STUDIES ON PROTEIN BOUND IODINE, VITAMIN A, LIVER FUNCTION TEST AND MICROSCOPIC FEATURES OF SOME ENDOCRINE ORGANS IN PULLETS, LAYING AND NON-LAYING WHITE LEGHORN HENS

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Dated, the 2nd Dec. 1969

Certified that the work described in this thesis entitled "STUDIES ON PROTEIN BOUND IODINE, VITAMIN A, LIVER FUNCTION TEST AND MICROSCOPIC FEATURES OF SOME ENDOCRINE ORGANS IN PULLET LAYING AND NON-LAYING WHITE LEGHORN HENS" is bonafide work of Ramadhar Singh carried out under my guidance and supervision for the award of M.Sc. (A.H.).

A. K. Ray 2 Dec '69.

(A. K. Ray).

D E D I C A T I O N

Dedicated to my revered Professor, Dr. A.K. Ray, Professor, Head of the Post-Graduate Department of Physiology, Bihar Veterinary College, Patna, whose unfathomable knowledge and timely inculcation have been source of immense inspiration.

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express his sincere feelings for the inspiration and encouragement from his brothers to take up the study.

Ramadhar Singh.
2.12.69

(Ramadhar Singh).

C O N T E N T S

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C H A P T E R - I.

General Introduction.

GENERAL INTRODUCTION

With the very advent of the beings, man has been endeavoring with all his inquisitiveness to unveil the truth of Nature by breaking through the barrier of its complexes. Production and reproduction, two of the most complex phenomenon of Nature, have seized the attention of scientists in various fields and much light has ostentatiously been flashed on it since then. In broad spectrum of their findings, much of the mechanism has been unfolded to a great extent and still incessant efforts are being made to stand face to face with the truth of these very many complexes to make maximum gain out of it for better subsistence and better existence.

Although, almost in all discipline, these aspects have been the arena of scientific investigation but the commendable support, lent by the works of physiologists, pharmacologists, biochemists and nutritionists as well, have revealed much as to how these phenomenon come to happen and are regulated under the various factors, of which major roles played by the endocrines are of paramount importance.

The attributes to avian physiology although scanty, but flourishing poultry husbandry in the recent past, with an aim at contribution to bridge up the gap in national economy, has encompassed the field of endocrines activities to an appreciable degree. With the awakening to the significance of poultry industry, the marked upsurge in scientific investigation is vivid on

the screen of epoch-making period of experimental enlignment in avian physiology. The object of scientific researches has well been characterised as "to render more intelligible the world we live in". This appeals both to the spirit of wonder and to the spirit of service and embraces the realm of Francis Bacon's mind when he said that science is both for enlignment of the mind and for the amelioration of man's estate. Undoubtedly, amelioration of man's estate aims at service both to his economic condition on the one hand and, on the other, to a sound state upon which depends the opportunity for cultural development and bright horizon of his life - the embodiment of comfort and happiness.

Physiologically, the whole estate of well being of the living organism is often spoken of as nutritional. This concept or the principle of "Natural Wholes" has bearing upon the problem, both of food value and of the nutritive welfare of entire life cycle. Since the baby kisses the ground, the primary concern to the achievement of this aspect lies in the provision and production of nutritive food, economic to the bare necessity and want for the betterment of one's own, betterment of the nation and wold community as whole. From this view point, the poultry share in this field safeguards the interest of vast population of the nation hovering around the fact that "Half the struggle of life is a struggle for food". Even behind the giant leap of a man from land to the moon lies the sapience of whole human race in exploration of rich source of new potentials so as to face the challenge of this struggle.

The prevailing food shortage in greater part of our

country has now led to the recognition of the great importance of poultry production as a measure of removing this shortage. The lack of appreciation of the nutritive value of eggs, previous to the 'Second World War' is now no more impregnated with sentimental prejudices against it. Poultry production, once the Cinderella of agriculture in the country, now occupies an important position in the livestock industry since recognition of its contribution to the prevaling food shortage. Even with the present rate of average production of 50 eggs per bird per year by 36 millions hens, in our country, its contribution in the building of national economy goes to the magnitude of Rs. 150 million, worth the price of 1841 millions eggs. There can be no denial to superimposition of the fact that mighty contribution of table poultry is worth the monetary equivalent of its egg product. Despite this seemingly large production, it is far from adequate to meet the nutritional as well as food shortage of the vast majority of the population.

Present day science is strongly imbued with the concept that we live in a dynamic world and that the chief significance of things is to be found not through static description but through the study of their functioning. In laying hens growth of ova proceeds at an almost unbelievable speed, ovulation and oviposition occur with a high degree of regularity and the rapid ovarian development at sexual maturity in pullet, in some way or other, are functional aspects of physiological phenomenon where as the converse aspect is reflected in non-laying birds. These different behaviors at different stages are subject to the influence of various organs as well as their excretion and secretion

in the body. All these are attributable to changes in their role under various phases. Thyroid, pituitary and adrenal which function either to expedite the process or to mobilize the material for it are conspicously significant by their interrelation as well as independent role of hormonal secretion.

The dynamism of thyroid with respect to its activities commanding the development and egg production, therefore, necessiates the study of Protein Bound Iodine which measures the level of thyroxine concentration in blood.

It has well been established that growth as well as reproductive processes are dependent upon adequate supply of Vitamin A. The increased physiological efficiency during growth is coincident with tremendous increase in the size of the oviduct of the hen at sexual maturity under the influence of estrogen elaborated by maturing ovary. Estrogen governs the increase in blood lipids of laying hens whereas increase in its synthesis is principally the function of liver which is store house of Vitamin A. Higher level of Vitamin A in liver and serum in laying birds than the broilers at all level of intake has not been an uncommon finding as is evident from a positive correlation between Vitamin A content of the egg yolk and chick liver (Laden et al. 1957).

Increase in the level of Protein Bound Iodine during pregnancy is apparent from the findings of various workers. As such it was considered reasonable to study the role of liver function which has a direct relation with storage of Vitamin A, the essence of whose requirement has a primary concern with the

growth and egg production.

Moreover, variation in growth, weight and microscopic structure of endocrines during different phases of life cycle, in turn, with their characteristic command in having influence over production and reproduction signify the respective features Payne (1946).

As such, the subject matter of study in pullets, laying and non-laying White Leghorn birds, was to see the physiological feature of thyroid activity which stands four square upon full recognition of scientific values of Protein Bound Iodine, Vitamin A, Liver function test and some endocrine organs under microscopic field.

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C H A P T E R - II.

Recent state of knowledge in the field.

RECENT STATE OF KNOWLEDGE IN THE FIELD

It is apparent from the early study of serum or plasma Protein Bound Iodine that it has been a single most reliable test to give a true reflection of thyroid activity. Salter *et al.* (1941) has impressed upon the authenticity of PBI value for a better correlation with clinical diagnosis than Basal Metabolic Rate.

Taurog *et al.* (1945) in a comparative study of PBI in different species is credited with furnishing the PBI value in plasma of domestic fowl.

Table showing protein bound iodine and total iodine level in plasma of different species.

Animal	Condition of animal.	Plasma PBI u/100 ml.	Plasma total iodine u/100 ml.	Estimated iodine intake per day mg.
1	2	3	4	5
Dog.	Post absorptive.	2.8	36	10 - 15
,,	,,	3.1	14	10 - 15
,,	,,	-	41	10 - 15
,,	,,	-	52	10 - 15
Chicken.	Post prandial.	3.6	8.4	90 $\frac{u}{}$ 100
,,	,,	3.8	6.4	90 - 100
,,	,,	3.3	-	90 - 100
Mouse.	Post prandial.	3.8	4.5	2 - 3
,,	,,	3.8	-	2 - 3

Contd...

Animal	Condition of animal.	Plasma PBI u/100 ml.	Plasma total iodine u/100 ml.	Estimated iodine intake per day mg.
Rat.	Post absorptive.	3.5	3.5	3 - 4
,,	,,	3.4	3.3	3 - 4
,,	,,	3.5	3.3	3 - 4
Human.	Post absorptive.	5.8	7.6	
,,	,,	5.7	7.4	
,,	,,	5.5	5.9	
,,	,,	7.8	6.7	
,,	,,	6.8	7.2	
,,	,,	6.8	-	

From perusal of the aforesaid data it is evident that despite large differences in level of total iodine in plasma the PBI values in dog, rat and mouse and chickens stand parallel except human in which it is appreciably high.

The swift in scientific trend to study the physiology of domestic animals with respect to their importance on economic aspect since then has attracted the attention of several workers towards their blood PBI value. Adoptation of traditional judging procedure to predict the future gain in young animal did not serve the fruitful objective in the past.

Efforts of Hankins et al. (1938) showed little rather no relationship between feeder grade and subsequent gain of

cattle in the feed lot. But a fairly high correlation between feeder grade and the number of days required to reach a certain slaughter grade was obviously marked.

A similar finding has been reported by Johnson (1944) and Patterson et al. (1949). The report of Knapp et al. (1951) are in fair agreement with the above findings.

Baker et al. (1951) conclusively remarked that individual differences do not account for the rate of gain existing between the animal rather it lies in their ability in utilization to make gain of the food absorbed.

Studies on other constituents of blood as urea, non-protein-nitrogen, creatine and creatinine, glucose or cholestrol level did not reveal any significant correlation with rate of gain as reported by Colby et al. (1950). That serum PBI reflects the thyroid activity in cattle as in human beings is in conformity with the conclusive views expressed by Salter et al. (1950).

Burns et al. (1952) studied the correlation between serum PBI and metabolic rate in male bovine. In course of experiment aimed at physiology of their growth they observed a considerable variation in metabolism with each animals similar to those of Benedicts et al. (1935).

