# INVESTIGATIONS CONCERNING THE EFFECTS OF CORTISONE IN THE DOMESTIC FOWL

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Joseph John Kudzia

1952

This is to certify that the

thesis entitled

INVESTIGATIONS CONCERNING THE
EFFECTS OF CORTISONE IN THE DOMESTIC FOWL

presented by

JOSEPH JOHN KUDZIA
1952

has been accepted towards fulfillment
of the requirements for

M.S. degree in Poultry Husbandry

Major professor

Date August 6, 1952

# INVESTIGATIONS CONCERNING THE EFFECTS OF CORTISONE IN THE DOMESTIC FOWL

by

JOSEPH JOHN KUDZIA

A THESIS

Submitted to the College of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Poultry Husbandry

1952

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ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. Lloyd R. Champion of the Department of Poultry Husbandry under whose inspiration, constant supervision and unfailing interest this investigation was conducted and for his assistance in the preparation of this manuscript.

Grateful acknowledgment is also due to Professor Carl G. Card of the Department of Poultry Husbandry for his continual encouragement, unfailing cooperation and for making available the facilities of the Department of Poultry Husbandry.

The author also wishes to thank Dr. William D. Baten, Professor of Mathematics for his assistance and kind guidance in the analysis of these data.

The cooperation and helpful suggestions given by Dr. Albert C. Groschke[1] of the Department of Poultry Husbandry are deeply appreciated.

The writer is deeply grateful to Merck and Company for providing the cortisone acetate used in the investigation.

[1]Now with the Borden Co., New York, New York.

# INTRODUCTION

The compound adrenal glands of mammals have been studied extensively since the discovery that the syndrome today known as Addison's disease involved these specific endocrine organs. Subsequent investigations have substantiated this pioneer study and further demonstrated that the adrenal cortical principles elaborated by the outer shell of the adrenals, the cortex, were concerned in the manifestation of this disease. These essential adrenal cortical principles are illustrated by desoxycorticosterone, a cortical compound which exerts its action predominantly upon water and salt metabolism, and corticosterone which exerts a strong effect upon carbohydrate metabolism. An influence upon growth has been demonstrated for desoxycorticosterone by Hartman and Thorn (1930).

Brown-Séquard (1856) was the first to demonstrate the rapidly fatal outcome resulting from extirpation of the adrenals. That the cortex of the adrenal is essential to life was demonstrated when Swingle and Pfiffner (1930a, 1930b, 1933) prepared an extract from the adrenal cortex and found that this extract was capable of

maintaining life in adrenalectomized cats. Studies by Wintersteiner and Pfiffner (1936), Reichstein (1936) and Kendall (1936) revealed that several hormonal compounds were present in cortical extracts. Intensive investigations by these researchers and others resulted in the isolation of 28 separate compounds from the adrenal cortex of which cortisone is generally considered as being the most active and critical compound of the group.

The isolation of cortisone, principal hormone of the adrenal cortex, by Edward C. Kendall (1938) has produced highly significant advances in medical science. The structural formula for cortisone is given in Figure 1.

A large number of clinical experiments have been conducted with cortisone on human patients for the treatment of various diseases. Most of the emphasis in the investigations has been directed toward the therapeutic value of cortisone but little, if any, research work has been done to determine the effects of cortisone on healthy normal animals. Research work with normal animals receiving cortisone indicated the possibility of deleterious effects occurring. This is the first attempt known to investigate the effects of cortisone with normal

chickens. Further information is herewith presented for an additional understanding of the problem.

## The Pituitary - Adrenal Relationship

The present concept is the adrenocorticotropic hormone designated hereinafter as ACTH stimulates the adrenal cortex to secrete cortisone and other cortical steroid hormones. This hormone is elaborated by the anterior pituitary and reaches the adrenals via the systemic blood circulation. It is believed that ACTH has the specific function of regulating the activity of the adrenal cortex.

The function of the anterior pituitary is presumably controlled to some degree by the hypothalamus which, in turn, can be influenced by the higher brain centers. The adrenal medulla which, in response to stress, releases epinephrine is another regulator mechanism. Epinephrine, reaching the anterior pituitary via the circulatory system, stimulates the anterior pituitary to produce ACTH. It is known that an increased titer of circulating adrenal cortical steroids has an inhibitory effect on ACTH secretion by the anterior pituitary.

Since cortisone suppresses the activity of the anterior lobe of the pituitary, it is evident that a balance exists between the pituitary, the adrenal cortex and the adrenal medulla which is maintained by the respective hormones of these glands.

Effect on the Pituitary - Adrenal Mechanism Produced by the Administration of Cortisone or ACTH

Under normal conditions, the sensitive regulatory mechanisms that have been described operate within a relatively narrow range. These delicate mechanisms are temporarily thrown out of balance if exogenous cortisone or ACTH is administered.

The systemic administration of cortisone causes a depression of anterior pituitary production of ACTH and depresses adrenal cortical secretion. Prolonged administration of cortisone in large doses has been shown to cause atrophy of the adrenal cortex.

Exogenously administered ACTH depresses endogenous pituitary secretion of ACTH and stimulates the secretion of adrenocortical hormones. It has been shown that when under the influence of larger than normal amounts of ACTH,

hypertrophy results. A transient period of relative adrenal cortical insufficiency occurs when the administration of exogenous ACTH is discontinued.

Auto-regulation of ACTH secretion by the normal variations of titer of circulating adrenal cortical steroids is by administration of exogenous cortisone or ACTH.

## Principal Effects of Cortisone

Cortisone is a potent hormone capable of affecting many body functions and many tissues. It has produced profoundly beneficial and often dramatic effects in the treatment of various diseases.

The predominant physiologic properties of cortisone are its strong effect on the metabolism of carbohydrates and proteins, and its rather mild influence on the metabolism of water and electrolytes. Gluconeogensis is increased partly at the expense of protein. Since alterations of the metabolism of two of the chief sources of energy, proteins and carbohydrates, are almost certain to modify that of the third source of energy, the fats, it is not surprising to find fat metabolism altered. The absorption and storage of fat may be increased.

There is also a tendency to produce a negative nitrogen balance either as a result of increased protein

catabolism, decreased protein anabolism, or possibly both. The excretion of potassium, phosphate, calcium, and chloride may likewise be increased.

A highly desirable biochemical effect is produced frequently with increases in hemoglobin values and erythrocyte counts and a return toward the normal ratio of serum proteins. Increases of as many as 1,000,000 red cells per ml. of blood have been observed in anemic patients during clinical trials for two weeks or longer.

The favorable nutritional effect with increased appetite, strength and weight gains has been reported frequently.

Another important effect is the increase of resistance of the organism to certain forms of stress such as: exposure to cold, starvation, and physical exertions.

Undesirable effects such as the retention of sodium and water may develop when using cortisone. These undesirable effects are to be respected but not feared as the undesirable effects are reversible and may be minimized or eliminated by reduction or discontinuance of cortisone administration.

## LITERATURE REVIEW

It has been reported that the route of administration of cortisone has an influence on the effects produced by this hormone. Mushett, Porter and Silber (1951) found that when dogs were administered 10 mg/kg. of cortisone subcutaneously over a three week period of time, polyuria and polydipsia resulted. Each animal also developed a slight anemia. Autopsies revealed an enlargement of the livers and atrophy of the thymus, lymph nodes and adrenal glands. However, when the dogs were given daily oral doses of 10 mg/kg. of cortisone these conditions were not observed.

Molomut, Spain and Haber (1950) demonstrated that the spleen size of mice was significantly decreased at the end of two days upon the injection of 1 mg. of cortisone intraperitoneally twice a day. Continued treatment beyond the second day did not further decrease the size of the spleen, indicating that this effect of cortisone is rapid and maximal.

Spain, Molomut and Haber (1950) experimenting with mice, suggested that the effect of cortisone on the healing of wounds was in many respects similar to that seen in vitamin C depletion.

An increase in the susceptibility of mice receiving cortisone to experimental tuberculosis was reported by Hart and Rees (1950). A high mortality prevailed during the stable period of this chronic pulmonary infection and the lungs contained large numbers of tubercle bacilli.

W. W. Smith, F. Smith and Thompson (1950) found that the survival time of normal mice which had been irradiated was not increased by treatment with cortisone and that the mortality rate was not reduced.

Further research work with pregnant mice conducted by Glaubach, Antopol and Graff (1951) substantiated the growth inhibition effects of cortisone on developing embryos. Mammary glands of the pregnant mice were greatly affected 24 to 48 hours after cortisone injections. White masses were seen through the skin in the mammary region. These white masses became most extensive within two days nearly encircling the mouse and extended from the skull region to beyond the pubic region. Histological studies of this tissue showed that the mass was breast tissue with the distended glands being filled with milk.

An experiment to determine the effect of cortisone on protein metabolism in the rat was conducted by Clark

(1950). He found that cortisone markedly increased urinary nitrogen excretion and that a decrease in protein synthesis occured in rats receiving cortisone.

Daily doses of 40 or 50 mg/kg. of cortisone administered subcutaneously to young rats by Follis (1951) resulted in a plateauing of the growth curve after an initial gain for one or two days. Greater amounts of cortisone led to a prompt plateauing of the growth curve followed by a gradual loss of weight. A dense zone composed of neddle-shaped bodies of calcified cartilogenous matrix encased in bone was found in the growing ends of the bones. This area of increased density was attributed to disturbances in normal bone resorption activity.

The retardation of wound healing by cortisone has been observed by Howes, Plotz, Blunt and Rogan (1950) with rabbits and rats. Cortisone prevented or delayed the formation of granulations and retards epithelization. It was hypothesized that cortisone retards granulation of wounds either by changing vessel permeability, interfering with some enzyme system or by having a direct effect on the sprouting of blood vessels and the proliferation and differentiation of fibroblasts.

In a study of the effects of cortisone on rabbit gestation, Courrier and Collonge (1951) reported that cortisone has a deleterious effect on the gestation period. Daily injections of 25 mg. of cortisone given four to seven days between the tenth and twenty-third days of gestation resulted in abortions in several animals and hemorrhages in other animals. Injections given between the tenth and fourteenth days of pregnancy resulted in living fetuses being reduced in size from 24 to 20 mm., the placentas were pale and evidence of reabsorbed fetuses were observed. All fetuses were found to be macerated and 15 to 38 mm. in size instead of the normal 54 mm. size when injections were given between the fifteenth and the twenty-first day of pregnancy.

Witschi and Chang (1950) found that the dosage of cortisone required to produce sex reversal was high in comparison with the other steroids tested in larval frogs.

Landauer (1947) injected an adrenal cortex extract into the yolk sac of early developing chick embryos and observed growth retardation. Karnofsky, Patterson and Ridgway (1949) and Karnofsky, Ridgway and Patterson (1951) found that cortisone acetate produced the characteristic

growth-inhibition upon injection into the yolk sac of four day old S. C. White Leghorn embryos. Greatest cortisone activity was found when the injection of cortisone was given via the chorioallantoic membrane route. Karnofsky, Ridgway and Patterson (1951) observed a selective inhibition of feather follicle formation in developing chick embryos when the eggs were injected with cortisone. These researchers suggested that the effect on feather formation may be due to a local action of cortisone on a highly sensitive tissue, the dermal papillae, and that cortisone may possibly function in altering embryonic metabolism.

