Studies on Some Endocrine and Reproductive Organs Weight, Serum Alkaline Phosphatase and Ascorbate Activity In Laying and Non-laying White Leghorn Hens

A THESIS

Submitted to the Faculty of
Veterinary Science and Animal Husbandry
Magadh University
In Partial Fulfilment of the Requirements
for the Degree of
Master of Science (Animal Husbandry)

December, 1967

BY

Badri Nath Gupta, B.V.Sc. & A.H.

Post-Graduate Department of Physiology

BIHAR VETERINARY COLLEGE, PATNA.

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Dr. A. K. Ray,
Professor and Head of the Post-graduate
Department of Physiology,
Bihar Veterinary College,
P A T N A.

P A T N A,

Dated 31st December, 1967.

Certifid that the work described in this thesis entiled " Studies on some Endocrine and Reproductive Organs weight, Serum alkaline phosphatase and ascorbate activity in laying and non-laying White Leghorn hens" is the bonafide work of Shri Badri Nath Gupta, carried out under my guidance and supervision for the award of MASTER OF SCIENCE (A.H.).

(A. K. Ray).

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It is a pleasure once more to attest to the unfailing patience of my wife, Malti.

It is with great appreciation that author expresses his indebtedness to the Government of Bihar for financial help during the period of this study.

PATNA, December, 1967.

(B. N. GUPTA).

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CHAPTER - I.

GENERAL INTRODUCTION.

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CHAPTER - I

GENERAL INTRODUCTION.

The work on various physiological attributes in livestock production is not scanty. Similar work related with poultry production is comparatively meagre. Any flourishing poultry industry requires the knowledge of the basic physiological processes which could be helpful for desired production.

Reproduction in birds, as in other classes of livestock, does not depend upon one factor. The correlated interplay of different hormones in various stages of reproduction may cause increased mobilisation and utilisation of certain usual constituents of blood and tissues with the results that the general composition of these tissues and body fluids cannot remain undisturbed. Such fluctuations are more obvious when the animal undergoes influence of any stress condition, physiological or otherwise. The original concept on which Seyle's General Adaptation Syndrome was based has been varified and experimented upon in various classes of livestock. Apparently therefore, there might exist some basic physiological mechanism which could offset the general tissue and body fluid composition, in an effort to bring about, the general adaptability of various stress reactions in living beings.

Stress, in general, denotes wide range of conditions, external and/or internal, which influence the rate of wear and tear in the body. Seyle's General Adaptability Syndrome presents three stages of body reactions (i) Alarm which is the initial response of the animal under the influence of stressor, (ii) Adaptation - the animal withstands satisfactory adjustments with the stressor and (iii) Exhaustion - when the capability of the animal to resist the stressor decline. In alarm reaction there is an immediate response of Pituitary Adrenal Axis.

Parturition in ruminants is a type of physiological stress. In ruminants, ketosis, incident to normal parturition and other stresses such as early lactation has been ascribed by Shaw (1956) to be due to adrenal insufficiency.

It is reasonable to believe that egg laying in poultry is also a kind of physiological stress. That the birds conserve energy and try to adjust their general posture prior to and during the process of egg laying is a common feature.

Though in mammals, there is marked depletion of adrenal ascorbic acid under the influence of stress; same is a doubtful condition in birds (Sayers, 1950). The general site of ascorbic acid synthesis in birds is kidney and not liver. Ascorbic acid activity is directly related

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with the level of Oestrogen in blood (Souders and Varozza, 1958) which in turn is also related to calcium and phosphorus mobilization for egg shell formation in laying birds (Sturkie, 1954c). This necessitates a study of serum alkaline phosphatase activity in laying and non laying condition, where the effect of oestrogen is expected to vary a good deal.

Reproduction in poultry differs from that in mammals in one special feature of increased mobilisation of calcium from bones for egg shell formation, in the process of egg laying. Alkaline phosphatase activity in serum is also influenced by the calcium level in blood, so that when there is increased mobilisation of calcium from blood during egg shell formation, the activity of the enzyme in serum could be expected to be decreased. Rako et al (1964) have pointed out that because the capacity of the hen to deposit calcium and phosphorus in egg shell formation is related with the efficiency of egg production, the assessment of alkaline phosphatase activity may be assumed to be one of the decisive points in the estimation of the hens reproductive capacity.

Reports are also available to show that under the influence of increased level of oestrogen the organic weights of certain other endocrine glands and organs vary much. In mammals, adrenal ascorbate activity is alsorelated

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to adrenal size under the influence of ACTH (Sturkie, 1954[a]).

It was considered desirable to study the ascorbate activity in adrenal, kidney and ovary both in laying and non-laying conditions so that the influence of oestrogen (in laying birds) may be studied on these glands in situ and under the normal physiological stress of egg laying. Simultaneously the serum alkaline phosphatase activity has also been studied. With a view to study the activity of different endocrine glands in these conditions, the organic weights of certain reproductive organs and endocrines has also been recorded.

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CHAPTER - II.

RECENT STATE OF KNOWLEDGE IN THE FIELD APART FROM ALREADY PRESENTED ELSEWHERE.

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CHAPTER - II

RECENT STATE OF KNOWLEDGE IN THE FIELD APART FROM ALREADY PRESENTED ELSEWHERE

Within the recent years the domestic birds have been used to an increasing extent for many physiological and endocrinological studies, on account of the inherent differences in its physiology from the mammals. Many investigators have taken organic weights and organ growth rate as parameters of primary importance in experimental biology. However, due to large variations of both genetical and environmental origin it has not been possible to establish any weight standard for specific tissues at specific age of the chicken (Nestor & Jaap, 1963). Nestor and Jaap (1963) during the course of their experiment found that tissue weight in day old chick may differ significantly in different hatches which occured inspite of attempts to provide idential incubation conditions. Difference between the gland weight of chicks hatched at different times have been reported at 62 days of age. Siegel and Siegel (1960) observed hatch differences in weights of adrenal but not in thyroid weights at 4 weeks of age, when the chicks were hatched at different times.

Sex difference in endocrines has been reported by many workers among which Nestor & Jaap (1963) found

that the adrenal weights in the female were higher than those of males at hatching and lower at all succeeding ages. However, Hoffman et al (1953) and Oakberg (1951) observed heavier adrenals in males at 8 weeks and 4 months respectively. But there are majority of the reports that there was no sex difference in the weights of chicken adrenal on body weight basis (Nestor & Jaap, 1963). Size of adrenal glands vary considerably with age, sex, state of health and other factors within and between species. However, as reported by Hartman and Brownell (1949) no effect of sex difference was found on the size of the adrenal in certain species of birds exclusive of chicken, pigeon and duck though the variation within individuals of some of the same species was great.

In embryos and young birds the size of the adrenals in proportion to body weight has been reported to be larger. Crile and Quiring (1940) and others have shown that in chicks weighing 40-50 gms. the adrenals are approximately as large in proportion to body weight as in chicks weighing 300 gms. No difference in the size of the right and left adrenals in the chicken has been reported (Sauer and Latimer, 1931).

According to Riddle (1923) the male pigeon adrenal is slightly heavier than that of the non-laying female but the glands of the females enlarge preceding and

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during ovulation and regress in size following ovulation. The relationship between the adrenal size and reproductive cycle in the chicken has received scanty attention, Nagel (according to Riddle) stated that adrenal size in pigeon, ducks and chickens did not vary with the reproductive cycle.

Effect of insulin injection in pigeons has also been found to increase the adrenal size in intact birds and to a lesser extent in those hypophysectomised (Miller and Riddle, 1941). Formaldehyde, ACTH and other anterior pituitary hormones injections increase adrenal size in pigeon while DCA decrease it and causes a marked atrophy of the interrenal tissue (Miller and Riddle, 1942). Ascarids infection or infection with T. B. in pigeons enlarge adrenals (Riddle, 1923). Hypertrophy and hyperplasia of adrenals and thyroids has also been reported to be the result of avian leucosis in chicken as reported by Arvy and Gabe,(1961). Also Vit. B_1 deficiency in pigeon and chicken (Beznak, 1923) and fasting (Sure, 1938) have been reported to cause adrenal enlargement.

Response in thyroid gland has also been observed by many workers among which Riddle (1947) found considerable variation in thyroid weight of pigeon and dove depending upon the breed, age, and season. Seasonal variations in thyroid weights have been reported in which the weight of the gland were usually greater in fall and

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winter and less in summer which has been confirmed by Cruickshank (1929) and Galpin (1938) but of course with exception. In contradiction to the above seasonal variation, Turner (1948) reported that there was little or no difference in thyroid weights of hens in the fall, winter and early summer. However, it has been well known that lower environmental temperature causes increase in the size of thyroid which does usually happen in fall and winter as reported above. Gland weight increases with the age of the bird(Sturkie, 1954).

Diet also has been reported to play an important role in influencing the thyroid size in animals and birds. Deficiency of iodine in diet causes goitre or enlarged thyroid in chicken (Patton et al, 1939). Diet extremely high in carbohydrate and deficiency of Vit. A or B also causes enlargement of the bird's thyroid gland (Greer, 1950). Goitrogenic or antithyroidal agents has also been reported to produce enlargement of thyroid and inhibition of thyromine secretion (Sadhu, 1948 and Blaxter et al , 1949).

Studies with pituitary gland have revealed that there appears to be no difference in the gland weight of normal male and female of comparative body weights at least upto 120 days of age (Oakberg, 1951).

Works on the level of serum alkaline phosphatase

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in relation to various physiological and pathological conditions have also been reported by many workers.

It is generally recognised that ricket and other disturbances of the normal process of bone formation are usually accompanied by an increase in the alkaline phosphatase of the blood plasma (Motzok and Wynne, 1950) which was later confirmed by others that the degree of activity of the alkaline phosphatase of the plasma is directly related to the severity of the rachitic condition (Bodansky and Jeffe, 1934a, b; Barnes and Carpenter, 1937; Klasmer, 1944 and Moog, 1946). Also determination of plasma phosphatase activity has been employed as a rapid procedure in assaying the potency of antirachitic substances used in poultry feeding in comparison to the tedious and time consuming process of determining the ash content of the bones (Motzok and Wynne, 1950).

The interpretation of the increased activity of the enzyme in the plasma phosphatase as evidence of increased concentration of the enzyme in the plasma, however, was questioned by Thanhausher et al (1938) who suggested that the abnormally high level of activity might be due to the effects of an activator which is present in the abnormal, but not in the normal plasma. This explanation was however, challenged by other workers (William and Watson, 1940, 41; Delory and King, 1944 and Gould, 1944). The majority of

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workers support the view that the relative phosphatase activities can safely be interpreted in terms of relative concentrations of enzyme, provided that the activities are measured under standardised conditions (Motzok, 1950).

Among the observations on the effect of physiological conditions on alkaline phosphatase activities, oestrogenic influence has been extensively studied also by Landauer et al (1941) in ducks. According to Bell (1960) exogenous sex hormones have not been shown to influence the plasma alkaline phosphatase activity in immature birds.

In the course of an investigation of the effects of gonadal hormones on enzyme levels, it was found that in the fowl exogenous oestrogen, increases serum alkaline phosphatase and this effect is reduced by concomitant administration of progesterone (Brown & Badman, 1961).

Alkaline phosphatase level in the serum of laying hens has been reported to be higher than that of cock (Common, 1934) which may be due to the presence of higher level of endogenous oestrogen level in laying hen than cock. Its' level in serum is extremely high in the young chicken and reaches a low level in the adult (Common, 1936; Tanabe and Wilcox, 1960). A considerable amount of work has been done with mammals on the effects of various hormones on the serum alkaline phosphatase.

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According to Tanabe and Wilcox (1961) the administration of thyroxine significantly increases the serum alkaline phosphatase level and thiouracil feeding significantly decreases the level in both in immature and mature chicken.

Similarly, in birds, works on various angles in relation to ascorbic acid metabolism has been reported by many workers. Thornton & Moreng (1958 and 1959) reported that addition of ascorbic acid to the hen's diet maintain or improve shell quality and also interior egg quality during heat stress which they concluded to be due to mediated through thyroid gland. Further studies by Thornton & Deeb (1961) have suggested that the response to ascorbic acid may be influenced by factors other than heat stress namely dietary protein level, calcium level and bird's metabolic rates. In contradiction to above Pepper et al (1961) and Harmes & Waldroup (1961) showed that during heat stress supplemental ascorbic acid did not significantly alter the egg production or shell thickness. In support of above Heywang & Kemmerer (1955) found that there was no difference in egg weight and egg shell thickness when Leghorn hens maintained at high environmental temperature were fed suplementary ascorbic acid as compared with birds not received this extra vitamin.

