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)

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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

TABLE I. Comparative Effect of Nithiazide and 2-amino-5-nitrothiazole in Experimental Enterohepatitis Produced with Cultures of *Histomonas meleagridis.*

Medication	% in feed	No. poults	Mean wt (g) Initial	Mean wt (g) Terminal	% infection	Mean lesion score Ceca	Mean lesion score Liver	% mortality
Controls	.00	256	209	341	91	3.4	3.0	73
Nithiazide	.0125	65	213	368	86	3.2	.4	35
	.025	119	218	445	43	1.2	0	4
	.05	115	209	466	7	.07	0	0
	.1	59	217	466	2	.03	0	0
2-amino-5-nitro-thiazole	.025	30	192	361	73	2.4	1.1	23
	.05	20	181	373	30	.6	0	0
	.1	30	194	342	3	.1	0	0

after withdrawal of medicated ration. In all experimental turkeys, enterohepatitis was demonstrated as the cause of mortality and the surviving turkeys were sacrificed and autopsy examinations were made for pathological lesions characteristic of infectious enterohepatitis. The presence and severity of the lesions in ceca and/or livers were scored as follows: 0 = normal, 1 = minimal detectable and 4 = maximal. Other criteria used for assessing the extent of disease in untreated control and treated experimental turkeys included incidence of infection, mortality rate and relative growth rates.

Results. The data obtained with graded concentrations of nithiazide in the feed are summarized in Table I. The non-medicated control turkeys had an infection rate of 91% and the mortality rate was 73%. In addition, control poults had well-developed cecal and hepatic lesions of enterohepatitis and survivors had markedly depressed rates of growth. A dose-response effect was observed with the various levels of nithiazide medication. The feeding of 0.0125% nithiazide to turkeys, starting 3 days after exposure to infection and continuing for 11 days, had little effect on decreasing percentage of birds infected. However, the mortality rate was reduced to slightly less than 50% of that in controls. The severity of liver lesions was also significantly reduced by feeding a diet containing 0.0125% nithiazide. The infectivity and mortality rates were further decreased when the level of medication in feed was increased to 0.025% nithiazide. When this dosage was used, cecal lesion scores were significantly less than in untreated control poults and none of the 119 nithiazide-fed poults examined had characteristic lesions of hepatic histomoniasis. There was no mortality in turkeys fed 0.05% or 0.1% nithiazide and cecal lesions were present in 7% and 2%, respectively. In most instances these lesions were very small; they may have represented the residual of partially healed lesions. No attempt was made to demonstrate the presence of viable histomonads in these lesions.

Comparatively, the results with graded concentrations of 2-amino-5-nitrothiazole have shown that nithiazide was approximately twice as potent as the former compound. In addition to greater therapeutic potency, nithiazide also was significantly better tolerated than 2-amino-5-nitrothiazole.

The effect of nithiazide on enterohepatitis induced in turkeys by inoculation of cecal

TABLE II. Effect of Nithiazide on Enterohepatitis Induced with *Heterakis gallinae* Ova Administered Orally.

% nithiazide in feed	No. poults	Mean wt (g) Initial	Mean wt (g) Terminal	% infection	Mean lesion score Ceca	Mean lesion score Liver	% mortality
.00	18	154	344	100	4	3.6	83
.0125	18	155	528	67	2	1.1	0
.025	18	155	589	61	1.2	.5	0
.05	17	150	616	0	0	0	0

TABLE III. Effect of Nithiazide Administered in Feed or Water on Mortality and Relapse in Experimental Enterohepatitis.

Relation of start of medication to infection	Days of medication	% in feed or water	No. poults	Enterohepatitis mortality (%) During medication	After medication	Total
1 day before	21	.025 feed	58	2	3	5
3 days after	18	.025	57	11	8	19
Controls	—	None	59	—	—	86
5 days after	9	.025 feed	40	25	37	62
Idem	"	.05	40	0	37	37
Controls	—	None	39	—	—	85
7 days after	14	.1 feed	12	—	—	8
Idem	"	.2	11	—	—	0
Controls	—	None	12	—	—	50
3 days after	18	.005 water	5	—	—	40
Idem	"	.01	20	—	—	0
"	"	.02	5	—	—	20
"	"	.04	10	—	—	0
7 days after	14	.01	10	—	—	0
Idem	"	.04	18	—	—	11
Controls	—	None	56	—	—	73
10 days after	11	.01 water	5	—	—	40
Idem	"	.04	5	—	—	0
Controls	—	None	20	—	—	100

worm (*Heterakis gallinae*) ova is presented in Table II. In this experiment treatment was started 3 days after infection and continued for 18 days. The results show that feed concentrations of 0.0125% and 0.025% nithiazide markedly reduced the incidence of infection and severity of intestinal and hepatic lesions. Nithiazide in a feed concentration of 0.05% was completely effective in eradicating all evidence of infection and in preventing mortality from enterohepatitis. The greatest weight gains were made by poults fed 0.05% nithiazide.

