SOME EFFECTS OF STEROID HORMONES AND THIOURACIL ON STORAGE AND MOBILIZATION OF VITAMIN A AND RIBOFLAVIN IN THE DOMESTIC FOWL

A Thesis

by

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GENERAL INTRODUCTION

The effects of the administration of gonadal hormones to immature pullets and cockerels have been studied widely. Most of the biochemical work has been carried out during the past ten years, and certain resultant changes in the composition of the blood and liver are well known.

The effect of gonadal hormones on the mobilization and storage of vitamins in the immature fowl is of special interest. Increases in the level of serum riboflavin and vitamin A have been reported in the immature pullet receiving gonadal hormones. Estrogen administration to riboflavin deficient chicks resulted in an increased serum riboflavin concentration. The levels of thiamine and nicotinic acid in the serum, however, remain unchanged in estrogenized pullets.

Many of the changes evoked in the immature pullet by gonadal hormone administration have also been observed at the normal approach of the laying period. Increases in the concentration of serum riboflavin were found in pullets at the onset of lay, and also in laying hens. It appears possible, therefore, that some of the vitamins are mobilized to the blood in the fowl by endogenous gonadal hormone activity.

In the work about to be described, the response of serum carotenoids and serum vitamin A, and their storage

in the liver, was studied in the immature pullet receiving testosterone, estrogen and testosterone plus estrogen. A similar investigation was made on the effect of estrogen administered to vitamin A deficient pullets and cockerels maintained on different dietary levels of vitamin A.

In addition, certain roles of the thyroid in the pullet were also investigated. Injections of thyroxine have been found to decrease the response of serum calcium, serum riboflavin, and liver crude protein to estrogen in the immature pullet. It became desirable, therefore, to study the influence of thiouracil on some of the responses to estrogen in the immature pullet. Also, there are reports that indicate an influence by the thyroid on the conversion of carotene to vitamin A in the intestinal wall of the rat and other animals. To establish a possible influence by the thyroid on the vitamin A response in the fowl, serum and liver levels of carotenoids and vitamin A were determined in estrogenized pullets receiving thyroxine and thiouracil, while maintained on diets containing either carotene only or preformed vitamin A.

PART I

Effects of Exogenous Gonadal Hormones upon the Serum and Liver Vitamin A and Carotenoids in the Sexually Immature Pullet

HISTORICAL INTRODUCTION

Administration of estrogens to sexually immature pullets and cockerels results in various marked changes in the composition of the blood and other tissues. Increases in serum calcium, serum lipid and the plasma phosphorous fractions in estrogenized birds have been reported by many workers. The earlier studies have been reviewed by Riddle (1). Increases have been reported also in serum neutral fat and cholesterol (2) and in serum riboflavin while serum thiamine has been said to remain unchanged (3). The crude blood volume, or more correctly, the drawn blood volume, is increased and there is a decrease in the haematocrit value (4). Serum riboflavin is also elevated in riboflavin deficient chicks receiving estrogen, the elevation being related to the dietary level of riboflavin. The serum nicotinic acid level, however, is not altered (5).

Treatment of immature pullets with estrogen increases the liver weight and the weight of the liver crude protein per kg. live weight (4,6,7,). Hypertrophy of the oviduct is also observed in the estrogenized pullet, and this effect, unlike that on the liver, is enhanced by concurrent small doses of androgen (8).

Chapman (9), working in this department, carried out

a series of experiments on male and female birds from hatching to maturity, and found that as the bird approaches sexual maturity, the composition of the blood and other tissues was greatly changed. There was an increase in liver fat, protein, and ribonucleic acid per kg. of live weight over that of the male. Chapman ascribed these changes to the increased estrogen production which occurs at this stage of development. In a further study, Chapman injected gonadal hormones into immature pullets and found responses similar to those observed in a normal pullet as it approached sexual maturity. He concluded that these changes in the normal pullet were evoked by endogenous gonadal hormones, estrogen in particular. During the course of this study, Chapman et al. (10) observed a three to four-fold increase in serum vitamin A in immature pullets receiving injections of estrogen plus testosterone.

Curiously enough, no previous observations appear to have been recorded in the literature as to the effects of estrogen on serum vitamin A in the fowl. Williamson (11), however, reports that estrogen injections lowered the plasma vitamin A of the normal rabbits. Williamson's study was prompted by the observation that the serum vitamin A level falls continuously during pregnancy in women and quickly rises to normal at parturition.

In a preliminary communication, Maw et al. (12) reported that injections of an anterior pituitary extract in chickens resulted in a slight decrease in serum calcium and serum vitamin A levels and an increase in liver vitamin A reserves. However, the recorded effects were later shown to be reproducible by injection of alcohol in amounts equivalent to the level of alcohol in the pituitary extract used (13).

Kimble (14) found a sex difference in vitamin A levels in human subjects. He reported that plasma carotene levels were slightly higher and more variable in women and that plasma vitamin A was distinctly higher in the men. Brenner et al. (15) observed that female rats stored and retained more vitamin A in their livers than did males, while males had higher blood levels. Temperton and Dudley (16) suggest that male chicks are less resistant to vitamin A deficiency than female chicks during the first four weeks of life.

Dietary factors influence the vitamin A and carotenoid storage levels in the bird. Taylor et al. (17) found that plasma vitamin A levels in the laying hen decreased when the vitamin A content of the diet was reduced. They suggested that plasma vitamin A levels in the bird are primarily a function of body storage. Mattson et al. (18) reported that a diet high in preformed vitamin A fed to chicks resulted in a decrease in the carotenoid concentration of the blood and liver. Rubin et al. (19) suggested the

existence of an antagonism between vitamin A and carotenoids in the fowl. They observed that chicks with low vitamin A stores accumulated yellow pigment in their shanks at a faster rate than those having high vitamin A stores. Rubin *et al.* also noted that large quantities of vitamin A in the diet, or stored in the body of the hen, have a depressing effect on the pigmentation of the egg yolk.

It is well known that there is an increased requirement for vitamin A by birds at the onset of lay. In view of the changes at this time, known to be associated with gonadal hormone activity, it appears reasonable to suspect a vitamin A - gonadal hormone relationship.

The work carried out in the present part of this thesis was designed to study more fully the effect of testosterone and estrogen, singly and combined, on the vitamin A and carotenoid level in the serum and liver of the immature pullet.

EXPERIMENTAL

1. Method of Experiment

The birds used in each experiment were of the same strain and hatching and were reared together under the same conditions. Sex-linked, cross-bred pullets (New Hampshire ♂ x Barred Plymouth Rock ♀) were used whenever they were available because of their greater uniformity. Individuals whose weights were much greater or much less than the group average were rejected when birds were taken for experiment. Care was taken that all birds would be sexually immature at the conclusion of the experiment. Pullets, and especially cross-bred pullets, may begin to lay before they are twenty weeks of age. In order, therefore, to exclude endogenous gonadal hormone activity, it is inadvisable to use birds whose age will exceed fifteen weeks at the end of the experiment.

The experimental birds were housed in an individual cage laying battery and treatments were assigned at random. Food intake as between all birds in a given experiment was kept constant. This precaution was considered necessary as birds receiving gonadal hormone injections have increased appetite. The daily ration was fed in two portions, one in the morning and one in the afternoon. Clean, fresh drinking water was available at all times.

The gonadal hormone preparations used in each experiment were estradiol benzoate ("Progynon B", Schering), 3.33 mg. per ml., and testosterone propionate ("Oreton", Schering), 5.0 mg. per ml. The hormonal treatments were administered by injection into the breast muscle. The total amount of the sesame oil base injected into all the birds in each experiment was maintained equal.

The birds were killed by decapitation after fasting over-night and were allowed to bleed completely as possible. The livers were removed, weighed, and stored immediately in screw-capped bottles at approximately -5°C pending analysis.

a. Experiment 1 *

Sixteen cross-bred (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets were assigned at random to four groups each of four pullets. They were 80 days of age at the beginning of the experiment and 92 days at the conclusion. A commercial chick starter ration was fed.

Hormonal treatments were administered in six equal doses on alternate mornings. Group A received the oily base, Group B 6 mg. testosterone propionate, Group C 18 mg. estradiol benzoate and Group D received the combined treatments of Groups B and C.

The birds were sacrificed on the second morning following the last injection.

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* The author is indebted to D. G. Chapman for the data reported in this experiment exclusive of vitamin A and carotenoid values.

b. Experiment 2

In this experiment twenty-four cross-bred (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets 83 days of age, were placed at random in four equal groups. The pullets were 95 days of age at the conclusion of the experiment.

Treatments in this experiment were similar to those in Experiment 1, with the exception that the total estradiol benzoate injected into the birds in Groups C and D was reduced to 12 mg.

2. General Analytical Methods

a. Moisture

One to two grams of minced liver was dried to constant weight at 105°C in an air oven.

b. Crude Protein

One to two grams of finely minced liver was weighed on a tared cigarette paper and transferred to a 800 cc. Kjeldahl flask. Twenty-five ml. of concentrated sulphuric acid, a pinch of selenium powder and a small crystal of copper sulphate were added. The flasks were heated for two hours, cooled and the contents transferred to a 100 ml. volumetric flask. The Kjeldahl flask was rinsed several times with small portions of distilled water which were added to the volumetric flask. The solution was made to volume with distilled water.

Aliquots of 3 to 5 ml. of this solution were used for the distillations, which were carried out in duplicate in a micro-Kjeldahl apparatus. The distillate was collected in 5 ml. of a 2 per cent boric acid solution containing 10 ml. per l. of a 0.1 per cent solution of methyl red. The distillate was titrated with a 0.02 N hydrochloric acid solution. The factor 6.25 was used in calculating the crude protein.

c. Serum Calcium

Serum calcium determinations were made by Halverson's method as described by Peters and Van Slyke (20).

d. Liver Lipid

(i) Experiment 1

Liver fat was determined by the rapid method used by Holcomb and Maw (21) for analysis of poultry meat. Chapman (9) has shown that this method is unreliable as a guide to total liver lipid, and the method was subsequently abandoned for this purpose.

(ii) Experiment 2

Five grams of fresh finely minced tissue was weighed on a watch glass and quantitatively washed into a Waring Blendor jar with 150 ml. of Bloor's mixture. The liver was disintegrated in the Blendor for one minute. The suspension was then transferred to a 250 ml. ground-glass Erlenmeyer flask and the Blendor jar washed out with small portions of Bloor's

mixture. It was then refluxed for ten minutes on a steam bath. The solvent was filtered through a Buchner funnel using suction, with the filter paper covered with a thin mat of filter aid. The residue was disintegrated again in the Blendor with 150 ml. of Bloor's mixture and once more refluxed and filtered. The combined filtrates were taken to dryness and the residue taken up in 100 ml. of ethyl ether. This was filtered into a tared Erlenmeyer flask and evaporated to dryness. The flask was dried at 105°C for one hour and weighed.

3. Determination of Vitamin A and Carotenoids

a. Reagents

Anhydrous alcohol-free chloroform was prepared by washing reagent grade chloroform three times with an equal volume of water, drying over sodium sulphate and distilling. The purified reagent was stored in the dark over anhydrous sodium sulphate.

Peroxide-free ethyl ether was prepared freshly before use, by distilling reagent grade ethyl ether over iron wire, discarding the first and last ten per cent of the distillate.

Antimony trichloride was prepared by dissolving 250 g. of the reagent in 1 l. anhydrous alcohol-free chloroform and filtering. The solution was stored in a dark bottle.

b. Serum Vitamin A

Serum vitamin A was determined by a slight modification

of the method of Dann and Evelyn (22). The amount of 60 per cent potassium hydroxide was tripled and the refluxing was continued for thirty minutes. This is necessary because of the greater amounts of protein and lipid in the sera of estrogenized birds.

c. Liver Vitamin A

Liver vitamin A was determined by the method of Chichester et al. (23), except that the absorption values of the antimony trichloride blue color were measured in an Evelyn photoelectric colorimeter, using a 620 mμ filter, and the vitamin A values were read from a calibration curve prepared with the Canadian Reference (1948) Standard Vitamin A solution. A carotene correction was made on the basis that 20 mcg. carotene produced the same amount of absorption in the antimony trichloride reaction as 1 mcg. vitamin A.

d. Serum and Liver Carotenoids

The carotenoid contents of sera and livers were determined by the absorption in a chloroform solution of the unsaponifiable fraction, read as "carotene" against a calibration curve prepared from a solution of beta-carotene (B.D.H., recrystallized from methanol) in chloroform.

e. Chromatographic Removal of Interfering Pigments from the Serum Unsaponifiable Fraction

In Experiment 2, carotenoids and the other interfering pigments in the serum unsaponifiable fraction were

removed by the chromatographic method of Narod and Verhagen (24). The development of the antimony trichloride blue colored complex with vitamin A was more typical in the absence of these interfering substances. Values for vitamin A in the purified extracts are almost certainly more reliable than those obtained by applying the carotene correction factor. Losses of vitamin A on the magnesium oxide-diatomite columns used were found to be negligible, and no particular difficulty was experienced in tracing the vitamin A bands on the column by their fluorescence in ultra violet light.

4. Results and Discussion

The data for individual birds in Experiments 1 and 2 are recorded in the Appendix. The treatments and average results are shown in Tables I and II.

a. General Results

The general results include the initial and final weights of the birds, and the weights of the organs removed. Also included in these results are data on crude blood volume, serum calcium, liver dry matter, liver fat and liver crude protein. These determinations were carried out when possible as they serve to define the state of the bird and to indicate the intensity of the treatments administered.