Furthermore egg specific gravity was neither influenced by the dietary ascorbic acid alone in hens under

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conditions of high environmental temperature nor did the vitamin alter the specific gravity significantely when fed in conjunction with low calcium diet or diets containing ammonium chloride or methyl thiouracil. Food and Oxygen consumption was reported to be slightly higher in hens receiving ascorbic acid under cool environmental conditions but which differed significantly in warm environmental temperature (Thornton & Moreng, 1959).

Treatment with ACTH has been demonstrated to elicit adrenal ascorbic acid depletion in laying hens (Perek & Eckstein, 1959). Adrenal hypertrophy an another measure of interrenal activity has been shown in male chicken by Brown et al (1958), in quail by Zarrow and Baldini (1952), and in chicks by Jailer & Boas (1954) following ACTH injection, although no effect was noted in chicks by Conner (1959) or in quail by Flickinger (1959). Adrenal hypertrophy in birds has also been observed as a result of cold exposure (44°F.) and muscular fatigue (Garren & Shaffner, 1952, '54, '56) and population density (Siegel, 1959, '60). Depletion of adrenal cholesterol in birds was also observed by Siegel (1959, '60) as a result of population density. Adrenal cholesterol or adrenal ascorbic acid depletion was not observed due to single intravenous injection of ACTH. However, intramuscular injections twice daily for four consecutive days caused 67.9% reduction in the amount of cholesterol per 100 mg. of adrenal tissues.

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Pepper et al (1961) found that ascorbic acid reserve of the high producing birds were some what less than those producing at lower level from which they concluded that this low reserve in high produces was inspite of an excess of ascorbic acid intake.

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CHAPTER - III.

METERIALS & METHODS IN GENERAL.

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CHAPTER - III

MATERIALS AND METHOD IN GENERAL

(a) EXPERIMENTAL BIRDS:

Throughout the experiment White Leghorn laying and non-laying birds were procured from Government Central Poultry Farm, Patna. All the birds were healthy, active, alert and consuming the same ration which is given in the Farm. The composition of the mash was as follows:-

Ingredients	% incorporated in mash	Protein %	Fats %	Carbohydrate %
Maize	50	11.11	4.39	82.56
G.N. Cake	25	40.00	6.00	40.00
Wheat bran.	15	11.39	1.79	76.98
Fish meal	5	70.00	4.00	5.00
Bone meal	2	-	-	-
Minerals	3	-	-	-
Terramycin - 5	100 Kg. of feed mixed with 100 gms. of Terramycin - 5.			

Water supply given was adlib.

All the birds brought for experimental work were under the same housing, feeding, and managemental conditions in the Farm.

The birds were brought from the Farm in the

afternoon and kept on the same mash given in the Farm with adlib water. Next day morning the birds were sacrificed for experimental work.

(b) GROUPING OF BIRDS:

A total of 28 White Leghorn birds obtained from Central Poultry Farm, Patna, were divided into three groups-active layers, resting layers and non-layers. This nomenclature of active and resting layers was done on the basis of the laying stage of the birds of the layer group. When the birds were in laying stage or in other words an egg was found in any part of the reproductive tract and oviduct was heavy and enlarged, they were grouped as active layers. But when the birds had stopped laying or were in resting stage after a sequence of laying, they were grouped as resting layers. In these birds there was no ova in any part of the oviduct, and the oviduct was atrophied and small. The ovary was also atrophied. Among non-layers only those birds were taken which were in growing stage and had till not laid eggs.

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CHAPTER - IV.

COMPARATIVE STUDY ON ORGANIC WEIGHTS OF ENDOCRINES AND REPRODUCTIVE TRACTS.

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C H A P T E R - IV

COMPARATIVE STUDY ON ORGANIC WEIGHTS OF ENDOCRINES AND REPRODUCTIVE TRACTS

I N T R O D U C T O R Y

Since the size and weight of the different organs and glands inside the body varies considerably depending upon a number of factors, e.g. season, environmental temperature, diet, age, functional stage and the different hormones level, the present study was directed towards the study of the status of different reproductive organs and endocrine glands between the laying and non-laying birds. Also, the expected oestrogenic difference in the birds of the laying and non-laying groups inspired to note the changes in the organic weights, as it was reported from other workers that oestrogen exerts some influence over the adrenal enlargement (Kumaran & Turner, 1949 a). Hypertrophy of adrenals of pigeon and chicken with oestrogen was also reported by Breneman (1942), Stamler et al (1950) and Miller & Riddle (1939). Oestrogen however was not found to influence the size of thyroid and pituitary (Kumaran & Turner, 1949b). Oestrogen was found to affect a tremendous increase in size of the oviduct of the hen (Sturkie, 1954). Thyroidal activity has been reported to be associated with serum alkaline phosphatase activity in birds (Common, 1936; Schultze & Turner 1945;

Tanabe & Wilcox, 1960) and also the ascorbate activity of the adrenal gland in mammals was reported to be related to adrenal size under the influence of ACTH (Sturkie, 1954[a]). With the above aim in view it was thought necessary to study the changes in the different reproductive organs and some of the endocrine glands.

EXPERIMENTAL PROCEDURE:

The birds were weighed in spring balance and were killed by decapitation. Weights of the adrenals, thyroids, pituitary and different segments of the oviduct were obtained within two hours of killing. Endocrine organs were weighed on torsion balance and segments of the oviduct on chemical balance to the nearest accuracy. Weights of the uterus and vagina could not be taken seperately as it was not possible to seperate them in non-layers. Each tissue was cleaned of extraneous tissue by dissection on a moist paper and weighed as soon as possible after removal from the body. The weights were expressed as percentage of the body weight to obviate the chances of error due to difference in body weight of birds varying from individual to individual and also for the fact that organic weights were dependent on the body weights of the birds.

RESULTS AND DISCUSSION:

The results obtained are presented in Tables I, II, III, IV, V, VI, VII,& XII along with their analysis of

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variance and critical difference vide Tables VIII, IX, X, XI, XIII, XIV, XV, XVI, XVII, XVIII, XIX & XX. The results show that the difference in adrenal weight between active, resting and non-layer groups were not significant. However, the weight of thyroid gland in non-layers was significantly greater than resting layers at 1% level but there was no significant difference between the thyroid weight of the active and resting groups and so also between active layers and the non-laying brids.

In so far as the pituitary weight was concerned, there was also no difference between the pituitary of the birds of different groups. Next, there was a marked significant difference in the weight of the different segments of the oviduct between the three groups of birds. The weight of infundibulum, magnum and isthmus of the active layers were significantly higher than non-layers and resting layers, but the same were not significantly different in resting layers than non-layers. However, the weight of uterus and vagina taken together were significantly higher in resting than the birds of the non-layer group.

The absence of any increase in adrenal gland weight in the active layers than either of the resting and non-layer group was contradictory to the findings of other workers (Kumaran & Turner, 1949a; Breneman, 1942; Miller & Riddle, 1939) which was perhaps due to non-influence of the anticipated higher level of oestrogen in laying birds

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in causing enhancement in adrenal weight. This was in agreement with the findings of Kar (1947b) who reported that adrenal weight was not increased due to oestrogen. The insignificant difference between the adrenal weight of resting and non-laying birds might also be due to the same non-influence of oestrogen on adrenal weight.

Next, the significant increase in thyroid weight in non-layers than the birds of laying group might have been due to increase thyroidal activity in non-laying condition during which birds are in growing stage. This is confirmed by the findings of other workers (Schultze & Turner, 1945) who reported that thyroidal hormone secretion rate is higher in young growing chicken than in adult.

Similarly, the insignificant difference in the weights of pituitary between the layers and non-layers might be due to the fact that oestrogen level in layers has got no effect on the weight of pituitary gland as reported by Kar (1947b) and Kumaran & Turner (1949b).

The weight of different segments of the oviduct e.g. infundibulum, magnum and isthmus were significantly higher in active than both the resting and non-laying groups. This might be the result of higher oestrogenic level in layers than in non-layers as also reported by other (Sturkie, 1954b) that oestrogen affects the increase in size of the oviduct. The increase in the oviduct weight of active layers than the resting layers might

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be the effect of higher level of oestrogen in active layers than resting ones. Also, the increase in uterine and vaginal weight taken together might also be due to the same elavated level of oestrogen in resting than non-layers.

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T A B L E (I)

Table showing body weight (gm) and Endocrine weight in mg./100 gm. body weight of active layers.

Body weight	Adrenal	Thyroid	Pituitary
1500	-	9.74	0.73
1500	-	9.35	0.73
1750	-	11.42	0.57
2000	7.70	11.30	0.50
2000	7.70	10.20	0.50
2000	12.40	9.75	0.55
1750	13.60	9.60	0.57
Average with S.E.		10.35±1.54	10.19±0.31	0.59±0.002

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T A B L E (II)

Table showing body weight (gm) and Endocrine weight in mg./100 gm. body weight of resting layers.

Body weight	Adrenal	Thyroid	Pituitary
1937	-	11.40	0.52
1500	-	8.53	0.66
1750	12.98	6.00	0.57
2000	9.35	7.70	0.55
1500	6.93	9.20	0.53
2000	8.15	12.35	0.55
1750	12.74	8.32	0.51
Average with S.E.	10.03±1.21	9.07±0.82	0.56±0.01

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T A B L E (III)

Table showing body weight (gm) and Endocrine weight in mg./100 gm. body weight of non-layers.

Body weight	Adrenal	Thyroid	Pituitary
1000	-	14.40	0.70
1000	-	12.20	0.70
875	-	17.14	0.69
1000	-	13.00	0.70
1500	-	11.93	0.53
1250	8.32	12.00	0.64
1250	8.96	13.24	0.72
1250	8.45	10.32	0.72
1125	9.15	11.64	0.62
1250	8.36	8.96	0.64
1125	8.53	8.71	0.71
1000	13.30	11.10	0.70
1000	11.40	9.90	0.60
1250	8.40	10.00	0.48
Average with S.E.	9.43±0.58	11.75±0.603	0.65±0.001

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T A B L E (IV)

Table showing the weights of the segments of the reproductive tract expressed in mg./100 gm.body weight in active layers.

Infundibulum	Magnum	Isthmus	Uterus and vagina
53.30	833.30	236.60	750.60
190.60	1523.30	186.66	991.30
52.57	894.85	188.28	730.56
35.50	174.00	25.50	340.75
31.25	140.50	39.50	292.00
160.50	817.50	73.00	699.50
75.43	517.14	146.86	530.85
Average with S.E.	85.59±24.05	700.08±181.03	128.05±33.32	619.36±93.67

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TABLE (V)

Table showing the weights of the segments of the reproductive tract expressed in mg./100 gm. body weight in resting layers.

Infundibulum	Magnum	Isthmus	Uterus and Vagina.
19.31	207.64	43.66	247.49
32.66	213.66	92.00	306.33
12.29	53.71	10.29	82.28
27.00	100.50	14.00	174.00
20.80	155.46	35.46	206.39
16.40	42.75	13.50	71.75
23.25	113.20	34.17	226.19
Average with S.E.	21.67±2.55	126.70±25.95	34.72±10.75	187.77±32.51

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T A B L E (VI)

Table showing the weights of the segments of the reproductive tract expressed in mg./100 gm. body weight in non-layers.

Infundibulum	Magnum	Isthmus	Uterus and vagina.
8.40	21.00	7.50	51.00
9.00	20.00	7.50	49.50
4.57	9.71	4.57	8.00
2.00	8.00	4.50	16.20
2.40	8.40	3.13	10.80
1.92	10.80	4.00	8.40
2.88	14.32	3.60	14.24
2.24	9.60	2.40	11.20
8.00	17.77	6.66	44.00
6.00	11.20	4.00	29.20
4.09	9.07	2.84	23.91
3.80	5.90	1.80	18.40
2.40	5.70	2.80	9.00
1.12	3.12	0.72	9.12
Average with S.E.	4.20±0.703	11.05±1.43	4.00±0.54	21.64±4.19

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T A B L E (VII)

Summary of the average endocrine weights (gms) of different groups.

