ENTEROHEPATITIS (BLACKHEAD) IN TURKEYS
V. FURTHER EXPERIMENTS ON CHEMOTHERAPY

By W. E. SWALES *

WORK DESIGNED to indicate possible roles of drugs in the control of enterohepatitis has been continued in parallel with other projects dealing with this disease. The earlier tests of various drugs by Swales and Frank (1) did not indicate any practical value for drug treatment. However, DeVolt and Holst (2) showed that iodochloroxyquinoline ("Vioform") had a definite preventive action and (3), that chlorohydroxyquinoline was equally effective. Waletzky et al (4), discovered that two thiametroimidines ("Enheptin-P" and "Enheptin-T") were effective in preventing mortality from experimental infections, and that turkeys were completely protected even when treatment was delayed for 120 hours after oral inoculation with eggs of *Heterakis gallinae*. When rectal inoculations with infected liver emulsions were employed the results were not so clear-cut, but still indicated a definite action, presumably histomonacidal. Waletzky's detailed studies on these promising compounds also indicated lack of toxicity in dosage levels below 0.2% of the feed.

McGregor (5) tested a number of compounds on turkeys that were exposed to infective material and found in a single test group that "Enheptin-T" prevented enterohepatitis when given as 0.05% in the feed continuously through the period of exposure. In McGregor's test the parallel use of Metachloridine, tetramethylthiuram disulphide, tetraethylthiuram disulphide, hexachlorethane, sulphaquinoxaline, bacitracin and two other compounds ("P-29" and "5051") allowed mortalities not significantly different from the 57.1% suffered by the untreated controls.

Sautter & Pomeroy (6) confirmed the value of "Vioform" and also indicated that "Stovarsol" (=Acetarsone USP) had an appreciable preventive action when used before, and on certain days during, the development of infection from rectal inoculation with infected liver emulsions.

In order to add to the list of drugs screened for anti-enterohepatitis value, and to make further observations on "Enheptin-T" (2-amino-5-nitrothiazole), one of the compounds studied by Waletzky *et al*, twenty-one experiments were conducted.

MATERIAL & METHODS

Broad-breasted Bronze turkey poults were used. As each test was commenced the birds were weighed, tagged and in most cases were given, *per os*, a capsule containing a pre-determined number of embryonated eggs of the caecal worm, *H. gallinae*, from sources known to be infective. In Experiments Nos. 1 and 3, the method of exposure to soil pens containing feces from common fowl was used. In all others the number of caecal worm eggs varied according to the known infectivity of any group of eggs. In preparing the

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dose of eggs it was found necessary to remove them from the glass bottom of the "culture" dishes by means of a rubber "sweep", then to pipette them quickly onto a small piece of hard gelatin (half an empty capsule) and to make the approximate count under a stereoscopic microscope. The piece or pieces of gelatin were then placed in a larger capsule, and a little ground turkey feed added to absorb the water. This capsule was then given *per os* before it became too soft. This technique avoided the gross inaccuracies which result from caecal worm eggs adhering to the glass pipettes etc.

When direct dosing of poults with caecal worm eggs was used the birds were kept in wire-floored turkey batteries.

The results obtained from the experiments are tabulated. (See Table I). These experiments are shown in the order in which they were conducted and apparent variations in procedure are due to modifications deemed necessary as the work proceeded. Many of the preliminary tests relied on determining any effect upon enterohepatitis of a drug given on the 12th day after inocution, when the first signs of the disease appeared, and when the first signs might be detected under farm conditions. However, it is realized that such tests are probably too severe, as hepatitis is usually established by the 12th day; hence the drugs so tested are not necessarily eliminated from future consideration by this or other laboratories.

Some of the test compounds do not appear to warrant discussion at this time, as the results did not show promise of success. In the case of 2, 2-Bis-(p-nitrophenyl)-1, 1-dichloroethylene the treated poults appeared to receive some degree of support, as they were active when the untreated controls were severely affected. However, the effect was, in most cases, temporary, as is evident from the mortalities.