The general results are in agreement with those recorded by many workers (1,2,3,6,8,). It is noteworthy

TABLE I - EXPERIMENT 1. EFFECTS OF ANDROGEN AND ESTROGEN ON THE VITAMIN A AND CAROTENOID CONTENT OF THE SERUM AND LIVER OF THE IMMATURE PULLET. AVERAGE RESULTS

Group	A	B	C	D
Number of birds	4	4	4	4
Total dosage estradiol benzoate, mg.	nil	nil	18	18
Total dosage testosterone propionate, mg.	nil	6	nil	6
Live weight, initial, kg.	0.95	0.98	0.93	0.95
Live weight, final, kg.	1.18	1.22	1.16	1.19
Food consumption, kg.	0.93	0.92	0.93	0.94
Oviduct weight, g.	0.20	0.15	12.4	16.8
Ovary weight, g.	0.39	0.29	0.32	0.28
Thyroid weight, mg.	102	99	77	81
Crude blood volume, ml.	37	37	48	56
Liver weight, g.	19.5	20.8	31.3	30.8
Liver weight, g./kg. live weight	16.5	17.1	26.9	25.7
Serum carotenoids, mcg./100 ml.	95	255	1072	1115
Serum vitamin A, mcg./100 ml.	33	54	134	142
Liver carotenoids, mcg./g.	7.1	7.7	11.3	11.2
Liver carotenoids, mcg./kg. live weight	117	132	305	286
Liver vitamin A, mcg./g.	88	102	56	52
Liver vitamin A, mcg./kg. live weight	1455	1758	1515	1327
Liver dry matter, %	27.7	27.6	27.7	27.2
Liver crude protein, %	21.2	21.2	20.0	19.7

TABLE II - EXPERIMENT 2. EFFECTS OF ANDROGEN AND ESTROGEN ON THE VITAMIN A AND CAROTENOID CONTENT OF THE SERUM AND LIVER OF THE IMMATURE PULLET. AVERAGE RESULTS

Group	A	B	C	D
Number of birds	6	6	6	6
Total dosage estradiol benzoate, mg.	nil	nil	12	12
Total dosage testosterone propionate, mg.	nil	6	nil	6
Live weight, initial, kg.	0.92	0.90	0.94	0.93
Live weight, final, kg.	1.22	1.21	1.18	1.23
Food consumption, kg.	0.85	0.85	0.85	0.85
Oviduct weight, g.	0.17	0.16	10.5	16.0
Ovary weight, g.	0.30	0.25	0.28	0.26
Thyroid weight, mg.	109	116	84	93
Liver weight, g.	23.9	22.2	31.7	30.0
Liver weight, g./kg. live weight	19.7	18.3	26.9	24.4
Serum carotenoids, mcg./100 ml.	222	311	856	823
Serum vitamin A, mcg./100 ml.	32	32	126	107
Serum calcium, mg./100 ml.	11.7	12.3	70.6	79.3
Liver carotenoids, mcg./g.	7.1	7.2	9.9	11.0
Liver carotenoids, mcg./kg. live weight	140	131	268	269
Liver vitamin A, mcg./g.	90	107	81	70
Liver vitamin A, mcg./kg. live weight	1758	1948	2178	1716
Liver fat, %	5.9	5.7	6.9	7.1

that testosterone propionate administration had not any effect on the composition of the tissues examined, nor on the organ weights except for a slight depressing effect on the ovary. The increased hypertrophy of the oviduct when testosterone treatment is superimposed on estrogen, has been observed by others (3,8). While it is accepted that androgen does, in fact, have a slight effect in stimulating growth of the avian oviduct, this effect is very feeble compared either with that of estrogen by itself, or with its effect when superimposed upon estrogen.

b. Vitamin A and Carotenoids

The marked increase in the serum vitamin A levels of the birds receiving estrogen plus androgen agrees with the observation of Chapman et al. (10). It is clear that injection of estrogen alone also increases serum vitamin A to a similar degree, while testosterone alone or combined with estrogen had no effect. Similar responses in serum riboflavin (3,5) and serum biotin (25) in the estrogenized pullet have been observed.

In contrast to these observations is the decrease in the serum vitamin A of the estrogenized rabbit, noted by Williamson (11). The present observations are also in contrast with the finding by Kimble (14) that males have higher levels of serum vitamin A. Of interest is the drop observed

by Beher and Gaebler (26) in the blood vitamin A of adult bitches receiving injections of testosterone. The possibility of endogenous estrogen effects in the bitches, however was not excluded by these authors. Neither the effect of injecting abnormally high amounts of gonadal hormones on serum and liver vitamin A in the immature bird, nor the effect of injecting gonadal hormones into the mature laying hen, has been studied as yet.

The gonadal hormone treatments in these experiments were designed to simulate, within reasonable limits, the normal reproductive activity in the pullet.

The responses of serum carotenoids in the birds receiving gonadal hormones ran parallel to the responses of vitamin A. An increase in the serum lipochrome of estrogenized birds was noted by Common and Bolton (3). Testosterone, administered at levels calculated to simulate normal puberal changes in the comb and wattles of the pullet, led to a definite increase in serum carotenoids. This increase, however, was not so great as that observed in the estrogenized birds. On the other hand, testosterone when injected with estrogen did not augment the effect on serum carotenoid mobilization. The results suggest differing responses of serum vitamin A and serum carotenoids to testosterone.

Total liver carotenoids in each experiment were increased in the birds injected with estrogen and with estrogen plus

androgen. It is all the more remarkable to note that testosterone alone or combined with estrogen had no effect on liver carotenoid storage.

Gonadal hormone treatment had no marked influence on liver storage of vitamin A. There would appear to have been a slight increase in total vitamin A in the livers of birds injected with testosterone, but this increase is not regarded as significant. In Experiment 2, an increase in vitamin A storage is seen in the livers of the estrogenized birds, although birds in Experiment 1 receiving estrogen, and those in both experiments receiving estrogen and androgen, had liver vitamin A levels similar to those of the controls.

It would appear that the mobilization of vitamin A and carotenoids to the serum of estrogenized birds is not simply the result of a withdrawal from the liver. The increase in the level of serum carotenoids is associated with increased liver storage in both experiments. As remarked above, the distribution of vitamin A in the bird, as between liver and plasma, is less clearly defined than that of the carotenoids. Estrogenized birds have increased liver storage in some cases, while the birds injected with estrogen plus androgen have unchanged, if not slightly decreased, liver vitamin A storage. According to Common et al. (7) the increase of riboflavin in the serum of estrogenized pullets, is not accompanied by a significant decrease in total liver riboflavin, although the concentration of riboflavin in the liver is decreased.

It seems reasonably certain from the present experiments that there is increased utilization of dietary vitamin A and carotenoids in the estrogenized bird. Whether this effect is the result of greater retention from the gut or of enhanced absorption through the intestinal wall, is probably the crux of the question. Recently Stamler et al. (27) has reported data which indicate an increase in the weight of the gut in cockerels treated with diethyl-stilbestrol over a period of 15 weeks, although they do not draw attention specifically to the possibility that hypertrophy of the gut had taken place. A similar response in the pullets in these experiments would strengthen the view that there is increased absorption following gonadal hormone administration. There is also the question of enhanced absorptive capacity and of possible histological changes in the intestinal mucosa of estrogenized birds. No one appears to have investigated these possibilities as they deserve.

The absorption of carotene and vitamin A in different animals has been the subject of extensive research. Russel et al. (28) found that laying hens absorbed less carotene from a low fat (ether-extracted) ration, than from a normal ration containing 4 per cent fat. The absorption of vitamin A from the low fat diet, was not affected. However, the retention of vitamin A in the liver was greatly reduced when massive doses were fed to hens on the low fat diet.

Ingelfinger et al. (29) noted that the administration of atropine to human subjects delayed the appearance of vitamin A in the plasma, after the vitamin had been placed in the small intestine. They attribute part of the delay to an inhibition of pancreatic and biliary secretion. Halpern and Biely (30) report an increased utilization of vitamin A in chicks when it was fed in an emulsion. The emulsifying agents used were methyl cellulose or a mixture of methyl cellulose with Demal 14. They suggest a more rapid hydrolysis of vitamin A esters and absorption of the vitamin when it is administered in this form.

Eden and Sellers (31) concluded, after investigations in calves and sheep, that hydrolysis of vitamin A takes place in the lumen of the intestine and that there is re-esterification in the mucosa by the action of a lipase using some of the free fatty acids present. Eden and Sellers suggest that circulating vitamin A is in the free alcohol form and that excess vitamin A remains in the ester form. It would be desirable to ascertain which form is increased by the administration of gonadal hormones. Similar studies in fractionating the carotenoid pigments in estrogenized birds would be of interest also.

Sex influences in the distribution of vitamin A in the rat have been reported recently. Booth (32) has observed greater storage in the livers of females when depleted rats were fed vitamin A. Eden and Moore (33) found more vitamin A

in the kidneys of male than in female rats. Furthermore, Moore and Sharman (34) noted when depleted rats were fed small doses of vitamin A, there was an increase in the vitamin A level of the kidneys in the males and a decrease in the females. At the same time, there was greater storage of vitamin A in the livers of females. The increase in kidney storage in the male was not sufficiently large to balance the higher liver levels in the female.

The tendency of female rats to store more vitamin A in the liver is in agreement with the trends observed in estrogenized birds in the present experiments. To account for the increase in vitamin A observed when immature pullets are treated with estrogen, it is suggested that in addition to increased absorption there is an increase in the capacity of the liver to store vitamin A. However, if estrogen mobilizes vitamin A to the serum at the expense of liver stores, the net result on total liver storage would not be very great.

It appears reasonable to suggest that in a given experiment, increased liver storage of vitamin A in the estrogenized bird could exceed the amount withdrawn to the blood, while in another experiment an opposite effect might well occur. Thus, depending on experimental conditions, the mobilization of vitamin A to the sera of estrogenized birds may be accompanied by either an increase or a decrease in the vitamin A content of the livers.

SUMMARY

1. Two experiments are described in which some of the responses of the sexually immature pullet to gonadal hormone injection have been investigated.

2. In confirmation of previous work, estrogen produced a marked hypertrophy of the oviduct, an increase in serum calcium levels and an increase in the weight of the liver per kg. live weight. Concurrent administration of androgen enhanced the response of the oviduct and serum.

3. Treatment with estrogen resulted in a marked increase in serum vitamin A and serum carotenoid concentrations. These responses were not altered appreciably by concurrent treatment with androgen.

4. Testosterone injections brought about an increase in serum carotenoids. This increase was comparatively less pronounced than that induced by estrogen. A differing response of serum vitamin A and serum carotenoids to testosterone in the fowl is therefore indicated.

5. Estrogen did not appreciably affect the storage of vitamin A in the liver.

6. Testosterone had a tendency to increase vitamin A storage in the liver. Testosterone plus estrogen had a tendency to reduce liver vitamin A. The latter result suggests the possibility of a withdrawal of vitamin A from the liver to the serum.

7. Estrogen and estrogen plus testosterone produced a distinct increase in liver carotenoid storage. Testosterone injections did not have any influence on liver carotenoids.

PART II

The Response to Estrogen of Vitamin A Deficient Pullets and Cockerels

HISTORICAL INTRODUCTION

The relationship between the response to gonadal hormones and nutritional influences has been the subject of several interesting investigations. It was first demonstrated by Hertz (35) that there is a quantitative relationship in the chick, between the response of the oviduct to estrogen and the level of folic acid in the diet. The oviduct response is reduced greatly when the diet is deficient in folic acid. Hertz and Tullner (36) subsequently demonstrated that a similar reduction in oviduct response to estrogen could be induced by a number of folic acid antagonists.

The effects of gonadal hormones on the levels of vitamins in the fowl's serum presents other interesting features. It is now well established that estrogen increases the level of serum riboflavin (3,7). Furthermore, this increase does not appear simply to be a consequence of the mobilization of liver riboflavin reserves, a finding which has been supported by the observations of Hertz *et al.* (5). The latter workers have shown that serum riboflavin is elevated by estrogen in both normal and riboflavin deficient chicks, and that the response is related to the level of riboflavin in the diet.

It is of interest in this connection to note that Singher *et al.* (37) have shown that liver slices from riboflavin and thiamine deficient rats were unable to inactivate estradiol. By constrast, deficiencies of pyridoxine,

pantothenic acid, biotin and vitamin A did not appear to interfere with the capacity of liver slices to inactivate estradiol. No one appears to have investigated sufficiently the possibility that oviduct or other responses might possibly be enhanced by riboflavin deficiency.

Hague et al. (38) have carried out a fairly extensive preliminary survey of the responses of vitamin deficient chicks to gonadal hormones. They observed that comb responses to testosterone were not prevented by any of the deficiencies studied, except for a slight reduction in the case of vitamin D deficiency. Testosterone did not alleviate any of the symptoms of vitamin deficiency. Oviduct response was reduced in folic acid deficiency, as was to be expected from the results secured by Hertz (35). In the cases of the other deficiency states studied, oviduct response was unaffected or even slightly increased.

Mayer and Truant (39) have examined the effects of administration of testosterone on vitamin A deficient rats. Their investigation was prompted by the observation of Mason (40) that vitamin A deficiency in the male rat leads to a condition closely resembling that of the castrated rat. Mayer and Truant (39) have suggested two possible alternative hypotheses to account for these effects of vitamin A deficiency. In the first place, vitamin A deficiency might conceivably interfere with the synthesis of testosterone, either directly, that is chemically, or indirectly, by leading to pituitary

atrophy and defective production of gonadotrophin. In the second place, vitamin A deficiency might produce its effects by interfering with the capacity of target organs to respond to circulating androgen. Mayer and Truant found that the response of vitamin A deficient rats to testosterone was essentially normal. They concluded, therefore, that the "castration effect" was due to lack of circulating androgen, and that the second of the two foregoing hypotheses could be excluded.

Rubin and Bird (41) found that the ascorbic acid contents of the liver and duodenum of the vitamin A deficient hen were similar to those of the normal hen. They point out that this observation in hens is in contrast to that found in cattle and rats. It might be suggested on this basis, that vitamin A deficiency does not interfere with the biochemical functions of the mature hen in quite the same fashion as it does in cattle and rats.

The present part of this thesis deals with three experiments, carried out with the general objective of investigating the responses of vitamin A deficient pullets and cockerels and of depleted pullets to estrogen when maintained on various levels of vitamin A. The detailed objectives will be mentioned in the discussion of the results of this series of experiments.