Group	Adrenal with S. E.	Thyroid with S. E.	Pituitary with S. E.
Active Layers.	10.35±1.54	10.19±0.31	0.59±0.002
Resting Layers.	10.03±1.21	9.07±0.82	0.56±0.01
Non - Layers.	9.43±0.58	11.75±0.603	0.655±0.001

T A B L E (VIII)

Analysis of variance table of Adrenal weights.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	2.70	1.35	0.24 (Non-significant)
Within groups.	15	82.82	5.25	
Total:-	17	85.52		

T A B L E (IX)

Analysis of variance table of Pituitary weights.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	0.03	0.015	0.31 (Non-significant)
Within groups.	25	0.12	0.048	
Total:-	27	0.15		

T A B L E (X)

Analysis of variance table of Thyroid weights.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	35.87	17.93	4.54*
Within groups.	25	98.70	3.95	
Total:-	27	134.57		

* Significant.

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T A B L E (XI)

Table showing the mean difference of Thyroid weights with critical difference between the three groups of birds.

	Mean difference	C.D. at 1%	C.D. at 5%
Active vrs. resting.	1.08	2.93	2.16
Active vrs. non-layers.	1.56	2.51	1.85
Resting vrs. non-layers.	2.68	2.51**	1.85

** - Significant at 1% level.

T A B L E (XII)

Summary of the average weights of the segments of the reproductive tracts of three groups.

Groups	Infundibulum.	Magnum	Isthmus	Uterus & Vagina.
Active layers.	85.59±24.05	700.08±181.03	128.05±33.32	619.36±93.67
Resting layers.	21.67±2.55	126.70±25.95	34.72±10.75	187.77±32.51
Non-layers.	4.20±0.70	11.05±1.43	4.00±0.54	21.64±4.19

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T A B L E (XIII)

Analysis of variance of infundibulum weights.

Source of variation.	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	31404.45	15702.22	15.98**
Within groups.	25	24562.63	982.51	
Total:-	27	55967.08		

** - Highly significant.

T A B L E (XIV)

Analysis of variance of Magnum weights.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	2283889.00	1141944.50	20.40**
Within groups.	25	1399079.64	55963.19	
Total:-	27	3682968.64		

** - Highly significant.

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T A B L E (XV)

Analysis of variance of Isthmus weights.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	72412.13	36206.06	17.67**
Within groups.	25	51230.03	2049.20	
Total:-	27	123642.16		

** - Highly significant.

T A B L E -(XVI)

Analysis of variance of uterus and vagina weights.

Source of variation	Degree of Freedom.	S. S.	M. S.	F.
Between groups.	2	1673038.43	836519.21	50.46**
Within groups.	25	414397.10	16575.88	
Total:-	27	2087435.53		

** - Highly significant.

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T A B L E (XVII)

Table showing the mean difference of infundibulum weights with critical difference at 1 & 5 percent between the three groups of W.L.H. birds.

	Mean difference	C.D. at 1%	C.D. at 5%
Active vrs.resting	63.92	46.26**	34.15
Active vrs.non-layer	81.39	40.07**	29.58
Resting vrs.non-layer	17.47	40.07	29.58

** - Significant at 1% level.

T A B L E (XVIII)

Table showing the mean difference of magnum weights with critical difference at 1 & 5 percent between the three groups of W.L.H. birds.

	Mean difference.	C. D. at 1%	C. D. at 5%
Active vrs.resting.	573.38	349.22**	257.85
Active vrs.non-layer.	689.03	302.44**	223.30
Resting vrs.non-layer.	115.65	302.44	223.30

** - Significant at 1% level.

T A B L E (XIX)

Table showing the mean difference of isthmus weights with critical difference at 1 & 5 percent between the three groups of W.L.H. birds.

	Mean difference.	C. D. at 1%	C. D. at 5%
Active vrs. resting.	93.33	66.82**	49.34
Active vrs.non-layer.	124.05	57.86**	42.72
Resting vrs.non-layer.	30.72	57.86	42.72

** - Significant at 1% level.

T A B L E (XX)

Table showing the mean difference of uterus and vagina weights with critical difference at 1 & 5 percent between the three groups of W.L.H. birds.

	Mean difference.	C. D. at 1%	C. D. at 5%
Active vrs.resting.	431.59	190.05**	140.33
Active vrs.non-layer.	597.72	164.58**	121.52
Resting vrs.non-layer.	164.13	164.58	121.52*

* - Significant at 5% level.
** - Significant at 1% level.

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CHAPTER - V.

ALKALINE PHOSPHATASE IN SERUM.

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C H A P T E R - V

ALKALINE PHOSPHATASE IN SERUM

INTRODUCTORY:

Alkaline phosphatase has been shown to be distributed throughout the body in various tissues and the phosphatase in the serum may be one or more enzymes arising from many tissues (Martin & Patrick, 1961). This was the first enzyme demonstrated to be involved in the calcification mechanism in bones by way of liberating phosphate ions from the organic combination and there by making them available to combine with calcium (McLean & Budy, 1959). Another hypothesis for the role of phosphatase in bone has been advanced by Bourne (1956) that it participates in the production of calcifiable protein matrix and its function is concerned with calcifiability rather than with calcification itself.

The plasma alkaline phosphatase of the healthy fowl is demonstrated to originate in the bone, and particularly in the osteoblasts, the enzyme activity of the plasma therefore reflects the activity of a mechanism for skeletal calcification (Hall & King, 1930-31, Auchinachie and Emsle, 1934; Common 1934, 1936; Motzok, 1950a,b) but the egg shell calcification on the other hand is mediated by carbonic anhydrase in the shell gland (Common,1941; Gutowska & Mitchell, 1945) rather than any mechanism in

which alkaline phosphatase is concerned directly. Indirectly, however, a high proportion of the shell requirements have to be met from skeletal sources (Driggers and Connar, 1949; Jowsey et al, 1956) which was also confirmed by Bell (1960).

Early works on the serum alkaline phosphatase activity in the laying hens by Common (1936) showed that large variations occured between hens and even between sera obtained from individual bird.

Thyroid hormone secretion rate on the basis of 100 gms. of body weight is higher in the young chicken than that of the adult (Schultze & Turner, 1945). The high value in the alkaline phosphatase observed in the serum of young chicken may be due to high thyroidal activity. Kobayashi et al (1955) reported that thyroxine injection into the pigeon caused increases in acid and alkaline phosphatase in the skin of pigeon. It is possible that the administration of thyroxine increases the phosphatase in the body.

EXPERIMENTAL PROCEDURE:

The serum was collected from the sacrificed bird by allowing the blood to clot.

Serum alkaline phosphatase determination was carried out according to Wooton (1964) which was based on the principle that under defined conditions of time, temperature and pH, phenol is released on enzymatic hydrolysis of phenolphosphate which is estimated colorimetrically.

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REAGENTS:

1. Buffer (pH 10.0):

6.3 gms. of anhydrous sodium carbonate and 3.36 gms. of sodium bicarbonate per litre in water. Kept at 4°.

2. Substrate(0.01 M disodium phenyl phosphate):

Dissolved 2.18 gms. in 1 litre of water and brought the solution quickly to boil to kill any organism. Cooled the solution immediately and preserved with a little chloroform (4 ml./litre) kept at 4°.

3. Stock phenol standard (1 mg./ml.):

This was prepared by adding 1 gm. pure crystalline phenol per litre in 0.1 N hydrochloric acid. Kept at 4° in a brown bottle.

4. Working phenol standard (1 mg./100 ml.):

Diluted 1 ml. stock phenol standard to 100 ml. with distilled water. Preserved with a few drops of chloroform and kept at 4° in a brown bottle.

5. 0.5 N. Sodium hydroxide:

It was prepared by dissolving 20 gms. of sodium hydroxide per litre in distilled water.

6. 0.5 N. Sodium bicarbonate:

Was prepared by dissolving 42 gms. of sodium bicarbonate per litre in distilled water.

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7. 4 - Amino-antipyrine:

6 gms. of 4-amino-antipyrine was added per litre in distilled water and stored in brown bottle.

8. Potassium ferricyanide:

24 gms. of Potassium ferricyanide was dissolved per litre in distilled water and stored in brown bottle.

ANALYTICAL PROCEDURE:

Blood was allowed to clot and the serum was taken out. Experiments with bird's serum were carried as follows:-

I. TEST (UNKNOWN SAMPLE):

In the first test tube 1 ml. of buffer was mixed with 1 ml. of phenyl phosphate substrate and was placed in a water bath at 37°C for 3 minutes. To it 0.1 ml. serum was added, mixed gently and incubated for exactly 15 minutes. The reaction was stopped by the addition of 0.8 ml. of 0.5 N Sodium hydroxide.

II. CONTROL:

In the second test tube 1 ml. of buffer, 1 ml. of substrate and 0.8 ml. of 0.5 N sodium hydroxide were taken and in the last 0.1 ml. of serum was added to it.

III. STANDARD:

In the third test tube 1 ml. of buffer was added to 1 ml. of phenol standard (1 mg./100 ml.). After that 0.8 ml. of 0.5 N Sodium hydroxide was added.

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IV. BLANKS:

In the fourth test tube 1.1 ml. of buffer, 1 ml. of water and 0.8 ml. of 0.5 N sodium hydroxide were taken.

To all tube 1.2 ml. of 0.5 N sodium bicarbonate followed by 1 ml. amino-antipyrine solution and 1 ml. potassium ferricyanide solutions were added. Mixing in each tube was made thoroughly after each additions to avoid the irregular results. The successive additions adjusted the pH and developed the colour.

The red colour so developed was immediately measured colorimetrically in Klette-Summerson Colorimeter using green filter (No.54). Exposure to strong sunlight was avoided.

CALCULATIONS:

The amount of phenol present in the standard tube is 10 microgramme. Thus the phenol produced in 15 minutes in the TEST is $\frac{T - C}{S - B}$ x 10 microgramme where T, C, S & B stand for Test, Control, standard and Blank respectively.

Hence, 100 ml. of serum would liberate $\frac{T - C}{S - B}$ x 10 milligramme of phenol. Since, 1 King-Armstrong Unit is the production of 1 mg. of phenol in 15 minutes under the condition of test, serum alkaline phosphatase

$$= \frac{T - C}{S - B} \times 10$$

(K.A.U. per 100 ml.).

RESULTS AND DISCUSSION:

The results obtained are presented in Tables XXI, XXII and XXIII.

The serum alkaline phosphatase was significantly higher in non-layers than resting layers (P<0.05) but the difference between the non-layers and active layers was not significant although the enzyme activity level was lower apparently in active layer than birds of the non-layer group. Also, the same insignificant difference was observed between layers of the active and resting groups.

The higher level of serum alkaline phosphatase in non-layers than resting layers might be accounted for the difference in the level of different hormones in their body among which oestrogen and thyroidal hormones are of importance. Since the birds of the non-layer group were in growing stage, it is anticipated that in such growing condition, the level of thyroidal hormone might be extremely high as was also reported by other workers (Schultze & Turner, 1945; Bell, 1960). This high level of thyroidal hormone might be taken to be associated with the increase in serum alkaline phosphatase activity as also confirmed by Tanabe & Wilcox (1961) who reported that in young chicken the enzyme level is extremely high which diminishes in adult. Since the thyroidal hormone secretion rate is reported to be higher in young growing chicken than adult (Schultze & Turner, 1945), it is possible that decrease in serum

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alkaline phosphatase activity in resting layers (consisting of adult mature birds which has not yet started laying) in comparison to non-layers as was indeed observed might have been due to a diminished secretion of the thyroidal hormone in the resting layers as evidenced by a significant decrease in the mean thyroid weight of the resting layers than the birds of the non-layer group ($P < .01$).

The lower enzyme activity in the active layers was not found to be significant statistically than the non-layers. However, it may be mentioned that the bird of this group (active layer) did not seem to be homogenous as regards their alkaline phosphatase content which was due to wide range of individual variation between the birds. This might be one of the possible causes for the non-significant difference from the non-laying birds. Hence, the apparent lower level of the serum alkaline phosphatase in the active layers than non-layers might also be due to the same diminished thyroidal activity. This reduction in the enzyme activity of the active layers was in agreement with the findings of Rako et al (1964) who reported that alkaline phosphatase activity is considerably reduced in the phase of full productivity.

In the same way, the birds of the active group did not differ significantly from that of the resting as regards the above enzymatic activity which might be accounted for the similar thyroidal status of the two groups since

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both belong to laying group, the resting being preparatory to active laying condition.

Next, the anticipated higher level of endogenous oestrogen in the layers which are sexually matured under the high level of oestrogen liberated by the matured ovarian follicles (Sturkie, 1954[b]) has been repoted to enhance the calcium and phosphorus level in blood (Sturkie, 1954[c]). This enhanced level of blood calcium due to high level of oestrogen in layers might be the possible cause of diminished serum alkaline phosphatase activity since it has been reported that in laying phase, due to increased deposition of calcium in egg shell ~~which~~ is accompanied by a reduced activity of alkaline phosphatase (Rako et al 1964).