## MATERIAL AND METHODS

Three experiments designated herein as Experiment I, II and III were conducted to determine the effects of subcutaneous injections of various dosage levels of cortisone acetate[1] on yearling male and female chickens. The New Hampshire males and females used in this study were supplied by the Department of Poultry Husbandry. During the experimental period the females were housed in individual cages in laying batteries equipped with raised wire bottoms. The males were housed in special male cages in the same building. Both the experimental and control birds received the basal ration ad libitum as shown in Table 1, and fresh water was constantly available. All females were inseminated at weekly intervals, the method being essentially that described by Burrows and Quinn (1937).

Prior to the start of the experimental period, all individuals were observed daily for a five-week interval to obtain a "normal picture" on body weights, egg production, egg size, fertility and hatchability.

Experiments were initiated on November 12, 1951 and continued through May 1, 1952. Data were continually

[1]Provided through the courtesy of Merck & Co., Inc. Rahway, N. J.

collected during the entire experimental period to determine the effects of cortisone on adult body weights, growth, egg production, egg size, feather development, fertility and hatchability. Individual body weights to the nearest ten gram unit were obtained for all birds three times a week. The weighing procedure was performed at a set time each day. All eggs were numbered with the hen number and weighed to the nearest gram each day. The eggs were collected and held at a desirable holding temperature for seven days prior to incubation. Settings of eggs were made each week in a forced-draft incubator operated according to the manufacturer's instructions. At the conclusion of each hatch, all incubated eggs which did not hatch were broken and examined in an attempt to determine the approximate time of embryo mortality and any abnormalities which the embryos may have exhibited were recorded. All chicks were wing banded and weighed to the nearest gram at hatching time and individual chick weights at two weeks of age were taken. The chicks were fed the station starting ration as shown in Table 2. Weekly measurements and observations were made on all feathers removed (pectoral, cushion, primary) from the date of removal until the feathers reached full development. The volume of semen

was measured after collection and inseminations were made immediately. Any modifications of these procedures are indicated in the appropriate experiment.

In Experiment I, three males comprised the experimental group and three males made up the control group. The males in the experimental group were injected subcutaneously with 1 mg/lb. of cortisone acetate for four consecutive days, followed by a lag period of three days and then injections were resumed for four additional consecutive days. To eliminate the possibility of any effects which could be attributed to the carrier of the cortisone, the control males were administered a .1% saline solution in proportions based on 1 mg/lb. of body weight. Primaries I and II were removed from the wings of the males three days prior to the start of injections to determine the effects of cortisone on feather growth.

In Experiment II, twenty-four selected females and six selected males were divided into two groups, an experimental and a control group. The experimental and control groups were further subdivided into three lots each, with each lot consisting of one male and four females. The experimental and control groups were com-

parable with respect to body weight and egg production. In this experiment only the females received the subcutaneous injections of cortisone at the 1 mg/lb. level for four consecutive days followed by a lag period of three days, and then injections were resumed for four additional consecutive days. The standard saline solution was administered to the control females based on body weights. The anterior pectoral tract feathers were removed from the females when the injections were started and weekly observations on feather growth were made until feathers reached full development.

In Experiment III, the twelve hens comprising each of the two groups in Experiment II were reduced to six birds per group. The females were selected for uniformity of body weight, egg production and egg size. The experimental females received 3 mg/lb. of cortisone acetate injected subcutaneously on four consecutive days. A three day lag period followed the initial injections and then injections were resumed for four additional consecutive days. The standard saline solution in proportions based on 3 mg/lb. of body weight was administered to the control females during the injection period of the experimental females. Primaries I and II were removed from

each wing of every female and an area of four square inches of cushion feathers were removed about one inch from the uropygial gland.

The data were analyzed by means of analysis of variance and covariance according to the procedure of Fisher (1924) as presented by Snedecor (1946). Covariance was used to correct the final body weights by adjusting for differences between individual birds in initial weights. Further, all data were analyzed for the pre-injection, injection and post-injection periods.

# RESULTS AND DISCUSSION

## BODY WEIGHT

There were no significant differences in initial body weights of individual birds (Experiments I, II and III) three days prior to the injection period, four days after the last injection and twenty-eight days after the last injection, as analyzed by analysis of variance (Tables 3 and 4). However, an analysis by covariance showed that the loss of body weight by the males receiving cortisone in Experiment I during th injection period was highly significant ($P < 0.01$). Also, a highly significant difference ($P < 0.01$) was found for the gain in body weight by the females receiving cortisone in Experiment III for the period during and after injections as shown in Figure 2.

As shown in Table 5, the experimental males lost an average of 240 grams in body weight during the injection period while the control males lost an average of 10 grams per male. Further analysis of the data revealed that the weight loss was statistically significant for the injection period only.. The loss in weight was not significant twenty-eight days after the last injection of cortisone. The male experimental birds be-

gan to recover their body weight moderately when injections were discontinued but their original body weight was never attained. It is postulated that the highly significant loss in weight resulted from protein catabolism, alterations of carbohydrate metabolism, or both.

The average weight loss of 90 grams for the experimental females in Experiment II during the injection period was not statistically significant. The corresponding average loss for the control females was 50 grams per female, however, the control females recovered their original body weight twenty-eight days after the last injection of saline solution. The experimental females made an average gain of ten grams per female over and above their original weight but this gain was not significant. Egg production of the experimental females was reduced during and post-injection but the reduction in egg numbers was not significant.

In Experiment III, the females receiving 3 mg/lb. of cortisone acetate lost an average of 20 grams in body weight during the injection period. This loss was not significant. Four weeks after injections ceased, the experimental females had gained an average of 100 grams

in body weight. This gain in body weight over and above the initial body weight was highly significant, ($P < 0.01$, Table 4). The average body weight lost by the control females during the same period of time was 70 grams and final body weights approximated original body weights. Among the six females receiving cortisone only one female continued egg production. It is suggested that the gain in body weight by the experimental females over and above their initial body weight was due to the cessation of egg production and consequently an increased deposition of body fat.

## FEATHER GROWTH

An analysis of the data on primary feather growth of the males in Experiment I, which received cortisone at the 1 mg/lb. level, revealed that a highly significant retardation in primary growth occurred, ($P < 0.01$, Table 6). This retardation was observed only during the first week after the initiation of subcutaneous injections, the differences in primary growth rate being non-significant thereafter. Differences in primary feather growth rate between the control females and the experi-

mental females that received cortisone at the increased dosage rate (3 mg/lb., Experiment III) was not found, however. Likewise, no significant differences in pectoral feather growth were observed (Table 8). An analysis of the data on the growth rate of the more sensitive cushion feathers showed that cortisone administered at the 3 mg/lb. level inhibited feather development to a highly significant degree ($P < 0.01$) for the first three weeks after injections started. Cushion feather growth was inhibited to a significant degree ($P = 0.05$) during the fourth week of development, whereas differences in feather growth for the fifth and sixth week were non-significant. These results may be seen in Table 9.

The observations of Karnofsky, Patterson and Ridgway (1949) and Karnofsky, Ridgway and Patterson (1951), in chick embryos, that cortisone retarded feather formation is hereby confirmed and extended to mature chickens. It is suggested that tissues vary greatly in their susceptibility to the action of cortisone, the physiological mode of action being complicated. The effects observed may be the product of a chain of events in which metabolism is shifted.

## EGG PRODUCTION

Reduced egg production was observed (Table 17) for the experimental females receiving cortisone at the 1 mg/lb. level in Experiment II but this reduction in egg numbers was not significant. The increased dosage of 3 mg/lb. of cortisone (Experiment III) for the experimental females resulted in a highly significant difference ($P < 0.01$) for reduced egg production during the injection period. An analysis of the data (Table 15) on egg production for the pre-injection period for the experimental females and control females showed no significant differences. However, as may be seen in Table 16, a significant difference was found during the post-injection period for the increased egg production of the females which received cortisone. Further analysis of these data revealed that the significant difference for increased egg production at the post-injection period was temporary. Over a three week period of time egg production declined gradually from the higher level to the normal egg production level as the effects of cortisone diminished for the females in Experiment III.

Using two week intervals, the experimental females (Experiment III) during the pre-injection, injection and post-injection periods laid the following average number

of eggs: 4.2, 1.5 and 4.4 respectively. The corresponding average egg production for the control females during the same time was 3.3, 3.9 and 3.3, respectively.

The increased dosage of cortisone (3 mg/lb.) caused egg production to cease completely within four days after injections were started for five of the females out of the six females comprising the experimental group. These five females were out of egg production for seventeen days.

From the observed results, it is suggested that cortisone administered in large doses has an inhibitory effect (directly or indirectly) on the secretion of leuteinizing hormone by the anterior pituitary.

## EGG WEIGHTS

An analysis of the mean egg weights in Experiments II and III for the pre-injection and post-injection periods by both analysis of variance and covariance showed that no significant differences existed. The mean egg weights for the injection period were not analyzed since egg numbers were greatly reduced for the experimental females in Experiment III. The slight variations for egg weights are shown in Table 19.

## FERTILITY

A significant decrease ($P = 0.05$, Table 21) in fertility was observed only during the injection period when the males received cortisone at the 1 mg/lb. level in Experiment I. This difference was not observed for the post-injection period. Also, a significant difference ($P = 0.05$) for reduced fertility was observed at the post-injection period when the females were receiving cortisone at the 1 mg/lb. level in Experiment II as may be seen in Table 22. The individual and average variations in fertility are given in Table 23.

It was observed that a small amount of cortisone reduced fertility but larger amounts did not significantly affect fertility.

## HATCHABILITY

No significant differences were found for hatchability in Experiment I, II and III for the pre-injection, injection and the post-injection periods (Tables 24, 25 and 26). The individual and average variations in hatchability are given in Table 27.

## CHICK WEIGHTS

No significant differences were found using analysis of variance and covariance for mean chick hatching weights and mean chick weights at two weeks of age in Experiment II or III for both the pre-injection and post-injection periods. The hatching weight averages for the control and experimental chicks as shown in Table 30, closely approximate. The variations for chick weights at two weeks of age are shown in Table 31. It was concluded that the growth of the experimental chicks was not significantly different from the growth of the control chicks.

## SUMMARY AND CONCLUSIONS

1. Subcutaneous injections of cortisone acetate exhibited a strong effect on the body weights of mature chickens. Males receiving cortisone at the 1 mg/lb. level demonstrated a highly significant body weight loss during the injection period. However, the male birds recovered most of their body weight lost three months after injections were discontinued but their original body weight was never attained. It is postulated that the highly significant loss in body weight resulted from alterations of carbohydrate and protein metabolism.

2. Females receiving 1 mg/lb. of cortisone acetate showed a non-significant body weight loss during the injection period and made slight average gains over and above their original body weights when injections were discontinued. Reduced egg production of these females was non-significant for the injection and post-injection period.

3. The body weight loss of the females receiving cortisone at the 3 mg/lb. level was not significant during the injection period but a highly significant body weight gain over and above the initial body weight was

present four weeks after the injections ceased. Reduced egg production of these females was highly significant during the injection period. It is suggested that this gain in body weight ever and above their initial body weight was due to the cessation of egg production and consequently an increased deposition of body fat.