The commercial preparation of arsenic in solution was treated on request from several sources, and because of reputed success under practical conditions in Great Britain. It is apparently inferior to certain other compounds as a preventive, but when the mortality in birds treated on the tenth day was compared to that in untreated controls (46% vs. 87%), the difference was found to be statistically significant ($x^2=7.38$. $P=0.05$).

Antrycide produced an acute toxic effect when used in the doses shown, although all but one bird recovered within an hour.

ADDITIONAL STUDIES ON 2-AMINO-5-NITROTHIAZOLE

Compared to other compounds tested the compound 2-amino-5-nitrothiazole ("Enheptin-T") was very effective, and was, therefore, accorded special attention. Waletzky *et al* (4) had conducted numerous tests, and the protocols which he kindly made available to the author suggested a definite value. Even in the one experiment in our work (No. 17) in which two deaths occurred in treated birds the lesions were comparatively light, both in the caeca and livers, and some question arose as to other factors being involved in the deaths. Furthermore, it was noted in these two birds that the two deaths occurred somewhat later than usual for such young poults, (23

TABLE I.

TESTS OF MISCELLANEOUS COMPOUNDS IN ESTABLISHED ENTEROHEPATITIS.

Expt. No.	No. of Poults.	Av wt. (lbs.)	Drug. dose and method of administration	Treatment started (Days after exposure or inoculation)	Duration of Treatment (days)	% Mortality (entero-hepatitis)
1	5	6.4	Chloroquine diphosphate, 50 mg./poult /day. (Capsule)	12	10	100
1	5	6.4	Control	—	—	100
2	5	3.7	Chloroquine diphosphate, 50 mg./poult day. (Capsule)	12	5	100
2	5	3.6	Di-iodohydroxyquinoline, 200 mg./poult /day. (Capsule)	12	6	100
2	5	3.6	Control	—	—	100
3	10	7.0	Soln. phenyl mercuric nitrate, 0.002% as drinking water	9	3(2)3(2)3	100
3	10	6.9	Control	—	—	90
4	5	6.0	Streptomycin, 15,000 units/lb. daily, in 3 doses, intramusc.	12	6	80
4	5	5.8	Acetarsone U.S.P. 8 grs./poult/day—oral tablets	12	4	40
4	5	7.8	Soln. phenyl mercuric nitrate, 0.004%, as water	12	3-(2)-3	100
4	5	5.8	Control	—	—	100
5	5	3.1	Acetarsone U.S.P., 4 gr./poult/day, oral tablets	10	4-(2)-2	80
5	5	8.7	p-Bis-(carboxymethylthio)-arsinobenza-mide 100 mg./poult/day - in capsule	12	6	40
5	5	3.9	Carbarsone oxide, 100mg./poult/day.-in capsule	12	4	100
5	5	6.5	Control	—	—	40
6	5	6.8	Carbarsone oxide, 50 mg./poult/day-in capsule	10	2-(3)-3	80
6	5	6.5	I-Pentanearsonic Acid. 100 mg./poult/day —capsules	12	4	100
6	5	6.1	Arsphenamine diglucoside (1.28% W/V As_2O_3) 1.3 cc per poult intravenously	12	12th & 15th	60
6	4	7.2	Control	—	—	75
7	5	9.3	8-Amino-5, 7-diiodoquinaldinic acid 200 mg/poult/day—in capsule	10	4	60
7	5	9.0	4-(3′, 4′-Dichlorobenzylamino) benzoic Acid 200 mg./poult/day —in capsule	10	4	20
7	5	9.0	2, 2-Bis-(p-nitrophenyl)-1, 1-dichloroethy-lene 200 mg./poult/day—in capsule	10	4	20
7	5	7.9	Control	—	—	100
8	5	9.1	Neoarsphenamine, 1 mg./lb body wt. in-travenous	10	1	60
8	5	8.6	2, 2-Bis-(p-nitrophenyl)-1, 1-dichloroethy-lene, 250 mg./poult/day — capsule	10	5	80
8	5	9.0	4-(3′, 4′-dichlorobenzylamino) - benzoic acid, 250 mg./poult/day—capsule	10	5	80
8	5	7.4	Control	—	—	60

