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On the Chemotherapeutic Action of 3-Nitro-4-Hydroxyphenyl-arsonic Acid Against the Coccidium Eimeria Tenella in Chickens

By Neal F. Morehouse and F. McKay

Morehouse and Mayfield (1946) reported that 3-nitro-4-hydroxyphenyl-arsonic acid and its sodium salts were effective in reducing hemorrhage and mortality from the coccidium *Eimeria tenella* when administered in the drinking water. The present investigation was undertaken to determine the activity of this arsonic acid when medication was started at various times with reference to the time of infection and to determine the stage or stages in the life-cycle affected by the compound.

Materials and Methods

The New Hampshire or Barred Rock x White Wyandotte crossbred chicks used in these experiments were hatched and brooded in our laboratory, the usual precautions being taken to insure freedom from coccidiosis infection. The age of the birds at the beginning of the experiment varied from 24 to 51 days. Individual wire-floored cages were used for all experiments except No. 5, in which case a wire-floored multi-deck brooding battery was used.

The severity of the disease in treated and untreated chicks was based primarily on mortality, but a daily estimate of the amount of hemorrhage from individually caged chicks during the acute stage of the disease was also used for comparison. Records were kept on weight, water consumption, and feed consumption throughout the experiments.

The 3-nitro-4-hydroxyphenyl-arsonic acid used in these experiments was administered in a tableted composition containing, in addition to the arsonic acid, ammonium and sodium phenolsulfonates and boric acid. The tablets used in Experiment 1 also contained lactose. These additional ingredients, used as bulking, solubilizing, and lubricating agents in the process of tableting, under our experimental conditions, have proved inactive against *E. tenella*. Hardcastle and Foster (1944) reported that 2 per cent boric acid in the mash for 7 days, starting medication at the time of infection, prevented lesions of cecal coccidiosis but that 2 of their experimental birds died of toxicity. In our experiments, boric acid was given at a level of 0.002 per cent in the drinking water, a dosage which has proved inadequate for the control of *E. tenella* infection.

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Infection of the chicks was accomplished by the introduction of approximately 100,000 sporulated oocysts of *E. tenella* directly into the crop of each chick at specified intervals from the time of infection.

Experimental

Exp. 1. Twenty-four New Hampshire chicks were divided into six equal groups and placed in individual cages when they were 47 days old. Five of these groups received 9.6 grams of a phenolsulfonate-lactose-boric acid composition containing 4.048 per cent 3-intro-4-hydroxyphenyl-arsonic acid per gallon of drinking water. Medication in the respective groups was started 69, 51, 26, and 3 hours before and 24 hours after infection with approximately 100,000 sporulated oocysts of *E. tenella* and continued for 8 days following infection. An unmedicated-infected group served as a control for the experiment.

No mortality occurred in any of the groups receiving the medication prior to infection but half of the chicks in which medication was delayed until 24 hours after infection died of cecal coccidiosis. Mortality from coccidiosis in the unmedicated-infected group was 75 per cent. Hemorrhage was completely suppressed in the chicks receiving medication at least 26 hours before infection and in three of the four chicks receiving medication 3 hours before infection. The fourth bird in the latter group showed severe hemorrhage but did not die. No suppressions of hemorrhage occurred in the chicks where medication was delayed until 24 hours after infection.

Exp. 2. Twenty-four Barred Rock x White Wyandotte cross-bred chicks were divided into three equal groups and placed in individual cages when they were 27 days old. Two groups received 8.8 grams per gallon of drinking water of a phenolsulfonate-boric acid preparation containing 4.38 per cent 3-nitro-4-hydoxyphenyl-arsonic acid, while the third group (controls) remained unmedicated. Treatment was started 24 hours before infection in one group and 12 hours before infection in the other group, the medication being continued for 9 days. Each chick in the experiment received approximately 100,000 sporulated *E. tenella* oocysts.

In the group receiving medication 24 hours before infection there was no mortality, but only approximately 25 per cent control of hemorrhage was obtained. Control of hemorrhage in the group receiving medication 12 hours before infection was about 40 per cent, but one of the eight chicks died of coccidiosis. Only one of the eight control chicks died from this disease, indicating a comparatively low virulence of the culture.

