Proceedings of the Iowa Academy of Science

Volume 67 | Annual Issue Article 72

1960

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Abstract. A method for controlled evaluation of drugs against *Hexamita meleagridis* in turkeys is presented. Experiments evaluating selected drugs and reevaluating all compounds and antibiotics previously reported to be efficacious against this species are described. Most of these compounds were tested against two separate isolates of this parasite, one from Indiana (1950) and the other from Wisconsin (1959). All preparations found effective against one isolate showed similar efficacy against the other. Conversely, preparations ineffective against one isolate were also ineffective against the other. Among the compounds showing efficacy were several antibiotics and dibutyl tin salts. Dibutyl tin dilaurate was found to have suitable efficacy at non-toxic dosage levels.

Chemotherapy of hexamitiasis, a disease of economic importance to the turkey industry, has received relatively little experimental emphasis, even though the pathological potentialities were predicted by Hinshaw *et al.* (1938). Increasing numbers of reliable diagnoses have been reported in the meantime. In fact, Hinshaw and Rosenwald (1949) consider this disease to be a more serious problem than coccidiosis in turkeys. Both diseases often occur in poults of the same age and are symptomatically indistinguishable.

The rapid course of this disease may produce high mortality and severe morbidity demanding specific medication. McNeil (1948) first recommended that 0.05 percent copper sulfate and 3 percent dried whey be substituted for the drinking water as a control measure. Almquist and Johnson (1951) suggested that several antibiotic substances and the compound 2-amino-5-nitrothiazole might interrupt the fatal course of hexamitiasis. McGuire and Morehouse (1952) reproduced this disease experimentally and were able to confirm only the antibiotic efficacy while reporting excellent preventive efficacy for dibutyl tin derivatives. Wilson and Slavin (1955) confirmed this effect stating, "Di-n-butyltin dilaurate (Tinostat) appears to be the most promising drug so far tried." Mangrum *et al.* (1955) reported therapeutic effect in approximately half of the birds treated with N(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone. Fogg (1957) reported 1-ethyl-3(5-nitro-2-thiazolyl) urea as an effective preventive and treatment in field outbreaks.

This paper summarizes our work on these chemotherapeutic agents and compares the efficacy of several of them against two

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separate isolates of *Hexamita meleagridis,* a 1950 isolate from Indiana and a 1959 isolate from Wisconsin.

Materials and Methods

The turkey poults used in these experiments ranged in age from three to six weeks. Four or more poults were weighed and placed in each test group so that the mean weight of each group was approximately equal. These poults were kept in cages or in heated batteries, depending upon the age of the poults at the time the experiment was initiated. The birds were weighed and group feed and/or water consumption records were recorded weekly during the 21 to 42 days of the experiment. Medication was administered in feed or drinking water for a period of 14 days.

Infective material was administered on or before the third day of medication. This material consisted of (1) two to four pieces of freshly excised ileum and their contents from birds recently dead of hexamitiasis, or (2) 0.5 to 1.0 ml. of allantoic fluid harvested from chick embryo cultures established by the method of Hughes and Zander (1953), and containing 25-50 organisms per low power field. It has been found that multiple infections produce greater mortality than single inoculations.

To obtain the pieces for infection method (1) the entire ileum was removed and examined at each severed end, and any terminal portion not showing many active protozoa was discarded. The intestine was then cut into two equal lengths and these pieces further cut transversely to provide the required number of smaller pieces for introduction into the crop of each poult. Each infected bird received at least one piece of ileum approximately one inch long from the cephalad portion and one from the caudad portion of the parasitized intestine. The number of *H. meleagridis* microscopically demonstrable from each piece of ileum varied considerably and was not routinely calibrated. However, actual hemocytometer counts of protozoa immobilized with formalin varied from 100 thousand to 500 thousand organisms per ml. of diluted intestinal fluid, and an infective dose was estimated to be in excess of a million trophozoites.

