

**The bursa of Fabricius plays a key role in the development of lymphoid leukemia (LL) (Peterson et al., 1964, 1966).** Surgical removal of the bursa, even though delayed up to 4–5 months of age, prevented the occurrence of this neoplasm. However, bursectomy had no effect on occurrence of erythroblastosis or osteopetrosis, neoplasms also caused by LL virus. Removal of the thymus, a lymphoid organ similar in several respects to the bursa of Fabricius, did not significantly affect the occurrence of any of these neoplasms. This is consistent with the hypothesis (Cooper et al., 1968) that LL is a neoplasm of the bursa-dependent lymphoid system. The primary neoplasm occurs in the bursa follicles and the malignant cells metastasize to the vital visceral organs eventually causing death.

Androgens have been used to prevent the development of the bursa of Fabricius when administered to embryos (Meyer et al., 1959; Glick and Sadler, 1961; May and Glick, 1964) or to cause rapid involution in the chicken when given after hatching (Glick, 1957; Kirkaptrick and Andrews, 1944). The use of this procedure for ablation of the bursa of Fabricius and thereby preventing the induction of LL was investigated and is herein reported.

**EXPERIMENTAL**

**General Procedure:** Day-old chicks of the inbred White Leghorn line 151 were inoculated intra-abdominally with 0.2 ml. of a $10^{-5}$ dilution of the virus preparation L31 of strain RPL12 (Burmester et al., 1960). The chicks were brooded in pens with wire floors and infra-red electric lamps to provide supplemental heat and received a standard ration of the laboratory feed.

The experimental period was 245 days post inoculation. Chickens that died during the period were autopsied and a diagnosis established by gross findings when possible, and by microscopic examination when necessary. A histologic examination was made of the bursa of Fabricius or, when it was not visible, the area around the atrophied bursa. The extent of bursal atrophy and presence or absence of tumors was particularly noted.

**Trial 1:** The first experiment was designed to determine whether the egg dipping method of administering the hormone is satisfactory. Eggs at 3 days of
TABLE 1.—Hatchability and bursal atrophy after dipping eggs in solutions of testosterone propionate—Trial 1

<table>
<thead>
<tr>
<th>Testosterone in alcohol dip solution (gms. per liter)</th>
<th>Number of 18-day embryos</th>
<th>Percent hatch</th>
<th>Bursal weight1 (gms.)</th>
<th>Bursa follicle score range</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>176</td>
<td>21</td>
<td>0.5</td>
<td>1-5</td>
</tr>
<tr>
<td>10</td>
<td>178</td>
<td>33</td>
<td>0.3</td>
<td>1-1.7</td>
</tr>
<tr>
<td>7.5</td>
<td>157</td>
<td>29</td>
<td>0.2</td>
<td>1-5</td>
</tr>
<tr>
<td>5</td>
<td>137</td>
<td>39</td>
<td>0.5</td>
<td>1-5</td>
</tr>
<tr>
<td>0</td>
<td>66</td>
<td>67</td>
<td>2.0</td>
<td>1-5</td>
</tr>
</tbody>
</table>

1 Based on 3 birds selected at random at 6 weeks of age.

2 An arbitrary score indicating the relative number of follicles in sections of bursa with 5 representing the normal number, and 1 representing 1 to 5 follicles in an entire cross section. Based on 3 birds at 13 weeks of age.

incubation were dipped about 3/4 (small end down) of their length into an alcoholic solution of testosterone propionate (TP), after which they were returned to the incubator. The concentrations of the TP in the dipping solutions were 15, 10, 7.5, 5.0 gms. per liter of 95 percent ethanol. The chicks that hatched were wingbanded serially and were inoculated with RPL12 virus.

Hatchability was adversely affected by TP since a high proportion of the 18 day embryos that had been dipped in TP solution did not hatch (Table 1). Dipping in TP solution resulted in a marked reduction in bursa size; however, there was a great variation in the extent of this reduction. In a sample of 5 chickens from each lot, examined at 6 weeks of age, the average bursal weights of birds of the treated lots were 1/5 to 1/4 those of the lot dipped in plain alcohol, but some of the bursae of the treated lots were as large as, while others were 1/5 the size. The bursa follicle score also varied greatly. Furthermore, there was no correlation between concentration of TP in the alcoholic dip and the average or range of bursa weights or scores. The marked variation in effects on the bursa was probably due largely to differences in egg shell porosity which resulted in unequal effective dosage per egg. Because of this variation the data were separated so that chickens with a bursa were compared with those without evidence of bursal tissue even though atrophy due to aging had occurred.