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T A B L E (XXI)

Table showing the serum alkaline phosphatase values (King-Armstrong Units/100 ml. serum).

Active layer group.	Resting layer group.	Non-layer group.
28.3	10.0	20.5
28.3	11.1	20.5
15.5	10.0	29.4
18.3	10.0	16.6
10.0	13.8	29.4
10.0	19.0	21.6
10.5	21.6	20.5
-	-	26.1
-	-	22.2
-	-	17.2
-	-	17.2
-	-	17.2
-	-	16.4
-	-	18.3
Average with S.E. 17.27±3.08	13.64±1.81	20.94±1.19

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T A B L E (XXII)

Analysis of variance of serum alkaline phosphatase values.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	456.19	228.09	7.15**
Within groups.	25	789.12	31.92	
Total:-	27	1254.31		

** - Highly significant.

T A B L E (XXIII)

Table showing the mean difference of serum alkaline phosphatase values with critical difference at 1 & 5 percent between the three groups of W.L.H. birds.

	Mean difference	C. D. at 1%	C. D. at 5%
Active vers. resting.	3.63	8.31	6.14
Active vrs. non-layer.	3.67	8.17	6.04
Resting vrs. non-layer.	7.30	8.17	6.04*

* - Significant at 5% level.

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CHAPTER - VI.

ASCORBATE ACTIVITY IN BLOOD & TISSUE.

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C H A P T E R - VI

ASCORBATE ACTIVITY IN BLOOD AND TISSUES

INTRODUCTORY:

There are conflicting views in literature regarding the effect of stress condition on the general functioning of chick adrenals. While in mammals adrenal hypertrophy and increased outpouring of cortical hormones have been observed, under the influence of stress condition (Tepperman et al 1943), similar adrenal response in chicks was reported to be doubtful (Bates et al, 1940). On the other hand more recent observations (Jailer & Boas, 1950) indicate that the adrenal hypertrophy does take place under the action of ACTH and epinephrine in chicks (Response of Pituitary and Adrenal axis).

It was also observed by Sayers (1950) that in mammals there is increased secretion of Adrenaline and a marked depletion of adrenal ascorbic acid, under the influence of stress but that the same was not true in poultry (Jailer & Boas, 1950).

There are reports that sex hormones may play a part in the regulation of blood, tissue and urinary level of ascorbic acid (Souders & Varozza, 1958). Ascorbic acid is synthesised in the kidney of birds and not in the liver, as in mammals (Roy & Guha, 1958). Observations in

certain experiments indicate that certain amount of ascorbic acid is stored in the corpus luteum and interstitial tissues of ovary (Ramsteyn, 1941). It has been shown that cholesterol is an important precursor for oestrogen bio-synthesis in the ovaries and that conversion of cholesterol to oestrogen has been shown to be dependant upon the ascorbic acid (Claesson & Hillarp, 1947) Noach & Van Rees, 1958). Hence it is plausible to think that oestrogen, a hormone from ovary might be related with the ascorbic acid utilisation.

Due to the evidence of conflicting views as indicated above regarding the response of chicks adrenal gland, its secretion and ascorbic acid content under the influence of normal physiological stress (egg laying in present investigation), it was considered desirable to study the ascorbic acid activity in blood, adrenal, ovary and kidney of poultry during non - laying, actively laying and resting laying conditions.

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S E C T I O N - I

BLOOD ASCORBIC ACID.

The birds as classified in previous chapters in three groups namely, active layer, resting layer and non - layer were sacrificed by decapitation and the determination of ascorbic acid was carried out in the collected blood. The blood was not allowed to clot by the addition of sodium oxalate (B. D. H.). The ascorbic acid content was determined by the method of Roe (1961), a modification of Roe and Kuether (1943) which is based on the following principle:-

Roe and Kuether (1943) used the coupling reaction of dehydro-ascorbic acid with 2, 4 - dinitrophenyl-hydrazine for the determination of total ascorbic acid in body fluids. In this method, l-ascorbic acid is first oxidised to dehydroascorbic acid by the addition of 'Norit' to the filtrate. A three hours incubation period at $37^{\circ}C$ with 2, 4-dinitrophenylhydrazine in the presence of thiourea is followed by final colour development with 85% sulphuric acid. A reducing substance like thiourea is used in the incubation period to prevent Oxidants like Fe^{+++} and H_2O_2 from interfering in the final colour development. 85% sulphuric acid tends first to dissolve interfering substances like glucose, fructose and arabinose and then causes the colour produced by them to fade until little

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or no interference occurs at the wavelength used. Trichloroacetic acid is the best precipitating agent for blood proteins and it also stablizes the l-ascorbic acid and reduces the pH of the extracting media. Maximal colour development is obtained with 85% sulphuric acid.

REAGENTS AND SOLUTIONS:

(i) 4 percent trichloro acetic acid :-

4 gms. of trichloroacetic acid was dissolved in 100 ml. of distilled water.

(ii) Standard Ascorbic acid solutions:-

Solutions of different concentrations from 5 microgramme to 50 microgramme ascorbic acid (E. Merck) per c.c. were prepared in 4% trichloroacetic acid.

(iii) Acid washed "NORIT" :-

Suspended 100 gms. "Norit" in 500 ml. 10 percent hydrochloric acid. Heated to boiling, then filtered with suction. Removed the cake of "Norit", stirred it up with 500 ml. of water, and filtered again. Repeated this procedure until the washings gave a negative or faint test for ferric ions. Dried overnight in an air oven at 110-120°C.

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(iv) 2, 4-Dinitrophenylhydrazine reagent:-

Dissolved 2 gms. of 2, 4-dinitrophenylhydrazine and 4 gms. of thiourea in 100 ml. of 9N sulphuric acid.

(v) 85 percent sulphuric acid :-

To 100 ml. distilled water, added 900 ml. concentrated sulphuric acid (sp.gr.1.84).

EXPERIMENTAL PROCEDURE:

In a 50 ml. centrifuge tube, 5 ml. of whole blood was added to 15 ml. of 4 percent trichloroacetic acid. It was stirred to obtain a fine suspension. Allowed to stand for five minutes and then centifuged. 0.75 g. of acid washed "Norit" was added to the clear supernatant solution. It was shaken vigorously and filtered. Four ml. aliquot of the filtrate was taken in a test tube and 1 ml. of 2, 4-dinitrophenylhydrazine reagent was added. The tube was placed in the water bath maintained at 37°C for exactly 3 hours. It was removed and placed in ice water bath. Then 5 ml. 85% sulphuric acid was added to it with stirring. Read the colour in the photelectric colorimeter with a filter transmitting maximally at 540 mμ setting the instrument at 100 percent transmittance with distilled water. This was done in duplicate. A blank experiment was run simultaneously.

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This was also passed through all the stages except that in place of blood, trichloroacetic acid was taken and 2, 4-dinitrophenylhydrazine was added in the last.

CALCULATION:

A calibration curve by testing 4 ml. aliquots of appropriate standards containing 5 µg to 50 µg ascorbic acid per ml. throughout the entire procedure. Photometric density was plotted against micro gramme ascorbic acid per ml. From the curve, ascorbic acid content of the blood sample was known.

RESULTS AND DISCUSSION:

The results of the blood ascorbic acid is presented in TABLE - (XXIV).

From the results it was found that total ascorbic acid in blood for the three groups - active layers, resting layers and non -layers did not differ significantly from each other.

The whole blood ascorbic acid level has been suggested to be a better index of the state of ascorbic acid nutrition of the tissue than blood plasma level (Hawk et al , 1954a). The insignificant difference of the ascorbic acid between the groups was in agreement with the findings of Jailer and Boas (1950) who reported that there

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was no depletion of adrenal tissue ascorbic acid due to injection of stress hormones like epinephrine and ACTH though it was anticipated that in laying birds which is believed to be a physiological stress, the ascorbic acid content of the tissue and blood might have gone depleted.

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T A B L E (XXIV)

Table showing the blood ascorbic acid content (mg./100 ml.).

Non-layers		Resting layers		Active layers.	
Expt. No.	A. Acid	Expt. No.	Ascorbic acid	Expt. No.	Ascorbic acid.
II	1.5	VIII	1.8	IV	1.5
III	1.5	XI	1.7	V	1.5
VI	1.8	XVI	2.0	IX	1.4
VII	2.3	XXI	2.0	XVII	2.0
X	1.2	XXII	2.2	XVIII	2.0
XII	2.2	XXIV	1.8	XIX	2.0
XIII	2.2	XXX	2.2	XX	2.0
XIV	2.2				
XV	2.2				
XXIII	2.2				
XXV	2.2				
XXVI	2.2				
XXVIII	2.2				
XXIX	2.4				
Average with S.E.	2.02±0.09		1.87±0.12		1.78±0.10

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S E C T I O N - II

ASCORBATE ACTIVITY AS MEASURED BY TISSUE OXYGEN CONSUMPTION.

MATERIALS :

The classification of birds in three groups namely active layer, resting layer and non-layer, remained the same as in previous experiments.

The tissues of adrenal, ovary and kidney of which oxygen consumptions were determined, were cleared of connective fibres, blood clot etc. and kept moist under ice.

REAGENTS:

(i) M/15 Phosphate buffer (pH-7.4):-

Was prepared by mixing 80.4 ml. of M/15 disodium phosphate to 19.6 ml. of M/15 potassium acid phosphate.

(ii) Sodium ascorbate (0.114 M) :-

Ascorbic acid (E.Merck)	2.052 gms.
Sodium hydroxide (B.D.H.)	0.456 gms.
Water to make up.	100 ml.

(iii) Cytochrome - C (10^{-5}M) :-

Cytochrome - C.	0.013 gm.
Water.	100 ml.

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(iv) 20 percent KOH solution:-

KOH (B.D.H.)	20 gms.
Water.	100 ml.

METHODS:

The oxygen uptake of the tissues of adrenal, ovary and kidney was determined in a conventional Warburg's apparatus at 40°C and 80-120 oscillations per minute by the method of Umbreit et al (1949). For this purpose, the manometers with their corresponding vessels were calibrated with mercury to find out the entire volume of gas space in the empty vessel, manometer side arm and manometer capillary down to the level of manometer fluid in order to calculate the changes in gas content in a vessel from the manometer readings under a particular set of experimental conditions. Thus the vessel constants were calculated for all the manometers with their vessels. One manometer was kept as thermobarometric control to correct the pressure changes due to atmospheric pressure, and temperature of the water bath. The principle of Warburg's working procedure is briefly as follows:-

Tissue is incubated at body temperature in a suitable buffered medium, in a vessel with attached manometer, the gas phase being air. The centre well of the vessel contains a little of strong alkali solution which absorbs

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any carbondioxide produced, so that any pressure changes are due to Oxygen consumption alone. The side bulbs provide for the addition of substrates, activators, inhibitors etc. during an experiment if desired. The liquid phase in vessel and gas phase in vessel and manometer capillaries are kept at constant volume. Oxygen consumption is measured therefore by a fall in pressure which is read on the manometer. The pressure readings when multiplied by a constant ("vessel constant") give the Oxygen consumption, usually expressed in microlitres and is symbolized by Xo_2. Readings are made at suitable intervals, (every 15 minutes for 1 hour).

Throughout the whole experiment the same vessels and manometers were used for a specific tissue as described by Ray et al (1965) with slight modification. In each of the clean and dry Warburg's flask of known "flask constants", the fluid volume was kept constant at 2 ml., avoiding the central well. A homogenate of the desired tissue in 1:20 dilution (W/V) was prepared and 0.5 ml. homogenate was taken in the vessel. To it 0.5 ml. Cytochrome-C and 0.6 ml. of M/15 Phosphate buffer were added. Then 0.3 ml. of sodium ascorbate was taken in the side arm of the vessel. After that 0.1 ml. of 20 percent KOH solution was placed in the central well, using a pipette with fine tip. No alkali was allowed to get into the medium surrounding the centre well. A small roll of starch free filter paper (Whatman No. 40)

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was placed in the centre well so that the top of the roll projected a millimeter or two above the rim of the centre well, to absorb the carbondioxide produced from the tissues.