4. The reduced egg production of the females receiving 1 mg/lb. of cortisone acetate was not significant for the injection and post-injection period. The increased dosage of 3 mg/lb. of cortisone acetate for the females resulted in a highly significant reduction of egg production during the injection period and a temporary highly significant difference for increased egg production was observed for the post-injection period. After a three week period of time, egg production declined gradually from the higher level to the normal egg production level for the experimental females as the effects of cortisone diminished. The increased dosage of cortisone acetate (3 mg/lb.) caused egg production to cease completely within four days after the start of injections for five females among the six females receiving cortisone. These five females were

out of egg production for seventeen days. From these data it is suggested that cortisone administered in large doses has an inhibitory effect (directly or indirectly) on the secretion of leuteinizing hormone by the anterior pituitary.

5. A significant decrease in fertility was observed only during the injection period when the males received cortisone at the 1 mg/lb. level. Also, a significant decrease in fertility was observed during the post-injection period when the females were receiving cortisone at the 1 mg/lb. level. It was observed that a small amount of cortisone temporarily reduced fertility but larger amounts of cortisone did not significantly affect fertility.

6. A highly significant retardation of primary feather growth was observed for the first week when the males were administered cortisone at the 1 mg/lb. level. Differences in rate of primary feather growth were not observed after the first week.

7. An analysis of the data on the rate of growth of the cushion feathers of the females receiving cortisone at the 3 mg/lb. level showed that cushion feather development was inhibited to a highly significant degree

for the first three weeks after injections started. A significant difference between the growth rate of the cushion feathers of the experimental and control females existed for the fourth week of feather development. Feather growth differences for the fifth and sixth week were not significant. It is suggested that tissues vary greatly in their susceptibility to the action of cortisone, the physiological mode of action being complicated. It may be possible that the effects observed are the product of a chain of events in which metabolism is shifted.

8. No significant differences were found for hatchability, egg weights, day-old or two week chick weights when the sires or dams were receiving cortisone injections at the various levels.

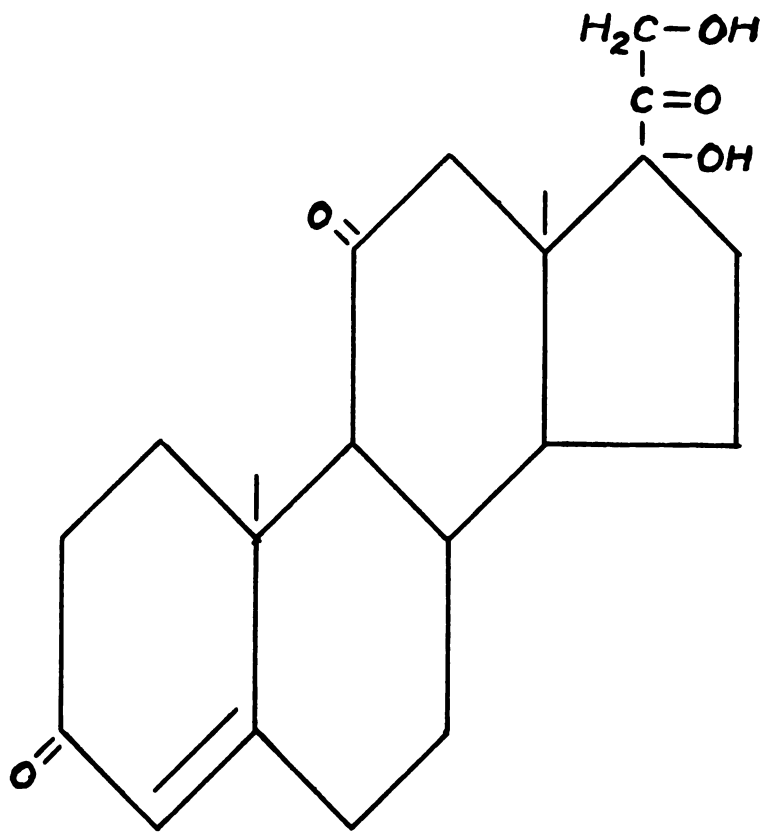
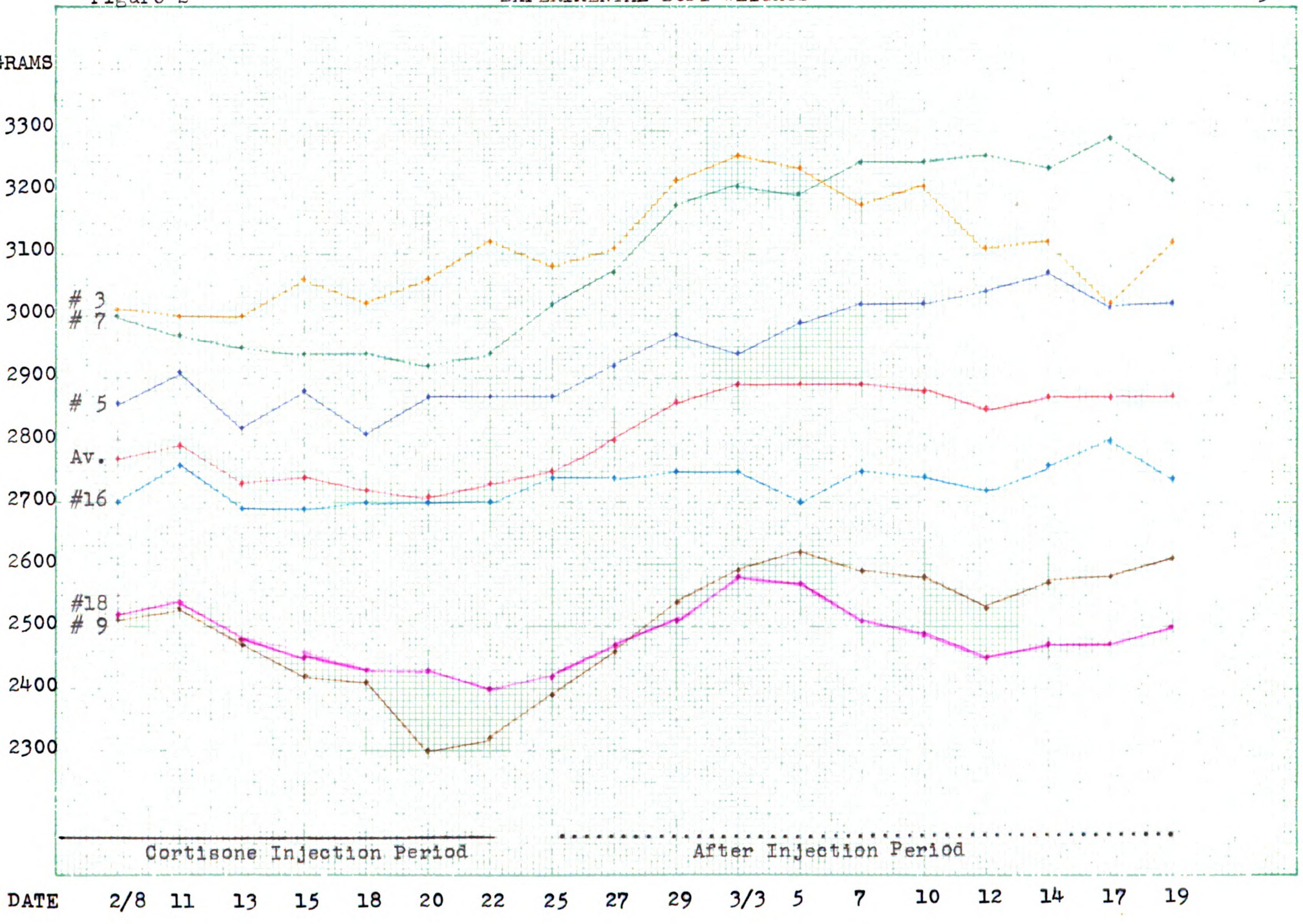



Figure 1. Structural formula of cortisone.

Figure 2 EXPERIMENTAL BODY WEIGHTS



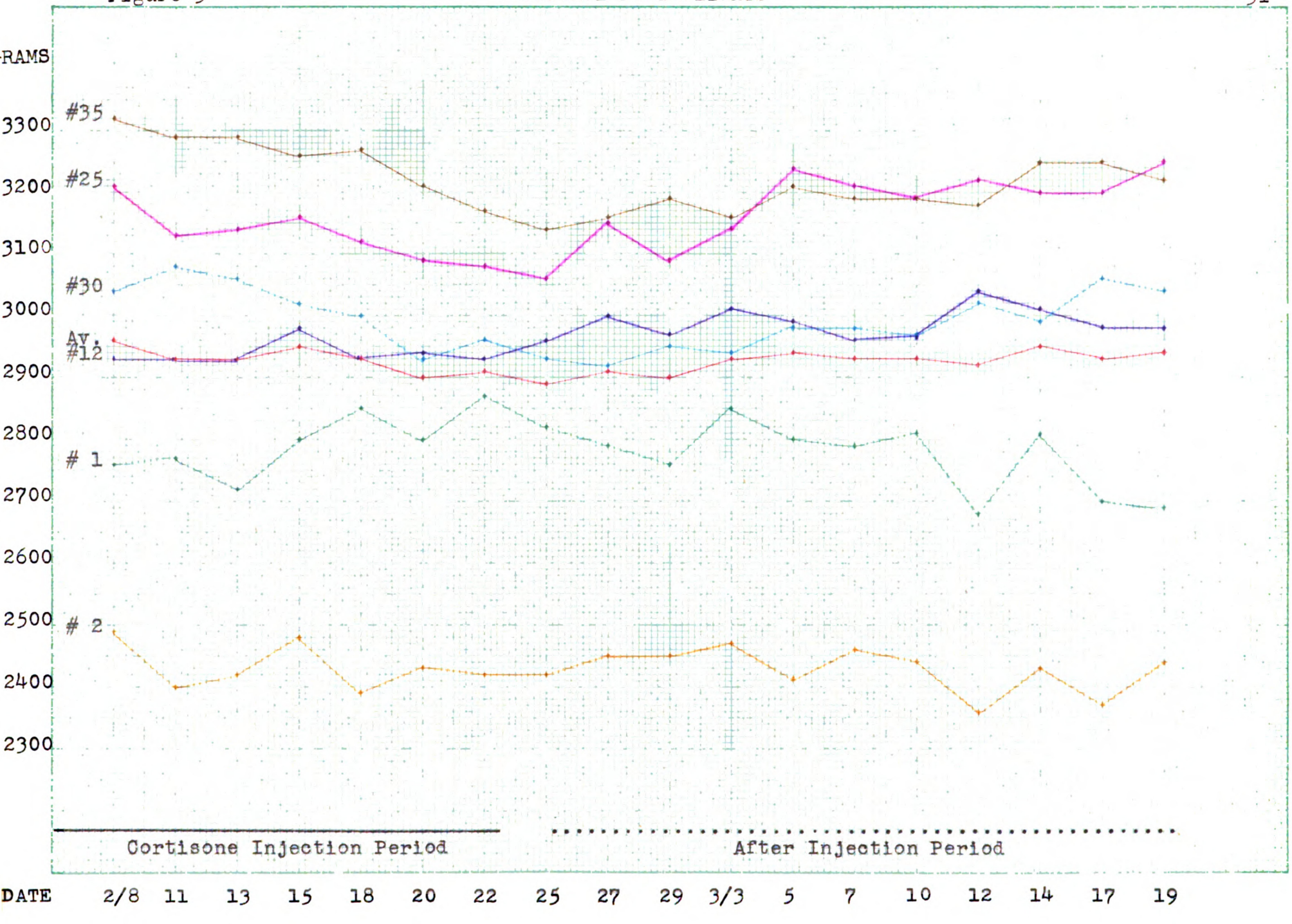

GRAMS
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
#35
#25
#30
Av. #12
# 1
# 2
Cortisone Injection Period
After Injection Period
DATE
2/8
11
13
15
18
20
22
25
27
29
3/3
5
7
10
12
14
17
19

