ENTEROHEPATITIS (BLACKHEAD) IN TURKEYS

VIII.—FURTHER OBSERVATIONS ON THE USES AND MODE OF ACTION OF 2-AMINO-5-NITROTHIAZOLE

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Following the discovery by Waletzky *et al* (1) that 2-amino-5-nitrothiazole was effective in the prevention of losses in turkey poults from enterohepatitis, and the work by several authors showing its effectiveness under field and laboratory conditions, this drug was widely adopted for practical use by the turkey raising industry. The results of its widespread use in Canada have been largely favourable, particularly when it was used to check outbreaks of the disease. However, its occasional failure under field conditions and some uncertainty on the part of poultrymen regarding the most efficient and economical means of using the drug made further observations by this Division necessary.

The observation by Swales (2) that the drug removed a high percentage of the larval *Heterakis gallinae* from poults to which these nematodes had transmitted the disease appeared to have some relationship to the absence or presence of renewed outbreaks in birds that had no further exposure. In order to confirm this larvicidal effect, and at the same time to make further controlled tests of the efficacy of the drug, a series of experiments was conducted.

EXPERIMENT No. I

Fifteen poults, four weeks old, each given 500 embryonated ova of *H. gallinae* (chicken origin), and were then divided into three equal groups. Group A was given A.N.T.* as 0.2% of feed on the 10th, 11th and 12th days, and as 0.1% for six more days following infection. Group B was given the same dosage, but treatment was commenced 48 hours later (on the 12th day). Results are shown in Table I. The one bird that died in Group B was too ill to eat by the 12th day, and therefore did not get any drug. The number of larvae in its caeca was 250, which is not included in the average figure of 8 derived from the other poults that were found, on being sacrificed with the other survivors on the 24th day, to harbour 0, 6, 1 and 24 larvae respectively.

During the post-mortem examinations it was found that the two controls, although living on the 24th day after infection, had severe caecal and liver lesions, whereas in the nine survivors in the treated groups the lesions were minor in character and good recoveries were evident.

EXPERIMENT No. II

This was a replicate of Experiment No. I except that the poults used

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* ANT—abbreviation for 2-amino-5-nitrothiazole used in the MS.

TABLE I

RESULTS OF EXPERIMENTS WITH 2-AMINO-5-NITROTHIAZOLE FOR TURKEYS

Expt. No.	Group No.	No. of Poults	Treatment	Period of Treatment	No. dead entero-hepatitis	No. of larvae of *H.gallinae* per bird (±S.D.)
1	A	5	A.N.T. 0.2% feed— 3 days+0.1%—6 days..	+10 to +19 days	0	2.2±3.0
1	B	5	A.N.T. 0.2% feed — 3 days+0.1%—6 days..	+12 to +21 days	1 (20%)	8.0±10.1
1	C	5	None.....	—	3 (60%)	86.3±69.4
2	D	5	A.N.T. 0.2% feed — 3 days+0.1%—6 days..	+10 to +19 days	0	13.8±14.9
2	E	5	A.N.T. 0.2% feed — 3 days+0.1%—6 days..	+12 to +21 days	1 (20%)	16.0±17.0
2	F	5	None.....	—	5 (100%)	55.0±30.7
3	G	5	A.N.T. 380 mg. 12th day +0.1% feed—9 days....	+12 to +21 days	0	8.8±14.1
3	H	5	A.N.T. 380 mg. 12th day +0.1% feed—9 days....	Clinical signs to +21 days.	0	23.8±20.0
3	I	5	None.....	—	2(40%)	55.8±52.3
4	J	3	A.N.T. 0.05% feed — 10 days.....	− 1 to +9 days	0	143.7
4	K	3	None.....	—	0	274.6
6	N	5	A.N.T. 0.1% feed—9days	+ 9 to +18 days	0	6.0±6.0
6	O	5	A.N.T. Soluble 0.03% water—9 days.....	+ 9 to +18 days	1 (20%)	0.6±0.8
6	P	5	None.....	—	5 (100%)	74.4±35.5
7	Q	5	A.N.T. Soluble 0.03% water—12 days.....	+10 to +22 days	0	—
7	R	5	A.N.T. Soluble 0.04% water—12 days.....	+10 to +22 days	2 (40%)	—
7	S	5	None.....	—	5 (100%)	30.2±24.0
8	T	7	A.N.T. 250 mg. by capsule on alternate days	+11(12)13(14) 15(16)17(18)19	0	6.0± 5.2
8	U	6	None.....	—	5 (83%)	71.3±40.2

were 8 weeks of age. One poult in Group E was too ill to eat on the 12th day, and did not get drug; in consequence it died, and was found to harbour 177 heterakid larvae which are not included in the average of 16.0 for the

remaining four birds. The nine survivors were all killed on the 24th day, and were found to have well healed caecal and liver lesions.