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Expt. No.	No. of Poults.	Av wt. (lbs.)	Drug. dose and method of administration	Treatment started (Days after exposure or inoculation)	Duration of Treatment (days)	% Mortality (entero-hepatitis)
9	6	2.2	1% Soln. arsenic (Commercial preparation) 0.35 cc./6 poults daily in feed.....	—1	3(3)3(10)3 &c.	50
9	6	1.8	1% Soln. arsenic (as above) 0.24 cc./poult/day, into crop.....	10	6(1)6	17
9	6	1.9	Control.....	—	—	50
10	6	2.0	2, 2-Bis-(p-nitrophenyl)-1, 1-dichloroethylene, 150 mg./poult/day.—capsule.....	5	3(3)4	33
10	6	2.0	4-(3', 4'-dichlorobenzylamino) - benzoic acid, 150 mg./poult/day.—capsule....	5	3(3)4	83
10	6	2.1	Control.....	—	—	83
11	6	3.7	1% soln. arsenic (as in Expt. 9), 0.35 cc./6 poults daily in feed.....	0	3(3)3(10)3	50
11	6	3.7	1% soln. arsenic (as in Expt. 9), 0.24 cc./poult/day, into crop.....	10	6(1)6	66
11	6	3.7	Control.....	—	—	100
12	6	1.2	Arsenious Acid Solution NF. 0.75 cc./6 poults daily in feed.....	0	3(3)3(3)3	100
12	6	1.2	Arsenious Acid Solution NF. 0.25 cc./poult daily into crop.....	10	7	83
12	6	1.2	Control.....	—	—	100
13	6	1.4	Arsenic diethyldithiocarbamate, 50 mg/poult/ day—capsules.....	5	3(2)3	50
13	6	1.4	2, 2-Bis- (p-nitrophenyl)-1, 1-dichloroethylene, 250 mg./poult/day—Capsules..	5	3(2)3	100
13	6	1.5	Control.....	—	—	66
14	6	1.7	Bismuth pentamethylene dithiocarbamate, 100 mg./poult/day—Capsules	5	3(2)3	50
14	6	1.7	Sodium diethyl dithiocarbamate, 100 mg./poult/day—Capsules.....	5	3(2)3	50
14	6	1.7	Control.....	—	—	83
15	6	2.8	1% Soln. arsenic (as in Expt. 9)—0.24 cc./poult/day, into crop.....	10	6(1)6	50
15	6	2.8	Control.....	—	—	100
16	6	2.7	1% Soln. arsenic (as in Expt. 9)—0.24 cc./poult/day, into crop.....	10	6(1)6	66
16	6	2.8	Control.....	—	—	100
17	6	3.0	"Enheptin-T" as 0.1% of feed.....	5	3(2)3	33
17	6	2.8	Bismuth pentamethylene dithiocarbamate, 200 mg./poult/day.....	5	3(2)3	83
17	5	2.7	Control.....	—	—	80
18	6	3.2	Bismuth diethyl dithiocarbamate 200 mg./poult/day—Capsules.....	5	3(2)3	66
18	6	3.2	"Enheptin-T", as 0.1% of feed.....	5	3(2)3	0
18	6	3.2	"Enheptin-T", as 0.1% of feed.....	5	12	0
18	6	3.3	Control.....	—	—	100

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TESTS OF MISCELLANEOUS COMPOUNDS IN ESTABLISHED ENTEROHEPATITIS.