Long et al. (1952) observed significant breed differencesin PBI value of dairy cattle. The calves were found to possess higher PBI value than cows.

Burns et al. (1952) found 30 to 90% variations in the extreme measured. Despite such variability they came to the

conclusion that there is a definite relationship between average rate of metabolism and level of serum PBI bearing a highly significant correlation as (ug. = 0.91, P ∠ .01).

Table showing relation of serum PBI level to rate of metabolism in male cattle. Burns et al.(1952).

Breed	Av.wt.of the animal Kg.	PBI/ug.per 100 ml.	Metablism	
			Observed value cal./Kg./day.	Average cal./kg./day.
H.	262	3.2	36	36
S.G.	408	3.4	34	34
S.G.	385	3.4	30, 37	34
S.G.	364	3.5	41, 41	41
S.G.	369	4.0	51, 58, 33	47
H.	300	4.1	58, 37	48
H.	347	4.2	37, 51, 54	47
Av.		3.69± 0.40		41± 6.1

A critical analysis of the data indicates that serum level of PBI does indeed reflect thyroid activity and hence of great value in respect of study of its functional activity on animal growth and livestock production. It appears, as such, that serum PBI level is a reflection on the metabolic rate of male bovine of similar age and weight. Since plasma protein bound iodine concentrations are proportional to plasma thyroxine concentration variation in plasma PBI reflects the status of circulating thyroid hormone, according to Bassett et al. (1941) and

Taurog et al. (1948).

Table showing the relation of serum PBI level to gains and feed efficiency in individually fed Hereford Bulls. Kunkel et al. (1953).

Animal no.	PBI ug/100 ml.	Daily gain lb.	Efficiency lb. feed/ lb. gain.
111	3.5	2.58	7.24
131	3.7	2.30	7.30
1	3.9	2.46	7.77
125	4.1	2.54	7.64
2	4.3	2.44	8.04
132	4.3	2.29	8.12
22	4.5	2.39	7.61
139	4.6	2.30	8.01
9	4.8	2.26	8.33
33	5.1	2.24	8.21
Av.	4.28	2.38	7.83
S.D.	0.50	0.26	0.38
C.V.	11.7%	10.9%	4.9%
Correlation	PBI/gain	PBI/Efficiency	Gain/Efficiency
	-0.69	0.84	-0.66
	P/ 0.05	P/ 0.01	P/ 0.05

The data indicates that PBI level has a significant negative correlation with daily gain and more so with feed

efficiency; low PBI level in this case is associated with animal making most efficient gain.

In another experiment designed to study the relation of PBI to rate of gain in group fed beef cattle of different breeds the result was as follows:-

Table showing PBI level and rates of gain in group fed Hereford bulls and heifers (Blue-bonnet Farms).

Sex	No.of animal	PBI ug/100 ml. serum			Gain lb./day			Correlation
		Av.	S.D.	C.V.	Av.	S.D.	C.V.	PBI/gain
Bulls	8	3.93	0.31	7.9%	2.58	0.18	6.8%	-0.36
Heifers	13	3.89	0.62	13.3%	1.59	0.21	16.0%	-0.55

The heifer PBI value showed a significant correlation with rate of feed lot gain but not with the bull owing to small variation both in PBI and rate of gain.

Result in another experiment indicated optimum level of PBI associated with highest gain, those deviating from the optimum showed lower rates of gain.

Table showing the relation of PBI to rate of gain in group fed beef bulls of different breeds.

Breed	Initial wt.lbs.	PBI ug %	Daily gain
Av. Both(Angus & Hereford).	534	4.48(0.83) S.D.	2.63(0.33)
Av. Angus.	539	4.78(0.71) S.D.	2.59(0.29)

Contd...

Breed	Initial wt.lbs.	PBI ug %	Daily gain.
Av. Hereford.	529	3.93 (0.86)	2.68 (0.38)
Coefficient of variation.	Both.	21.9%	14.2%

Analysis of the group data reveals a negative correlation existing only in those animals having optimal or higher level of PBI. Quite possibly is the association of low level of PBI with low rate of gains under influence of various endocrine secretion.

Their suggestion, therefore, projects the image of reports by several workers that serum PBI level may be used as a partial index of the potential efficiency of feed utilization or potential feed lot gain.

A negative correlation between PBI and average daily gain observed to be highly significant (P/0.01) in Hampshire pure bred quilts and barrow had a distinct bearing with body weight, length and back fat thickness. A decreasing trend in body length with increasing value for PBI was observed by Gawlenowski et al. (1953).

Long et al. (1951) in a much comprehensive study on PBI value in 116 dairy and beef cattle of 3 different breeds reported that age had a pronounced influence on the blood PBI level. They observed highest PBI values (av. 4.8 ug %) in calves of all

the breeds. Obvious decline with age upto 3 to 4 years (av. 3.1 ug %) followed by a gradual decline in 7 to 8 years (av. 2.6 ug%) was noticed.

Breed differences were also noted by them as is revealed by the data on PBI value for respective breed.

Breed	Number	PBI ug %	S. D.	Range.
Jersey.	17	4.11	1.2	2.6 - 7.5
Guernsey.	18	3.51	1.19	2.1 - 6.3
Brownswiss.	21	3.37	0.96	1.4 - 5
Ayreshire.	20	3.19	1.05	1.4 - 5.7
Holstein.	20	2.73	0.55	1.8 - 4
Beef breed.	20	2.19	0.58	1.3 - 4.7

They conclusively remarked that younger the animal in general, the higher the PBI. The breed differences indicate the PBI value having been influenced by the genotype of the cattle. Of course a large span of difference between the PBI value of dairy and beef does not seem to be bridged up merely by attributing all differences to factors of inheritance rather to the environmental ones.

Belated reports after Taurog *et al.* (1946) on PBI value of fowls appeared those of Katsh *et al.* (1955) in full agreement with the former. The reports of the latter are summarised below :-

Animal.	PBI ug %
Chicken.	2.6 ± 0.3
Rat.	4.5 ± 0.4
Rabbit.	3.3 ± 0.5
Sheep.	3.7 ± 0.3
Horse.	3.6 ± 0.4
Monkey.	6.0 ± 0.7
Opossmn.	0.4 ± 0.2
Hamster.	3.5 ± 0.4
Guinea pig.	2.5 ± 0.5

The report enables only to study the comparative PBI value of different species but revealing an interesting feature in opossum with the lowest PBI value of all. This low value reflects a low titre of circulating thyroid hormone in opossum which has been reported to have a peculiarly low body temperature about 95 to 97°F. These two phenomenon in opossum appear to account for a low level of hypophyseal thyroid interaction; but adaptation by the tissue to small amount of thyroxine capable of maintaining the efficiency of the animal can not possibly be ruled out.

The report does not focus any light on other aspect of physiological significance.

Mellen *et al.* (1957) made a specific study of the avian PBI value filling a big gap on the requirement of extensive data in domestic fowls to serve as base line of studying the thyroid

activity in a variety of physiological and genetic feature of economic aspect in chickens.

They conducted the experiment using alkaline ashing method. The also reported no difference in PBI value in any strain of the fowl whether or not the plasma used was normal, oxalated or heparinized; of course plasma used was quick frozen and stored at - 10°F. PBI value was measured on Evelyn colorimeter.

The experiment did not show any difference in PBI either in the control or in the experimental birds fed 0.1% thiouracil in the mash to act as an antithyroid potent agent. PBI value in 4-week-old New Hampshire chicks of two lines differing in responce to thiouracil has also been reported by Bumgardner and Shaffner (1957). They found no line difference. The mean PBI value was 1.12 ug percent.

The valuable data on PBI in different strains of domestic fowls by Mellen et al. (1957) are presented below :-

Table showing duplicate plasma PBI in female chickens of three breeds.

Breed		Approximate age in month.	PBI ug %.
New Hampshire	1	8	1.48 1.43
	2	8	1.08 1.98
	3	8	1.13 1.23

Contd....

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Contd....

Breed		Approximate age in month.	PBI ug %.
White Plymouth Rock.	1	8	1.17 1.07
	2	8	1.12 1.42
	3	8	1.27 1.17
	4	8	1.12 1.12
	5	8	1.22 1.46
	6	8	0.88 0.98
	7	8	1.12 1.02
White Leghorn.	1	20	1.22 1.07
	2	20	1.03 1.03
	3	20	1.22 1.22
Mean of all determination ± S.E.			1.16 ± 0.03

They also carried out the experiment to test the effect of ambient temperature on plasma PBI level of 10 cockerels of White Plymouth Rock. It was conducted by random selection and housing half of the birds in heated room and half in colder environment and were tested as weekly interval of 4 weeks. No significant difference in over all mean value of PBI due to different

treatment was found.

Table showing the effect of ambient temperature on plasma PBI of White Plymouth Rock cockerels.

Week	Heated room		Unheated pen	
	Mean temperature °F.	PBI ug %	Mean temperature °F.	PBI ug %
0			34	1.23
1	72	1.71	41	1.66
2	72	1.73	40	1.66
3	75	1.51	35	1.11
4	76	1.17	42	1.09
Mean (1-4 wt.)	74	1.53 ± .14	40	1.38 ± 0.14

In a separate experiment exclusively aimed at studying the effect of thiouracil feeding on plasma PBI value (of New Hampshire) of domestic fowl. They did not find difference either in the male or female sex.

Table showing effect of thiouracil 0.1% in feed on plasma PBI of New Hampshire chicken on 4 male and 4 female on each treatment.

Week	Age (week)	PBI ug %	
		Control	Thiouracil
0	9	1.11	
1	10	1.28	1.33
2	11	1.32	1.55
3	12	1.39	1.27
Mean (1-3 weeks)		1.33 ± 0.07	1.38 ± 0.07

The findings of Mellen et al. (1957) are not at all in agreement with the two previous reports discussed above.