Experiments I and II both indicated a high degree of efficiency for A.N.T. when used by the 10th or 12th day following infection, and only when there was no drug intake did it fail. In both experiments the larval heterakids had been removed to a statistically significant degree.

EXPERIMENT No. III

This experiment was an attempt to determine the value of the drug if given first as a large single dose directly into the crop at the first clinical sign of the disease (yellow droppings). Fifteen poults, eleven weeks of age, were divided into three groups following infection with 500 heterakid eggs each, but each individual was kept in a separate cage. At the end of the 12th day Group G was given 380 mg. A.N.T. per bird, in suspension, and was thereafter given feed containing 0.1% of the drug for 9 days. Group H was treated with the same amount, but the dose into the crop was given only when the appropriate bird ejected the first yellow droppings. Although all the treated birds recovered only 40% of the untreated controls died, making interpretation of results difficult. The number of larvae were apparently reduced by the treatment, but the differences between the treatd and untreated birds are, unlike those of the previous experiments, not statistically significant.

EXPERIMENT No. IV

This involved only six fully grown poults, divided into two groups of three, and was designed to indicate if a lower preventive level of A.N.T. would be effective against larval *H. gallinae*. Group J was given 0.05% A.N.T. in feed for one day before infection with heterakid ova, and for 8 days thereafter; Group K acted as controls. No deaths from enterohepatitis occurred, and the birds were sacrificed. The numbers of larvae in the treated birds were 0, 5, and 426 respectively, as compared to 388, 421 and 75 in the controls. This result is insignificant due to the large number of larvae retained by one bird.

EXPERIMENT No. V

This was an attempt to recover caecal worm larvae during the course of treatment, and thus to determine the mode and times of ejection. It had been noted that treated poults frequently excreted pieces of necrotic tissue, presumably caecal mucous membrane, and it appeared possible that the larval worms were discarded in such tissue.

Four large poults were used, two being given A.N.T. as 0.2% of feed on the 10th. to 12th. days following infection, and as 0.1% for 6 more days. The birds were caged separately, and all pieces of caecal cores and all the caecal feces were collected and kept separate for miroscopic examination. No larvae

were recovered during the first three days. Between the 5th. and 8th. days the treated birds ejected 65 larvae, 63 of which were in caecal feces. The controls discarded 19 larvae, all in shreds of caecal cores, between the 4th and 12th days. The birds were killed on the 14th day and 3 larvae were recovered from the caeca of the treated birds, 22 from the controls. Thus the treated birds lost 95.6% of their larvae, compared to 46.3% lost by the untreated birds.

EXPERIMENT No. VI

A new soluble form of A.N.T. became available early in 1951, and had the advantage of being administered in drinking water — a somewhat preferable method for birds that are already in the early clinical stages of enterohepatitis. Fifteen poults, 8 weeks of age, were used in an experiment to test this product for efficiency, and at the same time to make further observations on the larvicidal effect against *H. gallinae*. They were each given 1000 heterakid ova. Group N (5 poults) received A.N.T. as 0.1% of feed on the 10th and until the 18th day after infection; Group O received the soluble form which provided 0.03% of A.N.T. in the drinking water in parallel. During the experiment all the untreated controls died from enterohepatitis, whereas all the treated birds survived. However on the sixth day following cessation of treatment Group O showed clinical signs of the disease, and one bird died on the 28th day after being infected. Two days later all remaining birds were sacrificed, and it was found that while all lesions were in the healing phase, there was a greater degree of typhlitis in those in the group receiving the drug in water. The number of heterakid larvae in each treated group had been reduced to a highly significant degree.

EXPERIMENT No. VII

In view of the results of Experiment No. VI, it was decided to make a further test of the soluble A.N.T., this time extending the period of treatment and varying the concentration of drug in the drinking water. Fifteen poults, 8 weeks of age, were each given 1000 ova of *H. gallinae*, and were then divided into three groups of five. At the beginning of the 10th day thereafter Group Q was given the soluble drug to represent 0.03% of the water, while Group R was given the drug to make 0.04% of the water, in parallel; treatment was continued for 12 days.