Exp. 3. Twenty-four Barred Rock x White Wyandotte cross-bred chicks were divided into two equal groups when they were 41 days old and placed in individual cages. One of these groups received the same preparation at the same dosage used in Experiment 2, starting 6 hours before infection and continuing for 8 days. Each chick in the experiment was infected with approximately 100,000 sporulated *E. tenella* oocysts.

Mortality in the treated group was 8.3 per cent compared to 16.7 per cent in the control group. There was approximately 50 per cent less hemorrhage in the treated group than in the controls, 3 of the 12 treated birds having passed no blood following infection. All controls showed very severe hemorrhage.

Exp. 4. Twenty-four New Hampshire chicks were divided into three equal groups and placed in individual cages when they were 51 days old. Two of these groups of chicks received the same dosage of the preparation used in Experiments 2 and 3. Medication was started 6 hours before infection in one group and at the time of infection in the other group. In the latter group 15 cc. of the drinking water solution was introduced directly into the crop at the time of infection. Medication was continued for 8 days. The third group (controls) remained unmedicated throughout the experiment.

None of the chicks receiving medication 6 hours before infection died, but control of hemorrhage was only about 30 per cent. One of the 8 chicks in which medicated drinking water was introduced directly into the crop at the time of infection died of cecal coccidiosis. Control of hemorrhage in this latter group was about 10 per cent. The 8 unmedicated-infected chicks all showed very severe hemorrhage and five of them died of cecal coccidiosis.

Exp. 5. Eighty New Hampshire chicks were divided into four groups of 20 chicks each and placed in separate decks of a brooding battery when they were 26 days old. Three of these groups received the same dosage of the preparation administered to the treated chicks in Experiments 2, 3 and 4. Medication was started in one group 12 hours before infection, 6 hours before infection in another group, and at the time of infection in the third group. Ten cc. of the medicated solution was introduced directly into the crop of each chick in the latter group at the time of infection. Medication in these groups was continued for 8 days. One group of unmedicated-infected chicks served as a control for the experiment. Each chick in the experiment was infected with approximately 100,000 sporulated oocysts of *E. tenella.*

Since the birds on this experiment were not kept in individual

Table I

A summary of six cecal coccidiosis experiments showing per cent control and per cent mortality in infected control chicks and chicks receiving a preparation containing 3-nitro-4-hydroxyphenyl-arsonic acid, the medication being started or stopped at varying intervals from the time of infection.

Experiment Number	Chicks Used		Medication Administered		Results of Experiments		
	Number	Age in Days	Per cent Conc. in Water	Schedule* of Treatment	Per cent Control of Hemorrhage	Per cent Mortality	
						Treated	Control
1	4	47	0.0094	69 hrs— IW(11 days)	100	0	75
	4	47	0.0094	51 hrs— IW(10 days)	100	0	75
	4	47	0.0094	26 hrs— IW(9 days)	100	0	75
	4	47	0.0094	3 hrs— IW(8 days)	90	0	75
	4	47	0.0094	IW—24 hrs (7 days)	0	50	75
2	8	27	0.0102	12 hrs— IW(9 days)	40	12.5	12.5
	8	27	0.0102	24 hrs— IW(9 days)	25	0	12.5
3	12	41	0.0102	6 hrs— IW(8 days)	50	8.3	16.7
4	8	51	0.0102	6 hrs— IW(8 days)	30	0	62.5
	8	51	0.0102	IW(8 days)**	10	12.5	62.5
5	20	26	0.0102	12 hrs— IW(8 days)	....	25.0	80.0
	20	26	0.0102	6 hrs— IW(8 days)	....	55.0	80.0
	20	26	0.0102	IW(8 days)***	....	10.0	80.0
6	4	24	0.0102	67 hrs— IW(67 hrs.)	100	0	0
	4	24	0.0102	67 hrs— IW(91 hrs.)	95	0	0
	4	24	0.0102	67 hrs— IW(115 hrs.)	100	0	0
	4	24	0.0102	67 hrs— IW(139 hrs.)	95	0	0
	4	24	0.0102	67 hrs— IW(259 hrs.)	100	0	0

*I=Time of Infection; W=Medication given in water; x hrs.- IW=Interval of time medication was given before infection; IW (x days or hours)=Total length of time medication was given. IW- 24 hrs (7 days)=Medication in water was started 24 hours after infection and continued for 7 days.
**Fifteen cc. of the medication solution was introduced directly into the crop of each chicken in this group at the time of infection.
***Ten cc. of the medicated solution was introduced directly into the crop of each chicken in this group at the time of infection.