After properly fixing the flasks to the corresponding manometers, the flasks were placed in the Warburg's water bath at 40°C with manometer's stopcocks opened and temperature equilibrated for ten minutes. The manometer stopcocks were then closed and manometeric fluid were raised so that the level in the open limbs was several centimeters higher than in the closed limb. The reading in the closed limb was adjusted to 15.0 cm. and the reading of the open limb then read and recorded. The substrates in the side arm were then tipped into the main compartment. While removing manometers and attached flasks from the water bath for tipping substrates, a finger was placed over the open end of the manometer to prevent the rapid expansion or contraction due to temperature changes from either pushing out or sucking back the fluid in the manometer. One manometer with its flask containing 2 ml. distilled water, equilibrated for 10 minutes and set at 15.0 cm. mark was used as thermobarometric control. Manometric readings were taken at every 15 minutes for one hour incubation and oscillation 110 per minute, after readjusting the level in the closed limb back to 15.0 cm. All precautions outlined by Umbriet et al (1949) were observed.

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Datas were thus obtained on the oxygen uptake of tissues of adrenal, ovary and kidney. The difference between the initial and final manometric readings was corrected for the variation in thermobarometric readings. The microlitres of oxygen utilised were calculated by multiplying the manometric value with the corresponding flask constants.

RESULTS AND DISCUSSION:

The results of oxygen consumption by adrenal, ovarian and renal tissue of the active, resting and non-laying groups are presented in TABLES (XXV to XXXVI).

It was evident from the tables that the oxygen consumption of the adrenal tissue of the active laying and non-laying birds was significantly higher than the resting group and also the oxygen consumption was significantly different from each other at all the time intervals e.g., at 15 minutes, 30 minutes, 45 minutes and 60 minutes. However, the oxygen consumption of the ovary and kidney was not significantly different from each other group.

It is understood that ascorbic acid oxidation of the tissue is measured by oxygen consumption of the particular tissue (Ray et al, 1965) since utilisation of ascorbic acid by the tissues necessitates the uptake of oxygen. Therefore the higher oxygen consumption by the

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adrenal tissue of the laying birds shows that adrenals consume much oxygen in order to utilise the ascorbic acid to withstand the physiological stress of laying since metabolic state of the body is supposed to be very high and so particularly that of the adrenal gland. The higher concentration of vitamins are found in tissues of high metabolic activity, eg., adrenal, pituitary and intestinal wall as reported by Hawk et al (1954).

Hence, it is plausible to think that the higher metabolic activity in the active laying and non-laying (growing stage) birds might be related to the observed higher value for oxygen consumption by the adrenal tissue of the active laying and non-laying birds than the resting layers in which the metabolism is supposed to be less since the birds are in resting condition. In confirmation of above, Thornton & Deeb (1961) has reported that ascorbic acid synthesis is dependant on metabolic rate of birds because of its possible involvement in shell formation process which can be true only with the laying birds. And this higher rate of synthesis might be anticipated to be related with higher storage rate chiefly in adrenals as was in-deed observed.

There was no marked difference in the oxygen uptake by the ovarian and renal tissues between the three groups which might be due to the fact that these organs are neither concerned much with the ascorbic acid metabolism nor have been reported to possess high ascorbic acid storing capacity.

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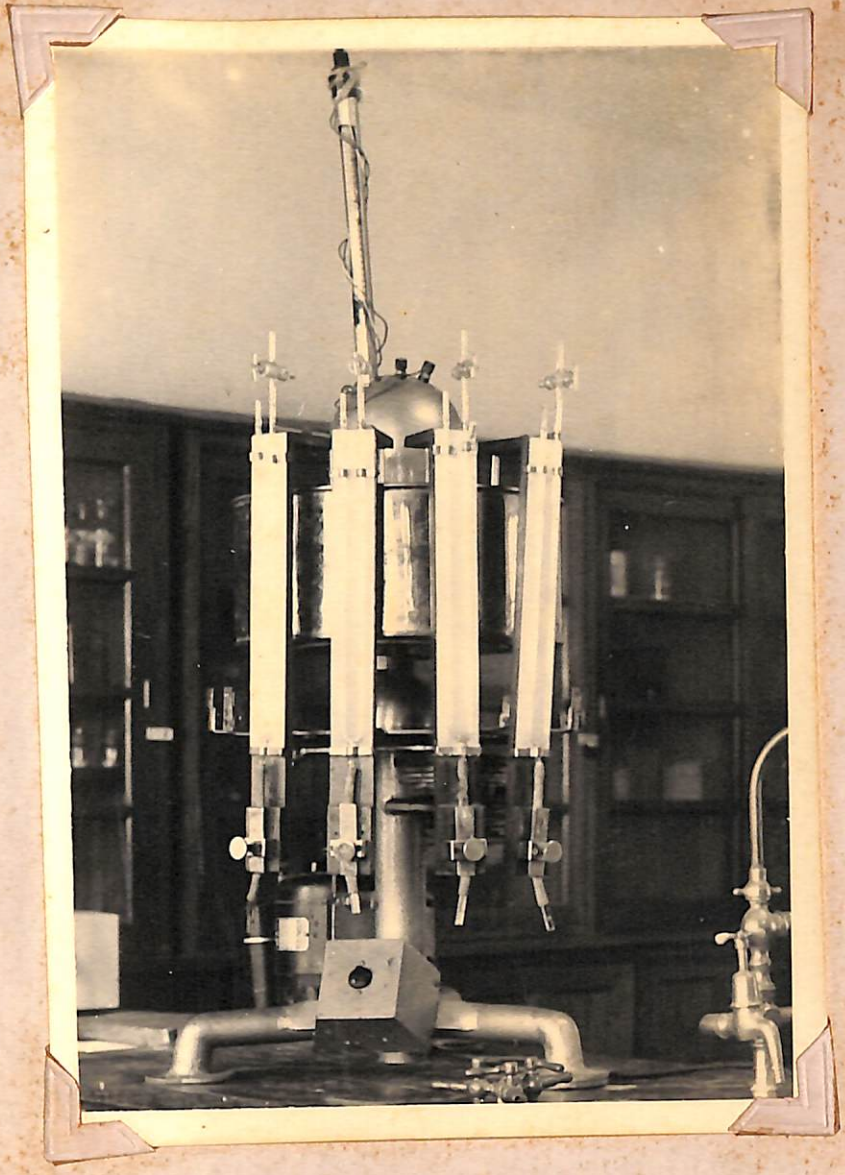
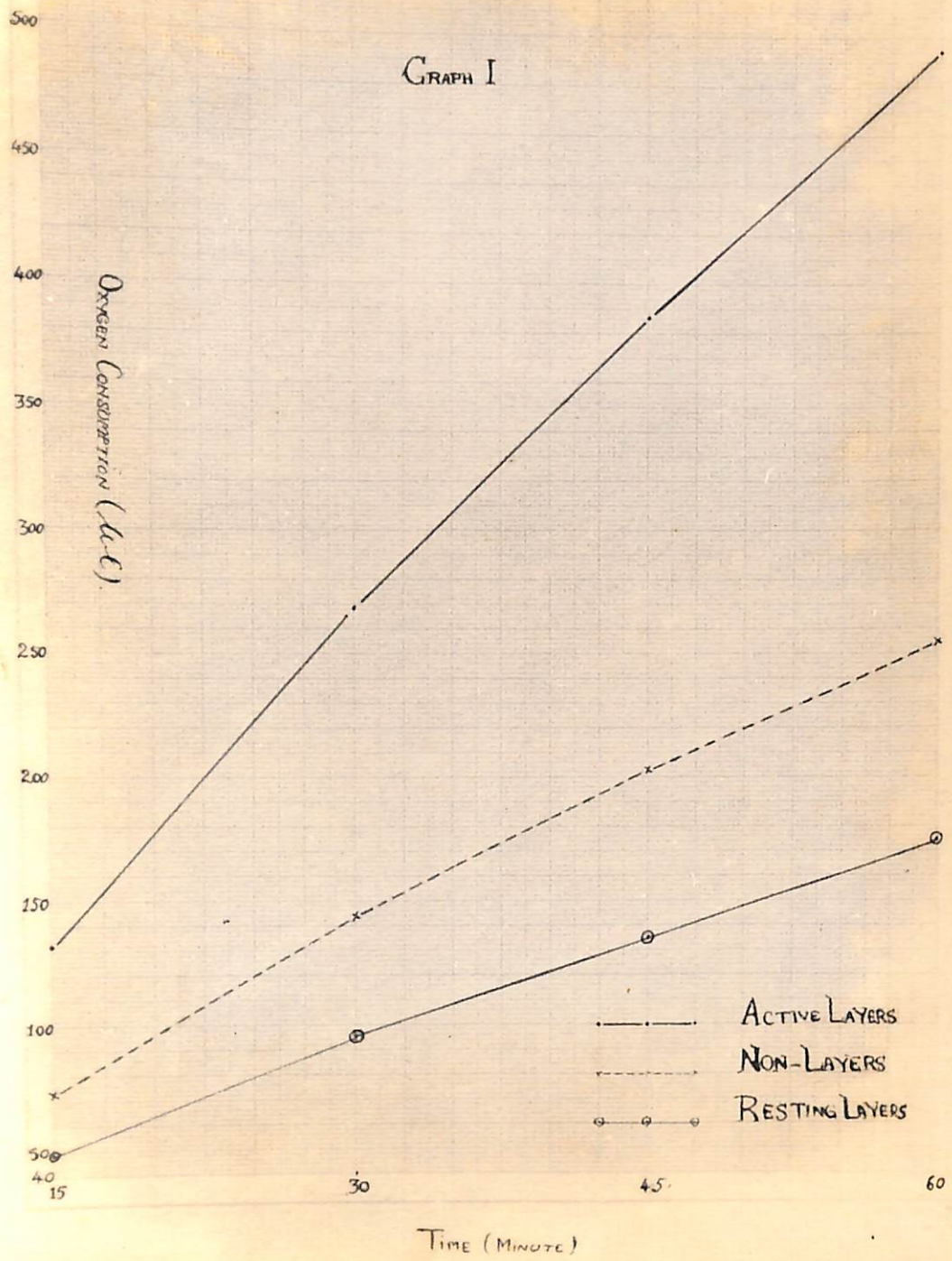
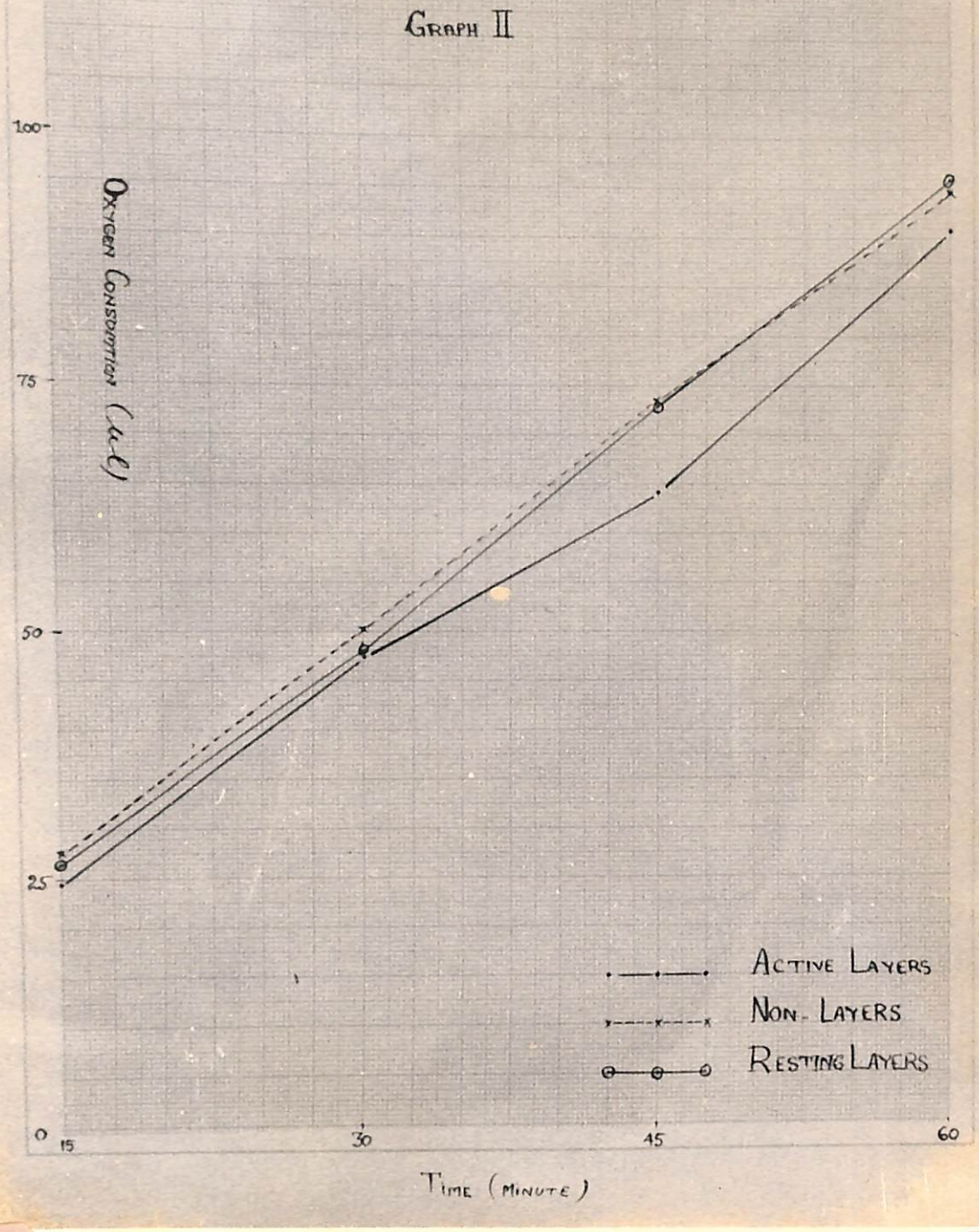


