THE IN VITRO AND IN VIVO RESPONSE OF MYCOPLASMA GALLISEPTICUM TO DEOXYCORTICOSTERONE

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BY

George A. Padgett

AN ABSTRACT

Submitted to the College of Veterinary Medicine of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Abstract George A. Padgett

The purpose of this study was to determine the *in vitro* and *in vivo* response of *Mycoplasma gallisepticum* to deoxycorticosterone (DOC) and deoxycorticosterone trimethylacetate (DOCT).

Attempts were made, using various dilutions of DOC to inhibit growth *in vitro* of four separate cultures of *M. gallisepticum*, an avian pleuropneumonia-like organism. Growth was inhibited by a minimum of 0.4 mg. of DOC; the growth characteristics of surviving colonies were modified.

In the *in vivo* experiments 1 cc. of cultures of *M. gallisepticum*, containing about 10^6 organisms, was injected via the trachea into chickens, causing chronic respiratory disease. The birds were treated either by injecting DOC into the abdominal airsacs or by injecting DOCT into the breast muscle.

To evaluate the response of birds infected with *M. gallisepticum* and treated with DOC or DOCT these criteria were used: (1) number of isolable organisms; (2) weight gains of the birds; (3) degree of gross pathology; (4) feed conversion.

When birds infected and treated were compared with infected and non-treated birds, the number of isolable organisms decreased, weight gains increased, gross pathology decreased, and feed conversion improved in the treated birds.

The most effective dosage level found was 20 mg. of DOCT. A statistical analysis using the t test was performed, and this dose was found to be significant at the 0.1% level. Treatment

at 0, 7, 14, or 21 days post-inoculation with the organisms appears to control the disease.

Toxicity of this hormone for the chicken, resulting from administration via either the infraorbital or intramuscular routes, with one exception, was not observed at the dosage levels used in these experiments.

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INTRODUCTION

Chronic respiratory disease (CRD) is a respiratory infection of chickens. It is characterized by coughing, tracheal rales and a pasty nasal discharge. The appetite of affected birds drops off, with a resultant loss in weight; the birds become depressed and egg production decreases in laying flocks.

Gross lesions of the disease consist primarily of a mucopurulent exudate in the nasal passages, trachea, bronchi, and airsacs. The airsacs may appear beaded and contain a caseous yellow exudate. The tracheal mucosa may be thickened and some degree of pneumonia may be observed.

The infective organism is transmitted by carriers, through the egg, by contact, and by airborne dust or droplets. The incubation period varies from 4 to 21 days.

Bergey's Manual classifies the etiological agent of CRD as Mycoplasma gallinarum.

Nelson (1935) described a "coryza of slow onset" caused by coccobacilliform bodies varying in size from 0.1 to 0.5 microns. This is probably the first report of the disease which Delaplane and Stuart (1943) called chronic respiratory disease. Markham and Wong (1952) identified the pleuropneumonia-like organisms (PPLO) as the etiologic agent of CRD. Both pathogenic and nonpathogenic species of PPLO have been isolated from the respiratory tracts of chickens. Edward and Kanarek (1960) suggest that the virulent pathogenic PPLO of avian

origin be given the species name galliseptícum.

It is estimated that CRD causes an annual loss of 25 million dollars to poultry raisers of the United States. The seriousness of this loss to poultrymen is reflected in the work of researchers in attempting to prevent, control, or cure the disease. As shown in Table 1 a wide range of antibiotics and chemotherapeutic agents have been used with varying results. More than 40 agents and/or combinations thereof have been tested in vitro, in ovo or in vivo, or by all of these methods, in attempts to discover effective therapy for CRD.

The results have been somewhat confusing and even at times contradictory. For example, Table 1 shows that at least 15 different investigators have worked with the antibiotic terramycin. With the in vivo work two of these researchers found the drug to be effective as a cure for the disease or at least to show excellent results in decreasing losses due to CRD. Four investigators obtained somewhat promising results from in vivo tests with the drug. Three, however, found it to be of little or no therapeutic value.

Several investigators have suggested possible explanations for variations in experimental results using terramycin and other antibiotics, among these being, strain differences in the parasite and host, variation in degree or seriousness of the infection, number and kinds of secondary invaders, variations in housing, light and nutrition, amount of stress to which the birds are subjected and differences in methodology employed in the research and interpretation of results. It is generally agreed by investigators in the field that these problems must be worked out before real correlative work can be accomplished.

Lester and Hechter (1958) have shown that deoxycorticosterone (DOC) inhibits Micrococcus aureus, Sarcina lutea, Bacillus megaterium, Bacillus subtilis, Corynebacterium pseudodiphtheriticum and Mycobacterium ranae as well as a number of Gram-positive yeasts and molds. They found that Gram-negative bacteria are generally insensitive to this steroid. Jefferson and Sisco (1959) have reported the inhibition of Aspergillus niger with DOC. Lester et al. (1958) with Neurospora crassa and Maxwell et al. (1960) with Saccharomyces fragilis have shown these organisms to be inhibited by steroids.

This thesis will report the in vitro and in vivo response of Mycoplasma gallisepticum, the agent of CRD, to the action of deoxycorticosterone and its trimethylacetate ester (DOCT).

This study was divided into six parts:

Experiment I: The effect of DOC on the growth of PPLO in vitro.

Experiment II: The toxicity of DOC for the chicken.

Experiment III: A pilot study on the effect of DOC on the growth of PPLO in vivo.

Experiment IV: The in vivo response of M. gallisepticum to DOCT.

Experiment V: The effect of dosage on the in vivo response of M. gallisepticum to DOCT.

Experiment VI: The effect of time of treatment on the in vivo response of M. gallisepticum to DOCT.

MATERIALS AND METHODS

Experiment I: The PPLO in this study were cultured using PPLO broth or agar (Difco) enriched with one per cent PPLO serum fraction (Difco).

DOC was added to 125 ml. Erlenmeyer flasks in 0.8, 0.4, 0.2, 0.1, and 0.05 mg. amounts per 20 ml. of culture. It was prepared by either one of two methods. In the first, (solvent 1) ethanol-chloroform in a 1:1 ratio was added as a solvent. The solvent utilized in the second method, (solvent 2) was 0.85 per cent NaCl-ethanol solution in a 4:1 ratio. In both cases the solvent was evaporated prior to inoculation of the flasks with the test organisms.

Four strains of PPLO were used in this experiment. CRD-1 and -2 ferment arabinose, dextrose, galactose, levulose and xylose. CRD-7 ferments arabinose, dextrose, dextrin, galactose, levulose, maltose, mannose, starch, trehalose and xylose. The fourth strain, isolated in the diagnostic laboratory at Michigan State University, is designated as CRD-11; fermentation reactions for this strain are not available. CRD-1 and -2 were used with solvent 1, CRD-7 and -11 with solvent 2. The PPLO were subcultured every 72 hours using 0.2 ml. of inoculum in 20 ml. of the PPLO broth. Following 72 hours of growth the cultures *in toto* were transferred to five flasks containing the various amounts of DOC. At the same time PPLO agar and the next broth subculture (which was used as a control) were inoculated. After 72 hours 0.2 ml. of both the control and the DOC flasks were transferred to PPLO agar plates. The plates were incubated for 72 hours, then observed.