Table II

Data of Table I rearranged so that length of treatment prior to time of infection of various groups in six experiments is presented in descending order.

Experiment Number	Chicks Used		Medication Administered		Results of Experiments		
	Number	Age in Days	Per cent Conc. in Water	Schedule* of Treatment	Per cent Control of Hemorrhage	Per cent Mortality	
						Treated	Control
1	4	47	0.0094	68 hrs— IW(11 days)	100	0	75.0
6	4	24	0.0102	67 hrs— IW(67 hrs.)	100	0	0
6	4	24	0.0102	67 hrs— IW(91 hrs.)	95	0	0
6	4	24	0.0102	67 hrs— IW(115 hrs.)	100	0	0
6	4	24	0.0102	67 hrs— IW(139 hrs.)	95	0	0
6	4	24	0.0102	67 hrs— IW(259 hrs.)	100	0	0
1	4	47	0.0094	51 hrs— IW(10 days)	100	0	75.0
1	4	47	0.0094	26 hrs— IW(9 days)	100	0	75.0
2	8	27	0.0102	24 hrs— IW(9 days)	25	0	12.5
2	8	27	0.0102	12 hrs— IW(9 days)	40	12.5	12.5
5	20	26	0.0102	12 hrs— IW(8 days)	....	25.0	80.0
3	12	41	0.0102	6 hrs— IW(8 days)	50	8.3	16.7
4	8	51	0.0102	6 hrs— IW(8 days)	30	0	62.5
5	20	26	0.0102	6 hrs— IW(8 days)	....	55.0	80.0
1	4	47	0.0102	3 hrs— IW(8 days)	90	0	75.0
4	8	51	0.0102	IW(8 days)**	10	12.5	62.5
5	20	26	0.0102	IW(8 days)***	....	10.0	80.0
1	4	47	0.0094	IW—24 hrs. (7 days)	0	50.0	75.0

*I=Time of Infection; W=Medication given in water; x hrs.- IW=Interval of time medication was given before infection; IW (x days or hours)=Total length of time medication was given. IW- 24 hrs (7 days)=Medication in water was started 24 hours after infection and continued for 7 days.
**Fifteen cc. of the medication solution was introduced directly into the crop of each chicken in this group at the time of infection.
***Ten cc. of the medicated solution was introduced directly into the crop of each chicken in this group at the time of infection.

cages, it was impossible to obtain reliable information on the relative amount of hemorrhage in the various groups. In the group where medication was started 12 hours prior to infection, mortality was 25 per cent; where medication was initiated 6 hours prior to infection, mortality was 55 per cent; and in the group where medication was introduced into the crop at the time of infection, mortality was 10 per cent. Mortality in the control group was 80 per cent.

Exp. 6. This experiment was conducted to determine whether stopping medication at the time of infection or at various intervals thereafter would affect the efficacy of the 3-nitro-4-hydroxyphenyl-arsonic acid preparation. Twenty-four New Hampshire chicks were divided into 6 equal groups and were placed in individual cages when they were 24 days old. Medication in these five treated groups was started 67 hours before infection and discontinued at the time of infection, or 24, 48, 72, and 192 hours, respectively after infection.

These treated chicks received 8.8 grams per gallon of drinking water of a phenolsulfonate-boric acid composition containing 4.38 per cent 3-nitro-4-hydroxyphenyl-arsonic acid. One group of unmedicated-infected chicks served as a control for the experiment. Each chick in this experiment received approximately 100,000 sporulated *E. tenella* oocysts.