Table 1. The ration that was fed to all birds in laying batteries during these tests.

| | Pounds |
|---|---|
| Ground yellow corn | 350 |
| Ground oats | 254 |
| Wheat bran | 300 |
| Wheat middlings | 300 |
| Alfalfa meal (17% dehydrated) | 150 |
| Soybean oil meal | 250 |
| Meat scraps (55%) | 100 |
| Fish meal | 100 |
| Dried milk | 60 |
| Ground oyster shell flour | 40 |
| Steamed bonemeal | 60 |
| Salt (iodized) | 20 |
| Fish oil (400D, 3000A) | 16 |
| Manganese sulfate 8 ozs. per ton | |
| | 2000# |

Table 2. The ration that was fed to all chicks during these tests.

| | Pounds |
|---|---|
| Ground yellow corn | 54.0 |
| Pulverized oats | 5.0 |
| Wheat Middlings | 5.0 |
| Dehydrated alfalfa leaf meal | 2.5 |
| Soybean oil meal | 25.0 |
| Meat scraps | 2.5 |
| Fish meal | 2.5 |
| Steamed bonemeal | 2.0 |
| Oyster shell flour or ground limestone | 0.5 |
| Salt (Iodized) | 0.5 |
| Fish oil (800D, 3000A) Nopco XXX | 0.15 |
| Lederle's Fortefeed (249-C) | 0.1 |
| *Lederle's Aurofac | 0.25 |
| Manganese sulfate 8 ozs. per ton | |
| | 100.0# |

*Vitamin B12 and antibiotic (aureomycin) feed supplement

Table 3. The results of an analysis of covariance of initial (x) and final (y) body weights (10 gram units) for experimental and control males and females during the injection period. Initial body weights observed three days prior to injections, final weights four days post-injection.

| Source of Variation | d/f | Sums of Squares and Products Sx 2 | Sxy | Sy2 | Errors of Estimate SS | d/f | MS |
|---|---|---|---|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | | | | | |
| Total | 5 | 2312 | 1706 | 2059 | | | |
| Between | 1 | 11 | -83 | 640 | 771 | 1 | 771** |
| Within | 4 | 2301 | 1789 | 1419 | 29 | 3 | 9.7 |
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| Total | 23 | 20912 | 20128 | 21108 | | | |
| Between | 1 | 1769 | 2094 | 2481 | 97 | 1 | 97 |
| Within | 22 | 19143 | 18034 | 18627 | 1638 | 21 | 78 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| Total | 11 | 8240 | 7740 | 8132 | | | |
| Between | 1 | 1102 | 785 | 561 | 68 | 1 | 68 |
| Within | 10 | 7138 | 6955 | 7571 | 794 | 9 | 88.2 |

* Significant
** Highly Significant

Table 4. The results of an analysis of covariance of initial (x) and final (y) body weights (10 gram units) for experimental and control males and females during and post-injection. Initial body weights observed three days prior to injections and final body weights 28 days post-injection.

| Source of Variation | d/f | Sums of Squares and Products $Sx^2$ | Sxy | $Sy^2$ | Errors of Estimate SS | d/f | MS |
|---|---|---|---|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | | | | | |
| Total | 5 | 2312 | 1318 | 1289 | | | |
| Between | 1 | 11 | -64 | 364 | 12 | 1 | 12 |
| Within | 4 | 2301 | 1372 | 905 | 87 | 3 | 29 |
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| Total | 23 | 20912 | 19433 | 22815 | | | |
| Between | 1 | 1769 | 1553 | 1365 | 2 | 1 | 2 |
| Within | 22 | 19143 | 17880 | 21450 | 4750 | 21 | 226 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| Total | 11 | 8240 | 8347 | 9536 | | | |
| Between | 1 | 1102 | 355 | 114 | 607 | 1 | 607** |
| Within | 10 | 7138 | 7992 | 9422 | 474 | 9 | 53 |

* Significant
** Highly Significant

Table 5. Body weights (10 gram units).

| Bird No. | Injection Period Pre | During | Post | Bird No. | Injection Period Pre | During | Post |
|---|---|---|---|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | | | | | |
| 37 | 416 | 388 | 398 | 42 | 406 | 405 | 403 |
| 45 | 380 | 361 | 366 | 43 | 428 | 420 | 414 |
| 40 | 432 | 406 | 399 | 44 | 386 | 392 | 394 |
| Total | 1228 | 1155 | 1163 | | 1220 | 1217 | 1211 |
| Av. | 409 | 385 | 388 | | 407 | 406 | 404 |
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| 3 | 270 | 264 | 290 | 1 | 255 | 256 | 270 |
| 5 | 280 | 266 | 281 | 2 | 245 | 232 | 239 |
| 7 | 319 | 308 | 306 | 6 | 286 | 291 | 293 |
| 8 | 255 | 269 | 292 | 10 | 286 | 277 | 285 |
| 9 | 233 | 232 | 235 | 11 | 294 | 296 | 291 |
| 14 | 240 | 230 | 237 | 12 | 286 | 278 | 276 |
| 15 | 279 | 268 | 292 | 13 | 212 | 216 | 224 |
| 16 | 233 | 229 | 249 | 25 | 286 | 288 | 296 |
| 17 | 230 | 222 | 222 | 28 | 253 | 250 | 247 |
| 18 | 250 | 229 | 243 | 30 | 300 | 282 | 295 |
| 29 | 284 | 280 | 285 | 35 | 332 | 318 | 315 |
| 33 | 230 | 207 | 193 | 36 | 274 | 264 | 275 |
| Total | 3103 | 3004 | 3125 | | 3309 | 3248 | 3306 |
| Av. | 259 | 250 | 260 | | 276 | 271 | 276 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| 3 | 301 | 308 | 312 | 1 | 276 | 283 | 269 |
| 5 | 286 | 287 | 303 | 2 | 249 | 242 | 244 |
| 7 | 300 | 302 | 322 | 12 | 293 | 296 | 298 |
| 9 | 251 | 239 | 261 | 25 | 321 | 306 | 325 |
| 16 | 270 | 274 | 274 | 30 | 304 | 293 | 304 |
| 18 | 252 | 242 | 250 | 35 | 332 | 314 | 322 |
| Total | 1660 | 1652 | 1722 | | 1775 | 1734 | 1762 |
| Av. | 277 | 275 | 287 | | 296 | 289 | 294 |

Table 6. The results of an analysis of variance of primary feather growth (mm.).

Experiment I. Males, 1 mg/lb.

| Source of Variation | d/f | Weeks 1 SS | MS | 2 SS | MS | 3 SS | MS | 4 SS | MS |
|---|---|---|---|---|---|---|---|---|---|
| Total | 5 | 3.6 | | 5.6 | | 8.3 | | 10.3 | |
| Between | 1 | 3.1 | 3.1** | 3.2 | 3.2 | 3.0 | 3.0 | 5.8 | 5.8 |
| Within | 4 | .5 | .1 | 2.4 | .6 | 5.3 | 1.3 | 4.5 | 1.1 |

* Significant
** Highly significant

Table 7. The results of an analysis of variance of primary feather growth (mm.).

Experiment III. Females, 3 mg/lb.

| Source of Variation | d/f | Weeks 1 SS | MS | 2 SS | MS | 3 SS | MS | 4 SS | MS |
|---|---|---|---|---|---|---|---|---|---|
| Total | 11 | 5.5 | | 10.5 | | 13 | | 12 | |
| Between | 1 | .7 | .7 | .7 | .7 | 2 | 2 | 1 | 1 |
| Within | 10 | 4.8 | .5 | 9.8 | .9 | 11 | 1.1 | 11 | 1.1 |

| Source of Variation | d/f | Weeks 5 SS | MS | 6 SS | MS | 7 SS | MS |
|---|---|---|---|---|---|---|---|
| Total | 11 | 17.4 | | 13 | | 7 | |
| Between | 1 | 4.8 | 4.8 | 3 | 3 | 1 | 1 |
| Within | 10 | 12.6 | 1.3 | 10 | 1 | 6 | .6 |

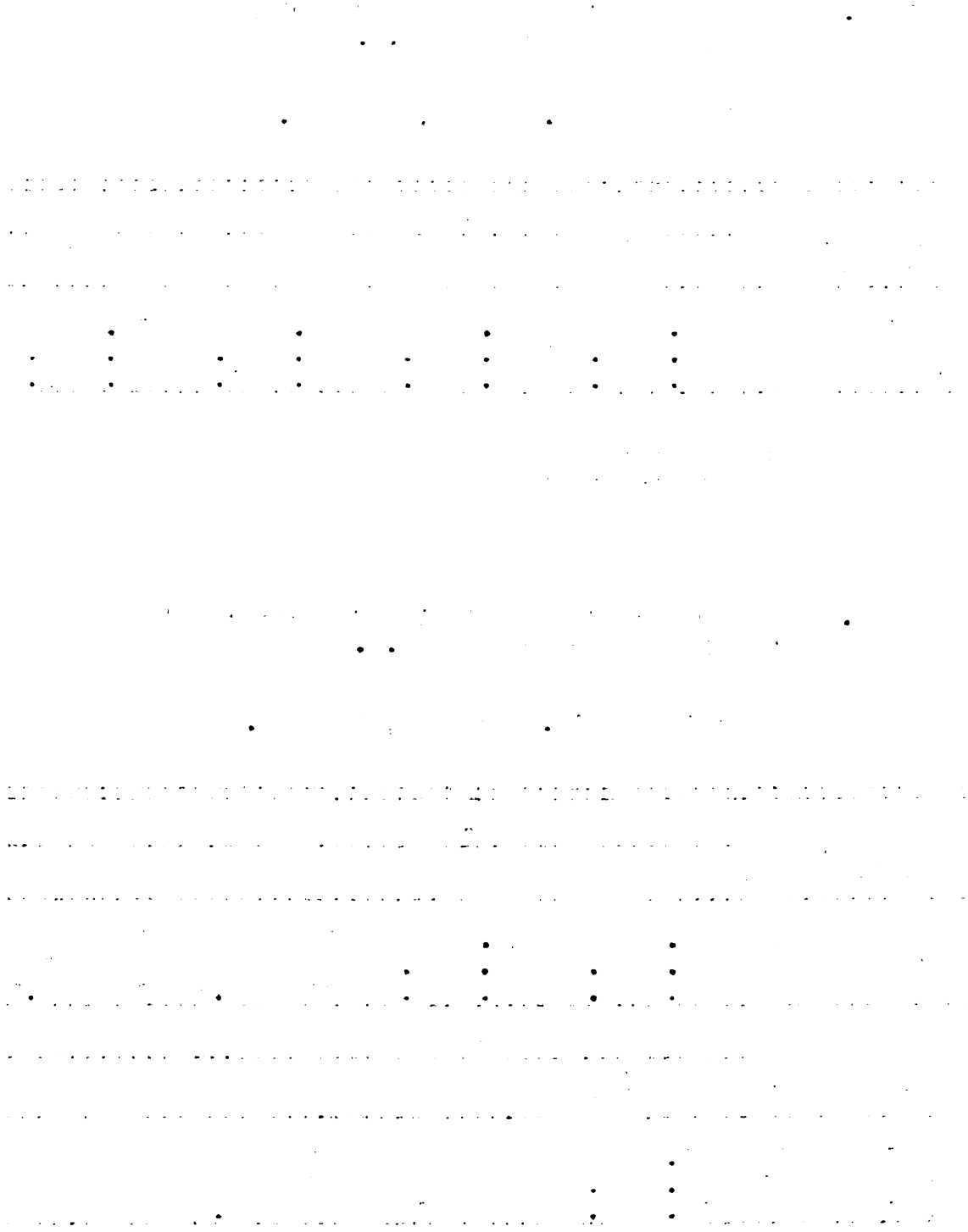


Table 8. The results of an analysis of variance of pectoral feather growth (mm.).