However, these investigators have concluded that PBI level in bird might not reflect relative thyroid activity nor does it fluctuate with the change in the thyroid activity.

The average PBI value in 70 days old cockrels maintained at low iodine content diet was reported to be 0.51 ug.%. While studying the thyroid metabolism of iodine (1^{127} and 1^{131}) in chicks and rats equilibration of injected iodine was brought at par with the existing thyroidal iodine (Rosenberg et al., 1964). Insignificant increase in PBI value of chickens following supplementation with low iodine diet was observed. The total and free iodide was largely increased both in rats and chickens.

PBI value in chickens, Bobwhite ëquail and Coturnix as 1.12, 1.76 and 1.26% respectively has been reported by Singh et al. (1968).

Gulati (1968) in his studies on "variation in thyroid secretion rate and plasma PBI in two breeds of poultry" found plasma PBI value as 1.75 ± 0.06 and 2.42 ± 0.17 ug. per 100 ml. in W. L. H. cockrels and Deshi chicks respectively at 14 weeks old age.

A critical analysis of his data showed PBI value in W. L. H. ranging from 0.92 to 4.10 with a normal trend of PBI level above 1.50 varying between 1.53 to 2.0 in 40% and 2.0 to 4.10 in nearly 25% of all the 50 experimental W. L. H. birds.

The values in Deshi birds were still more higher rangi-

-ng between 2.01 to 4.30 in 90% of all the experimental birds.

He adopted the dry ashing method of Faulkner et al. (1961). The results obtained by him are not in agreement with the reports of Mellen et al. (1957). It appears that differences are bound to play upon the technique adopted.

"Thyroid acts like a draught to the fire of life. The draught is necessary to get a better fire but without it the fire will still smoulder on. Life and its oxidation continue even when whole thyroid is removed but continues very sluggishly; the rate of oxidation falls to about 60% of its normal value. Only 1 mg. of thyroxine injected to a normal man will cause its chemical activity as measured by oxygen consumption and carbondioxide production to go up by 2% though the amount injected is only about one seventy millionth part of the man's weight" (H.G. Wells et.al.) The above facts eventually unfold the hidden clue of the potentiality of phenomenal activity of thyroid.

The existence of a specific hormone which stimulated the growth of thyriod gland and enhanced its function of supplying thyroid hormone to the whole organism was postulated in 1920 by Allen.

The thyroid gland of most species contains enough thyroid hormone for 2 to 10 weeks. Thyroglobin known since 1899 is an efficient means of storing large amount of thyroid hormone. In human a normal thyroid iodine content of 10 mg. will supply the daily requirement of iodine of about 60 ug. for 170 days. (Gutman et.al., 1932, Berson, 1956).

Money et al. (1952) reported that iodine content of diet has marked influence on thyroid iodine in rats so much so that with a daily iodine requirement of about 1.5 ug. over 2 weeks supply is available.

This feature in itself stands a top in a marked contrast to almost any other endocrine tissue in which a few hours supply of hormone is usually all that can be isolated from a single gland. (Robbins and Rall, 1960).

Thus it seems reasonable to assume that essentially all the thyroid iodine is eventually secreted as Tetra iodothyronine or Triiodothyronine. (Pitt-River et al., 1959).

Tixier-vidal (1958) stated partition of thyroxine and triiodothyronine between plasma and crythrocytes in birds with considerable variation between species. As compared to man the values in birds were found to be much higher.

Brown-Grant (1961) in his studies on extra thyroidal iodide concentrating machanism in domestic fowl concluded his findings as showing that yolk iodine was acquired during development in the ovary (9 to 11 days process) which accounted for the delay in appearance of tabled eggs. The iodine which appears much earlier and then disappears could be acquired by diffusion while the eggs were in oviduct just before laying.

It has also been confirmed and shown that the resting ovary does not concentrate iodide. Uptake of I^{131} by the thyroid gland of the developing chicks after 7th day of incubation has also been confirmed. The radio activity first appearing in the

embryo is followed by its concentration in the thyroid gland from 10th day of incubation onwards.

Concentration of iodine in the yolk would have the same end as the placenta and mammary gland iodide concentrating mechanism in mammals. The placenta of many animals are reported to concentrate the iodide and concentration in fetal blood is higher than in the maternal blood in later stages of pregnancy.

The mechanism of iodide transport by the placenta is said to be similar to that of iodide concentration by the thyroid gland. (Nataf et al., 1956).

Danowski et al. (1951) have shown that within 12 hours after birth the concentration of PBI in the blood of new born is equal to that of the maternal blood.

Serum protein are known to pass from blood into extra-vascular spaces and it can well embrace the supposition that thyroid binding protein are also derived from blood.

The ratio of PBI to total protein in normal bovine synovial fluid and amniotic fluid, all containing thyroid binding globulin and albumin was reported to be quite variable by Freinnel et al. (1957).

Tata et al. (1959) studied the thyroxine binding in the serum of chicken and duck and found avian serum to have a lower affinity for thyroxine in comparision to human. It was virtually so because of absence of a specific thyroxine binding factor - the alfa globulin.

Dowling et al. (1956), Robbins et al. (1958) reported

increase in TBG capacity compared to normal adults but not as high as mothers at terms. PBI was some times found to be elevated no doubt but the trend in elevation did not seem to be consistent.

Fall in PBI after oophrectomy was not observed by Stoddard *et al*. (1957). But a qualitative change in thyroid binding protein (TBP) due to genetic causes showing elevated PBI and elevated TBG has been reported by Beierwaltes *et al*. (1959).

Administration of methyltestostrone to human, brings about fall in serum PBI (Keital *et al*., 1957). Moreover Federman *et al*. (1958) reported that a striking fall also occurs in TBG capacity.

Nataf *et al*. (1957) reported triodothyromine to cross placenta in rats. Similar reports have also been made by Grumbach *et al*. (1956).

Thiouracil along with its good number of derivatives are reported to cross placenta all rapidly and exerting their effect on a fetal thyroid gland (Peterson, 1955).

Whereas affirmative views with respect to crossing of adrenocorticotropin in human placenta were expressed by Lanman (1953), the findings of Jones *et al*. (1953) in rats could not confirm it.

Data furnished by Danowski *et al*. (1962) showing increase in circulating PBI and thyroxine (Butanol Extractable Iodine) in pregnancy are presented below :-

	Control	Pregnant
Number of subject.	25	57

	Control	Pregnant
Number of sera.	89	81
PBI(mean ± S.D.) ug.%	5.4± 0.7	7.8± 1.3
Number of subject.	23	21
Number of sera	23	23
Thyroxine (mean ± S.D.) ug.%	3.7± 0.8	5.4± 1.0

Very little informations are available on animals other than man.

Ukita (1919) stated that thyroidectomy in rabbit prolongs the gestation period but was later on contradicted by Krohn (1950) who pointed out that removal of thyroid interfered with pregnancy. Jones et al. (1946) expressed the views that once pregnancy is established thyroidectomy has no descernible effect.

Ralph et al. (1961) studied the effect of two synthetic estrogen on the level of PBI in men and women with heart diseases. The increase in PBI concentration over the base line level was found to be 1.7 ug.% for women and 2.1 ug.% for men receiving ethinylestradiol and 2.0 ug.% for men receiving Manvene. The former in the doses used was about 5 to 6 times more effective in raising the PBI level in women than men.

The only report on thyroxine binding in pregnancy in animals appears to be reported by Annison et al. (1958, 1959). The TBG observed in sheep was found to be unaltered during pregnancy with no change in PBI level.

Zyl (1957) reported a slight change showing increase in the PBI value in baboons. He also observed a cyclical variation

in PBI in relation to menstrual cycle. Feldman (1958) reported no change in PBI value in rat.

Thus it appears that the difference in the nature of thyroxine binding protein among the species may be attributable to the difference in their response to physiological or pathological factors.

Niepce (1851) made his first observation on thyroid pituitary relationship in a goitrous cretin. In recent past association of hyperthyroidism with hypopituitarism has been confirmed by Fajans et al. (1958), Gurding (1959) and McCullagh (1957).

It is a well known fact that hypophysectomy results in a decrease in plasma PBI concentration (Taurog et al., 1946).

Lawrence et al. (1953) reported that hypophysectomy resulted in a fall in PBI from 1.0 ug.% to still lower level by 5th operative day against a PBI value of 2.6 ug.% in a normal dog. Miraculaus effect with a injection of 10 mg. of thyropopin in the hypophysectomized dog was observed with an increase in plasma PBI concentration within four hours. Repeated injection of thyrotropin resulted in an initial elevation of plasma PBI but it declined after 2-5 days despite maintaining the same dose and same frequency.

Zyl et al. (1961) reported rapid decrease in the serum PBI of the baboon after thyroidectomy, hypophysectomy and pituitary stalk section. They have also reported normal level of serum PBI in castrated baboon.

Robertson et al. (1955) found association of 17-

hydroxycorticosteroid level in plasma with low plasma PBI value.

Kydd et al. (1951) reported an usually elevated PBI in acute phases of infectious hepatitis. In hepatic cirrhosis although some instances of elevated PBI and mostly normal in another instances were observed but on the other hand low level of PBI value in some was found to be usually associated with low concentration of serum albuin.

Engstrom et al. (1955) found decrease in PBI level in many subjects with severe chronic illness.

They also put forth the explanation that lack of fall in PBI after surgery or acute illness could be due to insufficient time to develop the secondary effect (Engstrom et al., 1954).

Hakkila et al. (1960) reported that level of serum PBI was above normal in all but 7 in a group of 99 patient with thyrotoxicosis before treatment with radioactive iodine. But it fell afterwards at two later stages from 2 to 3 and 5 to 7 months.