All cultures were incubated at 37° C.

Experiment II: Solvent 2 was utilized in the toxicity tests. The birds were inoculated with 0.25 ml. of the solvent containing the DOC levels of 0.8, 0.4, 0.2, 0.1, and 0.05 mg. Two tests were run eight days apart on chickens. Two birds were used with each of the DOC levels. The route of inoculation was the infraorbitol sinus of the right eye. Toxicity was measured by inflamation and/or swelling of the sinus.

Experiment III: CRD-11, the PPLO strain used in this experiment, was serially passed three times in the allantoic fluid of ten-day-old chicken embryos, incubating five days in each passage, immediately prior to inoculation of the birds. The birds were inoculated intratrachially using sterile blunt 2½ inch 18-gauge needles. The DOC used in this trial was diluted with solvent 2 so that 0.5 ml. contained 0.4 mg. of DOC. The birds were treated by injecting the DOC into the abdominal airsac, alternating sides with each inoculation, using sterile 20-gauge needles. The design of this experiment is shown in Table 2.

To determine whether infection had occurred, tracheal swabs on all 39 birds were taken using six-inch sterile cotton swabs twelve days and twenty-two days after inoculation. The swabs were then streaked on PPLO agar plates containing one per cent PPLO serum fraction with thallium acetate at a concentration of 1:1000 added as an inhibitor.

When infection was established, twenty-two days after inoculation, treatment with DOC was started and the effect of DOC was ascertained on the basis of whether PPLO could be isolated two days after

the last day of treatment. Isolation attempts at the time of post mortem, were made by the swab method described above and by excising a section of the airsacs. Each portion of airsac was placed in a flask containing 20 ml. of PPLO broth with serum fraction and no inhibitors; the flasks were incubated for 24 hours at 37° C; the contents were then filtered through a Seitz-type S-3 filter. Identification of the PPLO isolates was based upon their characteristic growth and their inability to grow in or on medium not enriched with serum fraction.

Experiment IV: The M. gallisepticum used in this experiment were isolated in the diagnostic laboratory at Michigan State University from a field case of CRD. These organisms were typical of PPLO associated with CRD, as shown by serological, cultural, and pathogenic properties.

Prior to inoculation of the birds, the PPLO were serially passed eight times in the allantoic fluid of seven-day-old embryos.

Using a blunt 4-inch, 20-gauge needle, four-month old, apparently disease-free leghorn cockerels, found to be negative for Newcastle disease by the H.I. test, were inoculated intratracheally with 0.5 cc. of pooled PPLO-containing allantoic fluid from 25 twelve-day-old embryos. The birds were housed in isolation pens, with no contact between pens. They were fed a commercial antibiotic-free grower ration. Food and water consumption were measured in all pens beginning 22 days preinfection until termination of the experiment. All birds were weighed at the beginning of the experiment and immediately before they were sacrificed.

The experimental design is shown in Table 3.

Each group of birds (pens 2, 3, and 4) to receive treatment with DOCT (Ciba) (25 mg./cc. as commercially prepared) was divided, one half receiving 0.5 cc. of DOCT diluted with 0.5 cc. of a commercial diluent (Ciba) at each treatment and the other half receiving 0.6 cc. of DOCT with 0.4 cc. of the same diluent. All treated birds were given DOCT twice, 22 and 43 days after inoculation with the PPLO-containing allantoic fluid. The DOCT was injected into the right side of the breast, the left side serving as a control to determine degree of absorption of the DOCT.

Twenty-one and 41 days posttreatment, respectively, one-half the birds in each pen were sacrificed and examined for lesions of CRD. At the same time an attempt was made to isolate PPLO from the abdominal, diaphragmatic and thoracic airsacs of the birds. Portions of the airsacs were excised, weighed, and placed in 1 cc. of PPLO broth (Difco) enriched with one per cent serum fraction (Difco) and containing thallium acetate in a 1:2000 concentration. The tissue was then ground and three serial tenfold dilutions made. One-hundredth cc. of each dilution was dropped on PPLO agar (Difco) enriched with four per cent serum fraction and containing a 1:2000 concentration of thallium acetate. The plates were incubated at 37° C for 72 hours, after which the number of organisms contained in each 0.01 cc. drop was counted at 100X magnification. From this the number of organisms per mg. of tissue was calculated.

Experiment V: The M. gallisepticum used in this experiment was supplied by Dr. Henry Van Roekel (University of Massachusetts) and designated by him as 194. This culture has been and has continued to

be maintained in young chickens. The inoculum used to produce the disease was harvested from five infected birds by placing the airsacs and tracheas in 115 ml. of PPLO broth (Difco) containing 4% serum fraction (Difco) and 1800 units of procaine penicillin per ml. One cc. of this material containing approximately 10^6 organisms was inoculated intratracheally in 18 birds using a blunt 4-inch 20-gauge needle.

The birds utilized were five-to-six-week-old, apparently disease-free, leghorn cockerels. Prior to inoculation, serum plate agglutination tests using a standard PPLO antigen on blood samples from eight of these birds selected at random were negative.

The experimental design is shown in Table 4.

On the 21st day post-inoculation each group of birds to receive treatment with DOCT (pens 3, 4, 5, 6, 7) were given intramuscular injections of 1.6 ml. of diluent (Ciba) containing various quantities of DOCT (Ciba) in the right side of the breast.

At 0, 7, 14, and 21 days post-treatment, respectively, birds from each pen were sacrificed and examined for the lesions of CRD. At the same times, an attempt was made to isolate M. gallisepticum from the airsacs of the birds. Isolation, housing, and feeding of the birds werethe same as that described in Experiment IV.

Experiment VI: The M. gallisepticum used in this experiment was the culture 194 supplied by Dr. Van Roekel following four passages in young chickens since the previous experiment.

The preparation of the inoculum and method of inoculation were the same as in experiment V with the exception that each cc. of the

inoculum contained approximately 1.5×10^6 PPLO.

The birds used in this experiment were four-week-old, apparently disease-free leghorn cockerels.

On the day of inoculation and at 7 and 14 days thereafter 20 mg. of DOCT in 0.8 ml. of diluent (Ciba) (a total volume of 1.6 cc.) was injected intramuscularly in the right side of the breast of treated birds. The birds in pen 3 were treated with 1.6 ml. of diluent as a control at the time of inoculation.

At 7, 14, 21, 28, and 35 days post-inoculation, respectively, birds were sacrificed and examined for the lesions of CRD. At the same time an attempt was made to isolate M. gallisepticum from the airsacs of the birds. Isolation, housing, and feeding of the birds were the same as that described under Experiment IV.

RESULTS

Experiment I: The normal growth of all four strains of PPLO used in this study appears in two distinct colonial forms. One is a large colony, approximately 0.04-0.05 mm. in diameter, with an entire margin, and a "grainy" center. This form grows down into the agar. The other is a very small colony, approximately 0.01-0.02 mm. in diameter, with an entire margin, a less "grainy" appearing center, and less penetration of the agar than that characteristic of the larger colony form.