Experiment II. Females, 1 mg/lb.

| Source of Variation | d/f | Weeks 1 SS | MS | 2 SS | MS | 3 SS | MS | 4 SS | MS |
|---|---|---|---|---|---|---|---|---|---|
| Total | 18 | 2.83 | | 5 | | 3.1 | | 3.7 | |
| Between | 1 | .05 | .05 | .5 | .5 | .2 | .2 | .3 | .3 |
| Within | 17 | 1.78 | .1 | 4.5 | .3 | 2.9 | .2 | 3.4 | .2 |

Table 9. The results of an analysis of variance of cushion feather growth (mm.).

Experiment III. Females, 3 mg/lb.

| Source of Variation | d/f | Weeks 1 SS | MS | 2 SS | MS | 3 SS | MS |
|---|---|---|---|---|---|---|---|
| Total | 11 | 1 | | 1.5 | | 3.8 | |
| Between | 1 | .5 | .5** | .8 | .8** | 2.6 | 2.6** |
| Within | 10 | .5 | .05 | .7 | .07 | 1.2 | .12 |

| Source of Variation | d/f | Weeks 4 SS | MS | 5 SS | MS | 6 SS | MS |
|---|---|---|---|---|---|---|---|
| Total | 11 | 3 | | 6 | | 1.4 | |
| Between | 1 | 1.4 | 1.4* | 1 | 1 | .02 | .02 |
| Within | 10 | 1.6 | .16 | 5 | .5 | 1.38 | .14 |

* Significant
** Highly Significant

Table 10. Primary feather growth (mm.).

Experiment I. Males, 1 mg/lb.

| Experimental Bird Number | Weeks 3 | 4 | 5 | 6 |
|---|---|---|---|---|
| 37 | 3.3 | 7.6 | 10.8 | 13.0 |
| 45 | 3.1 | 6.8 | 9.6 | 11.5 |
| 40 | 2.4 | 5.5 | 7.8 | 10.8 |
| Total | 8.8 | 19.9 | 28.2 | 34.8 |
| Average | 2.9 | 6.6 | 9.4 | 11.6 |
| Control Bird Number | | | | |
| 42 | 4.5 | 7.8 | 10.1 | 13.1 |
| 43 | 4.4 | 8.2 | 11.0 | 13.3 |
| 44 | 4.2 | 8.2 | 11.3 | 14.3 |
| Total | 13.1 | 24.2 | 32.4 | 40.7 |
| Average | 4.4 | 8.1 | 10.8 | 13.6 |

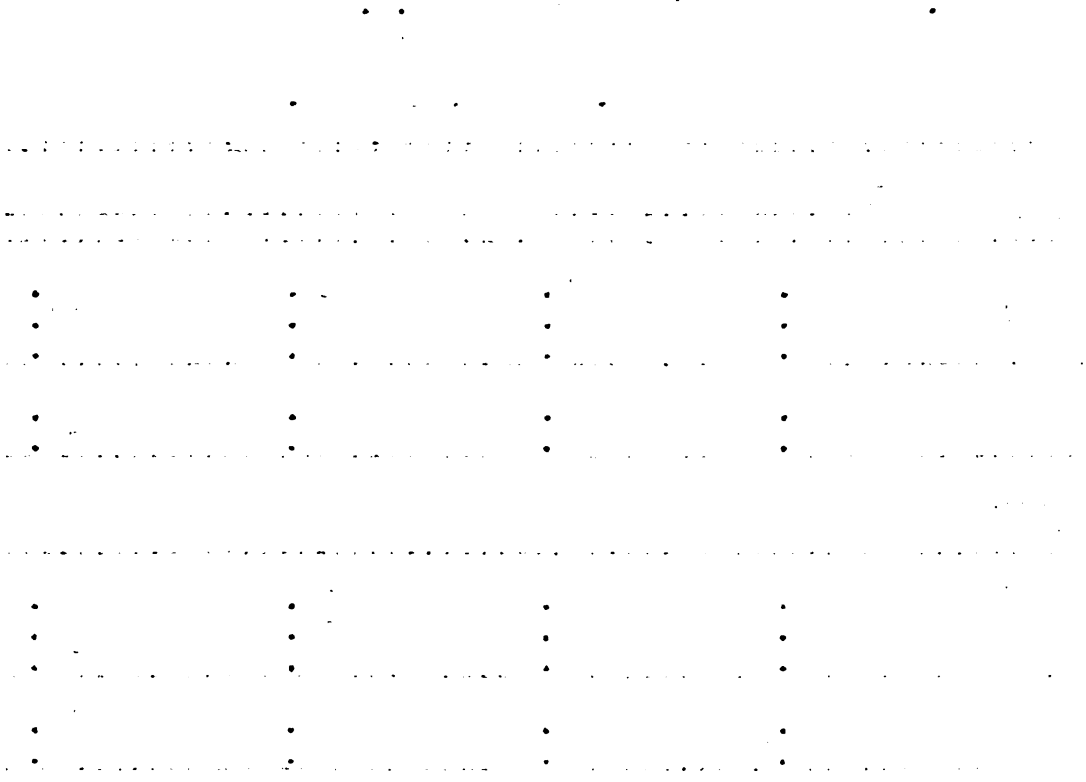


Table 11. Primary feather growth (mm.).

Experiment III. Females, 3 mg/lb.

| Experimental Bird Number | Weeks 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 3 | .3 | 4.4 | 8.6 | 10.8 | 12.8 | 14.1 | 14.9 |
| 5 | 2.6 | 5.6 | 9.4 | 11.3 | 11.3 | 12.4 | 15.0 |
| 7 | .0 | 2.3 | 5.8 | 7.9 | 9.8 | 11.8 | 12.8 |
| 9 | 1.2 | 5.4 | 9.2 | 11.6 | 14.0 | 15.0 | 14.9 |
| 16 | .4 | 4.5 | 7.6 | 10.0 | 12.4 | 13.8 | 14.2 |
| 18 | .9 | 4.0 | 7.5 | 9.7 | 12.1 | 12.2 | 13.2 |
| Total | 5.4 | 26.2 | 48.1 | 61.3 | 72.4 | 79.3 | 85.0 |
| Average | .9 | 4.4 | 8.0 | 10.2 | 12.1 | 13.2 | 14.2 |
| Control Bird Number | | | | | | | |
| 1 | 1.3 | 4.8 | 8.6 | 10.3 | 12.8 | 13.6 | 14.1 |
| 2 | 1.6 | 5.9 | 10.0 | 12.0 | 14.6 | 14.6 | 15.1 |
| 12 | 1.3 | 3.7 | 8.0 | 10.3 | 12.6 | 13.6 | 15.1 |
| 25 | 1.8 | 5.3 | 8.7 | 11.0 | 13.4 | 13.9 | 14.6 |
| 30 | 1.1 | 4.6 | 8.5 | 11.0 | 13.5 | 14.4 | 15.1 |
| 35 | 1.2 | 4.5 | 8.5 | 10.7 | 13.1 | 15.1 | 15.2 |
| Total | 8.3 | 28.8 | 52.3 | 65.3 | 80.0 | 85.2 | 89.2 |
| Average | 1.4 | 4.8 | 8.7 | 10.9 | 13.4 | 14.2 | 14.9 |

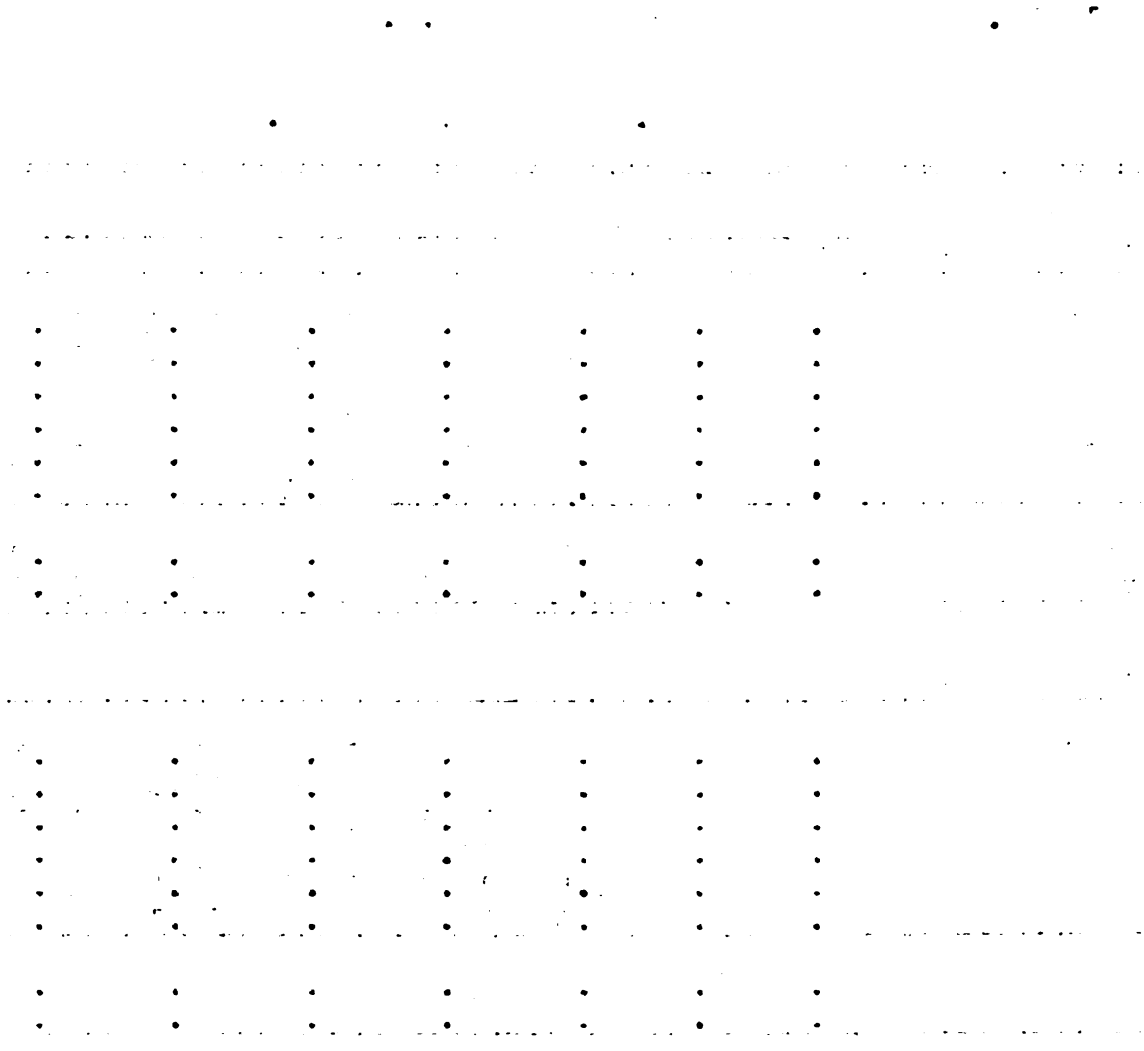


Table 12. Pectoral feather growth (mm.).