It has been found that heating infective material in a water bath for five minutes at 65° C. or freezing it overnight in a dry ice chest at –40° C. serves to kill the hexamita. Subsequent oral inoculation of appropriate controls with this deactivated material was occasionally utilized as a check against the possibility that bacterial or viral agents capable of surviving these treatments could be responsible for the pathology resulting from the hexamita-containing infective material.

When weight loss or mortality in the nonmedicated, infected

group indicated the termination of the clinical stage of the infection, which varied with the age of the test poults, samples of bursal and cloacal material were taken from the survivors and the causal organism microscopically identified. This provided a check on the experimental infection for each bird and determined the carrier state in birds which had recovered either with or without the aid of medication.

Results and Discussion

Figures 1 and 2 report two comparative experiments which show the compounds tested, the dosage levels used, the relationships of the infections to the daily mortality, and the experimental periods adopted for this test method. The birds in the first experiment (Figure 1) were initially infected by two separate oral inoculations with intestinal pieces containing hexamita from the Indiana (1950) isolate. Those of the second experiment (Figure 2) were initially infected by oral inoculation, on day zero, with ½ ml. per bird of allantoic fluid containing hexamita isolated from Wisconsin in 1959.

All groups received medication in feed at the indicated concentration administered two or three days prior to the primary infection and for a total of 14 days. The ten-day post-medication period, from day plus 11 to day plus 21, is utilized as a relapse observation period, as it has been proven that some agents appearing to have chemoprophylactic efficacy against hexamitiasis or other protozoan diseases, such as histomoniasis, merely tend to inhibit pathological development while medication is being given but allow rapid relapse on withdrawal of the drug.

Following this ten day relapse period a challenge infection was given to surviving birds, as is indicated on day plus 21 in the above figures. The challenge infection was utilized to determine resistance due to residual drug action, age immunity, or acquired resistance among surviving birds. The experiments were concluded on day plus forty.

It may be seen from these experiments that 100 percent mortality in the nonmedicated, infected controls was achieved by the end of the seventh post-infection day with either method of exposure. By the end of their medication period, nearly all birds with 0.025 percent chloromycetin, chlorotetracycline, bacitracin, neomycin, streptomycin, and 2-amino-5-nitrothiazole or 0.05 percent 1-ethyl-3(5-nitro-2-thiazolyl) urea had died showing typical lesions of hexamitiasis.

Only dibutyl tin dilaurate fed at 0.0375 percent provided 100 percent chemoprophylactic efficacy in these tests. Subsequent testing has revealed similar efficacy for other dibutyl tin salts including

maleate, dichloride, dioleate, distearate, oxide, and arsanilate. As summarized in Table 1, dibutyl tin dilaurate has repeatedly shown excellent chemoprophylactic efficacy at dosages innocuous to poultry.

Table 1

Summary of Dibutyl Tin Dilaurate Efficacy Against Both Indiana and Wisconsin Isolates of *Hexamita meleagridis*

Percent Concentration in Feed	No. dead of Hex/No. test poults		No. of Replicates	Average Percent Efficacy
	Treated	Nontreated		
0.1	1*/24	21/24	6	100
0.075	0/16	10/16	4	100
0.0625	2/20	19/20	5	85
0.05	3/25	23/25	6	80
0.0375	6/133	106/133	26	95
0.025	2/20	20/20	5	90
0.01	7/12	10/12	3	25
0.00625	8/8	8/8	2	0

*Dead of toxicity or other unknown causes—no demonstrable hexamita

We have been able consistently to demonstrate chemoprophylactic efficacy against both Indiana and Wisconsin isolates for only the dibutyl tin salts and the antibiotics or antibiotic-like substances shown in Table 2.