No mortality was obtained in any of the treated or control groups. Likewise, no hemorrhage occurred in the group where medication was discontinued at the time of infection or those groups where medication was discontinued 24 or 192 hours following infection. Approximately 95 per cent control of hemorrhage was obtained in the other two groups. Very severe hemorrhage was observed in each of the unmedicated-infected chicks. The data from these six experiments are summarized in Table 1.

One of us (McKay) has studied histological preparations of cecal tissues from medicated-infected, medicated-uninfected, unmedicated-infected, and unmedicated-uninfected chicks in order to obtain further evidence regarding the stage or stages in the life-cycle affected by 3-nitro-4-hydroxyphenyl-arsonic acid. The level of the arsonic compound used in the drinking water was 0.385 grams per gallon, medication being started three days before infection and continued until the birds were sacrificed. The chicks in the infected groups each received approximately 2,400,000 sporulated oocysts of *E. tenella*. Food was withheld from all chicks in the experiment for a period of 12 hours immediately before the coccidia oocysts were administered to the chicks of the two infected groups.

One chick in the unmedicated-infected group and one chick in the

medicated-infected group was killed and cecal tissues placed in Zenker's fixative at 6, 12, 18, 24 and each succeeding 6-hour interval from the time of infection to and including the 120th hour. Tissues from chicks of the medicated-uninfected group and from the unmedicated-uninfected group were obtained at intervals corresponding to the 24, 48, 72, 96, and 120 hour tissues from the medicated-infected and unmedicated-infected groups. Staining of tissues was by the buffered haematoxylin and eosin method described by Craig and Wilson (1937) and by the Mallory's methylene blue and eosin technique.

Neither gross nor microscopic changes, ascribable to the arsonic acid treatment, could be detected in the cecal tissues of the medicated-uninfected chicks at any time during the period of the experiment. The first sporozoites were found in the tissues of the unmedicated-infected group fixed 24 hours after infection. Tyzzer (1929) was unable to demonstrate sporozoites in the epithelial tissues either 20 or 26 hours following infection, although he found them in the cecal lumen as early as 20 hours after infection. By the 48th hour, numerous young first generation schizonts could be found in the unmedicated-infected group, and by the 60th hour the second generation schizonts were beginning to form. At 72 hours after infection, tissue destruction was extensive and numerous extravasated red blood cells were present in the submucosa.

No sporozoites could be found in the cecal tissues of the medicated-infected chicks during the first 72 hours following infection. However, first generation schizonts found in the 48-hour material showed that some sporozoites had entered the epithelium. These normal appearing parasites were isolated and very few in number compared to those which were found in the unmedicated-infected birds. Development of these thinly scattered parasites progressed normally, paralleling the development of those in the unmedicated-infected group.

Discussion

Goff (1941) reported that sulfur affected the sporozoite stage of *E. tenella*. Herrick *et al.* (1944) found that sulfadiazine prevented oocyst formation by this species and Ripsom and Herrick (1945) concluded that sulfadiazine exerted its influence on the second generation merozoites and possibly on the gametocytes. It has been observed by Swales (1944), Horton-Smith (1945), Horton-Smith and Taylor (1945), and by Farr and Wehr (1947) that the development of *E. tenella* in chicks receiving sulfamethazine is affected at the second generation merozoite stage. In their histological studies,

the latter authors observed normal penetration of the epithelium by sporozoites and partial development of the first generation schizonts. The greater part of the second generation schizonts and their merozoites were destroyed. The very young gametocytes were damaged or destroyed but the larger gametocytes were not affected. These observations on the ineffectiveness of sulfamethazine against the early endogenous stages of development and their effectiveness against the later stages supplement those of Horton-Smith and Taylor (1942), Swales (1944), and Wehr and Farr (1947) that this sulfanamide was effective in controlling *E. tenella* infection when medication was not initiated until two to three days following infection.

Early in our investigation, it became evident that the effect of 3-nitro-4-hydroxyphenyl-arsonic acid on *E. tenella* infection resembled that of sulfur. The arsonic acid was preventive and showed little, if any, so-called "curative activity" that has since been ascribed to certain sulfonamides. Reference to Table 2 will show that no mortality occurred in any of the chicks of our experiments receiving treatment at least 24 hours prior to infection.