The bursa of line 15I White Leghorns is grossly visible up to about 4-5 months of age. During this period the influence of hormonal treatment on bursa size could be determined easily. After 5 months, differentiation between normal and accelerated atrophy was more difficult; however, since a vestige of a bursa in 8-month-old untreated birds may be seen by careful examination, complete bursal atrophy within this period, as seen in hormone treated birds, would appear to be significant.

Mortality data of the chickens of the various treated and control lots which had been separated into those with or without bursa are presented in Table 2. Of the chickens in the TP treated groups,
about $\frac{1}{2}$ had no bursa. The 5 gm./liter group had the lowest proportion of birds without bursa; however, there was no consistent correlation between the hormone dose and absence of a bursa.

A total of 26 chickens died of causes other than neoplasms and one had Marek's disease. All but one of these were in the TP treated groups. A total of 18 chickens had osteopetrosis, and 13 developed erythroblastosis. The occurrence of these diseases was highly variable and there was no consistent difference in mortality from these causes between chickens with and without bursa at necropsy.

Lymphoid leukosis was confined almost entirely to chickens that had a bursa. Of 118 chickens treated with TP that survived to day 121, 31 were classified as having a bursa, or remnants thereof, at the time of necropsy, and of those, 15 or 48 percent, had LL. This may be compared with an LL incidence of 69 percent among the untreated chickens. In contrast, of 87 chickens treated with TP and without bursa at necropsy, only one had LL. Since the development of LL requires the presence of a bursa, the latter is bound to be present in chickens with LL. Thus, the foregoing classification and relation to presence or absence of a bursa is selective. If the classification is ignored and the percentage LL calculated on the basis of all chickens surviving to day 121, it is found that among the treated lots, the incidence varies from 8 to 15 percent. This is much lower than the 66 percent for the untreated controls.

**Trial 2:** Since the application of TP by dipping eggs in an alcoholic solution gave highly variable results, more direct methods of administration were investigated. For this trial TP was injected subcutaneously or methyl testosterone (MT) was fed in the ration. The TP was dissolved in warm safflower oil at the rate of 100 mg. TP per ml. of oil. Injections were given twice weekly starting at one day of age. One group of chicks received a total of 40 mg. in 4 injections and another, 80 mg. in 8 injections. The MT was mixed into the regular ration at the rate of 25 mg. or 100 mg. per pound. Mixing was facilitated by dissolving the hormone in 80 percent alcohol, and spraying the solution onto a thin layer of feed, and then mixing the sprayed feed. Chickens were fed the MT ration for the first 3 weeks and then they were placed on ration without hormone.

At 13 weeks of age the TP injected chickens weighed less than those of the control lot (Table 3). The mean weight of the bursa of the three injected birds was about $\frac{1}{2}$ that of the controls, and follicles were found in only one of the 6 birds examined. The thymuses were somewhat smaller than those of the controls; so also were the body weights.

Methyl testosterone fed at the 25 mg. level had no detectable effect on the bursa size or follicle score. The 100 mg. level reduced the weight of bursa by about one-half and also reduced somewhat the follicle score.

Egg production from the 7th to the 10th month of age was recorded and found to be quite low for the controls. This is not unusual for highly inbred chickens; however, it was much lower—8.5 percent—for chickens that received the high 80 mg. injection dose of TP.

There was considerable non-neoplastic mortality among chickens of the two groups that had received injections of TP, but none of them developed LL. This is in marked contrast to the untreated controls in which 58.2 percent of the chickens died of LL, and to the low dose MT fed group which had 61.4 percent LL. High level of MT feeding resulted in some
TABLE 3.—Effect of testosterone treatment on organ weights, egg production, and mortality—Trial 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight—gms.¹</th>
<th>Bursa follicle score²</th>
<th>Percent egg production³</th>
<th>Exptl. number</th>
<th>Non-neoplastic 121–245⁴</th>
<th>Marek's disease</th>
<th>Lymphoid leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intramuscular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mg. in 4 inj.</td>
<td>906</td>
<td>2.0</td>
<td>0.3</td>
<td>0.3</td>
<td>43</td>
<td>18.6</td>
<td>2.3</td>
</tr>
<tr>
<td>80 mg. in 8 inj.</td>
<td>813</td>
<td>1.7</td>
<td>0.1</td>
<td>0.0</td>
<td>8.5</td>
<td>44</td>
<td>40.9</td>
</tr>
<tr>
<td>Fed in ration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg. per lb.</td>
<td>1,113</td>
<td>2.8</td>
<td>2.7</td>
<td>5.0</td>
<td>—</td>
<td>44</td>
<td>4.5</td>
</tr>
<tr>
<td>100 mg. per lb.</td>
<td>1,018</td>
<td>2.9</td>
<td>1.5</td>
<td>4.0</td>
<td>—</td>
<td>46</td>
<td>10.9</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>1,025</td>
<td>2.5</td>
<td>2.3</td>
<td>5.0</td>
<td>36.0</td>
<td>43</td>
<td>9.3</td>
</tr>
</tbody>
</table>