EXPERIMENTAL

1. Method of Experiment

The selection and care of the birds and the general technique in these experiments were carried out as described in Part I. In this study, however, the birds were deficient or depleted in vitamin A prior to the actual experimental period.

a. Experiment 3

Sixteen cross-bred (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets were assigned at random to four groups of four birds each. These birds were maintained prior to the experiment on a vitamin A deficient diet fortified with alfalfa leaf meal computed to supply approximately 600 I.U. vitamin A potency per lb. The object of this treatment was to secure birds having no appreciable liver reserves of vitamin A and yet not suffering from severe avitaminosis A. Liver levels of approximately 0.3 mcg. per gram in birds sacrificed at the beginning of the experiment indicated marked depletion. The birds were 80 days of age at the start of the experiment and 92 days at the conclusion.

The basal ration was made up as follows:

Ground wheat	47 lb.
Ground barley	20 lb.
Ground wheat middlings	10 lb.
Fish meal	5 lb.
Meat meal	5 lb.
Soybean meal	5 lb.
Dried Brewer's Yeast	6 lb.
Ground limestone	1.5 lb.
Salt	0.5 lb.
Manganese sulphate	6.0 g.
Vitamin D_3	400 A.O.A.C. units per lb.

This ration has a very low vitamin A potency. Table III shows the dietary levels of vitamin A provided to the four groups. Groups A and B received the basal ration and Groups C and D received the basal ration fortified to contain 2400 I.U. vitamin A potency per lb. with a fish oil concentrate (2420 I.U./ g.).

The pullets in Groups A and B showed general unthriftiness and lack of vigor and a few birds suffered moderate loss of muscular control and became unsteady on their legs during the experimental period. In fact, some of these birds squatted most of the time.

The treatments of the four groups are shown in Table III. Each bird in Groups B and D received 12 doses of 1.5 mg. estradiol benzoate ("Progynon B", Schering) given by intramuscular injection each morning during the 12 day period. The control birds received similar injections of the oily base only. The birds were decapitated on the morning following the last injection.

b. Experiment 4

This experiment was similar to Experiment 3 except that sixteen immature cockerels were used and that higher levels of vitamin A were fed. The cockerels on the low vitamin A diet received 600 I.U. per lb. and those on the high level received 3000 I.U. per lb., added in the form of a fish oil concentrate (2420 I.U./g.).

The cockerels were 57 days of age at the onset of the experiment and 69 days of age when killed. They were reared from hatching on a vitamin deficient ration containing 500 I.U. vitamin A potency per lb. as Vitagrass. Analysis at the beginning of the experiment gave an average liver concentration of 5 I.U. vitamin A per gram which can be interpreted as a borderline value.

It was considered advisable to give a small amount of vitamin A to the birds on the low diet to prevent or delay further deficiency and thereby avoid difficulty in maintaining equality of food consumption as between all the treatments during the twelve day trial. The general condition of the birds was similar to that in Experiment 3, but the birds had improved appetites and the total food consumption during the trial was increased.

The hormonal treatment in this experiment was reduced to 1.0 mg. estradiol benzoate per day. Treatments are given in Table IV.

c. Experiment 5

Twenty four immature pullets (New Hampshire ♂ x Barred Plymouth Rock ♀) were alloted to four groups of six birds each. In contrast to Experiments 3 and 4, these pullets were reared on an adequate diet from hatching until 22 days before the trial started, when they were maintained on the following basal ration:

Ground wheat	50	lb.
Ground oats	10	lb.
Ground barley	15	lb.
Ground wheat middlings	10	lb.
Fish meal	2	lb.
Soybean meal	5	lb.
Dried Brewer's Yeast	6	lb.
Ground limestone	1.5	lb.
Salt	0.5	lb.
Manganese sulphate	6.0	g.
Vitamin D_3	400	A.O.A.C. units per lb.

The pullets were 72 days of age at the start of the experiment and 84 days of age at the conclusion. The livers of birds sacrificed at the start of the experiment had an average of 10 mcg. vitamin A per g. These data indicate that vitamin A storage, while not excessive, was not at a dangerously low level.

The levels of treatments are set out in Table V. One half of the birds remained on the vitamin A deficient basal ration throughout the experiment and the other half was given the basal diet fortified with fish oil (2420 I.U./g.) to contain 3000 I.U. vitamin A per lb. Six equal doses of 1.5 mg. estradiol benzoate were administered by intramuscular injection on alternate mornings during the experiment.

2. General Analytical Methods

The moisture and crude protein content of livers and serum calcium were determined by the methods as described in Part I.

3. Determination of Vitamin A and Carotenoids

The vitamin A and carotenoid content of the sera and livers was determined by the methods as described in Part I.

4. Determination of Serum Riboflavin

Serum riboflavin was determined by use of a macro-adaptation of the method described by Burch *et al.* (42).

5. Results and Discussion

The data for individual birds in Experiments 3, 4 and 5 are recorded in the Appendix. Treatments and average results are shown in Tables III, IV and V.

a. Experiment 3

Since it was known that estrogen increased serum vitamin A, it became of interest to investigate the extent to which this increase represented a drain on liver reserves or a deflection of absorbed vitamin A to the serum from storage. It seemed possible that the response of serum vitamin A, and of liver vitamin A to estrogen might depend on (a) the state of pre-existing liver stores and (b) the dietary supply of vitamin A. It was conceivable that, in partially depleted pullets receiving a low vitamin A intake, estrogen might elevate serum vitamin A at the expense of liver reserves, and that this effect would be more marked the more depleted these reserves were. Per contra, it was conceivable that similar birds receiving adequate vitamin A might respond to estrogen by showing an elevated serum

vitamin A, and at the same time an increase of liver reserves as compared with unestrogenized controls receiving the same diet. In short, the responses of liver reserves of vitamin A to estrogen might conceivably be in opposite senses accordingly as the dietary intake was low or high.

In view of the foregoing, it is necessary to consider in some detail the vitamin A status of the birds in these experiments. The vitamin A status of the birds in Group A is difficult to define in the absence of any concensus of opinion as to the level of liver vitamin A which constitutes the borderline between depletion and deficiency. This borderline, presumably, varies with age and growth rate. Taylor et al. (17) have suggested that in hens a drop to 70--100 I.U. vitamin A per 100 ml. plasma indicates marked depletion of body reserves, while an average value below 50 I.U. vitamin A per 100 ml. plasma indicates almost total depletion. The level of 16 mcg. per 100 ml. serum in Group A would appear to indicate a low status. Taylor et al. reported that hens maintained for four months on a ration containing 540 I.U. vitamin A per lb. as carotene, had plasma levels of 21 mcg. vitamin A per 100 ml., while birds of the same hatch on a ration containing 1170 I.U. per lb. had 93 mcg. per 100 ml. Thayer et al. (43) found, on providing a ration containing 700 I.U. vitamin A per lb., that the plasma vitamin A of hens fell from 40 mcg. to 20 mcg. per 100 ml. in ten weeks. The values of some hens fell below 11.6 mcg. per 100 ml.

TABLE III - EXPERIMENT 3. EFFECTS OF VITAMIN A DEFICIENCY ON THE RESPONSE OF IMMATURE PULLETS TO ESTROGEN. AVERAGE RESULTS

Group	A	B	C	D
Number of birds	4	4	4	4
Total dosage estradiol benzoate, mg.	nil	18	nil	18
Vitamin A in ration, I.U./lb.	nil	nil	2400	2400
Food consumption, kg.	0.67	0.66	0.68	0.67
Live weight, initial, kg.	0.79	0.84	0.78	0.81
Live weight, final, kg.	0.90	0.92	0.93	0.89
Oviduct weight, g.	0.16	12.3	0.15	11.7
Ovary weight, g.	0.22	0.17	0.23	0.16
Liver weight, g.	15.4	27.4	15.1	26.2
Liver weight, g./kg. live weight	17.6	30.0	16.5	29.9
Crude blood volume, ml.	29	36	31	43
Haematocrit value, %	32.8	22.6	34.8	22.1
Serum calcium, mg./100 ml.	11.3	27.2	11.8	76.4
Serum carotenoids, mcg./100 ml.	11.8	63.0	4.8	52.0
Serum vitamin A, mcg./100 ml.	17	19	23	34
Liver carotenoids, mcg./g.	1.5	1.6	1.3	1.4
Liver carotenoids, mcg./kg. live weight	25.3	48.2	21.0	41.2
Liver vitamin A, mcg./g.	0.3	0.3	1.6	0.7
Liver vitamin A, mcg./kg. live weight	5.3	10.3	25.0	20.7
Liver crude protein, %	20.6	18.4	21.2	20.2
Liver dry matter, %	26.7	25.4	27.9	26.9

Liver levels of 0.3 mcg. per g. in Group A likewise indicate insufficiency, if not deficiency. Johnson et al. (44) have examined the livers of chicks and have noted that growth was good when the liver contained 3--10 I.U. vitamin A per g., (corresponding to 0.9--3.0 mcg. per g.). Bolin et al. (45) have studied the gradual decline of liver vitamin A in chicks receiving a deficient diet from hatching onward. They have reported values as low as 2 I.U. per liver at 19 day, after which there was a slight increase to 10 I.U. per liver at 56 days.

The values for Groups A and C indicate that the supplementary vitamin A increased both serum and liver vitamin A. It may be concluded that Groups C and D were receiving adequate vitamin A. Thus, the objects of the dietary treatments were secured with the exception that the intake of the birds on the basal ration was slightly lower than the experimental ideal.

The response of serum vitamin A to estrogen in the deficient birds maintained on the vitamin A-free ration was negligible. A substantially greater response to estrogen was observed in the birds receiving dietary vitamin A, (comparison of Group C and D). The relative increase, however, was less than has been observed in normal birds, even when the observed increase in blood volume is taken into account, and when the crude blood volume is assumed to be 50 per cent of the actual blood volume (46). The increase in serum vitamin A in the

estrogenized birds (Group D), is associated with a decrease in liver vitamin A, while the opposite effect is found in the birds maintained on the vitamin A deficient diet. It would appear, from a comparison of Groups C and D, that the estrogen treatment tended to withdraw vitamin A from the liver at this level of vitamin A intake and storage. Thus, the effect of dietary vitamin A on the response of liver vitamin A reserves to estrogen treatment appears to have been exactly the contrary to that envisaged in the argument which inspired the experiment.

In comparison with the responses of serum vitamin A to estrogen, the responses of serum carotenoids were relatively greater. It might be suggested that the higher serum and liver carotenoid values observed in the birds on the deficient ration are the result of a withdrawal of carotenoid pigments from the shanks and other body stores to the liver and serum, and that this withdrawal is decreased when vitamin A is available in the ration. On the other hand, it is possible that the observed predominance of carotenoids in Groups A and B, is a consequence of a lesser efficiency of conversion of carotene to vitamin A in the vitamin A deficient bird. Also, Rubin and Bird (19) have reported an antagonism between vitamin A and carotenoids in the fowl.

A striking and unexpected feature of the results was the much smaller response of serum calcium to estrogen in the birds receiving the vitamin A deficient diet, as compared

with that in the birds receiving the supplemented diet. This is the more astonishing in that liver and oviduct hypertrophy in response to estrogen did not appear to be similarly affected. The present observation provides evidence of the relative independence of the responses of serum calcium and of the oviduct to estrogen treatment.

b. Experiment 4

This experiment was essentially a repetition of Experiment 3 except that cockerels were used instead of pullets. The average results are set out in Table IV.

The vitamin A status of the cockerels in Group A was similar to that of the pullets in Experiment 3. The vitamin A added to the low vitamin A diet was not reflected in any increase in the liver storage levels. It may be assumed, therefore, that the small vitamin A intake by Group A was being utilized completely. The birds in Groups C and D on the diet containing 3000 I.U. vitamin A per lb. displayed an increase of liver vitamin A storage as compared with Groups A and B. In comparison with Groups C and D in Experiment 3, it appears that the higher level of 3000 I.U. per lb. was desirable under these conditions.

The response of the cockerel on adequate vitamin A to estrogen was similar to that of the pullet under comparable conditions. The serum vitamin A response of the birds on the higher vitamin A intake could be accounted for by a withdrawal from the liver storage, as was observed also in Experiment 3.

TABLE IV - EXPERIMENT 4. EFFECTS OF VITAMIN A DEFICIENCY ON THE RESPONSE OF IMMATURE COCKERELS TO ESTROGEN. AVERAGE RESULTS

Group	A	B	C	D
Number of birds	4	4	4	4
Total dosage estradiol benzoate, mg.	nil	18	nil	18
Vitamin A in ration, I.U./lb.	600	600	3000	3000
Food consumption, kg.	0.76	0.76	0.76	0.76
Live weight, initial, kg.	0.57	0.60	0.61	0.52
Live weight, final, kg.	0.79	0.77	0.82	0.74
Testicle weight, g.	0.80	0.16	1.22	0.16
Liver weight, g.	16.2	25.0	16.2	21.6
Liver weight, g./kg. live weight	20.9	32.4	19.9	30.0
Crude blood volume, ml.	23	36	25	30
Serum calcium, mg./100 ml.	10.2	44.2	11.1	59.2
Serum carotenoids, mcg./100 ml.	81	172	89	203
Serum vitamin A, mcg./100 ml.	15	27	29	50
Serum riboflavin, mcg/ml.	0.02	1.57	0.01	1.74
Liver carotenoids, mcg./g.	0.64	1.39	1.27	1.24
Liver carotenoids, mcg./kg. live weight	13.2	45.6	25.0	37.2
Liver vitamin A, mcg./g.	0.3	0.3	5.5	2.0
Liver vitamin A, mcg./kg. live weight	7.2	8.5	110.0	59.5
Liver crude protein, %	21.2	19.6	21.8	20.1
Liver dry matter, %	28.0	27.4	29.4	28.3

In the estrogenized birds on the low vitamin A diet, the serum vitamin A concentration was not greatly increased, nor was the total vitamin A storage in the liver. The serum carotenoid levels in all treatments were greater than those in Experiment 3. Also, in contrast, the birds in this experiment on the high vitamin A ration had greater serum carotenoid concentrations than those on the low dietary intake, although the liver carotenoid levels are greater in the estrogenized birds on the low vitamin A diet. It is further noted that the serum calcium and riboflavin responses to estrogen were reduced in the low vitamin A birds, while liver and oviduct hypertrophy are of the same order, if not greater. In these respects, therefore, Experiment 4 confirmed the results secured in Experiment 3.

c. Experiment 5

In Experiments 3 and 4 the birds had been somewhat severely depleted. It was, therefore, decided to carry out a similar experiment using birds which were depleted but which did not display even slight clinical signs of avitaminosis. As explained above, suitable birds were secured by placing normal pullets on deficient diet for 22 days, a period calculated to give a reasonable degree of depletion without actually producing deficiency.