The protection afforded when medication was started from 3 to 12 hours before infection varied from a total absence of hemorrhage in some individual birds to very severe hemorrhage and death in others. It is believed that this variability is due to the failure of individual birds to start drinking the medicated water as soon as it was offered to them. This is substantiated by data obtained in Experiments 4 and 5 where the medicated water was injected directly into crops of the chicks at the time of infection. Mortality in these two treated groups was 12.5 and 10.0 per cent compared to 62.5 and 80.0 per cent in their respective controls, thus indicating considerable protection by the arsonic acid preparation. When the medication was withheld until 24 hours after infection there was no appreciable control of hemorrhage or mortality. Thus, the available evidence indicates that control of *E. tenella* infection by 3-nitro-4-hydroxyphenyl-arsonic acid in the drinking water is dependent on the individual chick having imbibed the medicated water at the time of or prior to the time of infection.

On examination of the data in Experiment 6 (Table 1), it becomes evident that in the case of a single infection the length of time medication is continued beyond the time of infection has little significance. The control of hemorrhage was as complete when medication was discontinued at the time of infection as when it was continued for eight days following infection.

A study of histological preparations showed very few parasites in the cecal tissues of the medicated-infected birds compared to the numbers observed in the tissues of unmedicated-infected chicks. In fact, they were so few in number that no sporozoites were found even after an extensive search. It is therefore believed that most of the sporozoites were inactivated by the treatment before they could gain entrance to the tissues. Having gained entrance to the tissues, however, the parasites apparently developed in a normal manner. These observations fully agree with the clinical observation (Experiment 1) that treatment with 3-nitro-4-hydroxyphenyl-arsonic acid was ineffective when medication was delayed until after infection. That a few parasites escape the action of the drug and undergo normal development appears to explain the acquisition of a substantial clinical immunity which has been observed in chicks receiving treatment (Morehouse and Mayfield [1946]). It was shown by Hall (1934) that a very light infection of the coccidium *E. miyairii* (=*E. nieschulzi*) conferred a high degree of immunity on the rat host. A single infective dose of four viable sporulated oocysts caused the rat to lose approximately 50 per cent of its natural susceptibility; 10 oocysts, approximately 75 per cent; 50 oocysts, approximately 98 per cent; and 1500 oocysts, approximately 100 per cent. While it is known that chickens require more than 1500 *E. tenella* oocysts to produce a solid immunity, it seems quite probable that a relatively small number of developing organisms may produce a clinical immunity.

These experiments indicate that 3-nitro-4-hydroxyphenyl-arsonic acid affects the sporozoite stage in the life-cycle of *E. tenella* and has little or no influence on subsequent stages. Consequently, for control of this parasite under practical field conditions of poultry raising, it would seem necessary to administer the treatment over a period of several days. Treatment cannot be expected to help those chicks which have already obtained a potentially fatal quantity of infective oocysts but exerts a protective action toward those chicks which may not yet have been exposed to the infection and toward those which may have acquired a sub-lethal infection. For practical application to poultry flocks, it is recommended that treatment be started at the very first appearance of blood in the droppings or other indications of the disease. Early treatment in such flocks, according to the results obtained in these experiments, can be expected to substantially control mortality but at the same time permit the development of a considerable protective immunity.

SUMMARY

1. The administration of approximately 0.01 per cent of 3-nitro-4-hydroxyphenyl-arsonic acid in the drinking water was effective for the control of *Eimeria tenella* infection in chickens when medicated was started at the time of or prior to the time of infection but was ineffective when medication was delayed until after infection.

2. Clinical and histological evidence indicates that 3-nitro-4-hydroxyphenyl-arsonic acid attacks the sporozoite stage in the life-cycle of *Eimeria tenella.*

3. A limited number of *Eimeria tenella* parasites developed normally in infected chicks which received a prophylactic dosage of 3-nitro-4-hydroxyphenyl-arsonic acid thus accounting for the resultant immunity which has been observed in such birds.

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