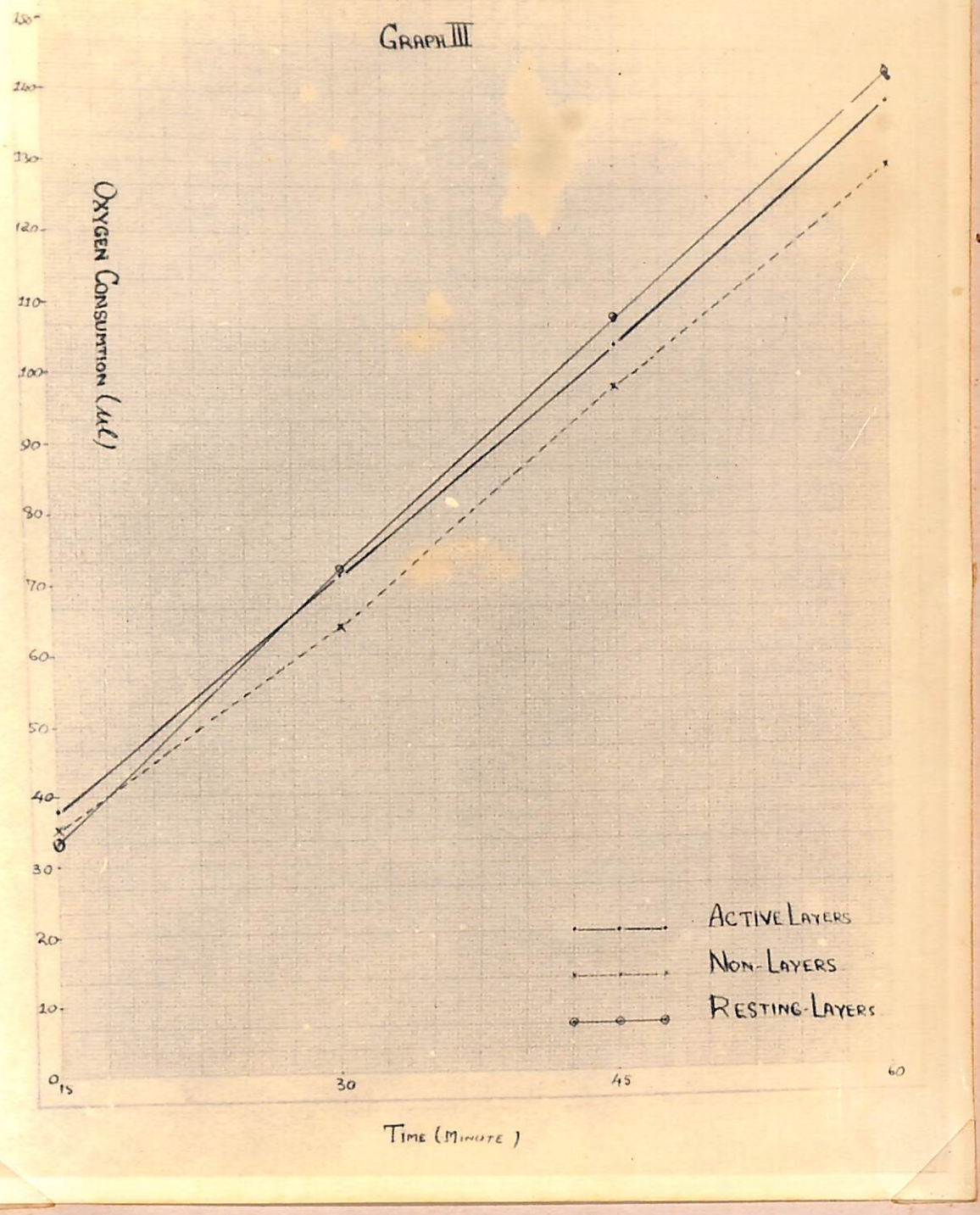
Photograph showing the Barcroft - Warburg apparatus on which ascorbate activity in the tissues was studied.



Ascorbic acid activity in the adrenal glands of active layers, resting layers and non-layers, expressed as the rate of oxygen consumption, with sodium ascorbate (0.114M) as substrate, against time in minutes. Each point is the mean value with its S.E.



Ascorbic acid activity in the ovary tissue of active layers, resting layers and non-layers, expressed as the rate of oxygen consumption, with sodium ascorbate (0.114M) as substrate, against time in minutes. Each point is the mean value with its S.E.



Ascorbic acid activity in the kidney tissue of active layers, resting layers and non-layers, expressed as the rate of oxygen consumption, with sodium ascorbate (0.114M) as substrate, against time in minutes. Each point is the mean value with its S.E.

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TABLE (XXV)

Table showing the ascorbic acid activity in adrenal tissue of active layers expressed in terms of oxygen consumption (micro-litres).

Experiment no.	Oxygen consumption at 15 mts. interval with	Oxygen consumption at 30 mts. interval.	Oxygen consumption at 45 mts. interval.	Oxygen consumption at 60 mts. interval
XVII	127.17	248.06	362.67	478.85
XVIII	136.59	288.88	405.06	499.26
XIX	130.31	263.76	394.07	508.68
Average with S.E.	131.36 ±2.7	266.90±12.1	387.26± 12.8	495.59± 9.05

TABLE (XXVI)

Table showing the ascorbic acid activity in adrenal tissue of resting layers expressed in terms of oxygen consumption (micro-mlitres).

Ept. No.	Oxygen consumption at 15 mts. interval.	Oxygen consumption at 30 mts. interval.	Oxygen consumption at 45 mts. interval.	Oxygen consumption at 60 mts. interval.
XVI	43.96	89.49	128.74	167.99
XXI	58.09	98.91	141.30	183.69
XXII	62.80	111.47	150.72	186.83
XXIV	39.25	86.35	131.88	164.85
XXX	43.96	89.49	127.17	167.99
Average with S.E.	49.61±4.6	95.14±4.6	135.96±4.5	174.27±13.9

T A B L E (XXVII)

Table showing the ascorbic acid activity in adrenal tissue of non-layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 mts. interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
XII	78.50	153.86	207.24	241.78
XIII	69.08	135.02	200.96	266.90
XIV	75.36	141.30	207.24	266.90
XV	81.64	160.14	213.52	263.76
XXIII	78.50	153.86	202.53	241.78
XXV	69.08	144.44	216.66	266.90
XXVI	58.09	152.29	221.37	266.90
XXVIII	67.51	105.19	149.15	222.94
Average with S.E.	72.22±2.70	143.26±6.10	202.33±8.03	254.73±6.03

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T A B L E (XXVIII)

Table showing the ascorbic acid activity in ovary tissue of active layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
IV	21.00	38.50	54.25	75.25
V	10.50	33.25	50.75	72.15
IX	15.75	36.75	63.00	80.50
XVII	26.25	54.25	78.75	110.25
XVIII	36.75	61.25	82.25	106.75
XIX	28.00	50.75	71.75	94.50
XX	33.25	57.75	77.00	96.25
Average with S.E.	24.50±3.5	47.50±4.3	68.25±4.7	90.81±5.8

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T A B L E (XXIX)

Table showing the ascorbic acid activity in ovary tissue of resting layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
VIII	12.25	31.50	54.25	71.75
XI	15.75	29.75	54.25	82.25
XVI	22.75	49.00	75.25	94.50
XXI	38.50	52.50	77.00	96.25
XXII	33.25	56.00	80.50	108.50
XXIV	38.50	67.50	96.25	120.75
XXX	24.50	50.75	75.25	98.00
Average with S.E.	26.50±4.03	48.14±5.1	73.25±5.6	96.00±4.9

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T A B L E (XXX)

Table showing the ascorbic acid activity in ovary tissue of non-layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
II	22.75	43.75	64.75	85.77
III	18.25	42.00	68.25	87.50
VI	21.00	47.25	71.75	89.25
VII	24.50	45.50	68.25	89.25
X	15.75	33.25	59.50	80.50
XII	26.25	50.75	71.25	92.75
XIII	26.25	50.75	70.00	96.25
XIV	22.75	40.25	63.00	82.25
XV	28.00	47.25	64.75	82.25
XXIII	31.50	61.25	85.75	108.50
XXV	29.75	50.75	73.50	96.25
XXVI	29.75	52.50	77.00	105.00
XXVIII	43.75	68.25	91.00	110.25
XXIX	47.25	71.75	98.00	119.00
Average with S.E.	27.67±2.3	50.37±2.8	73.37±3.3	94.62±4.6

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T A B L E (XXXI)

Table showing the ascorbic acid activity in kidney tissue of active layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
IV	28.48	51.62	72.98	106.80
V	21.36	49.84	81.88	108.58
IX	26.70	58.74	92.56	124.60
XVII	40.94	78.32	113.92	156.64
XVIII	42.72	80.10	117.48	153.08
XIX	64.08	99.68	137.06	178.00
XX	44.50	81.88	113.92	147.70
Average with S.E.	38.39±5.50	71.45±7.10	104.25±8.60	139.35±10.20

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T A B L E (XXXII)

Table showing the ascorbic acid activity in kidney tissue of resting layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
VIII	24.92	55.18	81.88	101.46
XI	37.38	71.20	113.92	158.42
XVI	26.70	67.64	103.24	142.40
XXI	55.18	87.22	131.72	169.10
XXII	35.60	67.64	97.90	131.62
XXIV	40.94	85.44	122.82	160.20
XXX	26.70	67.64	103.24	142.40
Average with S.E.	33.63±4.10	71.70±4.20	107.81±5.90	143.65±8.60

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T A B L E (XXXIII)

Table showing the ascorbic acid activity in kidney tissue of non-layers expressed in terms of oxygen consumption (micro-litres).

Expt. No.	Oxygen consumption at 15 minutes interval.	Oxygen consumption at 30 minutes interval.	Oxygen consumption at 45 minutes interval.	Oxygen consumption at 60 minutes interval.
II	35.60	67.64	99.68	128.16
III	44.50	65.86	103.24	138.84
VI	35.60	53.40	105.02	131.72
VII	40.94	72.98	105.02	135.28
X	23.14	48.06	90.78	121.04
XII	33.82	62.30	92.56	126.38
XIII	32.04	65.86	92.56	126.38
XIV	24.92	57.96	96.12	126.38
XV	42.72	71.20	97.90	121.04
XXIII	35.60	67.64	94.34	124.60
XXV	37.38	62.30	90.78	128.16
XXIX	39.16	80.10	115.70	151.30
Average with S.E.	35.45±1.80	64.60±2.50	98.64±2.10	129.94±2.10

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T A B L E (XXXIV)

Summary of the average ascorbic acid activity in adrenal tissue expressed in terms of oxygen consumption (micro-litres).

Group.	Oxygen consumption at 15 minutes interval with S. E.	Oxygen consumption at 30 minutes interval with S. E.	Oxygen consumption at 45 minutes interval with S.E.	Oxygen consumption at 60 minutes interval with S.E.	Percentage increase in Oxygen consumption at 1 hr. level.
Active layer.	131.35±2.7	266.90±12.1	387.26±12.8	495.59±9.05	285%
Non-layer.	72.22±2.7	143.26±6.1	202.33±8.03	254.73±6.03	146%
Resting layer.	49.61±4.6	95.14±4.6	135.96±4.5	174.27±13.9	-

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CHAPTER - VII.

SUMMARY AND CONCLUSION.