While some investigators (Vannoti et al., 1959) are in fair agreement with elevation of PBI in acute phase of a infectious hepatitis, views of other workers (Mueller et al., 1954) are not in agreement with them.

Judith and Andraskulscar (1965) observed a high catabolic activity on the part of the liver. To study the role of liver in regulation of thyro-ovarian combination, F. S. H. was administered to the thyroidectomized and castrated females rats but the hormone administration was found to be much less effective on these animals as compared to control which was evident from

the duration period of reaction as well as the number of reacting animals.

Brenesi et al. (1966) reported an interrelationship between the function of thyroid and liver. Subsequent upon thyroidectomy in rats increase in carbon tetrachloride tolerance of liver was observed; but decrease in tolerance on enhancing the thyroid function by thyroxine or triiodothyronine was characteristically reflected in absence of glycogen.

Silverstein et al. (1962) reported that rats made obese on high fat diet showed inactive thyroid gland marked histologically. The iodine concentration also was less in such rats as compared to those on normal diet. Of course PBI showed no consistent trend with respect to diet.

The thyroid gland of rats made obese on high fat diet had higher thyroidal iodine concentration and was found to be correlated with larger thyroid follicles more filled with colloid and distended. The thyroid secretion ratio was found in this case lower than the control showing lower thyroidal iodine concentration as reported by other workers.

The transient increase in the T/S ratio observed in rats and mice coincided with the period of sexual maturation which indicates an increase in thyroidal activity influenced by increase in T. S. H. secretion.

As regards the rats at 5 weeks age having a distinct lower T/S ratio the possibility that even lower T/S ratio could be found at earlier stages even in more immature stage of thyroid

can not be ruled out.

Aschkenasy et al. (1959) in his studies on thyroid function with radio active iodine in rats kept on synthetic diet deprived of protein and of low iodine content observed rapid iodine take up and sooner discharge than the glands of normal control. A subsequent rise in PBI especially butanol soluble iodine above those of normal control was noticed.

Post (1965) reported that plasma PBI increased by over 50% in 24 hours in bullocks on poor pasture with a supplement of 20 lbs. good quality lucern hay. In grazing spayed heifers given a supplement of 2 lb. ground nut meal per day over a 4 months period showed a higher PBI value than the control. Similarly observation in growing bullocks given a constant amount of lucern hay throughout the year revealed a decrease in PBI and T. S. R. consistant with a decline in feed intake per unit metabolic rate.

Marked differences in PBI value were also observed in free feeding on different types of ration. Significant lower PBI value in those fed on non-legume hay alone in contrast to those on leguminous hay was noticed. Thus it is apparent from the studies of the aforesaid investigation that various types of improved ration can have pronounced effect on thyroid though seasonal and animal differences may also reflect their influences on the difference in quality, on the nature of pasture and quantity of intake.

Pipett et al. (1962) observed lower PBI value in rats given protein free diet without added iodine than in those given a low iodine casein. The value for total iodine in thyroid iodine

per milligram gland was also found to be lower. The total iodine content of the protein deprived rat was less otherwise there was no difference between the values in rats given high iodine intake with or without protein. Thyroid of rats on amino acid supplemented diet without iodine showed extremely low iodine and parenchymatous goitre with less colloid.

Falconer et al. (1961) reported about changes in thyroid activity during growth. The early decline in PBI with age may be associated with change in the serum protein. The rate of secretion of thyroid per bodyw weight maximum at 6 months of age may suggest a relationship to energy expenditure on analogy with cattle.

The mean value taken as the chief parameter of thyroid activity for the suckling group showed PBI ug.% as 7.2, for ewe lamb of 5 to 8 months 4.9, for one year old 4.2 and 5.7 years old had the lowest value of 3.8 ug. only.

It indicates thyroidal activity variation with the age which tends to decline as it advances.

In 1899 Oswald described an inverse relationship between iodine and phosphorus concentration in thyroid gland. Association of increased thyroid activity with increased phosphorus content has also been reported. Borrell (1949) showed a direct quantitative correlation.

An inverse relationship between the inorganic phosphorus and carotene content of the blood plasma of Hereford cows maintained on adequate and low phosphorus ration was reported by

Ross and Gallup (1949).

Effect of phosphorus deficiency on metabolism of carotene and Vitamin A by beef cows was studied by Thomas, Gallup, and Whitehair (1953) and they found average plasma carotene level generally higher in the phosphorus deficient cows than the in those fed adequate phosphorus; whereas the plasma Vitamin A level appeared to be unaffected by phosphorus deficiency. 8 weeks postpartum the control and the test both group of cows were found to have lost similar amount of carotene and Vitamin A from the liver.

In another experiment on carotene and Vitamin A metabolism in cattle and sheep on phosphorus deficient ration they observed plasma carotene level to be conistently higher in cows on phosphorus deficient ration with decreased Vitamin A storage in the liver.

The trend in sheep was found to be different. The average Vitamin A content of the plasma and in liver in the phosphorus deficient diet was found higher. Thus they concluded suggesting that Vitamin A response of cattle and sheep to phosphorus deficiency might be attributed to the species differences.

Administration of Vitamin A to animal has been marked with a decrease in the size of thyroid gland and lower oxygen consumption. It has also been found effective in partially cancelling the hyper metabolic rate, the lethal effect of exogenous thyroxine and desiccated thyroid. It also accounts for depression in serum PBI. (Fasold and Peters, 1934 and Drill, 1943).

The above reports are suggestive to the fact that

excess of Vitamin A in animals may modify the thyroid function or may interfare with the peripheral effect of thyroxine and thereby the related hormones.

Therapeutic effects of Vitamin A response to hyperthyroidism cases have been reported by Logaras et al. (1938), Belasco et al. (1940)& Simkins (1947). Sadhu and Brody (1947) have reported that large amount of Vitamin A produce antithyroidal effect.

Danowski (1962) did not observe any change in the serum PBI of healthy control during massive dosage of Vitamins A therapy.

The basal ration being the same in respect of Vitamin A and protein supplement with one group of cattle given 45000 IU. of stabilized Vitamin A daily and other group as control Roussel et al. (1963) observed a highly significant increase in plasma Vitamin A in the Vitamin A supplement group. But no significant difference in carotenoid value of the two groups was found.

There was a slight increase in serum PBI value in the supplemented group over control but not significant statistically.

Constituent	Control	Vitamin A supplemented	Mean
Plasma carotenoid ug./100 ml.	186.3	193.5	189.9
Plasma Vitamin A ug./100 ml.	20.18	27.79	23.98
PBI ug./100 ml.	11.65	12.73	11.94

As regards deficiency Frape et al. (1959) reported that modifying effect on thyroid function may be attributed to Vitamin A deficiency.

The effect of total thyroidectomy in rabbit was noted with appearance of xeropthalmia by Kunde (1926). Vaneuler and Klussman (1932) also pointed out the existence of antagonism between carotene and thyroxine.

Drill and Traunt (1947) likewise reported that subsequent upon thyroidectomy depression in carotene and Vitamin A reaction can well be visualized from the inability of 10 mg. of beta- carotene daily to thyroidectomized rats to relieve the ocular symptoms caused by Vitamin A deficiency.

Johnson et al. (1947) pointed out very little storage of Vitamin A in the liver of rats treated with thiourea or thiouracil followed by administration of carotene. Thyroxine increased the ability of these animals to convert carotene to Vitamin A.

Kelley et al. (1948) stated decrease in Vitamin A deposition in the liver of rat fed carotene when treated with thiouracil and increase on thyroid supplementation.

That digestibility of carotene is a function of thyroid secretion is supported by the findings of Chanda et al. (1951) who demonstrated that it was higher during the administration of thyroxine and lower during thiouracil regimen.

According to Frape et al. (1959) the effect of Vitamin A on thyroid secretion is thought to be direct and not a reflection of its effect on growth in the young pig studied.

Boguth and Sari (1962) found that conversion of beta-carotene to Vitamin A is independent of thyroid function. Similar observations were made by Ascarelli *et al.* (1964) in White Leghorn chickens.

In light of utilization of Vitamin A by chicks it was found that the amount of Vitamin A present in the blood is related to Vitamin A intake. (Castano *et al.*, 1951).

Abdulnabi and Alneggur (1965) in their study on the biochemical pattern of thyroid disfunction observed that Vitamin A in serum of hypothyroid patient was significantly lower than in the serum of 14 euthyroid subjects or blood donors. Beta-carotene, total serum protein and cholestrol all were significantly higher than the control. On the other hand values in hyperthyroid patients were not significantly higher and different from the normal.

Chanda *et al.* (1955) made a study on the effect of deprivation of carotene and anterior pituitary hormone on the partition of Vitamin A in blood and milk of cows. By injecting 'Ambion' a commercial pituitary extract containing thyrotropic and gonadotropic hormone into the lactating cows on diet with or without carotene they observed that yield of Vitamin A alcohol in the milk and its content in the blood were increased both by omission of carotene and by the pituitary extract (content). The yield of Vitamin A esters in milk was increased by pituitary extract whether or not carotene was fed. It was found to be absent from the blood when carotene was not fed but present and

increased by pituitary extract on feeding carotene. Blood and milk carotene were not affected by pituitary extract. It is probable that pituitary hormone accelerated the mobilization of hepatic Vitamin A into blood as the alcohol form.

Excess of Vitamin A intake has been found to have a marked effect on the endocrines. Decrease in secretion of thyrotropic hormone as well as in metabolic rate has been reported by Sadhu (1947). The effect of excessive dosage of Vitamin A iodide and thyrotropic hormone on anterior pituitary was studied and large dosage of Vitamin A 3000 I.U. daily to individual rat was found to have reduced the thyroid size as a result of lower secretion of thyrotropic hormone. Increase in size of adrenal was also observed. It was confirmed by feeding iodized Vitamin A with no influence on thyroid size nor on thyrotropic secretion.