In this experiment, the possible effects of prolonged severe vitamin A depletion or deficiency, that cannot be ignored in Experiments 3 and 4, could be excluded.

TABLE V - EXPERIMENT 5. EFFECTS OF LIMITED DEFICIENCY OF VITAMIN A ON THE RESPONSE OF IMMATURE PULLETS TO ESTROGEN. AVERAGE RESULTS

Group	A	B	C	D
Number of birds	6	6	6	6
Total dosage estradiol benzoate, mg.	nil	9	nil	9
Vitamin A in ration, I.U./lb.	nil	nil	3000	3000
Food consumption, kg.	0.86	0.86	0.86	0.86
Live weight, initial, kg.	1.15	1.18	1.18	1.23
Live weight, final, kg.	1.30	1.35	1.36	1.38
Ovary weight, g.	0.38	0.37	0.34	0.33
Oviduct weight, g.	0.17	13.8	0.19	12.8
Thyroid weight, mg.	80	76	89	100
Liver weight, g.	26.3	34.3	23.9	34.1
Liver weight, g./kg. live weight	20.2	25.4	17.4	24.9
Serum calcium, mg./100 ml.	13.2	49.8	13.1	54.3
Serum carotenoids, mcg./100 ml.	48	100	54	121
Serum vitamin A, mcg./100 ml.	15	24	17	35
Liver carotenoids, mcg./g.	1.9	2.4	2.2	3.1
Liver carotenoids, mcg./kg. live weight	39	61	38	78
Liver vitamin A, mcg./g	2.1	3.4	18.6	11.3
Liver vitamin A, mcg./kg. live weight	42	88	325	275

The liver vitamin A in Groups A and C was appreciably higher than in the corresponding groups on the low vitamin A diets in Experiments 3 and 4. It is difficult to account for the fact that the serum vitamin A level in this experiment did not increase in proportion.

The response of serum vitamin A to estrogen in birds on the higher dietary source of vitamin A was of the same order as that in Experiments 3 and 4,where larger amounts of estrogen were injected. However, liver storage was much greater in this experiment. It is interesting that estrogen reduced total vitamin A storage in the liver just as in Experiments 3 and 4, even when there appeared to be an appreciable excess of vitamin A.

On the vitamin A-free diet, the response to estrogen was similar to that observed in the vitamin A deficient birds, with the exception that total liver carotenoid stores were lower than those on the higher vitamin A ration. It may be noted that in this experiment also, the average response of serum calcium to estrogen was apparently slightly depressed by partial depletion of vitamin A, although this effect is of questionable significance. This is, doubtless, a consequence of the lesser severity of the depletion of the birds in Experiment 5.

6. General Discussion

The data from these experiments indicate that liver and oviduct responses of vitamin A deficient pullets and

cockerels maintained on a vitamin A deficient diet or on a diet having low vitamin A potency, were normal or even slightly greater than normal so far as weight increase was concerned. Partially depleted pullets on a vitamin A deficient ration showed similar weight responses. However, in the depleted birds, the serum calcium response was not appreciably reduced. These observations suggest that vitamin A bears a specific relationship to estrogen activity in the bird. It may well be that liver hypertrophy in response to estrogen is quantitatively normal in the vitamin A deficient bird, but that vitamin A deficiency affects the capacity of the hypertrophied liver to perform its functions of phosphoprotein and phospholipid synthesis. Such a decreased functional capacity would be reflected in lower serum calcium responses (47).

It may be remarked that the livers of the controls were slightly larger than those on the adequate diet, although the statistical significance is doubtful. Singher et al. (48) state that the livers of rats on thiamine and riboflavin deficiency decrease considerably in size. Also, Mayer and Truant (39) working with rats, reported that kidney size was reduced in vitamin A deficiency, whereas hypertrophy of the kidneys by testosterone was of the normal order, although the actual size was never as great as in the case of rats receiving adequate vitamin A.

The observation that estrogen increases serum and liver vitamin A of vitamin A depleted birds on a deficient ration is difficult to explain. From these experiments, it can be suggested that the vitamin A status of the birds is one contributing factor and that the vitamin A intake is another factor. Comparison of the data for Experiments 4 and 5 shows that higher liver responses are obtained when the birds have higher body reserves of vitamin A. Comparison of data in all experiments between birds on the basal or low vitamin A diet and birds on the high vitamin A diet shows that the dietary factor is also involved.

Still more difficult to explain is the withdrawal of vitamin A from the liver of the estrogenized birds on a high dietary vitamin A intake, while the converse was observed in those on a low dietary vitamin A intake. This result, nevertheless, was obtained consistently in all three experiments, although contrary to the working hypothesis which led to this series of experiments.

SUMMARY

1. The responses of vitamin A deficient and depleted birds to estrogen and the influence of dietary vitamin A on these responses have been studied.

2. Liver and oviduct response to estrogen in deficient and depleted birds receiving no dietary vitamin A, was normal in respect to weight increases.

3. Serum calcium response to estrogen was greatly reduced in deficient birds maintained on a vitamin A-free diet. The reduction in serum calcium response was less marked in partially depleted pullets receiving similar treatment. Evidence is therefore secured that the response of serum calcium is independent of the liver and oviduct response.

4. Estrogen had a tendency to increase both the serum and liver vitamin A levels in vitamin A deficient and depleted birds receiving sub-optimal amounts of dietary vitamin A.

5. Estrogen administration to vitamin A deficient or depleted birds receiving adequate dietary vitamin A, brought about a moderate increase in serum vitamin A. This response was accompanied by a consistent withdrawal or deflection of absorbed vitamin A from the liver.

6. The response of serum and liver carotenoids to estrogen was not appreciably influenced by the vitamin A status of the bird, nor by the level of vitamin A in the diet. However, a slight antagonism of carotenoids by vitamin A in the birds was observed.

PART III

Effect of Thiouracil and Thyroxine on the Response of Vitamin A and Riboflavin in the Estrogenized Immature Pullet

HISTORICAL INTRODUCTION

Fleischmann and Fried (47) have shown that treatment with thyroxine depressed the response of the chick's serum calcium to estrogen, while the degree of hypertrophy of the oviduct was not appreciably affected by thyroxine. Common et al. (4) found that thyroxine administered to the immature fowl depressed the response of serum riboflavin to estrogen and at the same time depressed the increase in liver weight and weight of liver crude protein which are evoked by estrogen. Fleischmann and Fried (47) also found that thiouracil increased serum cholesterol and that this increase was antagonized by thyroxine. Thiouracil did not affect the levels of serum calcium and inorganic phosphorus. These workers offered the following interpretation of their observations: (a) estrogen mobilizes cholesterol from the tissues to the plasma and thiouracil has the same effect; (b) thyroxine tends to keep cholesterol in the tissues and in this respect antagonizes both estrogen and thiouracil; (c) the effects of estrogen and serum calcium and phosphorus are secondary to the effects of estrogen on the synthesis of phospholipid and phosphoprotein in the liver. Fleischmann and Fried (47) concluded that the effects of estrogen on serum cholesterol, serum calcium and phosphorus were separate and independent responses to that on hypertrophy of the oviduct. It became of interest, therefore, to ascertain whether thiouracil would influence the response

of serum calcium and serum riboflavin to estrogen.

The consideration of these possible effects of thiouracil prompted an extension of the investigation to the question of the part played by the thyroid in vitamin A metabolism. Considerable literature has accumulated on this subject, more especially in connection with the conversion of carotene to vitamin A in the intestinal mucosa. The earlier experimental and clinical observations have been reviewed extensively by Drill (49).

More recently, Drill and Truant (50) using the alleviation of ocular lesions as a criterion, failed to demonstrate vitamin A formation from carotene in thyroidectomized rats. Johnson and Baumann (51) found that rats treated with thiourea stored less vitamin A in their livers than the controls when carotene was fed. Rats on the same diet, receiving desiccated thyroid stored more vitamin A in their livers than the controls. Furthermore, the administration of thyroxine together with thiourea resulted in normal vitamin A storage in the liver. The latter observation showed conclusively that the decreased storage of vitamin A in the liver of thiourea-treated animals was due to an anti-thyroid effect. Kelley and Day (52) confirmed the observations of Johnson and Baumann (51).

Wiese et al. (53), by using biological assays based on body weight responses, concluded that vitamin A and carotene are equally effective in promoting the growth of normal and

thiouracil treated rats. These workers emphasized the limitations on growth response imposed by hypothyroidism. The latter authors also found that the growth rate, when limited by thiouracil feeding, could be restored to normal by administration of thyroxine, but not by large doses of vitamin A.

Cama and Goodwin (54), using rats, investigated the influence of the thyroid on the conversion of carotene into vitamin A. They claimed that the thyroid acts not by stimulating the conversion of carotene into vitamin A but by controlling the intestinal absorption of carotene. The antithyroid action of thiouracil inhibits the absorption of carotene and this effect is counteracted by the feeding of desiccated thyroid.

Lipsett and Winzler (55) studied the effect of vitamin A deficiency on thyroid function in the rat, by the use of radioactive iodine. The thyroid glands of rats deficient in vitamin A, were relatively heavier than those of the controls but the total uptake of radioactive iodine was equal in both groups. The inorganic iodine, however, attained values higher than normal, and these values decreased more slowly than in the controls. The rate of thyroxine formation was also decreased by vitamin A deficiency.

The thyroid is involved in many other functions in the fowl. Thus, Juhn (56) has observed that

cockerels fed thiouracil from one day of age exhibited continued growth but that normal attachment of the spur to the shank did not take place. Domm and Blivaiss (57) noted that thiouracil reduced comb growth in roosters, and to a lesser degree in hens. Haque et al. (58) studied the effect of vitamin deficiencies in chicks injected with large doses of thyroxine. High mortality was noted in such chicks when they received a ration low in folic and pantothenic acids. Thyroxine injection, however, produced a marked decrease in the deficiency symptoms of pantothenic acid deficient chicks, suggesting yet another hormone-vitamin relationship in the fowl.

The present and final part of this thesis deals with four experiments. The first two experiments deal primarily with the influence of thiouracil on the response of serum calcium and serum riboflavin in immature pullets receiving estrogen. The two latter experiments consist of an investigation of the effects of thiouracil and thyroxine on the utilization and storage of carotene and vitamin A in estrogenized immature pullets.

EXPERIMENTAL

1. Method of Experiment

The general experimental procedures and techniques used in the four following experiments were similar to those described in Part I.

a. Experiment 6

Twenty cross-bred immature pullets (New Hampshire ♂ x Barred Plymouth Rock ♀) were assigned at random to five groups each of four pullets. The birds were 82 days of age at the outset and 94 days of age at the end of the experiment. A commercial chick starter ration was fed.

The treatments are shown in Table VI. Group A received injections of the oily base only and served as the controls for the experiment. Each bird in the other four groups received six doses of 1.5 mg. estradiol benzoate ("Progynon B", Schering) administered by intramuscular injection on alternate mornings during the experimental period. In addition, Groups C, D, and E were given thiouracil by incorporation of the drug in their food at levels of 0.05, 0.10 and 0.20 per cent respectively.

The pullets remained in excellent condition and consumed their daily food allowance regularly. The birds were decapitated on the second morning following the last injection.

b. Experiment 7

Eighteen sexually immature Barred Plymouth Rock pullets were assigned at random between three groups each of six birds. The birds were 114 days of age at the beginning of the experiment and 128 days at the conclusion. The final age was greater than is desirable in such experiments, but the state of the ovaries of Group A at the conclusion of the experiment confirmed that the birds had not approached the puberal state.

The groups were given the treatments shown in Table VII. The estrogen dosage was the same as in Experiment 6 except that seven doses of 1.5 mg. estradiol benzoate were administered during the 14 day period. The level of thiouracil in the diet was increased to 1.0 per cent. The pullets remained in excellent condition, although the thiouracil diet was less readily eaten during the first three days of the experiment. No other effect of thiouracil on behaviour was noted. The pullets were killed on the second morning after the last injection.

c. Experiment 8

Twenty-five sexually immature (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets that had been maintained on a vitamin A depletion diet for 11 days prior to the experiment, were alloted at random to five equal groups. The pullets

were fed a basal vitamin A deficient diet (similar to that fed in Experiment 5) supplemented with 2400 I.U. vitamin A, added as carotene of dried cereal grass ("Vitagrass"). The carotene concentrate was stored at 10°C and was mixed into the basal ration daily. The birds were 74 days old at the beginning and 88 days old at the conclusion of the experiment.

The treatments are set out in Table VIII. The birds receiving estrogen were injected each morning with 1.0 mg. estradiol benzoate ("Progynon B", Schering) and the controls received equal injections of the oily base. Thiouracil was added to the ration of Groups D and E at a level of 0.75 per cent. Each bird in Group C received a daily subcutaneous injection of 1.0 mg. thyroxine sodium (B.D.H.). The thyroxine sodium was dissolved in the minimum amount of 0.1 N sodium hydroxide and made to the required concentration (2 mg. per ml.) with distilled water.

The birds were killed by decapitation on the day following the last injections.

d. Experiment 9

This experiment was essentially a repetition of Experiment 8.

Twenty-five immature (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets which had been maintained on a vitamin A depletion ration for 17 days prior to the experiment, were

alloted to five equal groups. The basal ration was similar to that fed in Experiment 8. In this experiment, the basal ration was supplemented with a fish oil concentrate to contain 2400 I.U. vitamin A per lb. The vitamin A concentrate was stored at 10°C and was added to the basal ration in daily amounts. The birds were 68 days of age at the beginning of injections and 80 days at the conclusion of the experiment.