Experiment II. Females, 1 mg/lb.

| Experimental Bird Number | Weeks 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 3 | 2.8 | 3.6 | 4.3 | 4.7 |
| 5 | 3.3 | 4.2 | 4.3 | 5.4 |
| 7 | 3.1 | 4.1 | 4.8 | 5.2 |
| 8 | 3.7 | 4.0 | 5.2 | 5.3 |
| 9 | 2.7 | 4.0 | 5.2 | 5.3 |
| 14 | 3.2 | 3.2 | 4.7 | 5.0 |
| 15 | 3.2 | 4.1 | 4.8 | 5.3 |
| 17 | 3.1 | 4.1 | 4.8 | 5.2 |
| 18 | 2.8 | 3.0 | 4.2 | 4.6 |
| 29 | 3.2 | 3.9 | 4.2 | 4.9 |
| 33 | 3.2 | 3.9 | 4.7 | 5.2 |
| Total | 34.3 | 42.1 | 51.2 | 56.1 |
| Average | 3.1 | 3.8 | 4.7 | 5.1 |
| Control Bird Number | | | | |
| 1 | 3.3 | 4.2 | 4.9 | 4.9 |
| 2 | 3.3 | 4.2 | 4.9 | 5.3 |
| 6 | 2.9 | 3.8 | 4.8 | 5.3 |
| 10 | 2.1 | 2.7 | 3.8 | 4.4 |
| 12 | 3.6 | 4.2 | 5.2 | 5.3 |
| 25 | 3.7 | 4.6 | 5.0 | 5.2 |
| 28 | 3.4 | 4.3 | 5.3 | 5.4 |
| 30 | 3.7 | 4.3 | 5.2 | 5.4 |
| Total | 26.0 | 32.3 | 39.1 | 41.2 |
| Average | 3.2 | 4.1 | 4.9 | 5.2 |

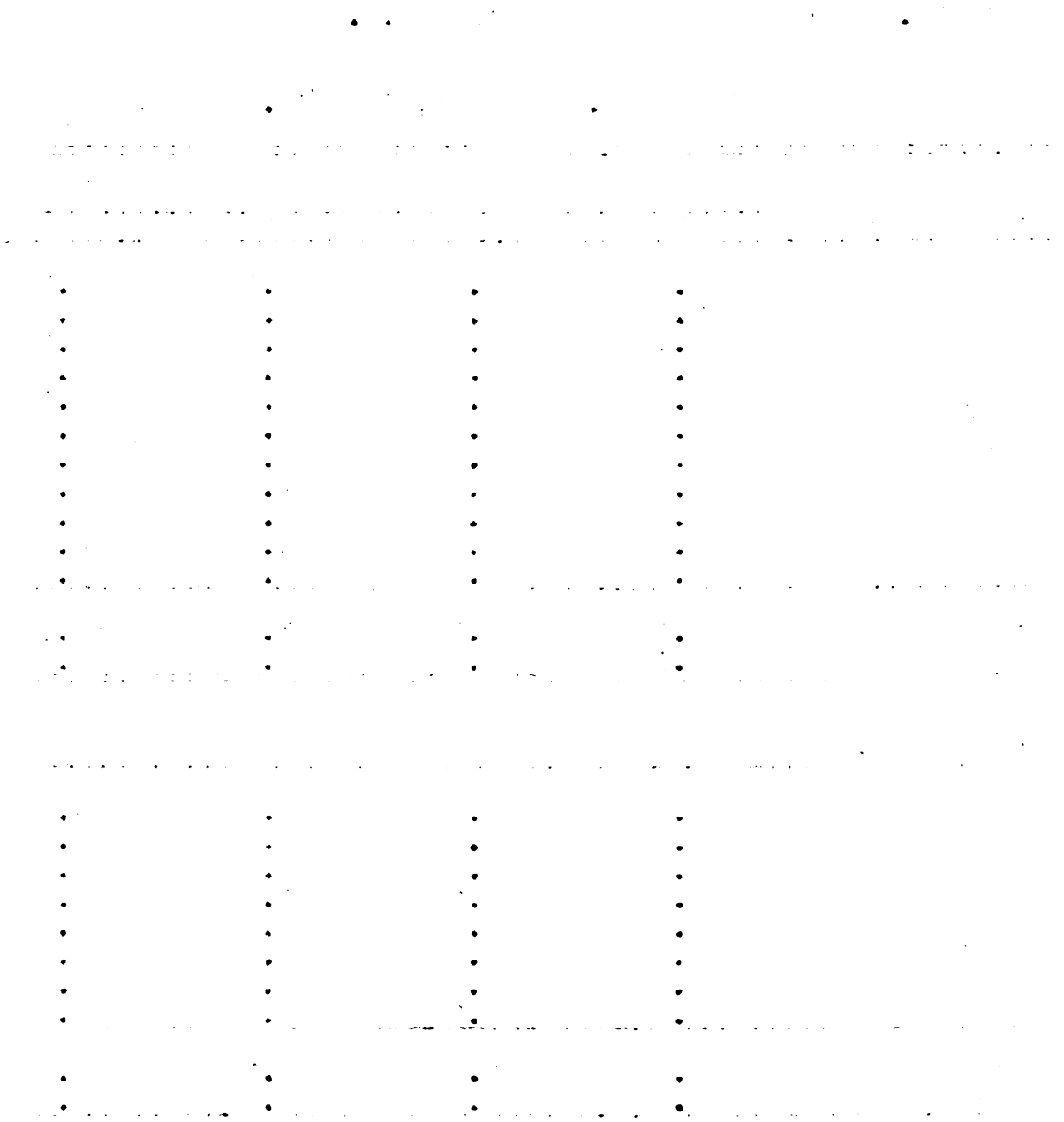


Table 13. Cushion feather growth (mm.).

Experiment III. Females, 1 mg/lb.

| Experimental Bird Number | Weeks 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 3 | .9 | 2.7 | 3.7 | 5.7 | 7.8 | 8.2 |
| 5 | .9 | 2.9 | 3.7 | 5.2 | 6.1 | 8.6 |
| 7 | 1.1 | 2.9 | 4.4 | 5.9 | 6.8 | 7.9 |
| 9 | .4 | 2.6 | 4.2 | 5.7 | 6.2 | 7.8 |
| 16 | .9 | 2.6 | 4.0 | 5.3 | 6.6 | 7.6 |
| 18 | 1.3 | 3.1 | 4.3 | 6.3 | 7.6 | 8.2 |
| Total | 5.5 | 16.8 | 24.3 | 34.1 | 41.1 | 48.3 |
| Average | .9 | 2.8 | 4.1 | 5.7 | 6.8 | 8.0 |
| Control Bird Number | | | | | | |
| 1 | 1.3 | 3.0 | 4.6 | 6.0 | 7.0 | 7.6 |
| 2 | 1.4 | 3.9 | 5.6 | 7.0 | 8.0 | 8.1 |
| 12 | 1.2 | 3.2 | 5.0 | 6.0 | 6.5 | 7.6 |
| 25 | 1.3 | 3.4 | 4.8 | 6.2 | 7.1 | 8.0 |
| 30 | 1.3 | 3.1 | 5.2 | 6.7 | 8.4 | 8.6 |
| 35 | 1.3 | 3.2 | 4.7 | 6.3 | 7.6 | 8.2 |
| Total | 7.8 | 19.8 | 29.9 | 38.2 | 44.6 | 48.1 |
| Average | 1.3 | 3.3 | 5.0 | 6.4 | 7.4 | 8.0 |

Table 14. The results of an analysis of variance of egg production during the injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 21 | 77 | |
| Between | 1 | .4 | .4 |
| Within | 20 | 76.6 | 3.8 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | | | |
| Between | | | self-evident ** |
| Within | | | |

* Significant
** Highly Significant

Table 15. The results of an analysis of variance of egg production for the pre-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 21 | 74 | |
| Between | 1 | .5 | .5 |
| Within | 20 | 73.5 | 3.7 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 11 | 113 | |
| Between | 1 | 24 | 24 |
| Within | 10 | 89 | 8.9 |

Table 16. The results of an analysis of variance of egg production for the post-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 5 | 1.16 | |
| Between Treatments | 1 | .06 | .06 |
| Between Weeks | 2 | 1.08 | .54* |
| Interaction | 2 | .02 | .01 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 23 | 39.3 | |
| Between Treatments | 1 | 8.0 | 8.0 * |
| Between Weeks | 1 | 8.0 | 8.0 * |
| Interaction | 1 | .3 | .3 |
| Error | 20 | 23.0 | 1.2 |

* Significant
** Highly Significant

Table 17. The egg production for the two week period prior to injections, the two week period during injections and the two week period after injections.

| Experimental | | | | Control | | | |
|---|---|---|---|---|---|---|---|
| Bird No. | Injection Period | | | Bird No. | Injection Period | | |
| | Pre | During | Post | | Pre | During | Post |
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| 3 | 10 | 7 | 9 | 1 | 4 | 9 | 7 |
| 5 | 7 | 5 | 9 | 2 | 5 | 4 | 8 |
| 7 | 9 | 4 | 6 | 6 | 6 | 2 | 2 |
| 8 | 0 | 0 | 0 | 10 | 1 | 1 | 7 |
| 9 | 6 | 8 | 8 | 11 | 7 | 8 | 5 |
| 14 | 7 | 4 | 6 | 12 | 4 | 7 | 6 |
| 15 | 9 | 3 | 7 | 13 | 6 | 6 | 8 |
| 16 | 9 | 8 | 9 | 25 | 11 | 10 | 8 |
| 17 | 7 | 6 | 7 | 28 | 4 | 7 | 7 |
| 18 | 9 | 8 | 7 | 30 | 8 | 7 | 7 |
| 29 | 7 | 5 | 2 | 35 | 8 | 8 | 7 |
| 33 | 0 | 0 | 0 | 36 | 6 | 7 | 6 |
| Total | 80 | 58 | 70 | | 70 | 76 | 78 |
| Av. | 6.8 | 4.8 | 5.8 | | 5.8 | 6.3 | 6.5 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| 3 | 10 | 2 | 10 | 1 | 8 | 8 | 8 |
| 5 | 10 | 2 | 9 | 2 | 9 | 7 | 6 |
| 7 | 5 | 1 | 7 | 12 | 7 | 7 | 7 |
| 9 | 9 | 2 | 10 | 25 | 6 | 7 | 9 |
| 16 | 8 | 9 | 8 | 30 | 2 | 10 | 3 |
| 18 | 8 | 2 | 9 | 35 | 8 | 8 | 6 |
| Total | 50 | 18 | 53 | | 40 | 47 | 39 |
| Av. | 4.2 | 1.5 | 4.4 | | 3.3 | 3.9 | 3.3 |

Table 18. The results of an analysis of covariance of mean egg weights for (x) pre-injection and (y) post-injection.