Table 2

Feed Concentrations of Antibiotics and Antibiotic-like Substances Effective Against the Indiana (1950) and Wisconsin (1959) Isolates of Hexamita

Percent Feed Concentrations (1950)	Compound	Percent Feed Concentrations (1959)
0.025-0.05	penicillin	0.02 -0.1
0.05 -0.1	chlortetracycline	0.03 -0.5
0.05	oxytetracycline	0.02 -0.1
0.05	chloromycetin	0.03 -0.1
0.05	magnamycin R	0.05
0.022-0.033	N-5-nitro-2-furfurylidine-3-amino-2-oxazolidone	0.016-0.033

Figures 1 and 2 show that drugs found ineffective against the 1950 Indiana isolate were also found ineffective aginst the 1959 Wisconsin isolate. Other drugs found ineffective against either or both isolates are reported in Table 3.

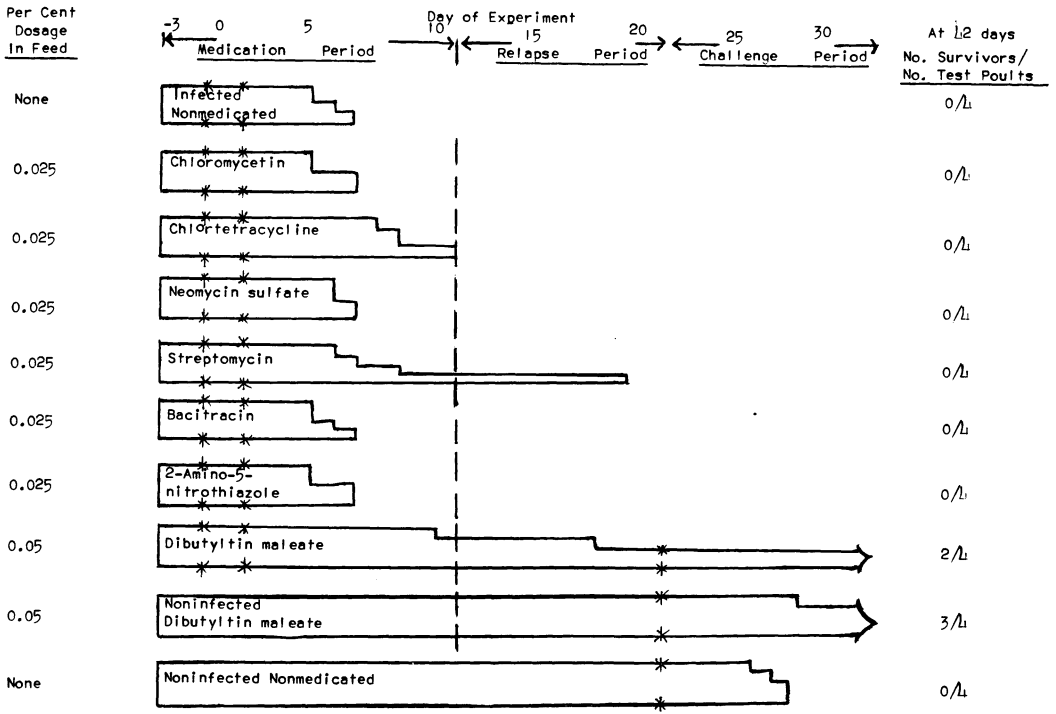

Figure 1. An experiment comparing antibiotic efficacy when infected with the 1950 Indiana isolate of *H. meleagridis*.