Treatments are summarized in Table IX. The pullets on estrogen treatment received six doses of 2.0 mg. estradiol benzoate ("Progynon B", Schering) injected intramuscularly on alternate mornings during the 12 day experimental period. Groups A and E received similar injections of the oily base only. Group C birds each received daily subcutaneous injections of 1.0 mg. thyroxine sodium (B.D.H.) prepared as described in Experiment 8. It was considered advisable to administer the thyroxine daily in order to ensure a uniform response. Groups D and E received a diet containing 0.75 per cent thiouracil.

The birds were killed by decapitation on the second day following the last injection of estrogen.

2. General Analytical Methods

Liver moisture, lipid and crude protein and serum calcium determinations were carried out as described in Part I.

3. Determination of Vitamin A and Carotenoids

The vitamin A and carotenoid determinations in sera and livers were carried out by the methods as described in Part I. The chromatographic separation of carotenoids and other pigments from the serum unsaponifiable fraction, as described in Part I also, was carried out in Experiments 8 and 9.

4. Determination of Riboflavin

a. Serum Riboflavin

Serum riboflavin was determined using a Coleman photofluorometer (Model 12) as described in Part II.

b. Liver Riboflavin

Liver riboflavin was determined fluorimetrically by the procedure of Kodicek and Wang (59).

5. Results and Discussion

Individual data obtained in the following four experiments are recorded in the Appendix.

a. Experiment 6

This experiment was an exploratory study of the effect of feeding various comparatively low levels of thiouracil on the response of the immature pullet to estrogen. The average results are summarized in Table VI.

TABLE VI - EXPERIMENT 6. EFFECTS OF THIOURACIL ON BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN. AVERAGE RESULTS

Group	A	B	C	D	E
Number of birds	4	4	4	4	4
Total dosage estradiol benzoate, mg.	nil	9	9	9	9
Thiouracil in food, %	nil	nil	0.05	0.1	0.2
Food consumption, kg.	0.83	0.83	0.83	0.83	0.83
Live weight, initial, kg.	0.87	0.93	0.89	0.84	0.87
Live weight, final, kg.	1.07	1.10	1.08	1.03	1.05
Ovary weight, g.	0.30	0.22	0.25	0.20	0.23
Oviduct weight, g.	0.08	7.4	8.4	6.0	6.7
Thyroid weight, mg.	84	65	93	120	141
Liver weight, g.	23.1	30.1	30.3	29.7	36.2
Liver weight, g./kg. live weight	21.6	27.5	28.0	28.9	34.5
Serum calcium, mg./100 ml.	11.3	41.8	39.4	31.4	31.0
Serum riboflavin, mcg./ml.	0.02	1.61	1.84	1.25	1.37
Serum carotenoids, mcg./100 ml.	113	665	676	404	346
Serum vitamin A, mcg./100 ml.	39	131	75	61	82
Liver riboflavin, mcg./g.	25.1	23.7	22.1	23.1	19.4
Liver riboflavin, mcg./kg. live weight	541	650	620	669	662
Liver carotenoids, mcg./g.	3.0	6.7	5.7	4.7	4.5
Liver carotenoids, mcg./kg. live weight	65	183	160	134	155
Liver vitamin A, mcg./g.	36	40	36	29	42
Liver vitamin A, mcg./kg. live weight	783	1109	1008	848	986
Liver crude protein, %	18.4	18.2	18.3	18.0	16.7

It was desirable to secure responses to estrogen of such a degree that an enhancement or a depression could be discerned readily. At the same time, it was desirable that thiouracil should not be administered in amounts that would have toxicological effects. From the results obtained, it can be concluded that the response to estrogen was of the desired order, and from the state of the birds receiving thiouracil, it was reasonably certain that the hypothyroid condition produced was not severe. In this connection, the uniform increase in thyroid hypertrophy as the dosage of thiouracil was increased, indicated a moderate hypothyroid condition.

In the estrogenized birds there was a decrease in the weight of the thyroid. Epstein and Wolterink (60) have found that estrogen depresses the turnover rate of iodine in normal chicks and in chicks made hypothyroid by thiouracil. It would appear that the decrease in weight of the thyroid in Group B is due to depression of normal growth and activity of the gland by estrogen, while the increase in weight associated with thiouracil treatment (Groups D and E) is a consequence of inhibition of thyroxine production with consequent abnormal production of colloid and hypothyroidism.

Thiouracil administered at these levels had no great effect on the response of the immature pullet to estrogen. The most marked effect was the increased response of liver

weight and liver crude protein in the birds fed 0.2 per cent thiouracil (Group E).

Contrary to expectations, thiouracil treatment produced a slight decrease in the response of serum calcium and serum riboflavin to estrogen. This observation suggests that thiouracil and thyroxine have a similar effect on serum calcium and riboflavin responses to estrogen. On the other hand, thiouracil affected liver weight and weight of liver crude protein response in the opposite sense to thyroxine.

Serum vitamin A response was depressed by thiouracil but no marked change in liver vitamin A resulted. Total liver riboflavin appeared to be slightly increased by thiouracil.

b. Experiment 7

In view of the results obtained in Experiment 6, a second similar experiment was carried out using a smaller number of birds but larger groups. Also, thiouracil in the diet was increased to 1.0 per cent. It was hoped that this experiment would provide more definite evidence regarding the effects of thiouracil indicated in Experiment 6. From the effects observed in the birds receiving 0.2 per cent thiouracil, it was felt that the level of thiouracil could be increased substantially without a danger of toxicity. This opinion was justified further by the apparent absence of toxicological effects in laying hens receiving up to 0.7 g. thiouracil per day over a long period reported by Domm and Blivaiss (57).

TABLE VII - EXPERIMENT 7. EFFECTS OF THIOURACIL ON BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN. AVERAGE RESULTS

Group	A	B	C
Number of birds	6	6	6
Total dosage estradiol benzoate, mg.	nil	10.5	10.5
Thiouracil in food, %	nil	nil	1.0
Food consumption, kg.	0.98	0.98	0.98
Live weight, initial, kg.	1.25	1.20	1.26
Live weight, final, kg.	1.37	1.29	1.36
Oviduct weight, g.	0.24	10.0	19.8
Ovary weight, g.	0.32	0.29	0.31
Thyroid weight, mg.	131	110	168
Liver weight, g.	25.1	28.6	40.2
Liver weight, g./kg. live weight	18.4	22.1	29.8
Serum calcium, mg./100 ml.	11.1	35.8	13.6
Serum riboflavin, mcg./ml.	0.02	1.46	0.37
Serum carotenoids, mcg./100 ml.	142	370	120
Serum vitamin A, mcg./100 ml.	44	53	43
Liver riboflavin, mcg./g.	29.0	25.9	23.3
Liver riboflavin, mcg./kg. live weight	531	570	689
Liver carotenoids, mcg./g.	1.7	4.4	2.0
Liver carotenoids, mcg./kg. live weight	31	98	59
Liver vitamin A, mcg./g.	37	42	22
Liver vitamin A, mcg./kg. live weight	665	906	652
Liver crude protein, %	21.1	19.2	17.8

The results of Experiment 7 are shown in Table VII. Thiouracil brought about a decrease in serum calcium and riboflavin confirming the indications secured in Experiment 6. However, the depression of the serum vitamin A response by the increased thiouracil dosage was not as great as that observed in the previous experiment. The mobilization of vitamin A to the serum by estrogen (Group B) was comparatively slight. It may be noted also, that estrogen had a tendency to increase liver storage of vitamin A and thiouracil depressed this effect.

A striking feature of the results was the greatly enhanced oviduct hypertrophy in estrogenized birds receiving thiouracil. Fleischmann and Fried (47) noted that neither thyroxine nor thiouracil inhibited the response of the oviduct to estrogen, but they have not suggested that thiouracil can enhance this effect of estrogen. The data in this experiment demonstrated that it is possible to produce experimentally a large degree of hypertrophy of the oviduct unaccompanied by any marked increase in serum calcium and riboflavin. In fact, two of the birds in Group C had serum calcium and riboflavin levels scarcely greater than those obtained in the controls, and yet their oviducts were strongly hypertrophied.

Thiouracil enhanced the effect of estrogen on the total amount of liver crude protein per kg. live weight as well as liver weight. This fact shows that the increase of liver

weight was not merely a reflection of fatty infiltration due to toxic effects of thiouracil. The increase in liver weight was not associated with increases in serum calcium and riboflavin but with decreases. In this respect, thiouracil and thyroxine may therefore decrease serum calcium and riboflavin by different mechanisms, for thyroxine decreases the effect of estrogen on the liver weight and liver crude protein.

It is difficult to account for the depressant effect of thiouracil on serum calcium and riboflavin, on the one hand, and its enhancement of oviduct and liver hypertrophy on the other. Fleischmann and Fried (47) suggested that thyroxine depresses serum calcium in the estrogenized chick either by inhibiting synthesis of plasma proteins and phospholipids in the liver or by increasing oxidative destruction of these constituents. The fact that thyroxine also depresses the increase of liver weight and liver crude protein in the estrogenized pullet favors the theory of synthesis inhibition. The effect of thyroxine could also be explained by an increased rate of destruction of estrogen due to thyroxine. A similar influence by thiouracil on estrogen would explain the decreased serum calcium and riboflavin responses observed in this experiment. This theory, however, cannot account for the enhanced oviduct and liver hypertrophy which accompanied the serum calcium and riboflavin responses in thiouracil-treated

birds. It may be inferred, therefore, that serum calcium and riboflavin responses are induced independently from those of liver and oviduct hypertrophy in estrogenized birds.

Further support is provided in these experiments for the view (4) that the increase in serum riboflavin in estrogenized pullets is not a reflection of liver riboflavin mobilization and that estrogen also tends to increase the total amount of riboflavin in the liver. It is interesting to note that in the estrogenized birds fed thiouracil in this experiment (Group C), the decreased serum riboflavin level is associated with an increase in liver storage. A similar tendency was observed in Experiment 6.

In both experiments, the amount of flavin adenine mononucleotide (FAM) and flavin adenine dinucleotide (FAD) were estimated on the sera of the estrogenized pullets by a macroadaptation of the method described by Burch et al. (42). In all instances the amounts were too small to be measured with any certainty in the presence of the relatively large proportion of free riboflavin. This finding has been confirmed subsequently by Bolton (61). It is of interest that Burch et al. (42) found the changes in total serum riboflavin in rats resulting from feeding different levels of the vitamin, reflect primarily the changes in free riboflavin.

c. Experiment 8

This experiment was designed principally to study the influence of the thyroid on the utilization of carotene in the pullet. Accordingly, the birds were maintained on a vitamin A deficient ration for 11 days prior to the beginning of the experiment. It was considered desirable to use partially vitamin A depleted birds in order that changes in liver storage levels would be detected more easily. The storage levels actually found in the birds were much higher than had been desired. Part of the vitamin A found in the livers was probably the result of the comparatively high level of carotene added to the diet during the experiment.

The influence of thiouracil administered at a level of 0.75 per cent on the response of the pullet to estrogen was very slight in contrast to that observed in Experiment 7. The responses of the oviduct and serum calcium to estrogen appear to have been unaffected by thiouracil treatment. More curious is the absence of hypertrophy of the thyroid glands in the estrogenized birds receiving thiouracil (Group D). The tendency for estrogen to depress thyroid weight apparently predominated over the opposing effect of thiouracil.

The effect of thiouracil in this experiment on liver weight response was as great as that observed in Experiment 7,

TABLE VIII - EXPERIMENT 8. EFFECTS OF THIOURACIL AND THYROXINE ON THE VITAMIN A AND CAROTENOID CONTENT OF THE ESTROGENIZED PULLET MAINTAINED ON A DIET CONTAINING PROVITAMINS A ONLY. AVERAGE RESULTS

Group	A	B	C	D	E
Number of birds	5	5	5	5	5
Total dosage estradiol benzoate, mg.	nil	13	13	13	nil
Total dosage thyroxine sodium, mg.	nil	nil	13	nil	nil
Thiouracil in food, %	nil	nil	nil	0.75	0.75
Food consumption, kg.	1.0	1.0	1.0	1.0	1.0
Live weight, initial, kg.	1.21	1.10	1.03	1.00	1.06
Live weight, final, kg.	1.44	1.36	1.19	1.30	1.37
Ovary weight, g.	0.39	0.28	0.26	0.25	0.34
Oviduct weight, g.	0.25	15.1	12.8	14.1	0.16
Thyroid weight, mg.	122	91	58	96	147
Liver weight, g.	26.1	37.2	25.0	56.2	37.8
Liver weight, g./kg. live weight	18.1	27.4	21.3	43.0	27.7
Serum calcium, mg./100 ml.	12.6	86.4	22.8	81.5	13.3
Serum carotenoids, mcg./100 ml.	410	970	-	760	466
Serum vitamin A, mcg./100 ml.	28	97	-	91	43
Liver carotenoids, mcg./g.	7.9	12.7	15.6	7.7	5.2
Liver carotenoids, mcg./kg. live weight	143	375	344	326	140
Liver vitamin A, mcg./g.	81	40	75	26	41
Liver vitamin A, mcg./kg. live weight	1476	1109	1580	1104	1080
Liver fat, %	6.0	8.6	5.7	6.2	5.2

when it was administered at a level of 1.0 per cent. Possibly the maximum response of liver weight to thiouracil in estrogenized birds is induced at levels well below 1.0 per cent. This response of the liver also suggests that liver and oviduct responses to estrogen are separately and independently influenced by thiouracil.

The effects of thyroxine administration on estrogenized birds are quite definite. The sharp decrease on serum calcium and liver weight agrees with the observation of other workers (4, 47).

Thiouracil did not affect serum vitamin A mobilization by estrogen, although a slight decrease was observed in serum carotenoid response. Unfortunately, the serum samples from the thyroxine-treated birds (Group C) for vitamin A determination were lost. The storage levels of vitamin A in the livers were variable but a decrease was indicated in the birds receiving estrogen. Thiouracil had no influence on liver vitamin A storage when fed to estrogenized birds, but it did slightly retard storage of vitamin A in the non-estrogenized birds (comparison Groups A and E). This result is probably due to impaired conversion of carotene to vitamin A. Liver carotenoid storage on the other hand was not affected by thiouracil.