| Source of Variation | d/f | Sums of Squares & Products $Sx^2$ | $Sxy$ | $Sy^2$ | Errors of Estimate SS | d/f | MS |
|---|---|---|---|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| Total | 20 | 243 | 148 | 164 | 74 | | |
| Between | 1 | 3 | 5 | 8 | 3 | 1 | 3 |
| Within | 19 | 240 | 143 | 156 | 71 | 18 | 4 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| Total | 11 | 138 | 157 | 188 | 10 | | |
| Between | 1 | 10 | 9 | 6 | -1 | 1 | -1 |
| Within | 10 | 128 | 148 | 182 | 11 | 9 | 1 |

Table 19. The mean egg weights for the two week period prior to injections and the two week period after injections.

| Experimental | | | Control | | |
|---|---|---|---|---|---|
| Bird No. | Injection Period Pre | Post | Bird No. | Injection Period Pre | Post |
| **Experiment II. Females, 1 mg/lb.** | | | | | |
| 3 | 51 | 52 | 1 | 57 | 62 |
| 5 | 55 | 54 | 2 | 60 | 60 |
| 7 | 63 | 59 | 6 | 62 | 61 |
| 9 | 56 | 59 | 10 | 65 | 59 |
| 14 | 65 | 64 | 11 | 59 | 57 |
| 16 | 60 | 58 | 12 | 61 | 63 |
| 17 | 57 | 58 | 13 | 56 | 57 |
| 18 | 61 | 60 | 25 | 55 | 55 |
| 29 | 59 | 58 | 28 | 59 | 61 |
| | | | 30 | 56 | 57 |
| | | | 35 | 61 | 60 |
| | | | 36 | 60 | 59 |
| Total | 527 | 522 | | 711 | 711 |
| Av. | 58.6 | 58.0 | | 59.2 | 59.2 |
| **Experiment III. Females, 3 mg/lb.** | | | | | |
| 3 | 56 | 55 | 1 | 66 | 65 |
| 5 | 58 | 57 | 2 | 64 | 63 |
| 7 | 66 | 67 | 12 | 67 | 67 |
| 9 | 61 | 61 | 25 | 58 | 56 |
| 16 | 62 | 64 | 30 | 59 | 60 |
| 18 | 61 | 61 | 35 | 61 | 61 |
| Total | 364 | 365 | | 375 | 372 |
| Av. | 60.7 | 60.7 | | 62.5 | 62.0 |

Table 20. The results of an analysis of variance of fertility for the pre-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I and II. Males and females, 1 mg/lb.** | | | |
| Total | 18 | 19088 | |
| Between | 1 | 1294 | 1294 |
| Within | 17 | 17794 | 1047 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 11 | 21513 | |
| Between | 1 | 374 | 374 |
| Within | 10 | 20139 | 2014 |

Table 21. The results of an analysis of variance of fertility during the injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | |
| Total | 21 | 29291 | |
| Between | 1 | 7287 | 7287* |
| Within | 20 | 22004 | 1100 |
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 21 | 19721 | |
| Between | 1 | 541 | 541 |
| Within | 20 | 19180 | 959 |

* Significant
** Highly Significant

Table 22. The results of an analysis of variance of fertility for the post-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | |
| Total | 21 | 19721 | |
| Between | 1 | 541 | 541 |
| Within | 20 | 19180 | 959 |
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 5 | 1727 | |
| Between Treatments | 1 | 1145 | 1145* |
| Between Weeks | 2 | 460 | 230 |
| Interaction | 2 | 122 | 61 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 11 | 9973 | |
| Between | 1 | 2002 | 2002 |
| Within | 10 | 7971 | 797 |

* Significant
** Highly Significant

Table 23. The fertility of the females in percent (%) with the number of eggs set (x) for the pre-injection, injection and post-injection period in two week intervals.

**Experiment I. Males, 1 mg/lb.**

| Experimental | | | | | | | Control | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Injection Period | | | | | | | Injection Period | | | | | |
| Bird | Pre | | During | | Post | | Bird | Pre | | During | | Post | |
| No. | % | x | % | x | % | x | No. | % | x | % | x | % | x |
| 3 | 100 | 9 | 0 | 12 | 71 | 7 | 1 | | | 100 | 4 | 100 | 9 |
| 5 | 50 | 6 | 89 | 9 | 80 | 5 | 2 | | | 83 | 6 | 100 | 4 |
| 7 | 71 | 7 | 54 | 11 | 50 | 4 | 6 | 100 | 6 | 100 | 7 | 100 | 2 |
| 8 | | | | | | | 10 | 0 | 2 | 100 | 1 | 0 | 1 |
| 9 | 78 | 9 | 0 | 8 | 38 | 8 | 11 | 33 | 3 | 44 | 9 | 62 | 8 |
| 14 | 75 | 4 | 100 | 7 | 75 | 4 | 12 | | | 100 | 5 | 86 | 7 |
| 15 | 100 | 2 | 0 | 10 | 0 | 2 | 13 | 100 | 2 | 100 | 8 | 83 | 6 |
| 16 | 86 | 7 | 0 | 10 | 100 | 8 | 25 | 100 | 3 | 100 | 3 | 100 | 10 |
| 17 | 100 | 1 | 70 | 10 | 100 | 6 | 28 | 0 | 1 | 80 | 5 | 86 | 7 |
| 18 | 86 | 7 | 91 | 11 | 88 | 8 | 30 | 67 | 3 | 100 | 9 | 100 | 7 |
| 29 | 86 | 7 | 78 | 9 | 80 | 5 | 35 | 100 | 7 | 60 | 10 | 38 | 8 |
| 33 | | | | | | | 36 | 100 | 2 | 50 | 8 | 83 | 6 |
| Total | 832 | | 482 | | 682 | | | 600 | | 1017 | | 938 | |
| Av. | 83.2 | | 48.2 | | 68.2 | | | 66.7 | | 84.8 | | 79.6 | |

**Experiment II. Females, 1 mg/lb.**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 100 | 9 | 71 | 7 | 15 | 13 | 1 | | | 100 | 9 | 100 | 12 |
| 5 | 50 | 6 | 80 | 5 | 71 | 7 | 2 | | | 100 | 4 | 67 | 12 |
| 7 | 71 | 7 | 50 | 4 | 42 | 12 | 6 | 100 | 6 | 100 | 2 | 89 | 9 |
| 8 | | | | | | | 10 | 0 | 2 | 0 | 1 | 40 | 5 |
| 9 | 78 | 9 | 38 | 8 | 21 | 14 | 11 | 33 | 3 | 62 | 8 | 57 | 7 |
| 14 | 75 | 4 | 75 | 4 | 100 | 8 | 12 | | | 86 | 7 | 80 | 10 |
| 15 | 100 | 2 | 0 | 2 | 0 | 2 | 13 | 100 | 2 | 83 | 6 | 100 | 10 |
| 16 | 86 | 7 | 100 | 8 | 38 | 13 | 25 | 100 | 3 | 100 | 10 | 100 | 15 |
| 17 | 100 | 1 | 100 | 6 | 90 | 10 | 28 | 0 | 1 | 86 | 7 | 100 | 12 |
| 18 | 86 | 7 | 88 | 8 | 83 | 12 | 30 | 67 | 3 | 100 | 7 | 100 | 13 |
| 29 | 86 | 7 | 80 | 5 | 80 | 5 | 35 | 100 | 7 | 38 | 8 | 50 | 12 |
| 33 | | | | | | | 36 | 100 | 2 | 83 | 6 | 44 | 9 |
| Total | 832 | | 682 | | 540 | | | 600 | | 938 | | 927 | |
| Av. | 83.2 | | 68.2 | | 54.0 | | | 66.7 | | 79.6 | | 77.2 | |

**Experiment III. Females, 3 mg/lb.**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 0 | 10 | | | 90 | 10 | 1 | 100 | 8 | | | 100 | 8 |
| 5 | 100 | 10 | | | 89 | 9 | 2 | 45 | 9 | | | 100 | 6 |
| 7 | 60 | 5 | | | 50 | 6 | 12 | 0 | 7 | | | 100 | 7 |
| 9 | 0 | 9 | | | 80 | 10 | 25 | 83 | 6 | | | 89 | 9 |
| 16 | 13 | 8 | | | 75 | 8 | 30 | 100 | 2 | | | 100 | 3 |
| 18 | 100 | 8 | | | 0 | 9 | 35 | 12 | 8 | | | 50 | 6 |
| Total | 273 | | | | 384 | | | 340 | | | | 539 | |
| Av. | 45.5 | | | | 64.0 | | | 56.7 | | | | 89.8 | |

Table 24. The results of an analysis of variance of hatchability for the pre-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I and II. Males and females, 1 mg/lb.** | | | |
| Total | 18 | 27880 | |
| Between | 1 | 244 | 244 |
| Within | 17 | 27636 | 1626 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 11 | 22292 | |
| Between | 1 | 209 | 209 |
| Within | 10 | 22083 | 2208 |

Table 25. The results of an analysis of variance of hatchability during the injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | |
| Total | 21 | 31828 | |
| Between | 1 | 4793 | 4793 |
| Within | 20 | 27035 | 1352 |
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 21 | 19305 | |
| Between | 1 | 110 | 110 |
| Within | 20 | 19195 | 960 |

Table 26. The results of an analysis of variance of hatchability for the post-injection period.

| Source of Variation | d/f | SS | MS |
|---|---|---|---|
| **Experiment I. Males, 1 mg/lb.** | | | |
| Total | 21 | 19305 | |
| Between | 1 | 110 | 110 |
| Within | 20 | 19195 | 960 |
| **Experiment II. Females, 1 mg/lb.** | | | |
| Total | 21 | 9890 | |
| Between | 1 | 198 | 198 |
| Within | 20 | 9692 | 485 |
| **Experiment III. Females, 3 mg/lb.** | | | |
| Total | 11 | 9177 | |
| Between | 1 | 833 | 833 |
| Within | 10 | 8344 | 834 |

Table 27. The hatchability of the females in percent (%) with the number of fertile eggs set (x) for the pre-injection, injection and post-injection period in two week intervals.