Expt. No.	No. of Poults.	Av wt. (lbs.)	Drug. dose and method of administration	Treatment started (Days after exposure or inoculation)	Duration of Treatment (days)	% Mortality (entero-hepatitis)
19	6	5.2	"Antrycide" Soln. 1 cc (=20 mg) per 2 lbs body wt. subcut.	10	10th & 14th	33
19	6	5.2	"Antrycide" Soln. 1 cc (=20 mg) per 2 lbs body wt. subcut. + 1 gm. phenothiazine *per os*	10	10th & 14th	50
19	6	5.2	"Enheptin-T", as 0.1% of feed	10	6	0
19	6	5.2	Control	—	—	33
20	5	3.5	"Enheptin-T", 0.2% of feed, 5 days, 0.1%, 6 days	10	11	0
20	5	3.4	Control	—	—	20
21	6	11.6	"Enheptin-T", as 0.2% of feed—4 days, 0.1%—6 days	10	10	0
21	6	11.0	"Enheptin-T", as 0.1% feed	5	12	0
21	6	11.1	Control	—	—	83*

* Killed while showing advanced enterchepatitis.

and 24 days after inoculation), and the immature *H. gallinae* were in a degenerate condition; this latter observation led to a special study on the effect of this compound on the caecal worm larvae. "Enheptin-T" was given as 0.1% of the feed of four chicks, two months of age, from the 2nd to the 5th day after they had each received 500 embryonated ova of *H. gallinae*. Four similarly infected birds were retained as untreated controls. Three days after the treatment the chicks were killed, and the caeca of the treated ones yielded an average of 50.5 larvae, whereas from the untreated chicks an average of only 5.2 larvae per bird was recovered. This experiment was unsatisfactory in that the small number of chicks was unevenly parasitized, but it indicated that the drug did not act as an anthelmintic against larvae of *H. gallinae* in the caecal mucosae of normal chicks.

Two adult fowl known to harbour adult *H. gallinae* were given "Enheptin-T" as 0.1% of their feed for four days. During this time, and for the three days following treatment, all the feces were carefully examined for caecal worms, with negative results. On post-mortem examination it was found that the two fowl harboured 121 and more than 1000 adult caecal worms respectively.

In spite of the above findings the opportunity was taken to examine more closely the effect of the drug on caecal worm larvae in turkeys. In Experiment No. 21 all the surviving birds were killed between the 23rd and 25th days after inoculation. The livers of the two lots that received the drug were free of necrotic lesions, and, except for a slightly paler than normal

colour in four cases, were macroscopically normal. In each case the caeca of the treated birds showed from four to eight very small lesions, not more than 1/8th" in diameter, and slightly raised above the surface of the surrounding mucosa. Severe enterohepatitis was present in five of the six untreated controls. All the caeca were carefully examined for larval or immature caecal worms and complete counts were made. In the untreated controls the numbers ranged from 31 to 1580, with an average of 520.0 (S.D. = 524.0). In the first treated group the number ranged from 0 to 15, with an average of 5.3 (S.D.=5.8) and in the second treated group the range was 1 to 64, average 21.0 (S.D. = 20.1). In spite of the very large standard deviation the differences between 5.3 and 520.0, and between 21.0 and 520.0, are statistically significant ($P = 0.05$). The lengths of the larvae varied between 3 and 8 mm., but there were no differences between groups in this respect, in spite of apparent differences between individual birds.

Observations were also made on the susceptibility of turkey poults that had been protected by "Enheptin-T" medication. In Experiment No. 17 three of the treated poults that had not shown clinical evidence of enterohepatitis were turned out to a soil pen previously used by common fowl. One bird died of enterohepatitis three months later; the other two remained healthy.

In Experiment No. 18 the two groups given this drug had remained free of any clinical evidence of the disease. As will be noted, one group was treated for three days, was without drug for two, and was again treated for three days, this regimen being chosen so that the drug intake would roughly coincide with the development of typhlitis and hepatitis respectively. The other group was treated continuously for twelve days. Thirty-five days after the start of the experiment three birds from each of these two groups were given 500 embryonated ova *per os*. By the 20th day thereafter the three birds that had been previously protected by continuous medication for 12 days were dead from typical enterohepatitis, while the three from the group previously given the drug over two periods of three days were only slightly affected by the challenge and they recovered quickly. One week later they were killed and found to be virtually free from lesions of enterohepatitis. Waletzky *et al* found that birds protected by this and a related drug remained susceptible to re-infection and while observations recorded here are on too small a scale to justify any decision, they do indicate that birds treated in the early stages of developing typhlitis will remain susceptible while those treated in such a way that the early pathological processes in the caeca are not completely suppressed may develop strong resistance to reinfection.

