 1-ethyl compound
1-methyl	H	CH_3	243.5° (dec.)	75
1,1-dimethyl	CH_3	CH_3	164–5°	25
1-ethyl	H	C_2H_5	228° (dec.)	100
1-isopropyl	H	$CH(CH_3)_2$	230–231° (dec.)	25
1-n-butyl	H	C_4H_9	200–202°	50
1-n-octyl	H	$n\text{-}C_8H_{17}$	156–7°	<10
1-n-octadecyl	H	$n\text{-}C_{18}H_{37}$	143–4°	<10
1-phenyl	H	C_6H_5	228° (dec.)	<10
1-p-nitrophenyl	H	$p\text{-}C_6H_4NO_2$	256° (dec.)	<10

Dimethyl-3-(5-nitro-2-thiazolyl) urea. A solution of 14.5 g (0.10 mole) of 2-amino-5-nitrothiazole and 16.1 g (0.15 mole) of dimethylcarbamyl chloride in ethylene dichloride (100 ml) was stirred at reflux for 2½ hours. On cooling the amber solution to room temperature, a heavy deposit of crystalline 1, 1-dimethyl-3-(5-nitro-2-thiazolyl) urea was obtained. The product was filtered, washed well with ether and dried. Yield: 21.3 g (98.5%); m.p. 164-5°. Anal. Calcd. for $C_6H_8O_3N_4S$: C, 33.33; H, 3.73. Found: C, 33.51; H, 3.90.

Biological. Experimental enterohepatitis was produced in young turkeys with cultures of *Histomonas meleagridis* as described in the second paper in this series. The 1-substituted-3-(5-nitro-2-thiazolyl) ureas were incorporated in the ration in concentrations of 0.1% and fed to the infected turkeys beginning on the third day after inoculation. The drug diets were fed until the 14th day after infection. The relative activity of the compounds was determined by their effect on preventing mortality and eradicating cecal and hepatic lesions of enterohepatitis. The effect of nithiazide on experimental trichomoniasis (*Trichomonas foetus*), trypanosomiasis (*Trypanosoma brucei*), coccidiosis (*Eimeria tenella*) and malaria (*Plasmodium gallinaceum*) was determined by standardized methods with appropriate reference standards for comparison(5).

Results. The comparative antihistomonad activities of nine 1-substituted-3-(5-nitro-2-thiazolyl) ureas are summarized in Table I. These data show that among the mono-substituted compounds the most effective were the 1-methyl and 1-ethyl derivatives. Increasing the length of this substituent beyond the butyl derivative decreased the activity of the compounds. Compounds with 8 or more carbon atoms in this substituent were relatively ineffective. The 1-methyl compound was slightly less effective and considerably more toxic (growth retardation) than the 1-ethyl derivative. The dimethyl substituted compound was less effective and less toxic than the mono-methyl compound. The 1-ethyl substituted compound was the most effective compound in enterohepatitis and, therefore, was selected for further evaluation in other parasitic protozoan infections.

In experimental trichomoniasis in mice, nithiazide eradicated body cavity infections of *T. foetus* when administered intraperitoneally in a dosage of 100 mg per kg on the 5th, 6th and 7th days of the infection. Under these conditions, nithiazide was equally as potent as aminitrozole, 2-acetylamino-5-nitrothiazole(5). However, when administered orally, nithiazide was less effective than aminitrozole. Nithiazide did not exhibit antimalarial, antitrypanosomal or anticoccidial

activity under appropriate standardized conditions of evaluation.

Summary. 1. The preparation of 1-monosubstituted and 1,1-disubstituted nitrothiazolylureas has been described. 2. Antihistomonad activity was demonstrated for monosubstituted thiazolylureas with not more than 4 carbon atoms. The 1-methyl compound was more toxic and less effective than the 1-ethyl derivative. The dimethyl compound was less toxic and also less effective than the mono-methyl compound. 3. In addition to therapeutic activity in enterohepatitis, nithiazide [1-ethyl-3-(5-nitro-2-thiazolyl) urea] was found to have antitrichomonal activity but antimalarial, antitrypanosomal or anticoccidial activity was not demonstrable.

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Nithiazide II. Effect on Enterohepatitis in Turkeys. (22520)

ASHTON C. CUCKLER AND CHRISTINE M. MALANGA. (Introduced by H. J. Robinson.)
Merck Institute for Therapeutic Research, Rahway, N. J.

Earlier studies(1) showed that nithiazide, 1-ethyl-3-(5-nitro-2-thiazolyl) urea, was an effective chemotherapeutic agent for histomoniasis and trichomoniasis, but ineffective for certain other parasitic protozoan infections. The present paper reports the results of studies on the effect of nithiazide on experimental enterohepatitis (blackhead disease) in turkeys.

Materials and methods. Day-old straight run Beltsville white poults were obtained from a commercial source. The poults were reared in electrically heated battery-brooders. They were fed an open-formula commercial turkey starter and water *ad lib.* When 2 weeks of age, they were sorted into balanced weight groups of 5 poults each. On the 15th or 16th day of age, the poults were inoculated intracloacally with 2 ml of a 48-hour culture suspension of *Histomonas meleagridis* grown in the Balamuth's(2) egg infusion medium. The number of histomonads in the inocula varied from 1 to 4 million per ml. In the various experiments, no attempt was made to adjust the number of organisms prior to inoculation since maximal infectivity and mortality were wanted. In the experiment in which enterohepatitis was induced with cecal worm infections, the poults were each inoculated orally with approximately 200 embryonated *Heterakis gallinae* ova. The groups of poults were then assigned randomly to unmedicated basal ration or to basal ration plus graded amounts of nithiazide or 2-amino-5-nitrothiazole. Graded concentrations of these compounds were thoroughly blended into turkey starter ration just prior to use. Nithiazide and the sodium salt of nithiazide, which was administered in graded concentrations in drinking water of the turkeys, were supplied by Merck Sharp and Dohme Research Laboratories. A total of 1267 poults were used in these studies. The graded levels of nithiazide required 707 poults whereas 480 poults served as untreated controls and 80 poults were utilized for comparative studies with 2-amino-5-nitrothiazole. The experiments in which poults were inoculated with *Histomonas* culture were terminated on the 14th day after infection. The experiment in which *Heterakis* eggs were used to produce the *Histomonas* infection was terminated on the 21st day after inoculation of the poults because the infection from this source develops more slowly. The experiments designed to determine relapse rate were ended 21 days