In the birds receiving estrogen and thyroxine, liver vitamin A levels were greater than those of the controls

and birds receiving estrogen. Cama and Goodwin (54) have reported that desiccated thyroid enhanced the absorption of carotene in the rat. To explain this effect in estrogenized birds, it might be suggested that thyroxine antagonizes the influence of estrogen in depressing liver vitamin A storage. The depression of vitamin A in the livers of estrogenized birds might be also interpreted as the result of the anti-thyroid action of estrogen.

d. Experiment 9

This experiment was carried out to investigate the influence of the thyroid on the utilization and storage of preformed vitamin A. The experimental birds received a depletion diet for 17 days before injections began. Thereafter they received the basal ration, (similar to that prepared in Experiment 5), supplemented with 2400 I.U. vitamin A per lb. added as fish oil. Otherwise, this experiment was similar to Experiment 8.

The vitamin A levels of the birds at the end of this treatment were much lower than in Experiment 8. The initial liver storage in these birds was no doubt appreciably smaller owing to differences in their diets during rearing. Johnson et al. (44) found that carotene supplied as dehydrated alfalfa was superior to vitamin A provided in fish oil for building storage of vitamin A in livers of young chickens.

TABLE IX - EXPERIMENT 9. EFFECTS OF THIOURACIL AND THYROXINE ON THE VITAMIN A AND CAROTENOID CONTENT OF THE ESTROGENIZED PULLET MAINTAINED ON A DIET CONTAINING ONLY PREFORMED VITAMIN A. AVERAGE RESULTS

Group	A	B	C	D	E
Number of birds	5	5	5	5	5
Total dosage estradiol benzoate, mg.	nil	12	12	12	nil
Total dosage thyroxine sodium, mg.	nil	nil	12	nil	nil
Thiouracil in food, %	nil	nil	nil	0.75	0.75
Food consumption, kg.	0.82	0.82	0.82	0.82	0.82
Live weight, initial, kg.	1.22	1.30	1.25	1.20	1.26
Live weight, final, kg.	1.42	1.49	1.39	1.42	1.43
Ovary weight, g.	0.41	0.34	0.37	0.32	0.44
Oviduct weight, g.	0.18	13.5	15.2	15.0	0.15
Thyroid weight, mg.	77	101	66	129	120
Liver weight, g.	26.6	36.5	27.6	48.6	35.2
Liver weight, g./kg. live weight	18.8	24.5	19.9	33.9	24.8
Serum calcium, mg./100 ml.	13.1	65.0	21.2	48.7	12.9
Serum carotenoids, mcg./100 ml.	54	127	67	115	60
Serum vitamin A, mcg./100 ml.	24	74	32	58	27
Liver carotenoids, mcg./g.	1.1	1.4	1.7	1.3	0.9
Liver carotenoids, mcg./kg. live weight	19.7	34.2	31.0	44.0	22.6
Liver vitamin A, mcg./g.	11.4	6.3	13.7	7.5	11.0
Liver vitamin A, mcg./kg. live weight	221	157	256	232	260

The effect of thiouracil treatment on estrogenized birds (Group D) was more in conformity with the results observed in Experiment 7, but the influence was less marked. However, thyroid weight response was inconsistent with that observed in other experiments. The thyroid weights were greater in estrogenized birds (Group B) and the thyroid weights in birds receiving estrogen and thiouracil were greater than those receiving thiouracil alone. It might be remarked, however, that thyroid weights are not a reliable indication of thyroid response or activity.

In comparing the effect of thiouracil with that in Experiment 8, the oviduct response to estrogen, although not great, is more enhanced, liver hypertrophy is slightly less marked and the reduction in serum calcium response is greater.

The effects of thiouracil and thyroxine on the response of vitamin A and carotenoids to estrogen for the most part were not great. Thyroxine appreciably lowered the serum vitamin A and serum carotenoid response to estrogen. The decrease in serum vitamin A was reflected in higher liver storage. Thiouracil treatment did result in a small decrease in carotenoid and vitamin A mobilization to the serum with corresponding small increases in liver storage.

6. General Discussion

From the results obtained in these four experiments, it is evident that the effects of thiouracil on the responses of the immature pullet to estrogen vary greatly with the level of the drug administered. It would appear also, that the maximum effect of thiouracil is exerted at a very critical level. At levels below 1.0 per cent, the effect of thiouracil on serum calcium and riboflavin response and on oviduct hypertrophy in estrogenized birds was not marked.

It is doubtful whether the greater effects of thiouracil fed at the 1.0 per cent level in Experiment 7, were due entirely to the additional amount of the drug administered. Thyroid activity has been found to vary according to strain in chickens. Glazener *et al.* (62) found that thyroprotein depressed growth of rapid-growing strains and accelerated growth of slow-growing strains. The thyroid activity of the Barred Plymouth Rock birds in Experiment 7 was conceivably greatly different to that of the cross-bred (New Hampshire ♂ x Barred Plymouth Rock ♀) pullets used in the other experiments. Mixner *et al.* (63) found breed and sex differences in the response of chicks to thiouracil and thyroxine. According to Hurst and Turner (64), the thyroid secretion rate in the mouse varied with sex, species, age, temperature and nutrition. These workers found that

thiouracil adversely affected gonad function in female mice and that injection of thyroxine above the physiological turnover had a similar effect. It is of further interest to note that the response of the oviduct, liver and serum calcium of Barred Plymouth Rock pullets, (Experiment 7) to estrogen were comparatively lower than similar responses in the cross-bred pullets used in the other experiments.

The effect of thiouracil on the liver was less variable. There was no appreciable difference between the 0.75 per cent and the 1.0 per cent levels of thiouracil on liver weight response to estrogen. The liver weight of non-estrogenized birds fed thiouracil was the same as that of estrogenized birds and the liver weight of the birds receiving estrogen and thyroxine was the same as that of the controls. Leathem (65) has reported an increase in liver size in rats fed thiouracil and that this increase was less marked when androgen was administered concurrently with thiouracil.

Thiouracil brought about increased liver storage of vitamin A in both estrogenized and non-estrogenized birds receiving preformed vitamin A. However, in birds fed carotene only, the effect of thiouracil alone or combined with estrogen was no greater in depressing liver vitamin A storage than that of estrogen alone. Serum vitamin A levels were similarly unaffected. These results fail to demonstrate

any great influence of thiouracil on vitamin A levels in birds fed carotene. Thyroxine had a greater influence on both serum and liver vitamin A. Thyroxine-treated birds on the vitamin A or carotene diets displayed increases in liver vitamin A which were associated with sharp decreases in the serum level. On the whole, the effects of thyroxine in estrogenized birds were more pronounced and less variable than those of thiouracil.

There are several views regarding the effect of the thyroid on vitamin A and carotenoid metabolism. As cited earlier, Cama and Goodwin (54) upheld the view that thiouracil inhibits the absorption of carotene from the intestinal wall of rats and that desiccated thyroid stimulates it. Heimer et al. (66) found that vitamin A storage in rats maintained on a diet deficient in vitamin A, was highest in thyroidectomized rats, lowest in the controls and intermediate in those injected with thyroxine. The control rats had superior growth and these investigators suggested that more of the liver stores of vitamin A were utilized in the controls. Kelley and Day (52) emphasize the necessity of recognizing that the amount of vitamin A found in tissues of thiouracil-treated animals may be the consequence of two opposing effects of thiouracil: (a) the impairment of carotene conversion to vitamin A and (b) the retention of vitamin A already stored in the liver. Cooper et al. (67) concluded from growth responses observed in chicks fed

thyroprotein and thiouracil that the metabolic requirement for vitamin A is decreased in hypothyroid chicks and that more of the dietary vitamin A is available for growth and storage.

In view of the limited and variable responses observed in these experiments, it appears desirable that a study of thyroid influence on vitamin A metabolism and on other responses in the estrogenized pullet, be made in a number of carefully planned stages. It might be suggested that experimental conditions be established as regards age, strain, and vitamin A status of the birds, as well as the level of vitamin A in the diet and the dosage of thiouracil, thyroxine and estrogen which will give optimum and reproducible responses. This might preferably be done on a statistical basis using a large number of birds.

SUMMARY

1. The effects of thiouracil and thyroxine on the response of the immature pullet to estrogen have been studied.

2. Thiouracil depressed the response of serum calcium and serum riboflavin to estrogen and in this respect resembled the effect of thyroxine.

3. Thiouracil enhanced the response of liver weight and the weight of liver crude protein to estrogen, while thyroxine depressed this response.

4. Thiouracil enhanced the hypertrophy of the oviduct. Thyroxine had no effect.

5. Thiouracil had a tendency to depress the mobilization of serum vitamin A and carotenoids in the estrogenized pullet. Thyroxine exerted a similar but more pronounced effect.

6. Estrogen treatment had a variable effect on liver vitamin A storage. In birds fed carotene, estrogen produced a decrease and estrogen plus thiouracil had a similar effect. Also, estrogen decreased liver vitamin A storage in birds fed preformed vitamin A and estrogen plus thiouracil increased it.

7. Thyroxine had a tendency to increase the liver vitamin A level in estrogenized birds fed either carotene or vitamin A.

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APPENDIX

EXPERIMENT 9. EFFECTS OF THIOURACIL AND THYROXINE ON THE VITAMIN A AND CAROTENOID CONTENT OF THE ESTROGENIZED PULLET MAINTAINED ON A DIET CONTAINING PREFORMED VITAMIN A ONLY. INDIVIDUAL RESULTS

Group	A					B					C					D					E				
Bird No.	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	Nil	Nil	Nil	Nil	Nil
Total dosage thyroxine sodium, mg.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	12	12	12	12	12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Thiouracil in food, %	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Food consumption, kg.	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Live weight, initial, kg.	1.22	1.11	1.30	1.29	1.17	1.34	1.35	1.31	1.22	1.29	1.15	1.19	1.40	1.32	1.17	1.16	1.11	1.17	1.24	1.34	1.26	1.18	1.16	1.40	1.31
Live weight, final, kg.	1.43	1.35	1.49	1.46	1.34	1.52	1.55	1.48	1.40	1.50	1.32	1.32	1.48	1.47	1.34	1.36	1.40	1.36	1.41	1.59	1.40	1.31	1.34	1.55	1.55
Ovary weight, g.	0.39	0.42	0.41	0.41	0.44	0.28	0.27	0.40	0.43	0.30	0.38	0.39	0.31	0.40	0.35	0.38	0.31	0.29	0.36	0.28	0.44	0.39	0.49	0.49	0.38
Oviduct weight, g.	0.17	0.19	0.20	0.19	0.15	12.5	14.3	13.8	11.1	15.8	14.8	18.8	15.7	13.0	13.8	19.0	13.6	14.1	16.8	11.7	0.15	0.13	0.13	0.20	0.15
Thyroid weight, mg.	75	85	60	90	75	130	75	100	100	100	35	60	60	85	90	110	85	160	130	160	120	80	130	150	110
Liver weight, g.	27.2	25.1	28.1	29.0	23.5	37.2	36.7	37.0	36.5	35.0	30.0	23.4	29.0	26.4	29.5	44.3	65.2	46.6	42.0	44.7	34.9	36.7	35.5	34.0	34.7
Liver weight, g./kg. live weight	19.0	18.6	18.9	19.8	17.6	24.5	23.7	25.0	26.1	23.3	22.7	17.7	19.6	17.3	22.3	32.6	46.5	34.3	29.0	27.2	24.9	28.1	26.6	22.0	22.5
Serum calcium, mg./100 ml.	12.8	12.8	14.1	13.3	12.6	74.5	45.5	79.0	51.0	75.0	18.8	17.3	22.4	18.6	28.9	77.6	32.6	55.6	50.6	27.3	13.5	13.0	12.1	12.6	13.1
Serum carotenoids, mcg./100 ml.	34	86	70	40	40	134	110	156	120	114	84	66	70	50	66	150	64	130	118	114	64	30	64	64	76
Serum vitamin A, mcg./100 ml.	20	22	20	24	32	76	54	62	88	88	24	52	20	24	38	88	36	54	64	46	26	22	30	32	24
Liver carotenoids, mcg./g.	1.0	1.4	1.0	0.9	1.0	1.4	1.6	1.5	1.3	1.2	1.2	2.5	1.0	2.1	1.6	1.2	1.1	1.5	1.2	1.5	0.9	0.8	1.3	0.9	0.7
Liver carotenoids, mcg./kg. live weight	19.0	25.3	18.9	17.8	17.6	33.1	37.0	38.3	34.7	28.0	27.9	43.8	20.2	36.0	34.6	39.8	52.1	50.0	34.2	41.8	23.2	21.4	33.5	18.9	16.2
Liver vitamin A, mcg./g.	6.6	9.9	13.4	24.5	2.9	3.8	2.1	14.0	5.8	5.9	7.3	14.7	16.0	28.3	2.3	9.5	1.6	6.2	8.2	11.9	10.5	7.1	3.4	23.0	10.8
Liver vitamin A, mcg./kg. live weight	125	185	252	490	51	96	49	355	151	138	167	260	313	488	52	311	74	212	239	324	260	200	90	505	242

EXPERIMENT 8. EFFECTS OF THIOURACIL AND THYROXINE ON THE VITAMIN A AND CAROTENOID CONTENT OF THE ESTROGENIZED PULLET MAINTAINED ON A DIET CONTAINING PROVITAMIN A ONLY. INDIVIDUAL RESULTS