Table showing the effect of potassium iodide and Vitamin A on the endocrine organs of the rats.

Group	No. of rats	Initial body wt. gms.	Final body wt. gm.	Gain in wt. per day.	Thyroid wt. per 100 gm. body wt.	Adrenal wt. per 100 gm. body wt.	Pituitary wt. per 100 gm. body wt.
Control.	9	89	167	4.3	6.56	11.89	3.46
Fed 1 gm. KI daily.	9	89	150	3.4	5.89	11.74	3.69
Fed 30,000 I.U. Vit.A daily.	9	93	139	2.6	4.74	16.70	4.22
Fed Vit.A 30,000 I.U. and KI, one gm. daily.	9a	86	112	1.4	6.61	18.44	4.78

a - 3 died during experiment.

With significant reduction in thyroid weight and significant increase in adrenal weight as compared to normal and also increase in anterior pituitary weight but a smaller thyroid weight,on Vitamin A and KI diet, conclusion was arrived at that iodine of anterior pituitary or of the protein elsewhere probably led to the formation of thyroxine, thereby depressing the formation of thyrotropic hormone by anterior pituitary and consequently decrease in thyroid size. Toxic effect was noted with Vitamin A and postassium iodide combination as was evident from the increase in adrenal size with no concomitant change in thyroid. Moreover the toxic effect was not marked with the same treatment in other rats.

From the results it was also gathered that effect expressed on pituitary was by Vitamin A alone and not by the Vitamin A thyroxine compound if any such formation (Sadhu, 1948).

The effect of Vitamin A deficiencies in biosynthesis of steroid hormone in rat was studied by Juneja et al. (1966). Intraperitoneal injection of retinal, retinoic acid or retinol into the deficient rat 24 hours before sacrifice was found effective in correcting the deficiency on this reaction. Vitamin A deficiency markedly affected synthesis of the deoxycorticosterone and corticostrone from pregnenolone in the adrenal of rats even at severe deficient stage. In vitro too, retinol or retinoic acid did restore the loss at mild stage of deficiency.

Synthesis of deoxycorticostrone from progestrone or of corticosterone from progestrone or deoxycorticosterone compared

with paired fed normal was found unaffected. Adrenal of deficient rats contained smaller amount of deoxycorticosterone and corticosterone.

Vuznyetsova et al. (1965) observed that prolonged feeding of cortisone to rats in dosage 3 mg./100 gm. body weight reduced the Vitamin A content of liver and also affected the hydrolytic activity of the tissue investigated.

Cohlan (1953) reported that deficiency of Vitamin A in the maternal diet have induced defective offspring whereas excess of Vitamin A in the maternal diet diminished litter rate with a high incidence of fetal resorption in utero of rat. Mature pregnant animals with hypervitaminosis A produced offspring with congenital malformation.

That Vitamin A is stored in fetal tissue as in adult has been revealed by a considerable increase in the amount present in the blood and tissue of fetus at birth with prepartum supplementation of the diet with Vitamin A to the mother. (Wise et al., 1946).

Similar reports were given by Esh et al. (1948).

A correlation between carotene intake and plasma carotene of calves was reported by Dolgi et al. (1953) and Roussean et al. (1954) also made observations in fair agreement with them.

Vedanayagam (1966) reported the Vitamin A and carotenoid content in blood plasma of 16 she buffalo nearing lactation or gone dry. The plasma Vitamin A value was 96.4 ± 12.9 I.U. and carotenoid 6.3 ± 0.8 ug per 100 ml. He attributed the low value

of carotenoid in the plasma of she buffalo to a better conversion of carotene to Vitamin A in their body.

Dennenburg et al. (1963) in their study on Vitamin A esters and alcohol of rats liver during pregnancy conclusively pointed out that low value for Vitamin A in plasma may be related to the high values for blood lipids found during pregnancy.

Landau et al. (1957) reported a positive correlation between Vitamin A content of the egg yolk and chick liver.

Plack et al. (1966) reported that plasma of laying hens contained about 8 ug. of retinal/100 ml. which was nearly 10 times more than found in the plasma of mature cockrel and immature pullet.

Frank et al. (1952) stated that percentage of protein in chickens egg white are influenced by the nature and quality of dietary protein of the laying hen as well as by genetical differences in lineage.

Stoewsand et al. (1961) reported that Vitamin A in serum and total in liver decreased as the protein in the diet of the chicks increased. The fall in liver Vitamin A was not found in association with the changes in liver lipids or Vitamin C.

As regards the utilization of Vitamin A influence on the level of protein on the storage and utilization of Vitamin A Bhattacharya et al. (1961) observed in 3 groups each of 24 littermate rats maintained on complete purified basal diet containing 6, 12, and or 18% casein with 710 I.U. Vitamin A palmitate (to half of each group of 12) that with larger intake of Vitamin A,

storage in liver was greatest with 12% protein. With lower intake of Vitamin A storage of Vitamin A in the liver was greatest with the lowest percent of protein.

They suggestively pointed out that when there is not sufficient protein in the diet Vitamin A is not fully utilized.

Similar observation were made by Lerner and Leathem (1963) who reported that growing rats on protein free diet lost about 25% of their initial weight in 14 days while other on diet of equal energy value containing 20% casein doubled their weight. Ratio of thyroid to body weight remained unchanged and no difference in hypertrophy of thyroid by graded dosage of thiouracil was found in both groups.

Malathi et al. (1963) advanced their reasoning after studies on biological activities of Vitamin A acid in rat that loss of weight in Vitamin A deficiency may be partly due to loss of water from the cell and that rapid early growth response to Vitamin A may be due to rapid rehydration.

Veen and Beaton (1966) studied Vitamin A deficient rats maintained on a diet consisting of 0, 4 or 20% protein. A repletion period of 7 days with 0, 10, 40, 80 or 160 ug. Vitamin A orally per day showed rise in plasma Vitamin A with increase in the amount of Vitamin A administration until plasma saturation with Vitamin A was reached. This saturation point was reached with 10 ug. Vitamin A in the 0% protein group and 40 ug. with another group. In latter case increased deposition of Vitamin A was found. It was observed that plasma Vitamin A was statistica-

-lly related to plasma albumin at all level of administration and to alpha-globulin after saturation of plasma with Vitamin A. As such they came to the conclusion that there exists a plasma protein carrier for Vitamin A.

Toxic effects with massive dosage of Vitamin A to albino rats have been reported by Raj et al. (1965). The weight of the hearts, livers, kidney and adrenal gland of the treated rats, expressed as percentage of their final body weight, were found greater than control owing to shift of fluid from the circulation to the tissue. It was also observed that the effect of hypervitaminosis was no more and the rats returned to normal 10 days after excessive administration of Vitamin A was stopped.

Table showing the mean organ weight, expressed as percentage of body weight of 20 rats suffering from hypervitaminosis A (group T) compared with pair fed control rats (group C).

Organ.	Group C	Group T	Mean difference with S. E.	t value.
Heart.	0.498	0.653	0.155 ± 0.0028	55.71*
Liver.	5.019	6.058	1.039 ± 0.00331	313.29*
Kidney.	1.039	1.453	0.414 ± 0.0051	80.92*
Adrenal gland.**	0.0396 18.61 mg.	0.0744 31.25 mg.	0.0348± 0.00026 12.64 ± 0.636 mg.	133.69* 65.38*
Brain.	2.798	2.791	0.007 ± 0.0023	2.21
Spleen.	0.821	0.809	0.012 ± 0.00175	3.42

* Significant at the 1% level.
** Value in parenthesis are the absolute weight.

C H A P T E R - III.

Materials and Methods.

MATERIALS AND METHODS

EXPERIMENTAL BIRDS AND DIETS:

Throughout the experimental period, White Leghorn birds maintained in an identical environment, were procured from the Central Poultry Farm, Patna. They were selected at random from the farm. The demarcation line, distinguishing between the laying and non-laying birds used in the experiment, was based on judging. But the hard and fast rule followed in making out differentiation of laying and non-laying was not merely the farm report rather actual verification of the reproductive tracts for, absence or presence of eggs in the tract, proliferation of ovary and enlargement or atrophied condition of the oviduct.

All birds were obtained from the farm and were divided in three groups according to their age and reproductive features. The birds in age group over 1 year considered as adult, were layers and non-layers and those in age group of 4 to 6 months were pullets.

The birds were brought from the farm directly to laboratory. In any case, after they were transferred from the farm to the laboratory, they were given at least 10 to 12 hours rest.

Throughout the experiment the birds received the same diet at an uniform schedule of the farm. They had an ad lib acess to drinking water.

The composition of the diet was as follows :-

T A B L E - 1.1

Table showing the feed ingredients supplied in Central Poultry Farm, Patna.

Ingredients	% incorporated in feed	Protein %	Fat %	Carbohydrate %	Ca %	Phosphorus %	Iodine content ug/Kg.	
							Fresh basis	Dry basis
Maize.	57	11.1	3.6	662.	0.01	0.33	27	43
Wheat crushed.	20	11.8	1.5	71.2	0.05	0.03	37	44
G.N. Cake.	17	26.7	40.1	20.3	0.05	0.39	-	200
Fish meal.	3	22.6	-	-	-	0.02	-	-
Mindiff Mixture.	3	-	-	-	-	-	-	-
Vitablend (Glaxo).	20 gm. Vitablend mixed with 100 Kg. of feed.							

The composition of Vitablend was as follows :-

(a) Vitamin A .. 40,000 I.U.

B_2 .. 25 mg.

D_3 .. 6,000 I.U.