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C H A P T E R - VII

SUMMARY AND CONCLUSION

A total of twenty eight hens of White Leghorn breed were used in this study which were divided into three groups namely active layers, resting layers and non-layers on the basis of presence or absence of egg in reproductive tract and the state of reproductive organs as regards their enlarged or atrophied condition. Difference in organic weights of endocrine and reproductive tract, serum alkaline phosphatase level, and ascorbate activity in blood, adrenal ovary and kidney were recorded between all the three groups which were as follows:-

1. The adrenal weight between the groups was not significantly different from each other. However thyroid weight in non-layers was significantly greater than resting layers at 1% level but no significant difference between the thyroid weight of active and resting groups and so also between active layers and the non-laying birds was observed. There was also no significant difference in pituitary weight between the groups. The absence of any significant increase in the adrenal weight of active layers than either of resting and non-layers might be due to non-influence of anticipated higher level of oestrogen in laying birds in causing an increase in adrenal weight. Similarly, the insignificant difference between the adrenal weight of resting and

non-laying birds might be accounted also for the same non-influence of oestrogen on adrenal weight. The significant increase in thyroid weight in non-layers than the birds of the laying group might have been due to increased thyroidal activity in non-laying condition on account of their being in growing stage. Likewise, the insignificant difference in the pituitary between the layers and non-layers might be due to the fact that high oestrogenic level (as anticipated in layers) has no effect on the pituitary weight.

2. There was a marked significant difference in the weight of the different segments of the oviduct between the three groups of birds. The weights of infundibulum, magnum and isthmus of the active layers were significantly higher than non-layers and resting layers but the same were not significantly different in resting layers than non-layer group. However, the weight of uterus and vagina taken together were significantly higher in resting than the birds of the non-layer group. The higher weight of oviduct in the active layers than non-laying birds might be the result of higher oestrogenic level in layers than in non-layers. The increase in the oviduct weight of active layers than the resting layers might be the effect of higher level of oestrogen in active layers than resting ones. Also the increase in the uterine and vaginal weight taken together might be accounted for the same elevated level of oestrogen in resting than non-layers.

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3. The serum alkaline phosphatase was significantly higher in non-layers than resting layers but the difference between the non-layers and active layers was not significant, although the enzyme activity level was lower apparently in active layers than birds of the non-layer group. Also, the same insignificant difference was observed between layers of the active and resting groups. The higher level of the enzyme activity in non-layers than resting layers might be accounted for the difference in the level of oestrogen and thyroidal harmone on account of the anticipated higher level of thyroidal hormone in the non-laying birds which are in growing stage than resting and active layers. The diminished serum alkaline phosphatase activity in layers might be due to the high level of oestrogen in laying birds mediated through an enhanced blood calcium level.

4. The blood total ascorbic acid did not differ significantly between the groups which was perhaps due to confirmed fact that there does not occur any depletion of ascorbic acid in birds due to stress.

5. The oxygen consumption of the adrenal tissue of the active laying and non-laying birds was significantly higher than the resting group and also the oxygen consumption was significantly different from each other at all the time intervals e.g., 15 minutes, 30 minutes, 45 minutes, and 60 minutes. However, the oxygen consumption of the ovary and kidney tissue was not significantly different from each other group.

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The higher oxygen uptake by the adrenal of the laying birds might be the results of increased utilisation of ascorbic acid by the adrenal gland to withstand the physiological stress of laying since ascorbic acid oxidation of tissues are indicated by their oxygen consumption.

Similarily, the insignificant difference observed in the oxygen consumption by the ovary and kidney between the three groups might be supposed to be due to the fact that these organs are neither concerned much with the ascorbic acid metabolism nor have been reported to possess high ascorbic acid storing capacity.

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CHAPTER - VIII.

B I B L I O G R A P H Y.

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B I B L I O G R A P H Y.

1. Arvy, L and M. Gabe (1951) Comp. Rend. Acad. Sci; Paris, 232:260.
   Cited by Sturkie, P. D (1954) in Avian Physiology (1st Ed.) Comstock Publishing Associates, Ithaca, N.Y. Pp.408.

2. Auchinachie, D.W & Emsle, A.R.G (1934) Biochem.J, 28:1993
   Cited by Bell, D.J (1960). Biochem. J.75:224

3. Barnes, D. J & Carpenter, M. D (1937). J.Pediat, 10:596
   Cited by Motzok, I and Wynne, A. M (1950). Biochem. J, 47:187.

4. Bates, R.W; Riddle, O; Miller, R.A. (1940)
   Preparation of adrenocorticotrophic extracts and their assay on two-day chicks.
   Endocrinology, 27:781.
   Cited by Sturkie, P. D (1954) in Avian Physiology (1st Ed.) Pp.407.

5. Bell, D. J (1960). Tissue components of the domestic fowl. 4 Plasma-alkalinephosphatase activity.
   Biochem. J, 75:224-229.

6. Beznak, A (1923). Biochem. Z, 141:1
   Cited by Sturkie, P. D (1954) in Avian Physiology (1st Ed.), Pp.408.

7. Blaxter, K.L; E.P. Reineke; E.W. Crampton and W.E. Petersen (1949).
   The role of thyroidal materials and synthetic goitrogens in animal production and an appraisal of their practical use. J.Animal Sci., 8:307
   Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.), Pp.367.

8. Bodansky, A and Jaffe, H.L (1934a) Amer.J.Dist.Child.48:1268.
   Cited by Motzok, I and Wynne, A.M.(1950) Biochem. J; 47:187

9. Bodansky, A and Jaffe, H.L (1934b), Arach. intern.Med. 54:88
   Cited by Motzok, I and Wynne, A.M (1950) Biochem. J; 47:187

10. Bourne, G.H. Ed. The Biochemistry and Physiology of Bone (Academic Press, Inc., New York, N.Y., 875 Pp.1956.
Cited from Annual rev. of Physiology, 21:76,1959

11. Breneman, W.R (1942) Action of diethylstilboestrol in the chick. Endocrinology, 31:179
Cited by Sturkie, P. D (1954) in Avian Physiology (1st Ed.), Pp.337

12. Brown, K. I; D.J. Brown and R.K. Meyer (1958)
Effect of Surgical Trauma, ACTH and adrenal cortical hormones on electrolytes, water balance and gluconeogenesis in male chickens.
Am. J.Physiol, 192:43-50

13. Brown, W.O & Badman, H.G (1961).
Effects of Oestradiol and Progesterone on serum alkaline phosphatase, Poultry Sci., 40:819

14. Claesson, L;& Hillarp (1947).
Acta Physiol. Scand., 14:102
Cited in Biol.Abs, 22:2390, 1948

15. Common, R. H (1934).
Serum Phosphatase in the domestic fowl.
Nature, 133:572
Cited by Tanabe, Y and Wilcox, F. H (1961),
Poultry Sci., 40:411

16. Common, R. H (1936).
Serum Phosphatase in the domestic fowl.
J.Agr.Sci. 26:492
Cited by Tanabe, Y and Wilcox, F. H (1961)
Poultry Sci. 40:411

17. Common, R. H (1941) J.Agric. Sci., 31:412
Cited by Bell, D. J (1960).
Biochem. J. 75:224

18. Conner, M. H (1959)
Effect of various hormone preparations and nutritional stresses in chicks.
Poultry Sci. 38:1340-1343

19. Crile, G and D. P. Quiring (1940)
A record of body weight and certain organ and gland weights of 3690 animals
Ohio. J.Sci. 40:219
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 405

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

20. Cruickshank, E.M (1929)
The iodine content of the thyroid and ovary of the fowl during growth, laying and molting periods. Bioch. J. 23: 1044
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.349

21. Delory, G. E & King, E.J (1944)
Biochem., J. 38:50

22. Dringgers, J. C and Connar, C. L (1949)
Poultry Sci. 28:420

23. Flickinger, D. D (1959)
Adrenal responses of California quail subject to various physiologic stimuli.
Proc. Soc. Exp. Biol. Med. 100:23-25
Cited by Wolford, J. H and Ringer, R. K (1962)
Poultry Sci. 41:1521

24. Galpin, N (1938)
Factors affecting the hatching weight of Brown Leghorn chickens.
Proc.Roy.Soc. Edinburgh, 58:98
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.349

25. Garren, H. W and C.S. Shaffner (1952)
The response of young New Hampshire chickens to conditions of stress. Poultry Sci. 31:917

26. Garren, H.W and C.S. Shaffner (1954)
Factors concerned in the response of young New Hampshires to muscular fatigue
Poultry Sci. 33:1095-1104

27. Garren, H.W and C.S. Shaffner (1956)
How the period of exposure of different stress stimuli affects the endocrine and lymphatic gland weight of young chickens.
Poultry Sci. 35:266-272

28. Gould, B.S (1944) Arch.Biochem. 4:175
Cited by Motzok, I. Biochem. J. 47:193-196, 1950

29. Greer, M.A.
Nutrition and Goitre. Physiol. Rev. 30:513
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.349

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

30. Gutowska, M. S & Mitchell, C.A (1945)
Poultry Sci. 24:159
Cited by Bell, D. J (1960) Biochem.J,75:224

31. Hall, G. E and King, E. J (1930-31)
Poultry Sci. 10:132
Cited by Bell, D.J (1960) Biochem.J. 75:224

32. Harms, R. H and Waldroup, P.W (1961)
The influence of dietary calcium level and supplementary ascorbic acid and/or Dienestrol Diacetate upon performance of egg production type hen, Poultry Sci. 40:1345

33. Hartman, F. A and K.A. Brownell (1949)
The adrenal gland. Lea and Fibiger, Philadelphia.
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 404

34. Hawk, P.B.; Oser, B.L and Summerson, W.H (1954a)
Practical physiological chemistry (13th Ed.)
McGraw Hill Book Company Inc. N.Y. Pp.1235

35. Hawk, P. B.; Oser, B.L and Summerson, W. H (1954b)
Practical Physiological Chemistry (13th Ed.)
McGraw Hill Book Company Inc.N.Y. Pp.1230

36. Heywang, B.W and A.R, Kemmerer (1955)
The effect of procaine penicillin and ascorbic acid on egg weight and shell thickness during hot weather. Poultry Sci. 34:1032

37. Hoffman, E; C.S, Shaffner and R.E, Comstock (1953)
Strain comparisons of gland weights of twelve week old broilers. Poultry Sci. 32:106-110

38. Jailer, J.W and Boas, N.F (1950)
The inability of epinephrine or adrenocorticotrophic hormone to deplete the ascorbic acid content of the chick adrenal. Endocrinology, 45:312
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.407

39. Jailer, J.W; N.F. Boas (1954)
The inability of Epinephrine or adrenocorticotrophic hormone to deplete the ascorbic acid content of the chick adrenal. Endocrinol, 46:314-318
Cited by Wolfor, J.H and Ringer, R.K (1962)
Poultry Sci. 41:1521

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

40. Jowsey, J. R; Berlie, M.R; Spinks, J.W.T & O'Neil, J.B (1956) Poultry Sci. 38:1234

41. Kar, A. B (1947)
The action of male and female sex hormones on the adrenals in the fowl. Anat. Rec. 97:551
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 337

42. Klasmer, R (1944) Amer. J.Dist.Child. 67:348
Cited by Motzok, I and Wynne, A. M (1950) Biochem. J. 47:187

43. Kobayashi, H;K; Muruyama and S.Kambara (1955)
Effect of thyroxine on the phosphatase activity of pigeon skin. Endocrin, 57:129-133
Cited by Tanabe, Y and Wilcox, F.H (1961) Poultry Sci. 40:411

44. Kumaran, J.D.S and C.W.Tuner (1949a)
The endocrinology of spermatogenesis in birds. I: Effect of oestrogen and androgen
Poultry Sci. 28:593
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 336 and 337

45. Kumaran, J.D.S and C.W.Turner (1949b)
The endocrinology of spermatogenesis in birds. II. The effect of androgens.
Poultry Sci. 28:739
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.337

46. Landauer, W; C.A.Pfeiffer; W.W.Gardner and J.C.Shaw(1941)
Blood serum and skeletal change in two breeds of ducks receiving oestrogen. Endocrinol.28:458
Cited by Brown, W.O and Badman, H. G (1961) Poultry Sci. 40:819

47. Martin, W.G and Patrick, H (1961)
The effect of oral doeses of CA^{45} to chicks on changes in serum alkaline Phosphatase.
Poultry Sci. 40:1360

48. McLean, F.C and Budy, M (1959)
Connective and supporting tissue; Bone.
Annual Rev. Physiol. 21:69

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

49. Miller, R.A and O. Riddle (1939)
Stimulation of adrenal Cortex of pigeons by anterior pituitary hormones.
Proc. Soc. Exp. Biol. & Med. 41:518
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 337

50. Miller, R.A and O. Riddle (1941)
Cellular response to insulin in suprarenals of pigeons. Proc. Soc. Exp. Biol. & Med. 47:449
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.408

51. Miller, R.A and O. Riddle (1942)
The cytology of the adrenal cortex of normal pigeons and in experimentally induced strophy and hypertrophy. Am. J. Anat. 71:311
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.408

52. Moog, F (1946), Biol. Rev. 21:41
Cited by Motzok, I. and Wynne, A.M(1950) Biochem. J; 47:187

53. Motzok, I and Wynne, A.M(1950)
Studies on the plasma phosphatase of normal and rachitic chicks. 1. General characteristic of the enzyme. Biochem. J, 47:187-192

54. Motzok, I (1950a)
Studies on the plasma phosphatase of normal and rachitic chicks. 2. Relationship between plasma phosphatase and the phosphatase of bone, kidney, liver and intestinal mucosa. Biochem. J, 47:193-196.