Group	A					B					C					D					E				
Bird No.	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	Nil	Nil	Nil	Nil	Nil
Total dosage thyroxine sodium, mg.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	13	13	13	13	13	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Thiouracil in food, %	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Food consumption, kg.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Live weight, initial, kg.	1.22	1.09	1.19	1.32	1.23	0.94	1.27	1.07	1.14	1.08	0.84	0.91	1.03	1.25	1.11	0.97	1.02	0.93	0.98	1.09	1.11	1.04	1.13	1.06	0.97
Live weight, final, kg.	1.44	1.30	1.51	1.55	1.42	1.24	1.51	1.22	1.45	1.41	1.02	1.08	1.21	1.40	1.22	1.29	1.29	1.28	1.33	1.34	1.43	1.38	1.39	1.38	1.29
Ovary weight, g.	0.33	0.51	0.41	0.40	0.34	0.21	0.23	0.35	0.38	0.25	0.21	0.21	0.25	0.25	0.38	0.24	0.31	0.18	0.28	0.24	0.42	0.27	0.41	0.31	0.31
Oviduct weight, g.	0.24	0.34	0.22	0.18	0.24	13.2	15.6	16.7	15.2	15.0	10.9	10.6	13.2	16.3	13.1	11.7	15.7	14.3	15.2	13.5	-	0.13	0.13	0.23	0.15
Thyroid weight, mg.	100	75	150	160	125	75	105	105	90	80	55	45	45	75	70	85	120	90	95	90	120	155	133	125	200
Liver weight, g.	28.0	22.7	25.8	27.5	26.8	31.5	41.2	37.6	38.6	37.0	27.5	23.4	22.7	28.9	22.7	52.2	46.0	54.2	62.3	66.4	34.0	34.5	35.8	38.1	46.6
Liver weight, g./kg. live weight	19.4	17.5	17.1	17.8	18.7	25.8	27.4	30.9	26.7	26.4	26.9	21.6	18.8	20.7	18.6	40.5	35.0	42.4	47.5	49.4	23.8	25.0	25.8	27.7	36.1
Serum calcium, mg./100 ml.	12.1	12.5	12.6	12.7	13.3	66.6	105.2	106.0	77.6	76.6	45.1	15.0	17.0	18.9	18.1	99.2	67.7	91.4	75.9	73.5	13.7	12.5	13.5	14.6	12.4
Serum carotenoids, mcg./100 ml.	470	480	400	300	400	900	1200	768	900	1080	-	-	-	-	-	760	740	500	900	900	400	510	480	470	470
Serum vitamin A, mcg./100 ml.	31	31	19	31	28	123	96	102	88	78	-	-	-	-	-	72	104	96	88	96	64	56	20	46	31
Liver carotenoids, mcg./g.	9.2	7.5	8.1	7.5	7.3	17.9	11.1	6.6	11.1	16.9	18.5	8.8	-	16.1	19.2	4.8	9.5	8.3	10.7	4.8	5.6	4.3	6.2	6.0	3.8
Liver carotenoids, mcg./kg. live weight	178	130	139	133	137	461	304	203	295	614	497	189	-	332	357	196	332	354	509	240	133	107	160	165	137
Liver vitamin A, mcg./g.	89	53	70	99	96	44	50	35	37	38	51	62	-	103	86	21	35	24	28	23	49	37	59	40	19
Liver vitamin A, mcg./kg. live weight	1730	920	1190	1750	1790	1130	1370	1078	980	990	1372	1340	-	2018	1590	850	1215	1022	1320	1145	1168	920	1520	1112	678
Liver fat, %	6.1	5.9	6.6	5.6	6.1	10.4	8.8	7.5	6.2	9.9	7.1	5.2	5.2	5.5	5.4	6.4	6.4	5.6	7.4	5.0	5.3	5.4	5.4	5.3	4.4

EXPERIMENT 7. EFFECTS OF THIOURACIL ON BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN.
INDIVIDUAL RESULTS

Group	A						B						C					
Bird No.	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	Nil	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Thiouracil in food, %	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.0	1.0	1.0	1.0	1.0	1.0
Total food consumption, kg.	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Live weight, initial, kg.	1.49	1.40	1.07	0.99	1.10	1.47	1.22	1.30	1.13	1.13	1.13	1.33	1.38	1.34	1.17	1.15	1.34	1.17
Live weight, final, kg.	1.55	1.48	1.25	1.17	1.24	1.55	1.38	1.37	1.25	1.19	1.23	1.37	1.50	1.41	1.30	1.25	1.42	1.27
Oviduct weight, g.	0.28	0.30	0.27	0.17	0.22	0.19	2.8	10.0	14.5	10.7	9.8	12.1	25.4	20.2	20.0	16.7	20.0	16.5
Ovary weight, g.	0.30	0.50	0.29	0.19	0.32	0.30	0.37	0.27	0.25	0.31	0.30	0.24	0.36	0.29	0.26	0.30	0.35	0.27
Thyroid weight, mg.	151	158	120	113	136	106	107	111	83	120	113	125	182	168	165	124	236	137
Liver weight, g.	24.5	29.6	24.9	23.6	23.9	24.1	30.4	27.0	28.5	28.0	26.7	30.9	36.7	40.7	41.6	35.9	41.5	44.7
Liver weight, g./kg. live weight	15.8	19.9	19.9	19.9	19.3	15.6	22.3	19.8	22.9	23.6	21.7	22.5	24.4	28.9	32.1	28.8	29.3	35.3
Serum calcium, mg./100 ml.	10.5	11.6	11.1	13.0	11.1	9.3	41.9	19.5	48.0	30.8	34.1	40.4	11.2	11.2	11.6	20.5	11.6	15.5
Serum riboflavin, mcg./ml.	0.02	0.01	0.01	0.03	0.02	0.01	2.12	0.33	2.02	1.18	1.12	1.98	0.14	0.09	0.17	1.10	0.09	0.63
Serum carotenoids, mcg./100 ml.	180	104	80	94	246	150	390	210	534	170	464	454	192	80	160	80	58	150
Serum vitamin A, mcg./100 ml.	55	35	68	29	38	38	43	30	90	33	30	92	71	22	72	28	29	34
Liver riboflavin, mcg./g.	30.6	28.1	30.0	28.2	24.5	32.5	26.4	32.3	24.2	24.9	24.8	22.9	26.4	25.6	21.1	20.7	23.2	22.6
Liver riboflavin, mcg./kg. live weight	482	560	597	570	473	504	583	640	555	587	538	515	645	740	675	596	680	797
Liver carotenoids, mcg./g.	1.7	1.5	1.1	2.1	2.3	1.4	3.9	5.2	3.4	4.5	4.8	4.8	1.6	1.7	3.3	2.3	1.1	1.8
Liver carotenoids, mcg./kg live weight	26.9	29.8	22.0	41.8	44.4	21.8	87.0	103.5	77.8	106.0	104.0	108.0	39.0	49.0	106.0	66.3	32.2	63.6
Liver vitamin A, mcg./g.	80	54	14	16	34	26	14	85	19	27	55	53	34	21	42	9	21	8
Liver vitamin A, mcg./kg. live weight	1265	1065	275	323	656	405	318	1680	435	632	1188	1185	817	604	1335	256	604	293
Liver crude protein, %	20.5	20.7	20.1	20.8	21.6	22.7	19.7	19.8	19.9	19.4	16.9	19.5	19.2	18.6	16.9	18.0	17.5	16.3

EXPERIMENT 6. EFFECTS OF THIOURACIL ON BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN. INDIVIDUAL RESULTS

Group	A				B				C				D				E			
Bird No.	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Thiouracil in food, %	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.05	0.05	0.05	0.05	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20
Total food consumption, kg.	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Live weight, initial, kg.	0.90	0.87	0.78	0.93	0.97	0.98	0.91	0.88	0.95	0.84	0.85	0.92	0.85	0.83	0.84	0.85	0.88	0.86	0.94	0.80
Live weight, final, kg.	1.10	1.05	0.96	1.16	1.14	1.16	1.09	1.02	1.16	1.04	1.01	1.12	1.01	1.05	1.03	1.02	1.06	1.04	1.12	1.00
Ovary weight, g.	0.31	0.36	0.26	0.26	0.16	0.34	0.18	0.21	0.31	0.25	0.16	0.28	0.17	0.21	0.24	0.19	0.20	0.21	0.24	0.25
Oviduct weight, g.	0.11	0.09	0.01	0.13	7.5	6.1	9.1	7.1	8.0	7.6	6.1	12.1	5.0	5.7	7.3	6.1	7.5	4.7	5.4	9.1
Thyroid weight, mg.	103	68	60	103	75	59	66	59	104	100	92	77	114	135	92	137	168	90	191	115
Liver weight, g.	25.1	24.8	18.7	23.7	31.2	30.4	29.4	29.6	31.4	29.2	30.2	30.3	30.5	29.2	29.4	29.7	32.6	42.6	33.4	36.1
Liver weight, g./kg. live weight	22.8	23.6	19.6	20.6	27.3	26.3	27.2	29.0	27.1	28.0	29.9	27.0	30.1	27.8	28.5	29.0	30.8	41.2	30.0	36.1
Serum calcium, mg./100 ml.	12.5	10.8	10.7	11.3	34.6	38.9	47.9	45.9	29.6	42.9	43.3	41.7	27.9	25.8	43.3	28.8	31.7	17.9	27.0	47.1
Serum riboflavin, mcg./ml.	0.03	0.03	0.00	0.00	1.1	0.98	2.25	2.1	1.21	2.20	2.25	1.75	0.92	0.87	2.08	1.12	1.40	0.30	0.83	3.01
Serum carotenoids, mcg./100 ml.	200	148	58	44	420	700	520	1020	510	575	885	735	260	320	600	436	506	146	166	566
Serum vitamin A, mcg./100 ml.	54	47	29	26	87	115	122	199	82	41	111	67	40	34	94	74	95	73	54	105
Liver riboflavin, mcg./g.	21.7	24.4	26.5	27.8	21.3	25.0	21.4	26.9	23.9	21.6	22.8	20.2	23.5	21.1	21.8	26.2	20.5	16.9	20.5	19.5
Liver riboflavin, mcg./kg. live weight	545	576	520	571	582	657	582	780	648	605	682	545	707	587	621	760	631	695	615	705
Liver carotenoids, mcg./g.	3.1	2.5	3.7	2.7	6.7	7.3	5.8	6.9	5.6	5.5	5.8	5.9	3.9	5.3	5.0	4.5	5.3	3.2	2.9	6.7
Liver carotenoids, mcg./kg. live weight	71	60	73	56	182	192	158	199	152	154	173	159	116	148	143	129	162	130	87	240
Liver vitamin A, mcg./g.	35	42	38	29	42	33	35	50	43	22	45	33	28	31	20	39	30	26	15	42
Liver vitamin A, mcg./kg. live weight	800	990	750	590	1145	860	990	1440	1166	627	1352	885	836	862	564	1130	915	1055	453	1522
Liver crude protein, %	17.3	17.8	20.0	18.7	17.3	17.8	18.9	18.9	18.4	18.7	18.0	18.3	17.6	18.2	17.9	18.5	17.7	15.3	18.4	15.4

EXPERIMENT 5. EFFECTS OF LIMITED DEFICIENCY OF VITAMIN A ON THE RESPONSE OF IMMATURE PULLETS TO ESTROGEN. INDIVIDUAL RESULTS

Group	A						B						C						D					
Bird No.	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	Nil	9	9	9	9	9	9	Nil	Nil	Nil	Nil	Nil	Nil	9	9	9	9	9	9
Vitamin A in ration, I.U./lb.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Food consumption, kg.	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Live weight, initial, kg.	1.11	1.10	1.12	1.18	1.18	1.22	1.18	1.22	1.19	1.21	1.26	1.01	1.21	1.10	1.28	1.16	1.20	1.23	1.33	1.31	1.08	1.20	1.35	1.08
Live weight, final, kg.	1.24	1.22	1.38	1.31	1.36	1.30	1.40	1.36	1.35	1.36	1.44	1.21	1.39	1.33	1.40	1.32	1.38	1.35	1.45	1.43	1.30	1.38	1.46	1.24
Ovary weight, g.	0.37	0.42	0.43	0.35	0.42	0.36	0.40	0.45	0.28	0.38	0.40	0.28	0.25	0.32	0.29	0.31	0.40	0.47	0.35	0.46	0.34	0.28	0.24	0.33
Oviduct weight, g.	0.09	0.21	0.23	0.17	0.17	0.18	16.4	15.0	13.7	13.4	13.5	11.2	0.23	0.15	0.22	0.19	0.18	0.18	15.0	12.9	11.9	10.0	12.7	14.1
Thyroid weight, mg.	82	100	70	50	75	100	55	80	70	90	75	83	90	70	55	110	120	90	110	115	100	100	90	85
Liver weight, g.	23.3	30.1	29.4	23.5	28.0	23.3	35.3	34.0	34.6	35.9	33.8	32.3	24.1	23.8	23.3	23.3	24.3	24.3	32.0	31.8	35.8	38.9	33.1	32.8
Liver weight, g./kg. live weight	18.8	24.8	21.4	17.9	20.6	17.9	25.3	25.1	25.6	26.4	23.4	26.7	17.3	17.2	16.7	17.7	17.6	18.1	22.2	22.4	27.4	28.3	22.7	26.6
Serum calcium, mg./100 ml.	12.0	14.2	13.2	13.4	12.9	13.6	54.0	63.5	55.0	43.5	31.5	51.0	13.3	13.8	13.2	12.9	12.8	12.4	61.0	34.0	43.5	65.5	56.5	65.0
Serum carotenoids, mcg./100 ml.	36	40	60	40	70	42	106	140	102	72	80	100	40	52	46	36	52	96	120	82	118	124	134	150
Serum vitamin A, mcg./100 ml.	18	14	10	6	22	18	14	18	38	20	18	38	21	25	16	15	10	18	42	26	28	42	36	35
Liver carotenoids, mcg./g.	2.3	2.4	1.4	1.3	1.9	2.1	2.0	3.1	3.1	1.6	1.9	2.7	2.2	2.3	2.1	1.2	2.5	3.0	5.0	2.6	2.8	3.3	2.4	2.9
Liver carotenoids, mcg./kg. live weight	43	58	30	23	40	38	50	76	77	42	45	73	38	39	34	22	44	53	111	57	74	93	53	77
Liver vitamin A, mcg./g.	6.0	1.9	0.5	0.8	2.6	1.0	0.8	10.8	0.7	2.0	0.8	5.5	9.9	17.3	46.0	8.5	14.1	16.8	10.9	14.4	10.0	7.0	18.4	7.4
Liver vitamin A, mcg./kg. live weight	113	47	11	13	52	18	20	270	17	53	19	148	171	298	764	150	248	318	242	320	274	198	417	195