(b) Each and every experimental bird was given 120 gm. mash mixture of the above ingredients daily.

The birds were killed in the same laboratory and with same instrument throughout. Just before they were sacrificed their liveweight was recorded along with any specific feature in their appearance and health as would appear worth noting.

T A B L E - 1.2

Table showing the liveweight of the birds recorded in Kg.

Layers.	Non-layers	Pullets
2.0	1.5	1.5
2.0	2.0	1.25
2.25	2.25	1.25
2.0	2.0	1.0
1.75	1.25	1.25
2.25	1.75	2.0
1.75	1.5	-
2.0	1.75	-
2.0	2.0	-
2.25	-	-
Average. 2.025	1.777	1.375

T A B L E - 1.3

Table showing average Iodine content of some animal Feedingstuffs. (Monte A. Greer).

Oilseed meals.	Iodine content ug/Kg.	
	Fresh basis	Dry basis.
(1) Linseed cake meal.	-	110
(2) Cottonseed meal.	-	149
(3) Soyabean meal.	-	170
(4) Groundnut cake meal.	-	200
Mean	-	157

T A B L E - 1.4

Table showing average iodine content of some every day foods.

	Iodine content ug./Kg. Fresh basis	Dry basis.
Eggs,		
Hen's egg -	93	-
Mean.	93	
Fish (a) Marine fish:		
(1) Sole.	163	1072
(2) Sea bass.	250	471
(3) Sardines	284	745
(4) Mackerel.	371	1031
(5) Halibut.	520	2225
(6) Herring.	520	1358
(7) Sea perch.	742	3105
(8) Cod.	1463	7493
(9) Haddock.	3180	15941
Mean.	832	3715
(b) Anadromous fish:		
(1) Sea trout.	320	1028
(3) Salmon.	341	1030
Mean.	330	1029
(c) Fresh water fish:		
(1) Carp.	17	68
(2) River bass.	30	115
(3) Lake Trout.	31	88
(4) River perch.	40	194
Mean.	30	116

TABLE - 1.5

Table showing average Iodine content of some everyday foods.

Cereal grains and products	Iodine content ug./Kg.	
	Fresh basis	Dry basis.
1. Rice.	22	39
2. Maize.	27	43
3. Wheat.	37	44
4. Flour.	42	-
5. Bread.	58	-
6. Barley.	58	92
7. Oats.	60	91
8. Rye.	72	84
Mean.	47	65

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C H A P T E R - IV.

Protein Bound Iodine.

PROTEIN BOUND IODINE

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

"Thyroid is Nature's device for drugging the higher animals upto a pitch of activity not found in lower forms".

(H.G.Wells).

The veracity of his statement is true of thyroid necessary for normal growth and development as is evident from the fact that growth is markedly retarded following thyroidectomy(Blivaiss et al., 1942; Winchester et al.,1949; and Clegg et al.,1959).

Removal of thyroid not only affects the growth and development but also hampers the reproductive efficiency to a great extent. Decrease from 10 to 29 percent in egg productions in pullets thyroidectomized at or near the age of sexual maturity against that of normal control has been observed (Winchester, 1939;Taylor & Burmester, 1940).

Protein Bound Iodine test measures the level of iodinated protein in the serum which, under normal condition of diet, is made up of principally of the thyroid hormone along with its other related substances (Long et al.,1952). Serum protein consists chiefly complexes of protein and iodine in the form of thyroxine(Schatz & Volpe,1959; and Taurog & Chaikoff,1948). Two of these complexes, the Protein Bound Iodine and the thyroxine like or Butanol Extractable Iodine show a high degree of correlation with regard to abnormal level of thyroid activity as estimated by

clinical and laboratory means (Danowski, 1962).

Preference of serum or plasma against the original usual method of measuring total blood iodine or organic or hormonal fraction of whole blood has now been given over the latter. It is so, because most of the Protein Bound Iodine is in the serum or plasma which gives high value (Danowski, 1962).

With the advancement of scientific technique and refinement in method from time to time, accuracy in the measurement of serum Protein Bound Iodine has been reached to the highest degree. Long before, when Protein Bound Iodine was no more a prevalent method of direct determination for measurement of functional activity of thyroid, BMR and Cholestrol were the test-stone for studying the physiological significance of thyroid. Magnus-Levy. A (1895) reported rise in BMR in early pregnancy. But sine BMR and Cholestrol level are subject to the influence of a wide range of physiological as well as pathological conditions, their use remains limited to act as a true index of thyroid activity.

Protein Bound Iodine test has a good number of advantages over BMR which the latter lacks under many circumstances. BMR test measures the oxygen consumption of the body which is but an end product of all the oxidative processes of the body. (Long et al. 1952), Boothby and Sandiford, (1922) and Boothby et al. (1936) clearly showed a significant relation with oxygen consumption to body weight but factors like age, sex, pregnancy, climate etc. were also reported to modify it.

The transport utilization and breakdown of thyroidal

hormone may or may not commensurate with the rate of oxygen consumption. Moreover in cases like hypertension as a sequence of non-thyroidal hypermetabolic state,oxygen consumption of the body may also be affected.

It has been estimated that with the increase in age BMR decreases 3 to 5 per cent units per decade and that ultimately sex differences become less evident (Miller *et al.*, 1956). Melville and Mezey (1959) reported BMR within normal range even in severe psychotic patient, but enhancement in oxygen consumption due to much more psychic sensitiveness in domestic animals is apt to cause BMR to lose its significance of reliability and accuracy to a great extent.

The role of thyroid hormone in regulation of oxidative metabolism of birds and mammals is of paramount importance. Depression in metabolic rate following thyroidectomy has been report in geese, by Lee and Lee (1937), Marvin and Smith (1943) reported a similar state in pigeon. In chickens also Winchester(1939) observed depression in metabolic rate following thyroidectomy. A rise in metabolic rate till thyroprotein supplementation was reported by McCartney and Shaffner (1950). Mellen (1958) observed increase in metabolic rate for a short period during first few hours of fasting in thyroprotein fed birds.

Singh *et al.* (1968) reported transient rise in metabolic rate of chickens following administration of thyroxine which reached the maximum after two hours.

Administration of triiodothyronine and tetraiodothyronine

with combination of thyroxine was found to have produced significant depressing effect at 24 hours of their administration as reported by Singh et al. (1968).

Uses of sophisticated scientific apparatus and radioisotope too stand beyond the scope of usual laboratory test owing to fiscal factors and specialization in the technique of PBI determination. Chemical determination of PBI though technically difficult but huge works done in the past have left seal on its relability as a true index of thyroid function.

Several methods for the estimation of PBI in biological materials have been developed by various workers.

Chaney (1940):

He employed wet ashing and distillation method involving oxidation of the serum or plasma by means of sulphuric or chromic acid and separation of iodine by means of distillation in a special apparatus. His method was followed by various other investigators.

Lein (1941):

He adopted the ceric sulphate aresenious acid reaction in the determination of blood inorganic iodine.

Talbot, Butler, Saltzmann, and Rodriguez (1944):

Procedure adopted was same of Chaney (1940) but ashing was combined with permanganate alongwith photoelectric determinatim of starch iodine.

Salter and McKay (1944):

Salter adopted dry ashing procedure and made use of

ceric sulphate arsenious acid colorimetric reaction.

Taurog and Chaikoff (1945):

Taurog followed the procedure of Chaney (1940) with a few changes in the method. He was critical to the dry ashing method introduced by Salter et al. (1944). He considered distillation over dry ashing as being more accurate with respect to making iodine free more effectively from the non-volatile constituents which interfere in the colorimetric reaction.

Barker (1948):

He introduced alkaline incineration method.

Zak, Williard, Meyers and Boyle (1952):

Zak (1952) adopted chloric acid oxidation.

Ackerman and Meyers (1959):

He introduced rapid screening method.

Acland (1957):

The method consists of dry ashing and ceric sulphate arsenious acid colorimetric reaction.

Faulkner, Richard and Leonard (1961):

The technique introduced for determination of PBI in serum incorporates dry ashing and use of trichlor acetic acid for precepitation and sodium arsenite as reducing agent.

Hugh, Katz and Jenset (1967):

The method is a improved semimicro one with advantageous feature including use of 0.1 ml. sample and removal of inorganic

iodide by an anion exchange resin obviating the protein precepitation.

After considering the pros and cons of applicability of the various methods for determination of Protein Bound Iodine in the serum of White Leghorn laying, non-laying hens and pullets, in the existing facilities, the method of Acland (1957) was found suitable and was followed in the present investigation.

EXPERIMENTAL PROCEDURE

REAGENTS:

The reagents were prepared with redistilled water which was prepared with potasium permanganate.

(1) Zinc Sulphate solution. .. 10%

(2) Sodium Hydroxide solution. .. 0.5 N

(3) Sodium Carbonate solution. .. 4 N

(4) Hydrochloric acid solution. .. 2 N

(5) Sulphuric Acid solution. .. 3.5 N

(6) Ceric Ammonium Sulphate solution ..

3.9 gm. of the salt was dissolved in 3.5 N sulphuric acid and volume made 100 ml. in a volumetric flask and kept in refrigerator.

(7) Arsenious Acid solution.

3.8 gm. of arsenious oxide was dissolved in 50 ml. of warm normal sodium hydroxide, cooled and then 50 ml. redistilled water was added. Then the volume was made 500 ml. with 3.5 N Sulphuric acid.

IODINE STANDARD:

Stock solution - I.

130.8 mg. of previously dried Potassium Iodide (A.R.) was dissolved in redistilled water in a volumetric flask and volume made upto 1 litre with addition of double distilled water. This solution contained 100 ug. iodine per ml.

Stock solution - II.