DOC in 0.8, 0.4, and 0.2 mg. amounts per 20 ml. almost entirely inhibited the growth of the small colonial form of PPLO. The large colonies treated with the same amounts of DOC lost their "grainy" appearance, the margin became jagged, and a reduction in size was noted. Inhibition occurred with 0.1 and 0.05 mgs. of DOC per 20 ml. of medium but to a lesser extent than when treated with the larger amounts. Controls using either DOC solvent showed no appreciable difference in growth when compared with the non-solvent controls.

Experiment II: Toxicity was determined by inflammation and/or swelling of the sinus. The toxicity tests, with two exceptions, were negative. The controls using either solvent showed no toxicity.

Experiment III: Tracheal rales were noted in the infected birds beginning on the 14th day following inoculation; none were observed in the control pen. A pasty nasal discharge was noted from some of the birds in the infected pen twenty-two days after inoculation. On post mortem examination of the birds in group 1, thirty days after

inoculation, the gross lesions were typical for CRD. Thirty-three days after inoculation and after the birds had received a total of 2.4 mg. of DOC the coughing and rales tended to decrease in the pen containing groups 2 and 3, although they did not completely stop throughout the course of this experiment. From group 2, which was posted 38 days after inoculation, four birds did not show the typical lesions of CRD. Their airsacs were clear and the birds appeared to be in good condition. In the six remaining birds of this group the typical cloudy thickened airsacs were present and a mucous exudate was found in the trachea; no pneumonia, however, was noted. Group 3 birds were posted 46 days after inoculation along with the nine control birds. With the exception of two of the control birds, all of birds examined at this time appeared healthy and showed no lesions. The two control birds showed involvement of the airsacs, but no tracheal exudate and no pneumonia.

Results of attempts to isolate PPLO from all the groups of birds both pre and post treatment are shown in Table 6.

Experiment IV: Approximately 12,000 organisms per mg. of tissue were isolated from the infected birds immediately prior to treatment. (See Table 7.) From this table it can be seen that at the first post treatment sacrifice PPLO was not isolated from eight of the 18 birds that had been infected and treated (pens 3 and 4); from the other ten birds an average of less than 100 organisms per mg. of tissue was recovered. At the same time, an average of 5,700 organisms per mg. of tissue was isolated from the infected, non-treated control birds (pen 5); all birds sacrificed from this pen were found to be infected.

In the final sacrifice, 41 days post treatment, PPLO could not be recovered from the infected, treated birds, while an average of 100 PPLO per mg. of tissue was isolated from six of the nine infected, non-treated birds. At this time no PPLO were isolated from the remaining three birds. (See Table 7.)

No organisms could be isolated from birds in either of the non-infected groups (pens 1 and 2) at any time during the course of the experiment.

The number of PPLO per mg. of tissue isolated before and after treatment, shown in Table 7, are presented in graphic form in Figure 1.

Except for slight enlargement of the testes, no evidence of toxicity due to DOCT was noted in any of the treated birds, infected or non-infected.

When the birds were sacrificed prior to treatment, the typical lesions of CRD, i. e., tracheitis and aerosacculitis with an accumulation of exudate in the trachea and airsacs, were marked in all of the inoculated birds. Twenty-one days later, when one-half the remaining birds were sacrificed, the airsacs and trachea of all except two of the infected, treated birds appeared normal; the airsacs of these two birds were slightly cloudy, but no exudates were present. The infected, non-treated birds showed the typical lesions of CRD at this time.

At the termination of the experiment, when all the remaining birds were sacrificed, all birds in the infected, treated groups appeared normal. Four of the nine infected, non-treated birds had slightly cloudy airsacs; the rest appeared normal. During the entire

experiment, no lesions of CRD were noted in the non-infected birds.

As shown in Table 8, average weight gains of the infected, non-treated birds were considerably less than in any of the other four groups of birds.

A considerably higher percentage of feed was required to produce a pound of gain in the infected, non-treated birds than was needed in the infected, treated birds when compared with the "normal" controls.(Table 8).

All of the DOCT did not appear to be absorbed at the time of the first post treatment sacrifice; the amount of DOCT remaining at the final sacrifice appeared considerably greater than that remaining at the first post treatment sacrifice.

No observable difference in effect between the 0.5 cc. and 0.6 cc. dosages of DOCT was detected throughout the course of the experiment.

Experiment V: The first symptoms of CRD in the inoculated birds occurred seven days post inoculation. Seven days later tracheal rales, coughing, depression, and listlessness were marked in these birds. Coughing did not stop in the treated pens throughout the course of this experiment; however, coughing was considerably alleviated in the treated pens as compared to the non-treated pens during the course of treatment.

An analysis of variance (F test) was completed on the data given in Table 9. An F ratio of 5.001 with 65 degrees of freedom allowed the conclusion that it is improbable that sampling variation alone could account for the observed differences between the treatment group means (Batson, 1956).

To compare each of the DOCT treated groups of birds with the untreated, infected control birds (pen 2) the t test was applied. Comparing the isolation results (Table 9) of pen 2 with pens 5, 6, and 7, with 22, 21, and 22 degrees of freedom respectively, it was found that the above three pens of birds differed significantly from the birds in pen 2 at the 0.5%, 0.1% and 1.0% levels respectively.

According to Randall (1958) if there is greater than a 2% chance that a value is a normal variation (in biological work) then that value is generally not considered significant. The levels of significance for pens 3 and 4 are 5% and 2.5% respectively; as they are above the 2% level, the values for these pens are not considered to be significant.

Weight gains and feed conversion were considerably better in the treated birds than in the non-treated birds (Table 10). The DOCT treated birds gained a minimum of 18% (2.5 mg. DOCT) and a maximum of 39% (20 mg. DOCT) better than the infected untreated controls when compared with the "normal" birds. Furthermore, less feed per pound of gain was required to produce this weight than was necessary for the infected and untreated controls (Table 10).

The airsacs of all of the infected, non-treated birds, except one, were extremely thickened and cloudy. The gross pathology of the airsacs of the infected, treated birds was considerably less extensive and marked than the non-treated birds; however, none of the airsacs of the infected birds returned to a completely normal appearance during the 21-day treatment period.

It should be noted that the airsacs of the noninfected,

non-treated controls remained clear.

With the exception of the noninoculated control birds (pen 1), M. gallisepticum was isolated from every bird sacrificed in this experiment.

At the final sacrifice, 21 days after treatment, a slight amount of DOCT remained unabsorbed in one bird in pen 3, one bird in pen 4 and two birds in pen 5. A greater amount was unabsorbed and was found in the four remaining birds sacrificed in each of pens 6 and 7.

No evidence of toxicity was observed in this experiment. The testicular enlargement mentioned in discussing experiment IV was not observed with these birds.

The PPLO per mg. of tissue isolated post-inoculation, shown in Table 9, are presented in graphic form in figure 2.