EXPERIMENT 4. EFFECTS OF VITAMIN A DEFICIENCY ON THE RESPONSE OF IMMATURE COCKERELS TO ESTROGEN. INDIVIDUAL RESULTS

Group	A				B				C				D			
Bird No.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	12	12	12	12	Nil	Nil	Nil	Nil	12	12	12	12
Vitamin A in ration, I.U./lb.	600	600	600	600	600	600	600	600	3000	3000	3000	3000	3000	3000	3000	3000
Food consumption, kg.	0.76	0.76	0.76	0.76	0.75	0.76	0.76	0.75	0.76	0.76	0.76	0.76	0.75	0.76	0.76	0.76
Live weight, initial, kg.	0.62	0.46	0.74	0.48	0.79	0.46	0.60	0.56	0.51	0.69	0.70	0.54	0.50	0.60	0.46	0.53
Live weight, final, kg.	0.84	0.67	0.95	0.70	0.94	0.68	0.81	0.66	0.77	0.87	0.90	0.72	0.67	0.82	0.66	0.76
Testicle weight, g.	0.42	0.50	1.80	0.50	0.17	0.17	0.17	0.13	0.56	0.60	3.50	0.22	0.16	0.22	0.10	0.17
Liver weight, g.	15.9	15.8	17.0	16.3	31.9	20.6	23.7	23.6	15.6	17.3	16.4	15.7	21.7	23.4	20.1	21.1
Liver weight, g./kg. live weight	18.9	23.6	17.9	23.0	33.9	30.3	29.5	36.0	20.2	19.5	18.2	21.8	32.6	28.6	30.8	27.8
Crude blood volume, ml.	23	23	22	24	41	37	35	28	25	27	-	24	23	36	32	29
Blood specific gravity	1.016	1.017	1.018	1.017	1.020	1.020	1.020	1.020	1.017	1.018	-	1.019	1.020	1.021	1.022	1.021
Serum calcium, mg./100 ml.	11.1	9.8	10.6	9.4	29.1	58.3	50.0	39.2	11.1	11.6	-	10.5	46.3	61.7	62.1	67.1
Serum carotenoids, mcg./100 ml.	136	46	112	28	102	240	154	192	62	90	-	114	186	154	230	240
Serum vitamin A, mcg./100 ml.	25	10	18	9	25	20	30	33	22	27	-	38	39	48	51	61
Serum riboflavin, mcg./ml.	0.02	0.02	0.1	0.02	0.7	1.9	2.0	1.7	0.02	0.02	-	0.01	1.8	1.7	1.6	1.9
Liver carotenoids, mcg./g.	0.2	0.4	1.0	0.8	1.4	1.2	1.3	1.7	0.7	0.8	2.4	1.2	1.3	0.9	1.5	1.3
Liver carotenoids, mcg./kg. live weight	5	10	18	19	46	36	38	62	15	15	45	25	42	26	44	35
Liver vitamin A, mcg./g.	0.2	0.3	0.4	0.4	0.2	0.3	0.5	0.1	4.5	6.0	5.4	6.3	2.1	0.8	2.8	2.2
Liver vitamin A, mcg./kg. live weight	3.8	8.0	8.0	9.0	6.8	8.8	15.3	2.9	91.0	117.0	98.0	138.0	69	22	86	61
Liver crude protein, %	21.5	21.7	21.1	20.7	17.8	20.4	20.4	19.9	22.1	22.2	21.1	21.6	20.1	19.8	20.0	20.7
Liver dry matter, %	28.4	28.7	27.6	27.2	27.2	27.6	26.4	28.2	29.3	29.7	29.0	29.4	27.9	28.4	27.3	29.4

EXPERIMENT 3. EFFECTS OF VITAMIN A DEFICIENCY ON THE RESPONSE OF IMMATURE PULLETS TO ESTROGEN.
INDIVIDUAL RESULTS

Group	A				B				C				D			
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	18	18	18	18	Nil	Nil	Nil	Nil	18	18	18	18
Vitamin A in ration, I.U./lb.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2400	2400	2400	2400	2400	2400	2400	2400
Food consumption, kg.	0.67	0.68	0.67	0.67	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.68	0.66	0.68
Live weight, initial, kg.	0.81	0.70	1.03	0.63	0.71	0.86	0.86	0.93	0.73	0.91	0.84	0.65	0.85	0.86	0.72	0.79
Live weight, final, kg.	0.92	0.82	1.14	0.71	0.80	0.99	0.91	0.97	0.89	1.07	0.97	0.80	0.93	0.97	0.77	0.88
Oviduct weight, g.	0.12	0.19	0.15	0.19	9.0	13.4	14.4	12.6	0.18	0.10	0.21	0.13	10.2	12.1	12.5	12.0
Ovary weight, g.	0.19	0.23	0.25	0.20	0.16	0.17	0.14	0.22	0.23	0.23	0.25	0.20	0.15	-	0.19	0.14
Liver weight, g.	14.7	14.5	17.3	15.1	24.0	28.9	25.3	31.6	13.6	16.2	15.6	15.1	27.0	26.2	26.8	24.7
Liver weight, g./kg. live weight	16.0	17.6	15.3	21.3	30.0	29.4	28.0	32.5	15.6	15.3	16.2	18.9	29.0	27.1	35.0	28.3
Crude blood volume, ml.	29	28	34	26	25	35	37	48	28	30	36	27	38	48	39	44
Haematocrit value,%	28.3	35.3	34.4	33.2	19.5	23.4	28.0	19.5	30.1	35.7	37.6	35.9	17.5	19.8	27.2	23.9
Serum calcium, mg./100 ml.	10.5	11.7	11.3	11.7	30.6	27.0	31.5	19.8	-	12.2	11.3	11.9	83.3	97.7	47.2	77.4
Serum carotenoids, mcg./100 ml.	5	10	28	4	10	44	168	28	5	5	4	5	22	110	46	26
Serum vitamin A, mcg./100 ml.	22	16	5	25	25	17	20	15	21	20	25	28	27	45	39	30
Liver carotenoids, mcg./g.	1.9	1.3	1.3	1.4	1.6	1.6	1.6	1.6	1.3	1.8	1.1	1.0	2.0	1.4	0.7	1.5
Liver carotenoids, mcg./kg. live weight	30	24	20	28	49	47	46	51	20	27	19	18	59	39	25	42
Liver vitamin A, mcg./g.	0.3	0.2	0.4	0.4	0.4	0.3	0.3	0.3	1.8	2.0	1.2	1.2	0.8	0.7	0.4	0.7
Liver vitamin A, mcg./kg. live weight	4.7	3.2	5.7	7.5	12.6	8.2	9.2	11.2	27.6	30.3	20.0	22.6	23.3	18.6	21.6	19.0
Liver crude protein, %	21.2	20.8	20.2	20.1	18.1	18.3	19.1	17.9	21.6	20.7	21.2	21.3	20.8	21.0	19.0	19.8
Liver dry matter, %	28.2	26.4	26.3	26.0	26.4	24.1	27.2	24.1	28.6	27.4	28.0	27.8	27.3	27.5	25.5	27.2

EXPERIMENT 2. EFFECTS OF ANDROGEN AND ESTROGEN ON THE VITAMIN A AND CAROTENOID CONTENT OF THE SERUM AND LIVER OF THE IMMATURE PULLET. INDIVIDUAL RESULTS.

Group	A						B						C						D					
Bird No.	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	12	12	12	12	12	12	12	12	12	12	12	12
Total dosage testosterone propionate, mg.	Nil	Nil	Nil	Nil	Nil	Nil	6	6	6	6	6	6	Nil	Nil	Nil	Nil	Nil	Nil	6	6	6	6	6	6
Live weight, initial, kg.	0.97	1.01	0.87	0.99	0.87	0.83	0.90	0.87	0.87	0.90	0.86	1.02	0.95	0.95	0.94	0.99	0.87	0.94	1.00	0.87	0.91	1.02	0.85	0.93
Live weight, final, kg.	1.25	1.25	1.15	1.24	1.29	1.13	1.23	1.18	1.18	1.19	1.22	1.27	1.24	1.18	1.16	1.23	1.15	1.12	1.26	1.24	1.19	1.27	1.19	1.23
Food consumption, kg.	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Oviduct weight, g.	0.17	0.21	0.14	0.17	0.17	0.17	0.09	0.19	0.24	0.15	0.13	0.15	12.8	10.9	9.5	10.0	9.5	10.4	18.0	15.4	15.3	14.6	17.8	14.8
Ovary weight, g.	0.25	0.38	0.27	0.27	0.38	0.23	0.24	0.20	0.22	0.32	0.24	0.30	0.27	0.31	0.25	0.25	0.32	0.31	0.41	0.20	-	0.20	0.22	0.26
Thyroid weight, mg.	68	140	90	135	160	60	90	120	95	195	85	110	80	75	70	80	100	100	85	90	130	70	85	100
Liver weight, g.	22.2	26.6	21.8	25.9	24.0	23.0	24.4	19.4	22.2	23.2	21.6	22.3	34.0	37.0	32.0	28.4	29.0	29.6	29.4	29.6	28.8	30.2	29.0	35.1
Liver weight, g./kg. live weight	17.8	21.3	19.0	20.9	18.6	20.4	19.8	16.5	18.7	19.4	17.7	17.6	27.5	31.6	27.7	23.2	25.4	26.4	23.4	23.9	24.2	23.9	24.4	26.9
Serum calcium; mg./100 ml.	11.4	11.7	11.0	12.1	11.1	12.9	12.6	12.5	12.5	12.4	12.2	11.5	74.8	74.8	69.6	66.0	59.2	79.2	69.2	109.6	48.8	74.0	80.4	94.0
Serum carotenoids, mcg./100 ml.	300	206	166	206	222	236	340	370	264	254	400	240	880	800	900	600	1080	880	768	800	670	880	860	964
Serum vitamin A, mcg./100 ml.	32	34	28	26	36	38	34	30	34	28	40	27	143	135	110	64	140	162	120	128	60	94	121	120
Liver carotenoids, mcg./g.	9.7	8.1	4.7	6.6	6.7	7.0	6.6	10.0	6.8	6.3	8.5	5.4	12.1	8.5	11.7	7.4	11.4	8.5	11.6	8.2	9.8	14.8	10.9	10.8
Liver carotenoids, mcg./kg. live weight	173	172	89	129	124	140	131	165	127	122	149	94	333	267	324	172	290	223	270	196	237	354	266	291
Liver vitamin A, mcg./g.	84	74	104	87	107	85	90	130	98	109	133	85	85	78	76	80	99	68	70	66	62	94	56	75
Liver vitamin A, mcg./kg. live weight	1485	1575	1970	1810	1980	1730	1770	2130	1840	2120	2340	1490	2330	2455	2100	1855	2525	1800	1630	1570	1490	2240	1358	2010
Liver fat, %	7.2	5.5	5.5	6.4	5.9	5.0	5.3	6.3	5.5	6.1	4.8	6.3	6.6	7.0	7.1	6.4	7.4	7.0	6.6	6.8	6.5	7.5	7.3	8.0

EXPERIMENT 1. EFFECTS OF ANDROGEN AND ESTROGEN ON THE VITAMIN A AND CAROTENOID CONTENT OF THE SERUM AND LIVER OF THE IMMATURE PULLET. INDIVIDUAL RESULTS

Group	A				B				C				D			
Bird No.	128	130	118	122	121	117	127	119	126	129	123	131	125	120	124	132
Total dosage estradiol benzoate, mg.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	18	18	18	18	18	18	18	18
Total dosage testosterone propionate, mg.	Nil	Nil	Nil	Nil	6	6	6	6	Nil	Nil	Nil	Nil	6	6	6	6
Live weight, initial, kg.	1.05	0.83	0.93	0.98	1.01	0.97	0.99	0.97	0.93	1.00	0.94	0.86	0.94	0.98	1.02	0.86
Live weight, final, kg.	1.28	1.04	1.20	1.21	1.27	1.21	1.25	1.16	1.16	1.31	1.10	1.09	1.15	1.19	1.28	1.14
Food consumption, kg.	0.93	0.94	0.92	0.95	0.94	0.89	0.95	0.89	0.92	0.93	0.94	0.94	0.94	0.93	0.94	0.94
Oviduct weight, g.	0.22	0.11	0.26	0.19	0.14	0.10	0.17	0.20	13.6	10.5	13.6	11.7	15.3	16.7	21.4	13.8
Ovary weight, g.	0.50	0.29	0.41	0.35	0.29	0.26	0.27	0.34	0.34	0.39	0.32	0.25	0.35	0.26	0.30	0.20
Thyroid weight, mg.	121	73	93	121	75	90	147	93	92	53	76	85	67	92	114	51
Crude blood volume, ml.	39	37	35	37	40	35	38	34	48	48	49	45	59	56	59	51
Liver weight, g.	20.9	17.2	20.3	19.6	22.3	23.1	18.7	19.1	29.7	35.1	31.7	28.7	31.6	28.8	32.6	29.0
Liver weight, g./kg. live weight	16.4	16.5	16.9	16.1	17.6	19.1	15.0	16.5	25.6	26.8	28.8	26.4	27.5	24.2	25.5	25.6
Serum carotenoids, mcg./100 ml.	80	140	60	100	280	200	320	220	1200	1080	1080	930	1185	1208	1000	1067
Serum vitamin A, mcg./100 ml.	30	27	41	35	38	52	66	59	118	141	94	114	112	162	160	135
Liver carotenoids, mcg./g.	7.8	6.3	7.8	6.3	7.9	7.7	8.9	6.4	10.6	11.1	13.8	9.6	7.9	12.0	14.6	10.4
Liver carotenoids, mcg./kg. live weight	128	104	132	102	139	147	134	106	272	298	398	253	214	290	372	266
Liver vitamin A, mcg./g.	105	71	105	71	100	120	90	99	55	61	68	40	46	52	59	50
Liver vitamin A, mcg./kg. live weight	1725	1170	1775	1150	1760	2290	1350	1632	1410	1635	1960	1055	1265	1259	1505	1280
Liver dry matter,%	28.5	27.9	27.6	27.1	27.5	28.2	27.5	27.4	27.5	28.1	27.6	27.5	26.2	27.3	28.2	27.2
Liver crude protein,%	21.2	21.1	21.4	21.3	21.3	21.2	21.1	21.3	20.1	18.6	20.3	20.6	19.8	19.0	20.1	20.0