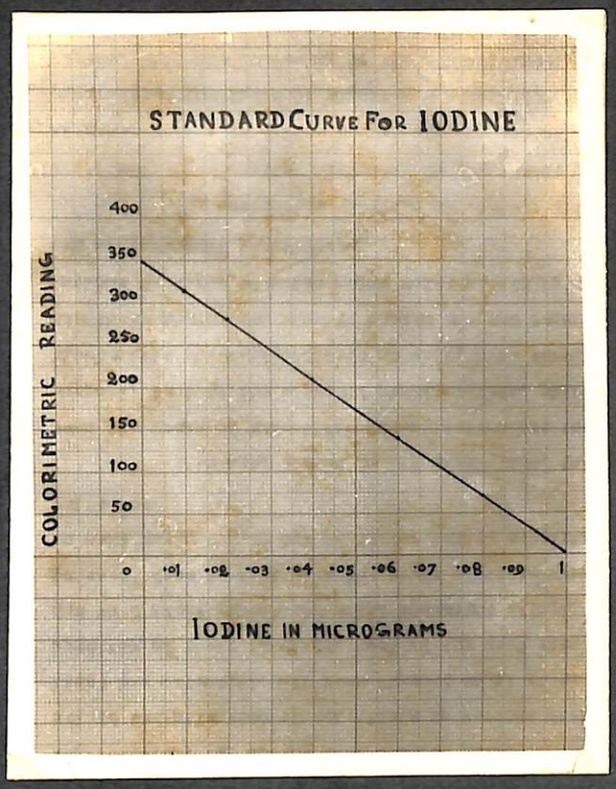
1 ml. of the first stock solution was diluted to 1000 ml. in a volumetric flask with redistilled water. This solution contained 0.1 ug. per ml. All the stock solutions were stored in refrigerator.

STANDARD SOLUTION:

Out of the stock solution containing 0.1 ug. Iodine per ml., 0.00 ml., 1 ml., 2 ml., 4 ml. and 8 ml. were taken in 5 different tubes. The volume of each tube was made 10 cc. by diluting with redistilled water. Therefore each tube contained 0.00, 0.01, 0.02, 0.04 and 0.08 ug. Iodine per ml. respectively. These solutions were prepared a fresh from time to time to run the standard.

CARE OF GLASSWARES:

Great care was taken to prevent the contamination of the room with mercury and salicylates which lower the PBI values. Particular attention was paid towards prevention of contact with inorganic iodide. All glasswares left in a detergent of dichromate


STANDARD CURVE FOR IODINE
COLORIMETRIC READING
400
350
300
250
200
150
100
50
0
·01
·02
·03
·04
·05
·06
·07
·08
·09
1
IODINE IN MICROGRAMS

cleansing solution overnight were thoroughly washed in running tap water first, and then rinsed with distilled water which were again rinsed with redistilled water.

All the tests were carried out in pyrex glass tubes (125 X 15 mm.). The tubes, once used, were discarded owing to bad etching. Preparation of iodine standard was done in a separate room.

PRECIPITATION AND WASHING:

Clear serum was separated from the blood of sacrificed bird after allowing it to stand at room temperature. To 1 cc. of the clear serum in a pyrex test tube 7 ml. redistilled water was added. Then 1 ml. of 10% zinc sulphate solution was added. It was mixed with a narrow glass rod. After adding 1 ml. of N/2 Sodium Hydroxide solution it was again thoroughly mixed and any material adhering to the wall of the tube was removed by rotating the glass rod against the inner wall of the tube. The tube was allowed to stand for 3 hours after which it was centrifuged for 10 minutes at 2000 revolution per minute in a centrifuging machine. The supernatant fluid was decanted. 10 ml. redistilled water was added to the tube. By light stirring with glass rod an uniform suspension of the residual protein precipitate was obtained. It was again centrifuged for 10 minutes at the same rpm. and the supernatant fluid was again decanted. Similar two more washings were carried out further.

DRYING AND INCINERATION:

After finally pouring off the supernatant fluid, 1 ml. of 4 N sodium carbonate was added to the washed precipitate. With the previously used glass rod it was gently stirred and the rod was washed drop by drop with 0.5 ml. redistilled water. The test tube (Pyrex) was then placed in a hot air oven at 95° to 98°C to drive off the water overnight. After thorough drying of the test tube, it was ashed for 3½ hours on an uniform bunsen flame till no charred particle was left unashed. Then the tube was allowed to cool to room temperature.

DISSOLVING IODINE FROM ASH:

To the ash 1 ml. 2 N Hydrochloric Acid was carefully added from the sides of the tube to avoid any excessive effervescence. The ash was completely dissolved by thorough mixing with a glass rod. 6 ml. redistilled water was added to it and was left to stand for 30 minutes. Within this period the tubes were stirred 3 times with glass rod and then centrifuged for 20 minutes at 3000 rpm.

COLOUR DEVELOPMENT:

Then 3 ml. portion of the clear supernatant fluid was pipetted out in duplicate to each of which 0.5 ml. of arsenious acid solution was added. After adding 1 ml. of 3.5 N Sulphuric Acid solution to each of the tube and mixing the content by flicking with fingers, the tubes were placed in a constant temperature bath

at 37° + 0.1°C for 10 minutes. The ceric ammonium sulphate solution was also warmed similarly. At the end of 10 minutes 0.5 ml. ceric ammonium sulphate solution was added to each tube and quickly mixed by flicking with fingers.

Exactly 12.5 minutes after beginning to add the ceric ammonium sulphate solution, reading was taken in a photoelectric colorimeter by using filter no. 42.

A reagent blank was run with each determination carrying out the same procedure as usual for the test except that instead of taking 1 ml. serum 1 ml. redistilled water was used.

CALCULATION:

The amount of iodine present as PBI in 3/7 ml. of serum was read from a calibration curve. Multiplication of this by 700/3 gave the microgrammes PBI per 100 ml. The sample taken for estimation consists of 1 cc. serum. After ashing is completed, addition of 1 ml. 2 N Hydrochloric Acid followed by 6 ml. of water make the volume 7 cc. in all. On centrifuging the same, only 3 ml. portion of the supernatant is taken which is 3/7th of the 1 cc. serum which is read from the curve. As such inorder to obtain the value in percentage it is multiplied by 700/3 which gives the PBI value in ug. per 100 ml.

RESULTS AND DISCUSSION

The results obtained are presented in Tables 2.1, 2.2 and 2.3.

T A B L E - 2.1

Table showing Protein Bound Iodine in ug. per 100 ml. serum in three different groups of birds.

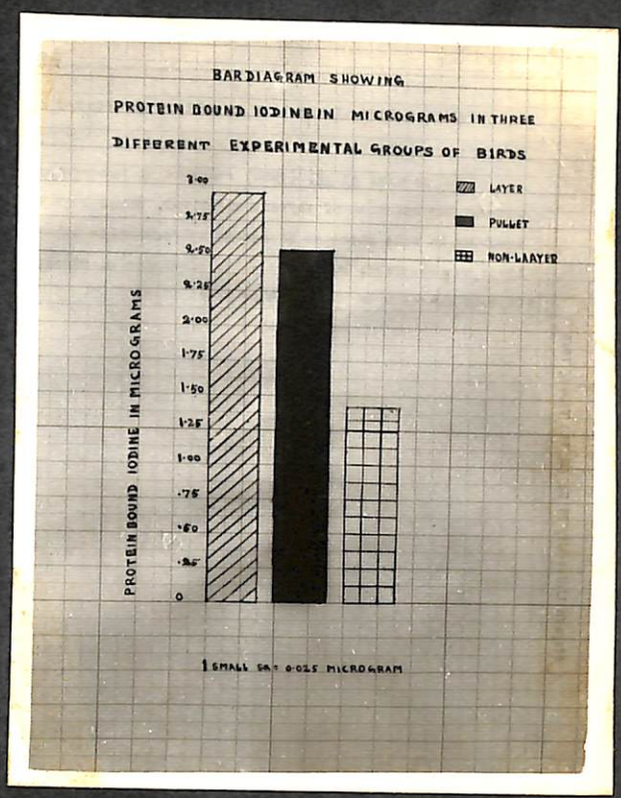
Layers.	Non-layers.	Pullets.
4.2	1.1	2.3
3.9	1.6	3.03
3.0	1.4	2.8
2.5	1.8	2.3
4.6	1.05	2.3
1.86	1.58	-
2.3	-	-
2.1	-	-
Average with S. E. 2.95± 0.342	1.42 ± 0.123	2.54 ± 0.155

T A B L E - 2.2

Table showing analysis of variance of Protein Bound Iodine.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	8.5813	4.2906	7.86*
Within group.	17	9.2705	0.5453	
Total:-	19	17.8518		

* Significant.


BAR DIAGRAM SHOWING
PROTEIN BOUND IODINE IN MICROGRAMS IN THREE
DIFFERENT EXPERIMENTAL GROUPS OF BIRDS
LAYER
PULLET
NON-LAYER
PROTEIN BOUND IODINE IN MICROGRAMS
2·00
1·75
1·50
1·25
1·00
·75
·50
·25
0
1 SMALL SQ = 0·025 MICROGRAM

T A B L E - 2.3

Table showing the mean difference of PBI with critical difference between the three groups.

	Mean difference	C.D. at 5%	C.D. at 1%
Layers Vs. Non-layers.	1.529 **	0.3182	1.0478
Layers Vs. Pullets.	0.405 *	0.3757	1.2589
Non-layers Vs. Pullets.	1.124 **	0.4198	0.6409

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

(a) Protein Bound Iodine value in two cases of liver damage in non-layer groups ---- 0.7 and 0.9 ug.%.

(b) Protein Bound Iodine value on storage under refrigeration of serum sample of the same bird in a layer group.