Experiment VI: Nine days following inoculation the first symptoms were observed: a slight amount of coughing from the birds in all pens except pen 1, feed consumption slightly off, water consumption normal. Six days later the typical CRD symptoms, tracheal rales, coughing, depression and relative listlessness, were noted in all pens except pen 1, where a slight amount of coughing had started. These marked signs of CRD persisted throughout the course of the experiment in the birds in pens 2 and 3. With the exception of the birds in pen 1 where marked symptoms were not seen at any time, the symptomatology (i.e. rales and coughing) tended to decrease eight to ten days after treatment although they had not stopped at the termination of the experiment. As can be seen from Table 11, treatment

appears to reduce the number of infecting organisms a minimum of ten-fold. Weight gains and feed conversion were considerably better in the treated birds than in either the non-treated birds or those treated with diluent (Table 12). Due to a shortage of pen space uninfected controls were not maintained for this experiment. The "normal" bird figures used in Table 12 have been taken from "Feeding Poultry" by Heuser (1955) and represent the normal gain and feed per pound of gain expected from four-week-old leghorns maintained for 35 days, the length of this experiment.

The DOCT treated birds gained approximately the same as the Heuser "normals" and 0.69 pounds more than the birds not treated with DOCT. The feed conversion results were not as good when the DOCT treated birds were compared with the "normals" requiring an average of 19% more feed per pound of gain. However, the birds not treated with DOCT required a minimum of 96% more feed per pound of gain than the "normal" birds.

With the exception of one bird treated at the time of infection (pen 1) and sacrificed 14 days later, M. gallisepticum was isolated from every bird sacrificed in this experiment.

DOCT was found in the right side of the breast of all treated birds (excluding diluent treated controls) sacrificed in this experiment except for two birds treated at time of inoculation (pen 1) and sacrificed 35 days later. The other two birds in this pen sacrificed at the same time had very slight amounts of DOCT remaining. It appears that a minimum of 35 days is required for absorption of 20 mg. of DOCT diluted to 1.6 ml.

No evidence of toxicity was observed in this experiment. The testicular enlargement remarked in experiment IV was not noted with these birds.

The PPLO per mg. of tissue isolated post-inoculation, shown in Table 11, are presented in graphic form in figure 3.

Treatment with 1.6 ml. of diluent (Ciba) produced results essentially no different from those obtained with complete absence of treatment in the infected, non-treated control birds (see Tables 11 and 12, figure 3).

DISCUSSION

From the results previously presented it can be said that DOC, a mineralocorticoid adrenal hormone, appears to behave as an antimicrobial agent *in vitro*, and *in vivo* in the chicken. In the mammal, DOC activity is said to be limited to an influence on sodium, potassium and water metabolism (Beckman 1958), any benefit accruing to an animal treated with this hormone being attributed to the "salt effect." In large doses DOC has been shown to be toxic to mammals.

In experiment II infraorbital injections of DOC did not elicit toxic responses when 0.25 ml. containing 0.4 mg. or less of the inoculum was used. Toxicity was noted when 0.8 mg. of DOC with either of the solvents was used and was characterized by both inflamation and swelling of the area around the infraorbital sinus of the birds. The author believes, however, that this reaction may not be entirely due to DOC toxicity, as 0.5 ml. of the solvent containing 0.8 mg. of DOC was used in this case. Thus, a bulging of the skin covering the sinus was produced mechanically. The rest of the birds were inoculated with only 0.25 ml. of the preparation and no swelling was produced.

When a total of 6.0 mg. of DOC per bird was administered via the abdominal airsac over a period of 24 days no deleterious effects upon the birds were observed (experiment III).

With the exception of a slight testicular enlargement when a total of either 25 mg. or 30 mg. of DOCT had been injected into the breast of both infected and noninfected birds, there was no evidence of toxicity

due to this hormone (experiment IV). No testicular enlargement or other evidence of toxicity was noted in experiments V and VI. This difference in glandular reaction may be due to the 2.5 and 3 months age difference between these birds and those in experiment IV at time of treatment. This apparent lack of toxicity for the chicken is supported by the work of both Boas (1958) and Conner (1959) who have reported a similar conclusion.

DOC appears to inhibit the growth of PPLO in vitro. In experiment I not only was the density of the colonial population markedly reduced but the appearance of the surviving colonies was altered and did not resemble the typical colonial morphology. The fact that DOC is an effective antimicrobial agent in vitro suggests that it also acts directly upon the microorganisms in vivo. Therefore, the beneficial therapeutic activity of DOC is probably not simply a result of the physiological response of the host. This conclusion seems to have merit in the light of the work of Maxwell et al. (1960) who have shown that 1-androstene -3, 17 dione is lethal, not just inhibitory to growing cells of S. fragilis.

All of the in vivo experiments III, IV, V, VI indicate that DOCT effectively inhibits the organisms associated with CRD at least to the degree that the pathological responses of host (i.e., sinusitis, tracheitis and aerosacculitis with exudate accumulation in the airsacs) are reversed. If the chief cause of the pathology in CRD is indeed the secondary invaders, especially Escherichia coli and Pseudomonas aeruginosa as suggested by many workers in the field, then any effect of DOCT in alleviating this pathology would be very difficult to explain, as

both of these organisms are Gram-negative. As pointed out previously, Lester and Hechter (1958) have shown that Gram-negative organisms are generally insensitive to this hormone. Since these experiments have shown that DOCT is effective in the treatment of CRD, it leads one to suspect that the secondary invaders may be saprophytes and when the primary etiological agent, PPLO, are reduced in numbers the normal defense mechanisms of the host are sufficient to overcome the secondary invaders. Price et al., (1957) in their work with CRD practically ignored PPLO when measuring the effect of terramycin on the disease (as was done in the present work as far as the secondary invaders are concerned). Their work was concentrated on measuring the response of the secondary invaders and they showed terramycin to be fairly effective in controlling CRD. In the light of the present work and that of Price et al. one may well wonder then about the role of both PPLO and the secondary invaders in CRD.

From figures 1, 2, and 3 it can be seen that DOCT reduces the normal recovery time of birds affected with CRD. As the name implies CRD is a chronic disease with a course varying from a minimum of about 30 days to a maximum of about 180 days. Any significant shortening of the term of the disease would, of course, be highly desirable to the poultryman.

Weight gains in the infected and DOCT treated groups of birds in experiments IV, V, and VI were respectively 80, 83 (at the 20 mg. level of DOCT) and 102 (average of 3) per cent of normal, the latter increase of some 20 per cent probably being due to the earlier commencement of treatment (Tables 8, 10, 12).

Considering all three experiments we find that treated birds gained an average of 41 per cent more weight than non-treated birds when compared with "normal" controls.

Feed conversion (i.e., the amount of feed utilized to produce 1 pound of gain) in the infected and DOCT treated groups of birds in experiments IV, V, and VI were an average of 7% higher (more feed) than the normal controls, while the infected, non-DOCT-treated birds required an average of twice as much feed (103% more) as the normal controls to produce 1 pound of gain.

The noninfected treated birds (experiment IV, Table 8) needed 9.8% less feed than the normal birds to produce a pound of gain. The fact that better feed conversion is obtained in those birds noninfected but treated suggests that DOCT may act as a growth stimulant in normal birds. However, considerably more experimentation would be necessary to establish this point. The use of number of isolable organisms as an indication of degree of infection and rate of recovery or even of just sickness and health may be questionable. In experiments IV, V, and VI about 12,000, 3700, and 6000 organisms, respectively, per mg. of airsac (see Tables 7, 9, and 11) were isolated from the infected, non-treated control birds. However, little or no difference in degree of gross pathology was noted between the birds of the three different experiments. The pathology was as marked in the birds in experiment V as in those of experiment IV even though fourfold the number of organisms was isolated from the birds in experiment IV as from the later experiment.