Further studies on this important point should be made with young poults.

DISCUSSION OF RESULTS

The results of greatest interest are those obtained through the testing of 2-amino-5-nitrothiazole ("Enheptin-T") the structure found by Walet-

zky *et al* to suppress experimentally induced enterohepatitis. In our limited tests the poults were protected from harmful enterohepatitis even when treatment was delayed to the 10th day after ingestion of infective eggs of *H. gallinae*. The finding that the larval heterakids in the caeca of infected turkeys were affected might prove to be of some significance insofar as an explanation of the mode of action of this drug is concerned. However, this drug was also shown by Waletzky *et al* to suppress enterohepatitis induced by the rectal inoculation of poults with infected liver tissue, so that a histomonicidal effect, as well as an anti-Heterakis action, appears to have been demonstrated.

The inoculation of the poults with embryonated eggs of the caecal worm brought about the disease in a similar way to natural exposure to contaminated ground, hence it is believed that the results indicated a definite role for the successful compound in the practical control of the disease. Outbreaks may occur in a flock over a period of several days or even weeks, and while the removal of the flock to new ground or to porches protects many birds there has, hitherto, been no highly successful way of saving those in which clinical signs of the disease are imminent. As the use of "Enheptin-T" under the conditions described has not resulted in any detectable toxic effect it seems that toxicity need not be considered if the drug is made available for use for the control of established disease. It has been shown by Waletsky and by McGregor that it will prevent the establishment of the disease when used continuously in the feed during exposure to contamination, but its long term use as a prophylactic would depend upon more and detailed studies on healthy poults given the drug continuously. If it was used as a preventive for poults on contaminated ground medication could probably not be stopped without risk of an outbreak, unless the flock was moved to quarters free of infective material. This also appears to be true in the case of the other drugs known to act as preventives in enterohepatitis. It is, therefore, believed that this new drug might more successfully fill the role of an emergency drug easily administered to a flock in which the disease has appeared, and in which a serious outbreak might be imminent.

SUMMARY

The results of twenty-one experiments with twenty-two chemical compounds in the chemotherapy of enterohepatitis of turkeys are presented.

The value of 2-amino-5-nitrothiazole ("Enheptin-T") against enterohepatitis, as discovered by Dr. E. Waletzky and coworkers, has been confirmed. Experiments with this compound have shown that it is effective even when treatment is delayed until the tenth day after inoculation of poults with caecal worm eggs. It was found to reduce the number of larval heterakids which survive in the caeca of the test poults to a low and statistically significant degree.

The possible roles of drugs in the practical control of enterohepatitis are discussed.

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HEMOLYTIC ANEMIA IN NEWBORN DOGS DUE TO ABSORPTION OF ISOANTIBODY FROM BREAST MILK DURING THE FIRST DAY OF LIFE

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FOUR different antigenic factors have thus far been identified serologically in dog erythrocytes. One of these which may conveniently be called the "Do factor" appears to be somewhat analogous to the Rh factor of human red blood cells. Dogs lacking this factor in their red corpuscles are accordingly labelled "Do-negative" and those whose cells contain this factor are called "Do-positive."

Do-negative bitches that have been immunized by transfusions of Do-positive blood develop Do-antibodies that appear in high concentration in the colostrum after pregnancy and persist for several weeks in the breast milk. Experience with nine litters has shown that Do-positive puppies regularly develop hemolytic anemia if they suckle such an immunized dam during the first day or two of life. The lack of antibody absorption from milk after the second day has not yet been explained. There is evidence that natural immunization of the dam by fetal red cell factors may occur during pregnancy, thus producing hemolytic disease in dogs similar to that recently discovered as a naturally occurring phenomenon in horses.

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