¹ Average body weight based on all birds; that for thymus and bursa based on 3 birds selected at random at 13 weeks of age.
² See footnote (2), Table 1.
³ On a hen-day basis for the period of 7–10 months of age.
⁴ Experimental number to day 121. Each lot was started with 60 chicks.
⁵ Deaths due to causes other than Marek's disease and lymphoid leukosis.

reduction in bursa size and follicles, and much lower incidence of LL (17.4 percent) than the controls. One case of Marek's disease occurred in each of three groups.

**Trial 3:** This trial was conducted to confirm the general results obtained in the previous trial, to reduce the number of injections required, and to attempt to reduce the adverse effects such as low egg production and high non-neoplastic mortality.

Experimental design and procedures were similar to Trial 2 except that a total of 60 mg. TP was administered, divided into 1, 2, or 3 injections, and one lot did not receive hormone injections until the 28th day.

The differences obtained when TP was administered in 1, 2, or 3 injections were small and inconsistent (Table 4). The bursa size and follicle score in birds given TP were reduced to about ½ that of the controls. However, this reduction was somewhat less than that obtained in Trial 2 with either 40 mg. or 80 mg. A dosage of 400 mg. given during the 5th week appeared to have about the same effect on the bursa size and follicle score as 60 mg. given during the 1st week. As in the previous trial, body weight and thymus weight were somewhat less than the controls.

The three groups that received 60 mg. TP starting at 1 day were combined to measure egg production during the period of 6–9 months of age. The average egg production for these three groups was only 13.3 percent. This is much lower than that of the control lot or of the other two treated lots.

As in previous trials, the mortality distribution was related to early bursa atrophy. The three groups that received 60 mg. in either 1, 2, or 3 injections responded similarly, having relatively high mortality from non-neoplastic causes (21–28 percent), and low death rates from LL (2–16 percent). The untreated control group had a low mortality due to non-neoplastic causes and a high (75 percent) mortality from LL. The groups which received 400 mg. in 2 injections starting at day 28, and the group that was fed a ration containing 400 mg. MT per lb. for the first 3 weeks, had LL mortalities about midway between the other groups.
Table 4.—Effect of testosterone treatment on organ weights, egg production and mortality—Trial 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight—gms.1</th>
<th>Bursa follicle score2</th>
<th>Percent egg production3</th>
<th>Expnl.4 number</th>
<th>Non-neoplastic 121-2453</th>
<th>Marek's disease</th>
<th>Lymphoid leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body</td>
<td>Thymus</td>
<td>Bursa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramuscular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>starting at day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 mg. in 1 inj.</td>
<td>714</td>
<td>1.7</td>
<td>.5</td>
<td>.6</td>
<td>46</td>
<td>23.9</td>
<td>21.7</td>
</tr>
<tr>
<td>60 mg. in 2 inj.</td>
<td>802</td>
<td>1.2</td>
<td>.4</td>
<td>1.5</td>
<td>43</td>
<td>20.9</td>
<td>11.5</td>
</tr>
<tr>
<td>60 mg. in 3 inj.</td>
<td>818</td>
<td>1.8</td>
<td>.2</td>
<td>1.4</td>
<td>51</td>
<td>27.5</td>
<td>15.7</td>
</tr>
<tr>
<td>starting at day 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 mg. in 2 inj.</td>
<td>776</td>
<td>1.9</td>
<td>.3</td>
<td>2.2</td>
<td>41</td>
<td>12.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Fed in ration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 mg. per lb.</td>
<td>917</td>
<td>2.0</td>
<td>.6</td>
<td>2.5</td>
<td>52</td>
<td>3.9</td>
<td>7.7</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>907</td>
<td>2.0</td>
<td>2.0</td>
<td>5.0</td>
<td>52</td>
<td>1.9</td>
<td>3.8</td>
</tr>
</tbody>
</table>

1 Average body weight based on 24 birds. Organ weights and bursa score based on 4 birds selected at random at 12 weeks of age.
2 Same as footnote (2), Table 1, except that averages are for 4 birds 12 weeks of age.
3 Same as footnote (3), Table 3, except the period was 6-9 months of age.
4 Same as footnote (4), Table 3, except each lot was started with 63 chicks.
5 Same as footnote (5), Table 3.