Differences in the results of these experiments may perhaps be

attributed to differences in age, susceptibility of the birds, or numbers and virulence of the organisms in the inoculums used. In addition, Adler and Shifrine (1960) have already pointed out many of the difficulties encountered when isolating M. gallisepticum.

If one uses the number of organisms isolated as an indication of the effect of DOCT in CRD, then feed consumption, weight gain, and gross pathology should probably be correlated with it. In these experiments when the number of isolatable infecting organisms decreased with DOCT treatment then the gain increased, feed per pound of gain decreased and gross pathology was considerably less marked. On the basis of the results presented for these four factors it appears that DOCT is effective in the treatment of CRD.

In experiment IV no organisms could be isolated 41 days post-treatment from previously infected and treated birds (Table 7). Whether M. gallisepticum were totally absent or whether they may have been present but in too few numbers to be isolated by the methods used is unknown. In experiments V and VI organisms were isolated from all infected and treated birds at the termination of the experiments; however, these experiments were terminated earlier than experiment IV.

The question arises as to whether DOCT is lethal or merely inhibitory to M. gallisepticum, and as to whether the disease will recur when treatment is stopped. The answer to neither of these points can be found in the data presented. Further work is necessary to determine lethality and recurrence.

Cholesterol has been shown to be a growth requirement for certain

PPLO. Considering the recent work of Smith and Rothblat (1960), who have found that the major portion of this sterol was located in the cell membrane and that only steroids possessing a side chain similar to cholesterol are utilized, indicating that the adsorption of the steroid occurs through the 8 carbon side chain, one may speculate as to the mode of action of DOCT. The present work has shown that DOCT inhibits PPLO both *in vivo* and *in vitro*. Cholesterol and DOCT differ structurally in their side chains and at the cholesterol 3-β-hydroxy site. The specificity of the 3-β-hydroxy group for growth has been shown by Smith and Lynn (1958). Its nonrequirement for initial adsorption (Smith and Rothblat, 1960), and the cholesterol esterase activity of the cells (Smith, 1959) suggest an additional metabolic function particularly for parasitic strains (Smith, 1960) as well as a probable structural role (Smith and Rothblat, 1960).

The utilization of cholesterol may be prevented by DOCT in much, but not necessarily, the same way that sulfas interfere with the utilization of para-aminobenzoic acid. Further evidence along this line is suggested by the fact that saprophytic PPLO (*i.e.* those not requiring cholesterol) are not inhibited by DOCT (Padgett and Schoenhard, unpublished data).

Negative evidence concerning this hypothesis is found in the work of Lester and Hechter (1958) who have shown that organisms not dependent on cholesterol are inhibited by DOC.

The mechanism of action of DOCT is unknown. The above hypothesis ties together some information about PPLO, cholesterol and DOCT, but further work will be necessary to determine the interactions of them.

It is hoped that the present thesis will stimulate further research on the subject.

SUMMARY

Avian pleuropneumonia-like organisms (PPLO) were inhibited by 0.4 mg. of deoxycorticosterone *in vitro*; the growth characteristics of surviving colonies were modified.

In three separate *in vivo* experiments avian PPLO were inhibited by deoxycorticosterone trimethylacetate; symptoms and pathology of chronic respiratory disease in chickens were reduced, with a resultant increase in weight in infected, treated chickens when compared with infected, non-treated control birds.

Toxicity of this hormone for the chicken, with one exception, was not observed at the dosage levels used in these experiments.

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Table 1

Antibiotics and chemotherapeutic agents used *in vitro*, *in ovo*, and *in vivo* to inhibit or reverse the effect of PPLO of avian origin

AGENT	IN VITRO			IN OVO			IN VIVO		
	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective
Aureomycin		9	6		9	2 3	1 4 7	6 11 17 18 20	5 10
Chloromycetin		6 13	16	2 9	3		1		
Terramycin			6 9		9 13 19	2 3 13	1 4 7	6 11 12 17	10 14
Streptomycin		9 13	16	9 13		2 3	4 8 11	4 20	
Bacitracin				2	3				
Penicillin	6			2 3			10 12 14		
Sulfamethazine				2					
Para aminobenzoic acid				2					
Neomycin				3	2				
Magnamycin			13		13	3		11	
Catenulin				3					
Rimocidin				3					

Table 1 - Continued

AGENT	IN VITRO			IN OVO			IN VIVO		
	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective
Polymyxin				3					
Viomycin				3					
Pen-Strep							8		4
Bicillen and Streptomycin							8		
Acetarsone				9					
Aldarsone	9			9					
Carbarsone	9			9					
Tryparsamide	9			9					
Furazolidone			16	9			11	11	
Nitrofurazone				9					
Erythromycin		9	13			9 13 19	20		
Viridogrisein						9			
Tetracycline			9 16		9			20	
Dihydrostreptomycin	9			9			11		

Table 1 - Continued

AGENT	IN VITRO			IN OVO			IN VIVO		
	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective
Bicillen							11		
Erythrocin							11		
Pabakay							11		
Actithiazic Acid				3					
PA 99				3					
PA 94				3					
G 8255				3					
Polyotic							11		
Hygromycin		13		13					
Dihydrostreptomycin Propylene-Glycol									15
Aureomycin + Terephthalic Acid								17 18	
Terramycin + Terephthalic Acid								17	

Table 1 - Continued

AGENT	IN VITRO			IN OVO			IN VIVO		
	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective	Not Effective	Somewhat Effective	Effective
Spiramycin								20	
Leukomycin								20	
Deoxycorticosterone			21						21

TABLE 1 - CONTINUED

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Table 2.

Design of Experiment III.

Pen 1 - Control			Pen 2 - Variable			
No. of Birds	Inoculum	Treatment	No. of Birds	Inoculum	Treatment	
5	U. A. F.*	None	Grp. 1 (7	I.A.F.**	0.4 mg.***	8 days
			(3	None	0.4 mg.	8 days
4	None	None	Grp. 2 (7	I.A.F.	0.4 mg.	16 days
			(3	None	0.4 mg.	16 days
			Grp. 3 (6	I.A.F.	0.4 mg. 0.8 mg.	16 days 8 days
			(4	None	0.4 mg. 0.8 mg.	16 days 8 days

*U.A.F. - Uninfected allantoic fluid

**I.A.F. - Infected allantoic fluid

***DOC when administered given on alternate days in all cases

Table 3.

Design of experiment IV.

Pen No.	Total No. Birds	Pretreat Sacrifice	Treatment*	Observation Posttreatment	
				21 days	41 days
1 (Non-inf., Non-treat)	14	3	11 birds - none	5	6
2 (Non-inf., Treat)	23	2	11 birds - 0.5 cc	6	5
			10 birds - 0.6 cc	5	5
3 (Inf. and Treat)	21	3	9 birds - 0.5 cc	4	5
			9 birds - 0.6 cc	5	4
4 (Inf. and Treat)	20	3	9 birds - 0.5 cc	5	4
			8 birds - 0.6 cc	4	4
5 (Inf. and Non-treat)	18 Removed from pens 3 & 4 post-infect.	0	18 birds - none	9	9
TOTALS	96	11		43	42

*Dosage each of two treatments.