The data shown in Table III were obtained to determine the influence and relationship of time of initiation and duration of medication on cure and relapse rates in enterohepatitis. When 0.025% nithiazide was fed starting 1 day before or 3 days after infection and continued for 18 or 21 days, mortality from enterohepatitis was significantly reduced. During a 21-day period of observation after withdrawal of medication, relapses were observed in 3% of poults fed nithiazide prophylactically. In the therapeutic trial started 3 days after infection, a relapse rate of 8% was observed. When treatment was begun 5 days after infection and continued for only 9 days, the relapse rate was 37% for each of the groups of poults fed 0.025% and 0.05% nithiazide. When dosage of nithiazide was increased to 0.1% or 0.2%, treatment was highly effective even when delayed until the 7th day after infection. Although clinical symptoms and mortality were observed in untreated control poults on the 7th day after inoculation, the use of nithiazide at this time, in concentrations of 0.1% or 0.2% in feed or 0.01% or 0.04% in water, significantly reduced or completely prevented mortality from enterohepatitis. Furthermore, administration of 0.04% nithiazide in water beginning on the 10th day after infection prevented mortality from an infection of enterohepatitis which was lethal for all untreated control birds.

Discussion. The data presented show that nithiazide is highly effective for prevention or treatment of enterohepatitis in turkeys. Although nithiazide (1-ethyl-3-(5-nitro-2-thiazolyl) urea) is related to 2-amino-5-nitrothiazole, the potency and toxicity of the compounds differ markedly(3,4). Nithiazide was approximately twice as potent as 2-amino-5-nitrothiazole for controlling mortality from enterohepatitis. The growth

of turkeys fed 0.1% 2-amino-5-nitrothiazole for only 2 weeks was greatly retarded whereas nithiazide in a feed concentration of 0.1% had little effect on growth of young turkeys.

The feeding of nithiazide at 0.025% concentration in the ration 1 day before or 3 days after infection with *H. meleagridis* was highly effective in preventing mortality from enterohepatitis. Furthermore, the feeding of 0.05% nithiazide 3 days after infection was completely effective in preventing mortality from infectious enterohepatitis which killed 73% to 83% of untreated controls.

When treatment was not started until 5 days after infection and continued for only 9 days, nithiazide in feed concentration of 0.025% was relatively ineffective. Under these conditions, a feed concentration of 0.05% was effective in preventing mortality during medication; however, the 9-day period of medication was insufficient and relapses occurred in 37% of the poults. When the concentration of nithiazide was increased to 0.1% or 0.2% and the period of feeding was extended to 14 days, mortality from enterohepatitis was effectively controlled even when medication was delayed until the 7th day after infection. The use of 0.01% to 0.04% nithiazide in the water, beginning 3 days or 7 days after infection, effectively prevented mortality from enterohepatitis. Mortality from enterohepatitis was also prevented with 0.04% nithiazide administered in the water 10 days after infection.

Summary. 1. The data presented show that nithiazide [1-ethyl-3-(5-nitro-2-thiazolyl) urea] was more potent and less toxic than 2-amino-5-nitrothiazole when fed to turkeys with enterohepatitis. 2. Nithiazide was most effective when administered prior to infection or beginning 3 to 7 days after infection. It prevented mortality from enterohepatitis produced either by oral exposure to cecal worm eggs or by rectal inoculation of *Histomonas meleagridis* cultures. Nithiazide may be administered in the feed or water and is equally effective in either form. 3. The therapeutic feeding of rations containing 0.05% nithiazide, starting 3 days after infection, prevented mortality from infectious enterohepatitis which was lethal for 73% to 83% of the untreated control turkeys.

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Inhibition of Experimental Nephrocalcinosis by Hypophysectomy.* (22521)

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Excessive administration of various mono- or dibasic phosphate solutions causes extensive renal calcification in rats. In unadrenalectomized animals (sensitized for the nephrotoxic action of mineralocorticoids by unilateral nephrectomy), this nephrocalcinosis is aggravated by desoxycorticosterone acetate (DOCA) and inhibited by cortisol acetate (COLA)(1-3). From this it was concluded that the corticoids exert an important regulating influence upon the ability of the kidney to handle an excess of phosphate. Yet nephrocalcinosis can be induced by phosphate even after complete adrenalectomy(4). The question now arose whether pituitary hormones are necessary for induction of nephrocalcinosis by combined treatment with DOCA and an excess of phosphate. This appeared of interest, because nephrocalcinosis is so markedly enhanced by DOCA, a steroid whose other nephrotoxic actions (the produc-

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