These mortalities correlate well with the bursa follicle score.

Results of this trial show that one injection is as effective as multiple injections in reducing LL, and that a delay of injection to 28 days, even at a dosage of 400 mg. reduces LL to about one-half that of the controls. However, there was an improvement in egg production and reduction in deaths from other causes.

Trial 4: Further reduction in the total amount of TP injected and a comparison of 1 and 2 injections were examined in this trial.

The mean bursa weight and bursa follicle score of 3 birds at 13 weeks were similar in groups receiving 30 or 40 mg. TP, whether they received it in 1 or in 2 injections (Table 5). For these treated groups the above statistics were about ½ those of the control group, and egg production was about ½ that of the controls.

Body weights of all treated groups were not appreciably lower than those of the control.

Mortality from LL occurred in all groups but was again directly related to bursa weight and follicle score. Groups that received 30 or 40 mg. TP in either 1 or 2 injections had similar LL mortality which was about ½ that of the control group. The group that received 20 mg. TP had an intermediate LL mortality. In this trial there was no consistent difference in the non-neoplastic mortality between the treated and control groups.

This trial provides further evidence that a single injection of hormone is as effective as multiple injections and suggests that the decreased mortality from LL and egg production and, to a lesser extent, increased non-neoplastic mortality are effects of TP. Since the desirable and undesirable effects are produced over the entire dosage range, there appears to be little or no possibility of separating these effects by adjusting the dosage.

DISCUSSION

The treatment of chickens with androgens effectively reduced, or in one trial, completely prevented lymphoid leukosis.
TABLE 5.—*Effect of testosterone treatment on organ weights, egg production, and mortality—Trial 4*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight—gms.</th>
<th>Bursa follicle score</th>
<th>Percent egg production</th>
<th>Exptl. number</th>
<th>Percent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body</td>
<td>Bursa</td>
<td></td>
<td></td>
<td>Non-neoplastic</td>
</tr>
<tr>
<td>Intramuscular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>disease</td>
</tr>
<tr>
<td>20 mg. in 2 inj.</td>
<td>1,209</td>
<td>1.7</td>
<td>2.7</td>
<td>40.4</td>
<td>41</td>
</tr>
<tr>
<td>30 mg. in 1 inj.</td>
<td>1,212</td>
<td>1.2</td>
<td>1.7</td>
<td>20.8</td>
<td>33</td>
</tr>
<tr>
<td>30 mg. in 2 inj.</td>
<td>1,150</td>
<td>1.0</td>
<td>1.7</td>
<td>20.3</td>
<td>37</td>
</tr>
<tr>
<td>40 mg. in 1 inj.</td>
<td>1,116</td>
<td>1.0</td>
<td>2.0</td>
<td>20.3</td>
<td>38</td>
</tr>
<tr>
<td>40 mg. in 2 inj.</td>
<td>1,143</td>
<td>2.1</td>
<td>2.1</td>
<td>20.3</td>
<td>41</td>
</tr>
<tr>
<td>Controls</td>
<td>1,212</td>
<td>3.0</td>
<td>5.0</td>
<td>45.5</td>
<td>40</td>
</tr>
</tbody>
</table>

1 Average of body weights based on 24 birds; bursa weight and score based on 3 birds at 13 weeks.
2 See footnote (2), Table 1.
3 See footnote (3), Table 4.
4 Same as footnote (4), Table 4, except each lot was started with 48 chicks.
5 Same as footnote (5), Table 3.

(LL). This result was apparently due to atrophic effect of androgens on the bursa of Fabricius. Chickens with completely atrophied bursa generally did not develop LL, whereas under similar conditions, most chickens with a normal bursa developed LL. A positive correlation between bursa size and occurrence of LL and its relation to testosterone dosage was found in the data of Trials 2, 3, and 4, which are graphically presented in Figure 1. The close relationship between bursa weight and percent LL is further illustrated in Figure 2.