Table 4.

Design of experiment V.

Pen No.	Total No. Birds	Pretreat Sacrifice (21 Days Postinoc)	Treatment	Observation Posttreatment		
				7 days	14 days	21 days
1. Non-inf. Non-Treat	14	2	0	4	4	4
2. Inf. Non-Treat	13	1	0	4	4	4
3. Inf. Treated	14	2	2.5 mg. DOCT	4	4	4
4. Inf. Treated	12	0	5 mg. DOCT	4	4	4
5. Inf. Treated	14	2	10 mg. DOCT	4	4	4
6. Inf. Treated	13	2	20 mg. DOCT	4	3	4
7. Inf. Treated	14	2	40 mg. DOCT	4	4	4
Total	94	11		28	27	28

Table 5.

Design of experiment VI.

Pen No.	Total No. Birds	Treatment	Time of Treatment	Observation Post Inoculation				
				7 days	14 days	21 days	28 days	35 days
1. Inf. Treated	20	20 mg. DOCT	At time of inoculation	4	4	4	4	4
2. Inf. Non-Treat	20	0	0	4	4	4	4	4
3. Inf. Treated	20	1.6 cc. diluent	At time of inoculation	4	4	4	4	4
4. Inf. Treated	18	20 mg. DOCT	7 days post inoculation	2	4	4	4	4
5. Inf. Treated	20	20 mg. DOCT	14 days post inoculation	4	4	4	4	4
Total	98			18	20	20	20	20

Table 6.

Results of experiment III.

Isolation of PPLO Pretreatment					Isolation of PPLO Posttreatment	
Days after Initiation of Exper.	Pen 1 - Control No. of Birds	Pen 2 - Variable No. of Birds		Days of Treatment	Pen 1 - Control No. of Birds	Pen 2 - Variable No. of Birds
12	$^{0}/_{9}$[1]	$^{20}/_{20}$	Receiving I.A.F.[2]	Grp. 1 - 8 days	--[4]	$^{10}/_{10}$
		$^{3}/_{10}$	Receiving No A.F.[3]	Grp. 2 - 16 days	--	$^{6}/_{10}$
22	$^{0}/_{9}$	$^{20}/_{20}$	Receiving I.A.F.	Grp. 3 - 24 days	$^{2}/_{9}$	$^{4}/_{10}$
		$^{10}/_{10}$	Receiving No A.F.			

1. Numerator indicates Number of Infected Birds
   Denominator indicates Total Number of Birds
2. I.A.F. - PPLO Infected Allantoic Fluid
3. A.F. - Allantoic Fluid
4. Not checked

Table 7.

Number of PPLO per milligram airsac. Experiment IV.

	Pen No. and Description	Dosage	Pretreatment No. Birds	Pretreatment Total* Count	Pretreatment Count/mg Tissue	21 Day Posttreatment No. Birds	21 Day Posttreatment Total* Count	21 Day Posttreatment Count/mg Tissue	41 Day Posttreatment No. Birds	41 Day Posttreatment Total* Count	41 Day Posttreatment Count/mg Tissue
I	(Non-Infect., Non-Treat)		5	0	0	5	0	0	6	0	0
II	(Non-Infect., Treat)	0.5 cc	1	0	0	6	0	0	5	0	0
		0.6 cc	1	0	0	5	0	0	5	0	0
III	(Infect., Treat)	0.5 cc	1	150,000	12,300	4	0 4,500 2,000 2,200	0 200 100 60	5	0	0
		0.6 cc	1	460,000	9,600	5	100 0 0 0 3,000	10 0 0 0 70	4	0	0
IV	(Infect., Treat)	0.5 cc	1	439,500	15,700	5	1,900 0 400 0 2,500	100 0 20 0 50	4	0	0
		0.6 cc	1	290,000	9,700	4	2,000 0 900 0	60 0 50 0	4	0	0
V	(Infect., Non-Treat)		2	827,200 426,400	13,300 12,000	9	15,000 90,000 140,000 250,000 45,000 1,200,000 55,000 300,000 950,000	1,000 4,000 5,500 5,400 1,400 15,600 1,500 7,200 10,600	9	0 2,500 0 1,500 7,000 7,200 3,600 0 1,500	0 100 0 20 100 150 200 0 100

*Average of 2 plates

Table 8.

Weight gain of infected and noninfected birds treated with DOCT. Experiment IV.

Pen No. and Description	Total Weight Gain Ave. per Bird in pounds	Increase over Infected, non-treated Controls in pounds	% of the Weight Gain of the "Normal" Controls	% Increase in feed per pound Gain over "Normal" Controls
1. Noninfected Nontreated	1.65	.86	--	--
2. Noninfected Treated	1.68	.89	101%	-9.8%
3 and 4. Infected Treated	1.32*	.53*	80%*	+18.9%*
5. Infected Nontreated	.79	--	47%	+85.6%

*Average of pens 3 and 4

Table 9.

Average number of PPLO per mg. of airsac per group of birds at each time of sacrifice. Experiment V.

Pen No. and Time of Sacrifice	Treatment	No. of Birds	Average No. of PPLO Per mg. Airsac
1	Pretreatment	2	0
2 3 4 5 6 7	Pretreatment	9	3,350
7 days Posttreatment			
1	0	4	0
2	0	4	3,300
3	2.5 mg.	4	2,000
4	5 mg.	4	550
5	10 mg.	4	1,200
6	20 mg.	4	400
7	40 mg.	4	1,600
14 days Posttreatment			
1	0	4	0
2	0	4	2,500
3	2.5 mg.	4	650
4	5 mg.	4	1,100
5	10 mg.	4	330
6	20 mg.	3	380
7	40 mg.	4	350
21 days Posttreatment			
1	0	4	0
2	0	4	3,600
3	2.5 mg.	4	320
4	5 mg.	4	140
5	10 mg.	4	120
6	20 mg.	4	220
7	40 mg.	4	380

Table 10.

Average weight gains and feed conversion. Experiment V.

Pen No. and Description	Treatment (21 days Postinoculation)	Total Food Consumed in pounds	Total Weight Gain in pounds	% of the Weight Gain of the "Normal Controls"	Feed per Pound of Gain In pounds	% Increase in Feed per pound Gain over "Normal" Controls
1. Noninfected Nontreated	0	6.68	1.68	---	3.97	---
2. Infected Nontreated	0	6.47	.74	44%	8.74	120%
3. Infected Treated	2.5 mg. DOCT	5.88	1.05	62%	5.60	41%
4. Infected Treated	5 mg. DOCT	6.38	1.25	75%	5.10	28%
5. Infected Treated	10 mg. DOCT	5.55	1.22	73%	4.54	14%
6. Infected Treated	20 mg. DOCT	4.63	1.44	83%	3.30	-17%
7. Infected Treated	40 mg. DOCT	5.61	1.12	67%	5.00	28%

Table 11.

Number of PPLO per milligram airsac
(average of birds sacrificed) Experiment VI.