| Experimental | | | | | | | Control | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Injection Period | | | | | | | Injection Period | | | | | |
| Bird | Pre | | During | | Post | | Bird | Pre | | During | | Post | |
| No. | % | x | % | x | % | x | No. | % | x | % | x | % | x |
| **Experiment I. Males, 1 mg/lb.** | | | | | | | | | | | | | |
| 3 | | | | | 80 | 5 | 1 | | | 100 | 4 | 100 | 9 |
| 5 | 67 | 3 | 100 | 8 | 100 | 4 | 2 | | | 100 | 5 | 100 | 4 |
| 7 | 100 | 5 | 100 | 6 | 100 | 2 | 6 | 83 | 6 | 100 | 7 | 50 | 2 |
| 8 | | | | | | | 10 | | | 100 | 1 | | |
| 9 | 71 | 7 | | | 100 | 3 | 11 | | | 75 | 4 | 80 | 5 |
| 14 | 33 | 3 | 100 | 7 | 100 | 3 | 12 | | | 100 | 5 | 100 | 6 |
| 15 | 100 | 2 | | | | | 13 | 100 | 2 | 100 | 8 | 100 | 5 |
| 16 | 83 | 6 | | | 100 | 8 | 25 | 100 | 3 | 93 | 13 | 100 | 10 |
| 17 | 100 | 1 | 71 | 7 | 100 | 6 | 28 | | | 100 | 4 | 83 | 6 |
| 18 | 67 | 6 | 100 | 10 | 100 | 7 | 30 | 100 | 2 | 100 | 9 | 100 | 7 |
| 29 | 83 | 6 | 100 | 7 | 100 | 4 | 35 | 86 | 7 | 100 | 6 | 100 | 3 |
| 33 | | | | | | | 36 | 100 | 2 | 100 | 4 | 100 | 5 |
| Total | 704 | | 571 | | 880 | | | 569 | | 1167 | | 1013 | |
| Av. | 78.2 | | 95.2 | | 97.8 | | | 94.9 | | 97.3 | | 92.1 | |
| **Experiment II. Females, 1 mg/lb.** | | | | | | | | | | | | | |
| 3 | | | 80 | 5 | 100 | 2 | 1 | | | 100 | 9 | 100 | 12 |
| 5 | 67 | 3 | 100 | 4 | 100 | 5 | 2 | | | 100 | 4 | 100 | 8 |
| 7 | 100 | 5 | 100 | 2 | 100 | 5 | 6 | 83 | 6 | 50 | 2 | 88 | 8 |
| 8 | | | | | | | 10 | | | | | 100 | 2 |
| 9 | 71 | 7 | 100 | 3 | 100 | 3 | 11 | | | 80 | 5 | 75 | 4 |
| 14 | 33 | 3 | 100 | 3 | 88 | 8 | 12 | | | 100 | 6 | 100 | 8 |
| 15 | 100 | 2 | | | | | 13 | 100 | 2 | 100 | 5 | 100 | 10 |
| 16 | 83 | 6 | 100 | 8 | 100 | 5 | 25 | 100 | 3 | 100 | 10 | 100 | 15 |
| 17 | 100 | 1 | 100 | 6 | 100 | 9 | 28 | | | 83 | 6 | 92 | 12 |
| 18 | 67 | 6 | 100 | 7 | 100 | 10 | 30 | 100 | 2 | 100 | 7 | 100 | 13 |
| 29 | 83 | 6 | 100 | 4 | 100 | 4 | 35 | 86 | 7 | 100 | 3 | 83 | 6 |
| 33 | | | | | | | 36 | 100 | 2 | 100 | 5 | 100 | 4 |
| Total | 704 | | 880 | | 888 | | | 569 | | 1013 | | 1138 | |
| Av. | 78.2 | | 97.8 | | 98.7 | | | 94.9 | | 92.1 | | 94.8 | |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | | | | | | | |
| 3 | | | | | 100 | 9 | 1 | 100 | 8 | | | 100 | 8 |
| 5 | 100 | 10 | | | 100 | 8 | 2 | 50 | 2 | | | 100 | 6 |
| 7 | 100 | 3 | | | 100 | 3 | 12 | | | | | 100 | 7 |
| 9 | | | | | 100 | 8 | 25 | 100 | 5 | | | 100 | 8 |
| 16 | 100 | 1 | | | 100 | 6 | 30 | 100 | 2 | | | 100 | 3 |
| 18 | 100 | 8 | | | | | 35 | 100 | 1 | | | 100 | 3 |
| Total | 400 | | | | 500 | | | 450 | | | | 600 | |
| Av. | 100 | | | | 100 | | | 90 | | | | 100 | |

Table 28. The results of an analysis of covariance of mean chick weights at hatching time.

| Source of Variation | d/f | Sums of Squares & Products $Sx^2$ | Sxy | $Sy^2$ | Errors of Estimate SS | d/f | MS |
|---|---|---|---|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| Total | 16 | 74 | 57 | 93 | 49 | | |
| Between | 1 | 4 | -3 | 2 | 9 | 1 | 9 |
| Within | 15 | 70 | 60 | 91 | 40 | 14 | 3 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| Total | 6 | 27 | 36 | 62 | 12 | | |
| Between | 1 | 1 | 0 | 4 | 4 | 1 | 4 |
| Within | 5 | 26 | 36 | 58 | 8 | 4 | 2 |

Table 29. The results of an analysis of covariance of the mean two week chick weights.

| Source of Variation | d/f | Sums of Squares & Products $Sx^2$ | Sxy | $Sy^2$ | Errors of Estimate SS | d/f | MS |
|---|---|---|---|---|---|---|---|
| **Experiment II. Females, 1 mg/lb.** | | | | | | | |
| Total | 16 | 1563 | 1308 | 2862 | 1767 | | |
| Between | 1 | .2 | 7 | 333 | 38 | 1 | 38 |
| Within | 15 | 1562.8 | 1301 | 2529 | 1729 | 14 | 123 |
| **Experiment III. Females, 3 mg/lb.** | | | | | | | |
| Total | 6 | 769 | 497 | 406 | 77 | | |
| Between | 1 | 241 | -50 | 10 | -.4 | 1 | -.4 |
| Within | 5 | 528 | 547 | 396 | 77.4 | 4 | 19.3 |

Table 30. Means of chick weight (grams) at hatching time.

| Experimental | | | Control | | |
|---|---|---|---|---|---|
| Bird No. | Injection Period Pre | Post | Bird No. | Injection Period Pre | Post |
| **Experiment II. Females, 1 mg/lb.** | | | | | |
| 5 | 37 | 34 | 1 | 38 | 40 |
| 7 | 45 | 40 | 2 | 41 | 40 |
| 14 | 43 | 44 | 6 | 42 | 42 |
| 17 | 41 | 42 | 11 | 41 | 40 |
| 18 | 41 | 40 | 12 | 42 | 43 |
| 29 | 41 | 38 | 13 | 37 | 38 |
| | | | 25 | 38 | 37 |
| | | | 28 | 41 | 42 |
| | | | 30 | 40 | 39 |
| | | | 35 | 42 | 41 |
| | | | 36 | 42 | 41 |
| Total | 248 | 238 | | 444 | 443 |
| Av. | 41.3 | 39.7 | | 40.4 | 40.3 |
| **Experiment III. Females, 3 mg/lb.** | | | | | |
| 5 | 40 | 39 | 1 | 43 | 42 |
| 7 | 45 | 49 | 2 | 43 | 44 |
| 18 | 41 | 42 | 25 | 40 | 39 |
| | | | 30 | 42 | 43 |
| Total | 126 | 130 | | 168 | 168 |
| Av. | 42 | 43.3 | | 42 | 42 |

Table 31. Chick weight means (grams) at two weeks of age.

| Experimental | | | Control | | |
|---|---|---|---|---|---|
| Bird No. | Injection Period Pre | Post | Bird No. | Injection Period Pre | Post |
| **Experiment II. Females, 1 mg/lb.** | | | | | |
| 5 | 103 | 70 | 1 | 118 | 104 |
| 7 | 126 | 104 | 2 | 123 | 100 |
| 14 | 125 | 107 | 6 | 119 | 112 |
| 17 | 107 | 107 | 11 | 121 | 119 |
| 18 | 136 | 118 | 12 | 123 | 116 |
| 29 | 116 | 89 | 13 | 113 | 112 |
| | | | 25 | 112 | 109 |
| | | | 28 | 115 | 113 |
| | | | 30 | 124 | 118 |
| | | | 35 | 138 | 118 |
| | | | 36 | 103 | 86 |
| Total | 713 | 595 | | 1309 | 1207 |
| Av. | 118.8 | 99.1 | | 119 | 109.7 |
| **Experiment III. Females, 3 mg/lb.** | | | | | |
| 5 | 102 | 106 | 1 | 122 | 109 |
| 7 | 120 | 116 | 2 | 132 | 126 |
| 18 | 128 | 123 | 25 | 122 | 106 |
| | | | 30 | 138 | 110 |
| Total | 350 | 345 | | 514 | 451 |
| Av. | 116.7 | 115 | | 128.5 | 112.4 |

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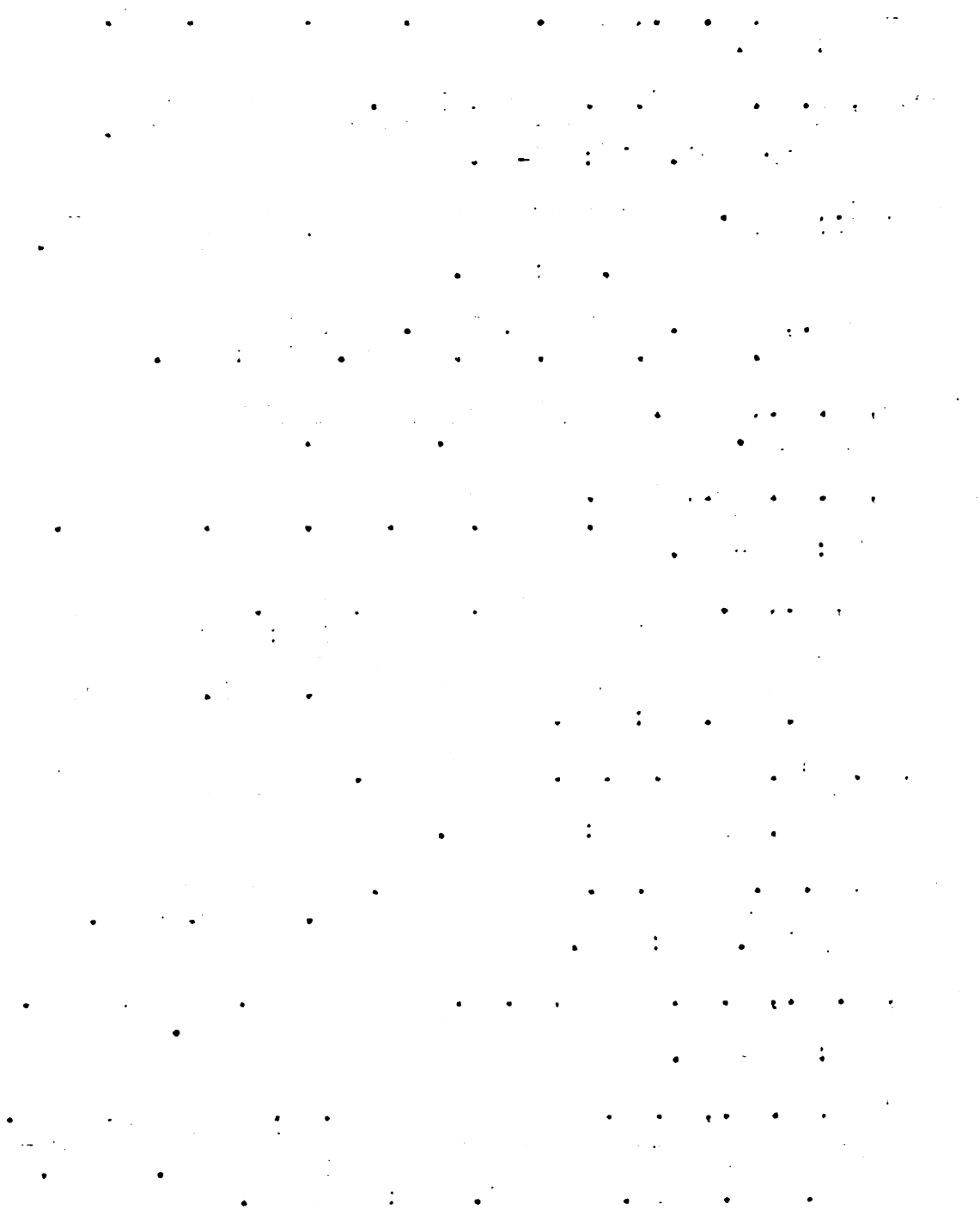

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