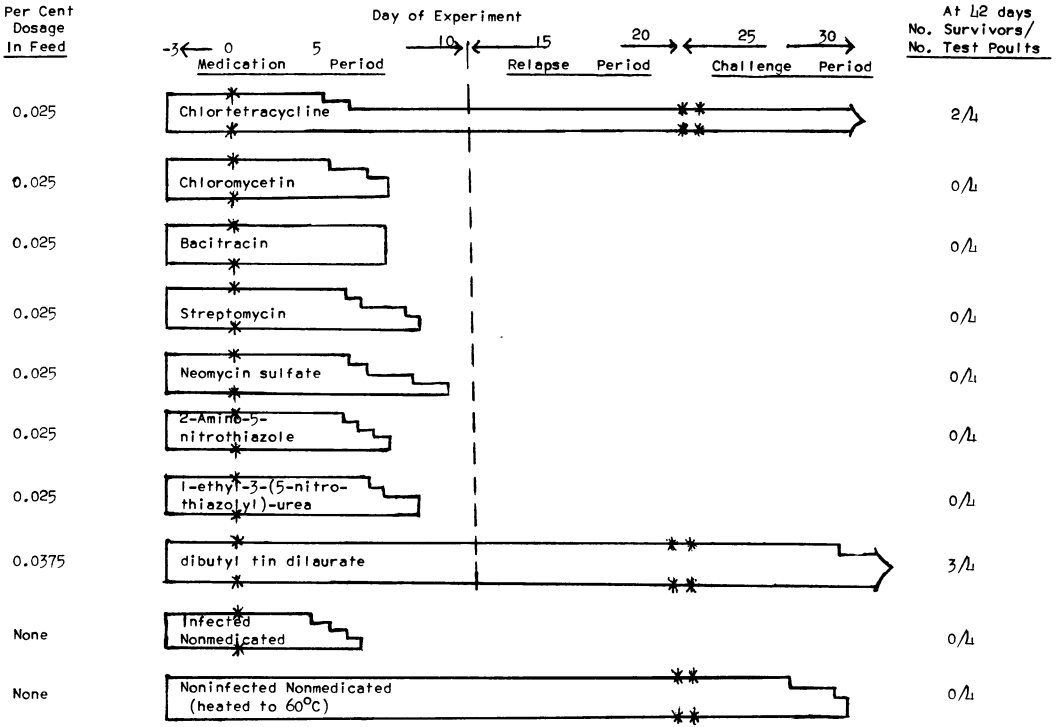

Figure 2. An experiment comparing antibiotic efficacy when infected with the 1959 Wisconsin isolate of *H. meleagridis*.

Table 3

Compounds Found to Be Ineffective Against the Indiana (1950) and Wisconsin (1959) Isolates of *H. meleagridis* When Administered for 14 Days in Feed (IF) or Drinking Water (IW) at Concentrations Shown

Percent (IF) or Percent (IW) (1950)		Compound Name	Percent (IF) or Percent (IW) (1959)	
0.05	IW	ccpper sulfate	0.05	IW
3-5	IW	whey	3	IW
0.05 + 3	IW	copper sulfate + whey	0.05 + 3	IW
0.015-0.05	IF	sulfaquinoxaline	0.015-0.05	IF
0.05	IF	sulfadiazine	—	
—		4,5 imidazole dicarboxamide	0.006	IF
0.015	IF	2-acetylamino-5-nitrothiazole	0.015-0.03	IF
—		2-hydroxymethylamino-5-nitrothiazole	0.05 0.03	IF & IW
0.011-0.022	IF	5-nitro-2-furfuraldehyde semicarbazone	0.022	IF
0.05	IF	arsanilic acid	—	
0.375	IF	4-ureido benzene arsonic acid	0.075	IF
—		3,5 dinitrobenzamide	0.05	IF
—		3,5 dinitro-o-toluamide	0.0125	IF
—		phenothiazine	0.1	IF
—		piperazine (as sulfate)	0.0013	
0.025		polymyxin B sulfate	0.025	
—		mycostatin	0.025	IF
0.025		fumagillin	0.025	IF
—		penicillin (autoclaved)	0.05	IF

Numerous compounds or mixtures, such as the ones listed above, have been suggested for treatment or prevention of hexamitiasis but tested according to our procedure, they have proven ineffective against both the Indiana and Wisconsin isolates of *H. meleagridis*. It has been suggested that failure of recommended treatment levels of certain drugs to control field infections of hexamitiasis has been due to species variation or drug resistance. We have found no evidence of measurable variability in drug response of two isolates of hexamita from widely separated areas.

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