Pen No. and Description	Total No. of Birds	Time of Treatment and Treatment		Observation Postinoculation			
			7 days	14 days	21 days	28 days	35 days
1. Infected Treated	20	At time of inoculation 20 mg. of DOCT	Total Count* 5,000	3,000	14,000	6,000	18,000
			Count/mg. Tissue 60	30	00	90	260
2. Infected Nontreated	20	0	Total Count* 5,250	502,000	436,000	567,000	750,000
			Count/mg. Tissue 60	6,200	3,100	1,700	3,600
3. Infected Treated	20	At time of inoculation 1.6 ml. of diluent	Total Count* 6,000	500,000	728,000	770,000	975,000
			Count/mg. Tissue 60	3,000	4,200	2,300	2,900
4. Infected Treated	18	7 days post inoculation 20 mg. DOCT	Total Count* 5,000	110,000	40,000	16,000	7,000
			Count/mg. Tissue 50	900	240	180	90
5. Infected Treated	20	14 days post inoculation 20 mg. DOCT	Total Count* 7,250	472,000	537,000	37,000	16,500
			Count/mg. Tissue 110	4,000	2,200	350	150

*Average of 2 plates

Table 12.

Average weight gains and feed conversion. Experiment VI.

Pen No. and Description	Time of Treatment and Treatment	Total Food Consumed in pounds	Total Weight Gain in pounds	Feed per pound of Gain in pounds	% Increase in Feed per pound Gain over Normals	% of the Weight Gain of the "Normal" Controls
Normals Heuser[1]			1.45[2]	2.80[3]	----	
1. Infected Treated	At time of inoculation 20 mg. DOCT	4.63	1.41	3.28	17%	97%
2. Infected Non-treated	0	5.27	0.88	5.99	114%	60%
3. Infected Treated	At time of inoculation 1.6 ml. of diluent	4.73	0.66	5.50	96%	45%
4. Infected Treated	7 days post inoculation 20 mg. DOCT	5.16	1.53	3.37	20%	105%
5. Infected Treated	14 days post inoculation 20 mg. DOCT	5.08	1.49	3.40	21%	103%

1. Heuser "Feeding Poultry" Table 19, page 350
2. Normal weight gains for 50% cockerels and 50% pullets (Leghorn) from 5 to 9 weeks of age inclusive (Heuser)
3. Pounds of feed per pound body weight for leghorns 9 weeks of age (Heuser)

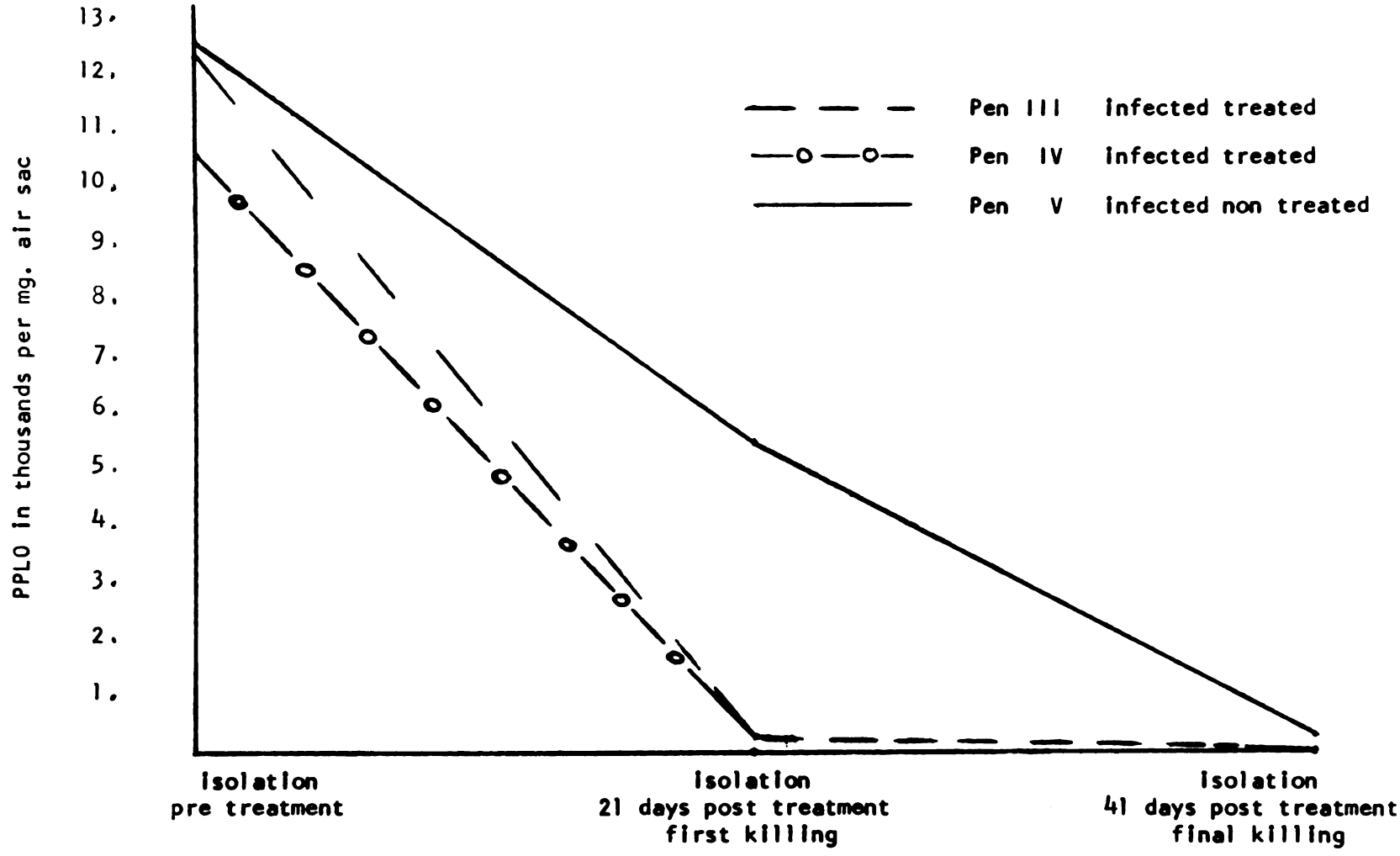

FIGURE 1. Average number of organisms per milligram of air sac recovered from birds inoculated with avian pleuropneumonia-like organisms and treated with deoxycorticosterone trimethylacetate.