The intake of liquid and food was measured for each group. During the test all the untreated controls died of enterohepatitis, as did two poults on the 0.04% solution. The survivors were kept under observation, and were not sacrificed, as observations on any secondary outbreak were needed. However, all remained healthy and were apparently resistant to enterohepatitis because they intermingled with common fowl and other recovered turkeys on soil pens for the rest of the season.

The reason for the mortality in Group R was, apparently, the lower drug intake due to unpalatability of the more concentrated solution. During the first three days the drug intake of the two groups was roughly the same, but thereafter those receiving the 0.03% solution took more drug than did those receiving the 0.04% solution (107 mgm/poult/day compared with 79 mgm/poult/day). Thus it appeared that the dosage of a solubilized preparation is fairly critical due to unpalatability of the stronger solution. Also, it was evident that treatment of birds, when started as an outbreak is imminent, should be continued for at least 12 days.

EXPERIMENT No. VIII

Numerous enquiries were made by turkey owners in regard to the possibility of using capsules or tablets of A.N.T. for the individual treatment of birds showing the first signs of enterohepatitis. This experiment was designed to test this method and to make further observations on the larvicidal effect of the drug on *H. gallinae*. Thirteen poults, 12 weeks of age, were each given 1000 heterakid ova and were then divided into Group T (7 birds) and Group U (6 birds) by random method. At the end of the 11th day thereafter yellow droppings were noted in both pens and a capsule containing 250 mgm. of A.N.T. was given to each bird in Group T. This dose was repeated at 48 hour intervals for a total of five treatments. All the treated birds recovered, while five (83%) of the controls died, and at one time the one surviving control was too ill to stand. The survivors were sacrificed on the 26th day after infection when all caecal and liver were in the healing phrase. The difference between the average of 6.0 ± 5.2 larvae in the treated birds and 71.3 ± 40.2 larvae per bird in the controls is statistically significant. The surviving control harboured 88 larvae.

DISCUSSION OF RESULTS

The results of these experiments are further evidence of the efficiency of 2-amino-5-nitrothiazole in the control of outbreaks of enterohepatitis; they also indicate that medication of drinking water with a soluble form of the drug, and individual treatment of affected poults at 48 hour intervals, are at least as effective as the drug incorporated into feed.

The effect upon the larvae of Heterakis has been confirmed by these tests, although the significance of this finding is not yet clear, insofar as the control of the disease is concerned. When a relatively inefficient preventive treatment with an arsenical was tested in other studies (Swales 3) it was found that there was no significant difference between numbers of larval heterakids in the caeca of treated and untreated birds; this comparison removes the possibility that the loss of the larvae was due to the factor of greater time between infection and post-mortem examination of test birds. Under conditions existing in eastern Canada, at least, field outbreaks appear to be initiated only by the ingestion of embryonated eggs of *H. gallinae*, and the Histomonas is not readily found in caeca until at least the seventh day after

exposure, and fatal damage to the caeca and liver is seldom present until the sixteenth day in a half-grown poult; this is in sharp contrast to the more rapidly developed histomoniasis produced by injections *per rectum* of emulsified liver lesions, as used by Sautter *et al* (4), for example. The difference is less likely to be due to greater numbers of Histomonas than it is to the probable role of the nematode larvae as reservoirs of the protozoa. Thus, the destruction of such reservoirs is very probably an important function of 2-amino-5-nitrothiazole.

SUMMARY

A series of experiments, using various forms of 2-amino-5-nitrothiazole, has added further evidence of the efficiency of this drug in controlling outbreaks of enterohepatitis in turkey poults, and has confirmed its anthelmintic effect upon the larvae of *H. gallinae* in affected caeca. It is suggested that this latter action is an important role of the drug in controlling naturally induced outbreaks of the disease.

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REFERENCES

(1) WALETZKY, E., CLARK, J. H., and MARSON, H. W., New chemotherapeutic agents in entero hepatitis (blackhead) of turkeys. Science, 111: 720-721. 1950.

(2) SWALES, W. E., Enterohepatitis (blackhead) in turkeys. V. Further experiments on chemotherapy. Can. J. Comp. Med. 14: 118-125. 1950.

(3) SWALES, W. E., Enterohepatitis (blackhead) in turkeys). IX. Miscellaneous tests of chemicals for possible therapeutic or prophylactic value. Can. J. Comp. Med. (in press).

(4) SAUTTER, J. H. and POMEROY, B. S., and ROEPKE, M. H. Histomoniasis (Enterohepatitis) in Turkeys. (1) A procedure for the screening and testing of drugs. Amer. J. vet. Res. 11: 115-119. 1950.