In Trial 1 where TP was given prior to hatching and 2 of the birds did not have a bursa, all birds, with one possible exception, that developed LL were among those with a notable bursa.

The central role of the bursa in the

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![Fig. 1](https://example.com/fig1.png)  
**Fig. 1.** Relationships between testosterone dosage and relative bursa weight and incidence of lymphoid leukemia.

![Fig. 2](https://example.com/fig2.png)  
**Fig. 2.** Relationship between relative bursa weight and incidence of lymphoid leukemia.
pathogenesis of LL is further evidenced by the observation that birds which develop LL have bursal involvement. Thus, of the 196 birds of Trials 2, 3, and 4 with typical lesions of LL, all but 8 were recorded as having a bursal tumor. Of these, five were not examined for bursal tumor and in only 3 cases was the absence of a tumor in the bursa verified.

Although androgen treatment has a definite beneficial effect in that it reduces the occurrence of LL, it also has several adverse effects which have not yet been resolved. Eggs dipped in an alcoholic solution of testosterone hatch ½ to 3 as well as those dipped in alcohol without the androgen.

Non-neoplastic mortality for most treated groups was appreciably higher than for the controls. Most of these differences can be accounted for by the fact that there were many more birds at risk in groups where mortality from LL was low than in groups where most of the birds died of LL. When the proportion of non-neoplastic deaths is expressed as the percent of survivors plus non-neoplastic deaths, differences between the treated and control groups are much less. Thus, for Trials 2, 3, and 4, the average non-neoplastic mortality for the 121–245 age period was 23.8 percent, and that of comparable controls, 18.0 percent.

Although the trials described herein were not originally designed to measure the effects of androgen treatment on subsequent egg production, some data were collected in three trials. For the groups which received sufficient testosterone to effect a marked reduction in bursa weight and in percent LL, there was also a marked reduction in egg production over that of the controls or of treated groups which showed only a partial reduction in bursa weight and percent LL.

In Trial 4 graded dosages of testosterone were administered in an attempt to determine the level which would result in a marked reduction in LL without the above adverse effects. This, however, was not accomplished since levels of testosterone that caused a reduction in LL also caused some adverse effects.

Since the foregoing effects are related to the same dosage levels, a common factor may mediate the effects. The reduction on LL mortality is no doubt due to atrophic effects on the bursa, and since the bursa plays a key role in the establishment of humoral immunity, the increase in non-neoplastic mortality is probably also due to the early induced bursa atrophy. However, the author is not aware of any other evidence relating gonad function to the activity of the bursa of Fabricius. It is more likely that the testosterone had a direct effect on the ovary.

Although the separation of the beneficial and adverse effects of testosterone was not accomplished, there are several avenues not explored in the trials that should be investigated, namely (1) delay treatment until after the bursa has established immune competence, (2) the use of other agents which have a more specific atrophic effect on the bursa, and (3) the use of a combination of agents which may result in a potentiating effect on the bursa.

The observed close correlation between bursa atrophy and reduction in the incidence of LL provides further evidence for the central role of the bursa in the causation of LL.

**SUMMARY**

Androgens were administered at an early age by three different routes in four trials to produce early atrophy of the bursa of Fabricius. Multiple or single subcutaneous injections of testosterone
propionate in safflower oil at total dosages of 30 to 80 mg. resulted in partial to complete atrophy of the bursa with a parallel decrease in occurrence of lymphoid leukemia. Similar inoculated groups of White Leghorns not treated with androgens had mortalities of up to 75 percent due to this disease. Adverse effects of the testosterone treatment were a decrease in hatchability and in egg production, and an increase in deaths due to causes other than lymphoid leukemia.

REFERENCES


Presence of Spermatozoa in Uterovaginal Fluids of the Hen at Various Stages of the Ovulatory Cycle

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INTRODUCTION

BOBR (1962), in studying the distribution of spermatozoa in the oviduct of the hen, identified in the uterovaginal region of the oviduct, tubular glands which contained large numbers of spermatozoa following natural mating or artificial insemination. The nomenclature of these structures, which have been called by a variety of names in recent years, has been reviewed by Gilbert et al. (1968). In this study the structures will be termed uterovaginal or UV glands.

BOBR (1962) and Bobr et al. (1964) studied the distribution of spermatozoa