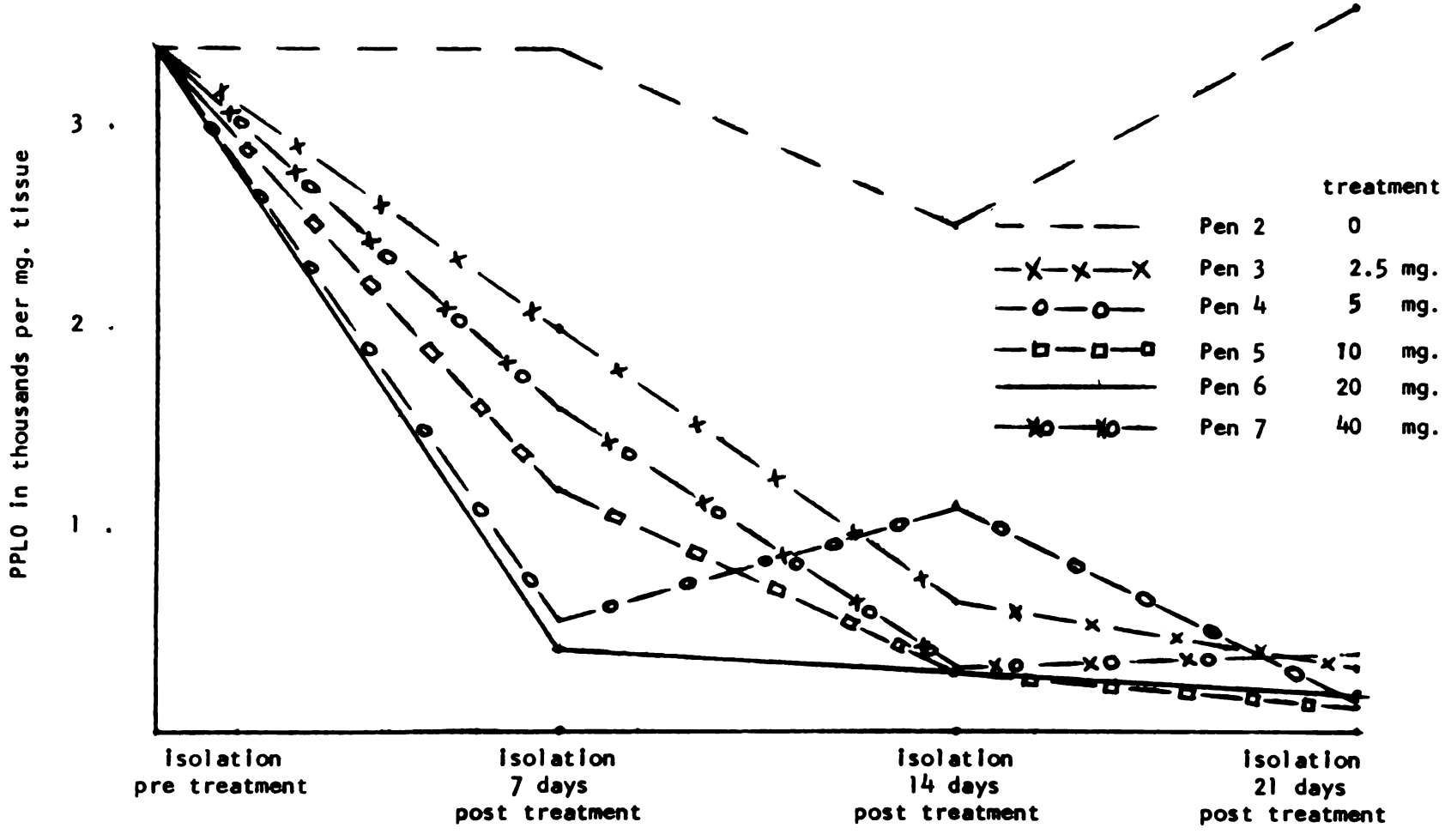

FIGURE 2. Average number of organisms per milligram of air sac recovered from birds inoculated with avian pleuropneumonia-like organisms and treated with deoxycorticosterone trimethylacetate.

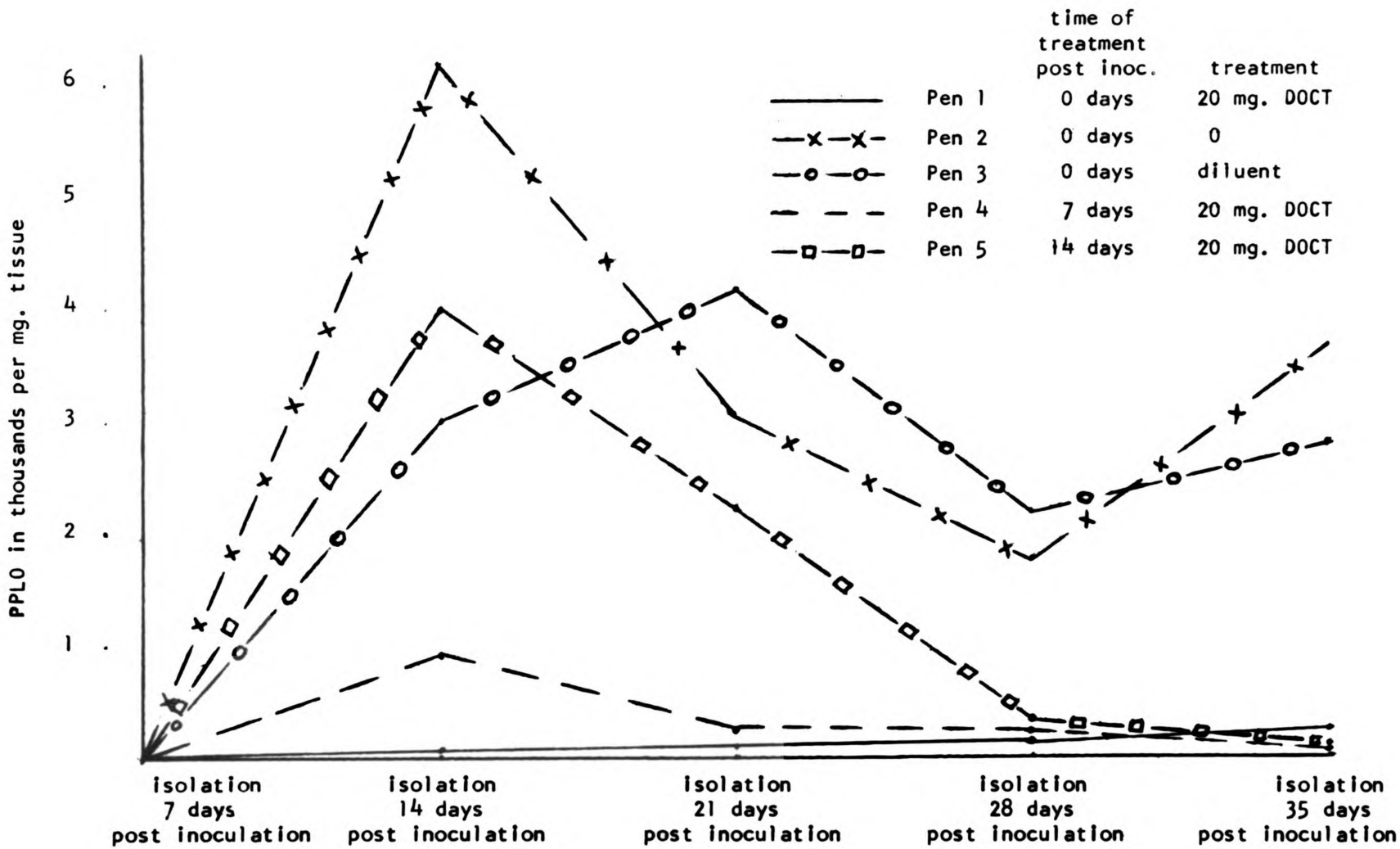

FIGURE 3. Average number of organisms per milligram of air sac recovered from birds inoculated with avian pleuropneumonia-like organisms and treated with deoxycorticosterone trimethylacetate.

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