55. Motozok, I (1950b) Biochem.J. 47:193-196

56. Nestor, K.E and Jaap, R.G (1963)
Bi-weekly gland and tissue weights in Young North Central Regional Randombered Leghorn Chickens. Poultry Sci. 42:989

57. Noach, E.E and VanRees (1958)
Acta Endocrinology, 27:502
Cited in Biol. Abs; 33:3414; 1959

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

58. Oakberg, E.F (1951)
Genetic differences in quantitative histology of the adrenal, organ weights and inter organ correlations in White Leghorn chickens.
Growth, 15:57-58
Cited by Nestor, K.E and Jaap, R.G (1963).
Poultry Sci., 42:989

59. Patton, A.R; H.S, Wilgus and G.S, Harshfied (1939)
The production of goitre in chickens.
Science, 89:162
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 349

60. Pepper, W.F; Winget C.M and Slinger, S.J (1961)
Influence of Calcium and ascorbic acid on egg quality. Poultry Sci. 40:657

61. Perek, M and B, Eckstein (1959)
The adrenal ascorbic acid content of Molting hens and the effect of ACTH on the adrenal ascorbic acid content of laying hens.
Poultry Sci. 38:996-999

62. Rako, A; Dumanovsky, F and Nikulec, K (1964)
On the relation between laying capacity and the activity of some enzyme, the level of serum protein and blood sugar in hens.
Poultry Sci. 43:201

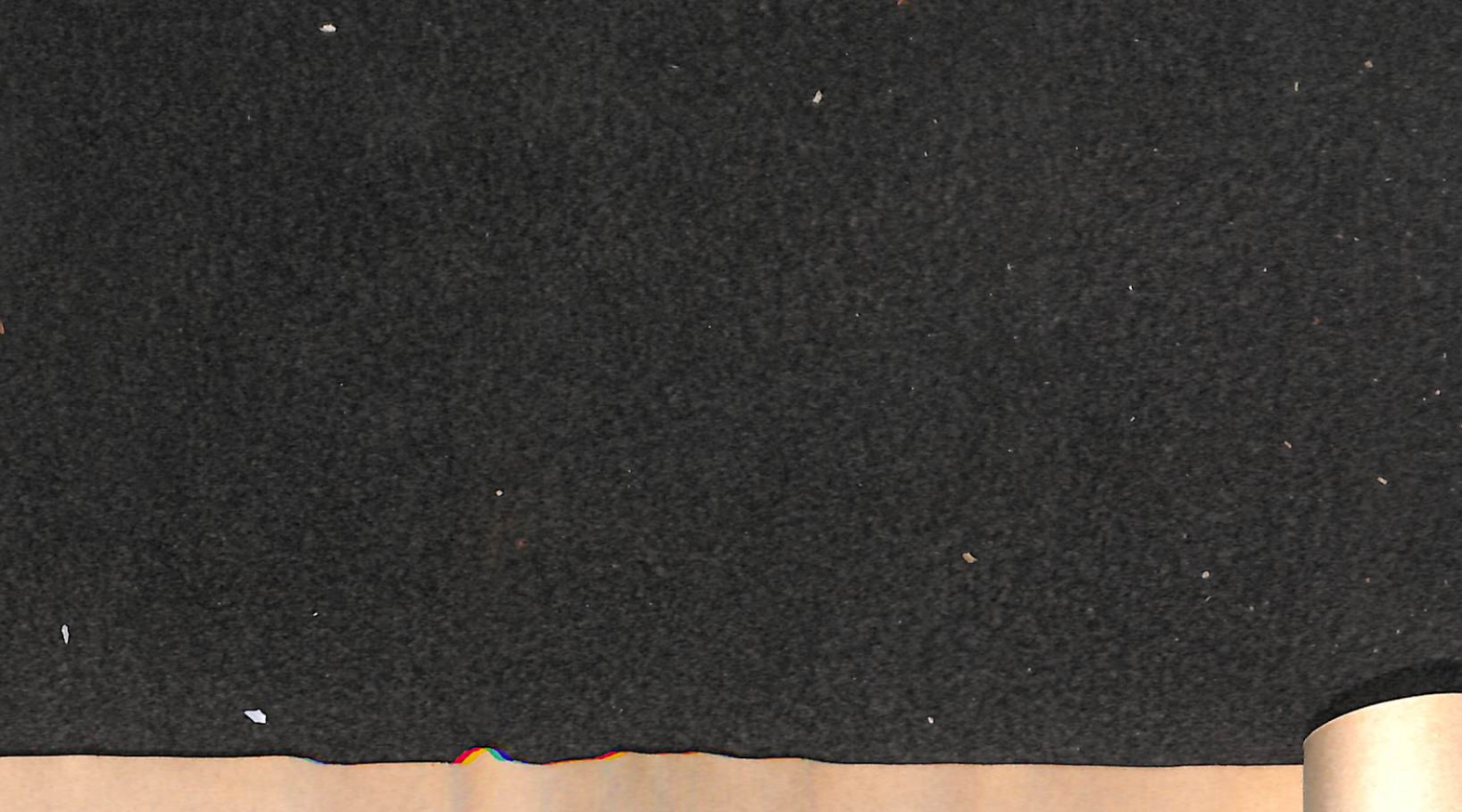
63. Ramsteyn, V, Th.L.W (1941)
Acta Morphol. Nurl; 285:45

64. Ray, A.K; Ray, N.R and Sadhu, D.P (1965)
Ascorbic acid activity in adrenal gland of rats with hypervitaminosis A. Brit.J.Nutr.19:321

65. Riddle, O (1923)
Studies on the physiology of reproduction in birds. XIV: Suprarenal hypertrophy coincident with ovulation. Am. J. Physiol, 66:322
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.405

66. Riddle, O (1947)
Endocrines and constitution in doves and pigeons. Carnegie Institution of Washington, Publication 572.
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.348

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.



67. Roe, J. H and Kuether, C.A (1943)
J.Biol. Chem. 147:399
Cited by Hawk, P.B.; Osers B.L. and Summerson, W.H.'Practical physiological chemistry'(13th Ed.) McGraw Hill Book Coy. Inc. N.Y (1954) Pp.1237

68. Roe, J.H (1961)
Comparative analysis for Ascorbic Acid by the 2-4-Dinitrophenylhydrazine with the coupling reaction at different temperature, a procedure for determining specificity. J.Biol.Chem.236:1611

69. Roy, R. N and Guha, B.C (1958)
Nature, 182:1689
Cited in Ann.Rev.Biochem. 29:413; 1960

70. Sadhu, D. P (1948)
Physiological mechanism of experimental goitrogenesis. Am.J.Physiol.152:150
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp.367

71. Sauer, F.C and H.B.Latimer (1931)
Sex differences in the proportion of cortex and medulla in the chicken suprarenal.
Anat.Rec. 50:289
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 405

72. Sayers, G (1950)
The adrenal and homeostasis. Physiol.Rev. 30:241
Cited by Wolford, J.H and Ringer, R.K (1962) in Poultry Sci., 41:1521

73. Schultze, A. B and C.W. Turner (1945).
The determination of the rate of thyroxine secretion by certain domestic animal.
Missouri Agr. Exp.Sta.Res.Bull. 392
Cited by Tanabe, Y. and Wilcox, F.H (1961)
Poultry Sci. 40:411

74. Shaw, J. C (1956)
Ketosis in dairy cattle. J.Dairy Sci. 39:402

75. Siegel, H.S (1959)
Egg production characteristics and adrenal function in White Leghorns confined at different floor space levels.
Poultry Sci. 38:893-898

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

76. Siegel, H. S (1960)
Effect of population density on the pituitary adrenal cortical axis of cockerels. Poultry Sci. 39:500-510

77. Siegel, P. B and H.S. Siegel (1960)
Genetic parameters of gland and body weights in White Rock Cockerels. J. Heredity, 51:59-62
Cited by Nestor, K.E and Jaap, R.G (1963) Poultry Sci. 42: 989

78. Souders, H. J and Varozza, A (1958)
Federation Proc., 17:493
Cited in Ann. Rev. Biochem. 28: 449; 1959

79. Stamler, J; C, Bolen; M, Dudley and E.Levinson (1950)
Effect of prolonged exhibition of diethylstilboestrol on plasma and tissue lipids in the chick. Endocrinol. 46:375
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 337

80. Sturkie, P.D (1954a)
Avian Physiology (1st Ed.)
Comstock Publ. Associates
Ithaca, N. Y. Pp. 308 & 309

81. Sturkie, P.D (1954b)
Avian physiology (1st Ed.) Pp. 332

82. Sturkie, P.D (1954c)
Avian Physiology (1st Ed.) Pp. 333 & 386

83. Sturkie, P.D (1954d)
Avian Physiology (1st Ed.) Pp. 349

84. Sure, B (1938)
Influence of avitaminosis on weights of endocrine glands
Endocrinol, 23: 575
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 408

85. Tanabe, Y and F.H. Wilcox (1960)
Effect of age, sex and line on serum alkaline phosphatase of the chicken. Proc. Soc. Exp. Biol. Med. 103:68-70
Cited by Tanabe, Y & Wilcox, F.H (1962)
Poultry Sci. 40: 411

MAGADH UNIVERSITY M.Sc.(A.H.) THESIS, 1967.

86. Tanabe, Y and Wilcox, F.H (1961)
Endocrine control of serum alkaline phosphatase activity in the chicken.
Poultry Sci. 40:411

87. Tepperman, J; Engel, F.L and Long, C.N.H (1943)
A review of adrenal cortical hypertrophy
Endocrinology, 32:373
Cited by Sturkie, P.D (1954) in Avian Physiology (1st Ed.) Pp. 407

88. Thanhausher, S.J; Reichel, M; Grattan, F & Maddock, S.J (1938). J. Biol.Chem. 124:631
Cited by Motzok, I (1960)
Bio.Chem.Journal, 47:193-196

89. Thornton, P.A and R.E. Moreng (1958)
The effect of ascorbic acid on egg quality factors. Poultry Sci. 37: 691-698

90. Thornton, P.A and R.E.Moreng (1959)
Further evidence on the value of ascorbic acid for maintenance of shell quality in warm environmental temperature. Poultry Sci.38:594

91. Thornton, P.A & Deeb, S.S (1961)
The influence of thyroid on blood ascorbic acid levels in chicken. Poutry Sci. 40:1063

92. Turner, C.W (1948)
Effect of age and season on the thyroxine secretion rate of White Leghorn hens.
Poultry Sci. 27:146

93. Umbreit, W.W; Burries, R.H and Stauffer, J.F (1949)
Manometric Techniques and tissue metabolism (2nd Ed.) Burgess Publ.Compy.Minneapolis, Minn.

94. Williams, H.L and Watson, E.M (1940).J.Biol.Chem.135:337

95. Williams, H.L and Watson, E.M(1941).J.Lab.Clin.Med.26:1333
Cited by Motzok,I(1960) Biochem.J.47:193-196

96. Wooton, I.D.P (1964) Microanalysis in Medical Biochemistry (4th Ed.). J.A.Churchill Ltd., 104 Glourcester place, London W-1. Pp. 101

97. Zarrow, M.X and J.F. Baldini (1952). Failutre of adrenocarticotropic and various stimuli to deplete the ascorbic acid content of the adrenal gland of the quail. Endocrinol. 50:555-561
Cited by Wolford,J.H & Ringer,R.K(1962).
Poultry Sci. 41:1521

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