Fresh serum - 2.1 ug.%

Over two months - 2.3 ug.%

In the present studies the Protein Bound Iodine values in three groups of White Leghorn birds found were 2.95 ± 0.342, 1.42 ± 0.123 and 2.54 ± 0.155 ug. per 100 ml. in layers, non-layers and pullets approaching sexual maturity respectively. The values reported by various workers in the domestic fowls are 3.3 to 3.8 Taurog *et al.* (1945), 2.6 ± 0.3 Katsh *et al.* (1955), 1.16 ± 0.03 ug.% (Mellen *et al.*, 1957). The value of PBI as reported by Mellen *et al.* (1957) ranged between 0.88 and 1.48 ug.% at 8 to 20

months of age respectively.

Reports on PBI value in this country by various workers shows as 1.75 ± 0.06 ug.% in White Leghorn cocks and 2.42 ± 0.17 ug.% in Deshi cocks (Gulati, 1968), and 1.12, 1.76 and 1.26 ug.% for chicks, Bobwhite Quail and Coturnix respectively (Singh et al., 1968).

The values of PBI in aforesaid three groups under study are comparable in general with those reported by other workers in domestic fowl. It is interesting to note the higher PBI value both in the laying and pullets which is significant at 1% and 5% levelas compared to PBI value in non-laying hens. The higher PBI value in laying and pullets appears to be due to increased functional activity of thyroid.

Increase in PBI could be induced by administration of estrogen to normal subject. Since estrogenic steroids are elevated in normal pregnancy it is probable that this is a factor in higher thyroxine level. Smith et. al. (1954), Peters et al. (1948) pointed out that uncomplicated pregnancy is characterised by a rise in serum PBI level within or above euthyroid range and that if there is no such increase in the first few weeks of pregnancy it follows abortion. Administration of thyroxine or desiccated thyroid is met with success in carrying out the terms(Man et al. (1951).

Krohn (1950) also reported that removal of thyroid interfered with pregnancy. Kessel and Politzer (1957) reported that immediately following delivery the serum PBI and thyroxine level decrease till finally come to normal.

Fogel (1965) reported that premature suppression of pregnancy was accompanied by the reduction in the quantity of PBI in blood plasma and that a threat to abortion was particularly in association with the low PBI value. The normalization of function of thyroid of such patient with preparation of thyroidine in combination with estrogen, progestrone alongwith gallascorbic acid brought about a rise in plasma PBI level.

Moreover increase in the thyroid binding globulin during pregnancy has been ascribed to and increase in estrogen secretion Smith and Smith (1948). These observations are in complete agreement with that of Dowling et al. (1959) who reported rise in thyroid binding globulin comparable to rise in pregnancy on administration of diethylboestrol to non-pregnant women. The increase in PBI as such seemed to be secondary changes in the thyroid binding globulin.

Due to estrogenic effect, increase in size of oviduct in chickens at sexual maturity has been reported by various workers. Hertz (1945) reported as much as 40 times increase in the size of oviduct following continuous administration of estrogen.

As such the plausible explanation to be put forth on the basis of above reports are very well applicable to a similar physiological condition in laying hens and sexually mature pullet showing significant PBI value over the non-laying birds under study. It would also be interesting to make a comparison here of the normal Protein Bound Iodine in birds with those of mamals reported by other workers.

The data on PBI values as reviewed earlier in preceding

chapters, indicate higher values in equine, bovine and man except baboon as compared to PBI value in avian. It shows that there are significant differences in thyroid function between birds and mammals. This difference appears to be due to important variation in the thyroid hormone circulating in the blood of chickens.

Newcomer (1957) observed that in contrast to other animals the volume of distribution of thyroxine is largely that of serum globulin in chickens. It was found to be bound to alpha albumin and alpha albumin to triiodothyronine as strongly as they bound thyroxine.

Wentworth and Mellen (1961) found circulating thyroid hormone in ratio of 60% thyroxine and 40% triiodothyronine in the blood of chickens. It is considered that in man alpha globulin and not the serum albumin is the main carrier of thyroxine Robbins et al. (1961). Whereas no alpha globulin rather thyroxine binding albumin and prealbumin have been reported in chickens Frare et al. (1962).

In man the thyroid binding globulin has a four fold affinity for thyroxine as compared to triiodothyronine but in case of birds the protein binding affinity is equal.

As such the disappearance of thyroxine from blood being equal they diffuse from the tissues at a similar rate (Shellabarger and Tata (1961).

In rat triiodothyroxine has been found to be 3 to 7 times more potent than thyroxine with respect to prevention of goitre (Sturkie (1965) but the biological activity of triiodothyro-

-nine and thyroxine are rather equal in birds Tata et al. (1957) and Singh (1967).

However, Newcomer (1957) reported that thyroxine is more potent than triiodothyronine as an antigoitrogenic substance in chickens.

Thyroxine in mammals stimulates the metabolic rate which lasts for several days Cyril and Neil (1965) in contrast with birds in which a single injection of thyroxine stimulates the basal metabolic rate lasting for a maximum duration of 2 to 3 hours only.

One characteristic feature observed in course of studies was low PBI value in two adult non-laying birds of the same group from same stock. These two birds were found to have damaged liver with crepitating lesions on it suggestive of chronic hepatic cirrhosis. Moreover they were apparently in debilitated condition and were anaemic with a poor outlook. Their PBI value was found to be lowest of all the birds in non-laying groups. In one case it was 0.7 ug.% and 0.9 ug.% in another.

Although no such reports are available as would throw light on this aspect particularly in the avian species under study but reports in human indicate that acute infections of liver have been followed by a rise in PBI whereas in chronic hepatic cirrhosis the PBI value in some cases was elevated, normal in most and low in some cases usually associated with low concentration of serum albumin Kydd et al. (1951). In case of later stages of impairment of liver the thyroid binding globulin may fall below normal. The

chronic illness and various other conditions may be associated with a decrease in thyroid binding prealbumin Richards et al.(1959).

This change might perhaps be the mechanism for the decrease in PBI. As such it is quite possible that decrease in thyroxine binding albumin and prealbumin in these two particular cases might have been the decisive factor to account for the low PBI value. It would not be unreasonable to presume that this feature of liver might have had adverse influence on estrogen affecting the laying ability of the bird which was apparent from the condition of the oviduct.

Similarly a peculiarly high PBI value in one another non-laying bird was observed which was 3.8 ug.% unlike all other non-laying birds. With all confidence it can be said that the very appearance of its ovary and oviduct bore ample proof to show that the bird was in the condition on the verge of laying as was apparent from the characteristic feature both external and internal noted before and after the sacrifice of each bird as usual.

It was also found that serum sample of the same bird on storage over 2 months period in refrigerator did not vary markedly in PBI value which is evident from the result obtained which was 2.1 ug.% in the beginning and 2.3 ug.% on estimation after two months.

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C H A P T E R - V.

Vitamin A.

V I T A M I N A

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

Correction of visual inability to see properly at night with vitamin A dates back to several thousand years (Bible). In very early days Chinese used to take advantage of the substances rich in vitamin A as remedies for night blindness. (Maynard and Loosli, 1956).

Hopkins (1906) stated that " no animal can live on a mixture of pure protein, fat and carbohydrate, and even when the inorganic material is carefully supplied, the animal still can not flourish".

Funk (1912) coined the term Vitamine. McCollum, Davis, Osborne and Mendel (1913) gave definite proof of the existence and specific physiological function of Vitamin A.

Rosenberg (1942) defined vitamins as"an organic compound required for the normal growth and maintenance of life of animals including man, who as a rule are unable to synthesize these compounds by anabolic processes that are independent of environment other than air and which compounds are effective in small amounts, do not furnish energy and are not utilized as building units for the structure of the organism but are essential for the transformation of energy and for the regulation of metabolism of structural units".

Maynard and Loosli (1956) summarized that a generally

recognized vitamin is one that has been proved an essential dietary constituents for one or more species. Some vitamins are metabolic essentials, but not dietary essentials for certain species, because they can be synthesized readily from other food or metabolic constituents. While metabolic needs are similar, dietary needs for the vitamins differ widely according to species. Vitamin C, a metabolic essential for many species, is a dietary essential only for man, guinea pig and monkeys.

Vitamin A exists only in the animal kingdom as free alcohol and esters of higher fatty acid and is related to unsaturated acids. In 1930 Thomas Moore gave proof that animal body transformed carotene into vitamin A. Four different kinds of carotenes which have vitamin A activity namely alpha, beta, gama and hydroxy beta carotene or cryptoxanthine have now been recognised. Vegetable plants are a rich source of carotene. Yellow corn which is rich in amount of provitamin A, also called beta hydroxy carotene, is as such, a rich dietary source of vitamin A to poultry.

The results of the experiment conducted on two strains of chickens by Hill *et al.* (1961) revealed a marked increase in the concentration of vitamin A with higher level of vitamin A. No close relationship between the vitamin A content of the liver and productive performance of the hen nor the ability to transferring to the egg yolk was observed at lower level. Adequate requirement for normal productive performance was met with lower level of vitamin A, no doubt, but was not sufficient to bring about high

level of storage of vitamin A in the liver.

T A B L E - 3.1

Table showing effect of dietary vitamin A in the vitamin A control of the liver of the hen. (Hill et al., 1961).

Vitamin A in U. S. P. units per lb. of diet	Vitamin A in U.S.P. units per gm. of liver in experiment-I	Vitamin A in U.S.P. units per gm.of liver in experiment-II.
800	Vitamin A not detectable.	16
1200	17	18
1600	70	-
2000	21	9
3600	-	330
5000	1540	3100
10000	-	3100

It is apparent from the above data that level of vitamin A in the hens liver was relatively low below the 2000 U.S.P. units per lb. or less than that. A similar study in previously conducted experiment by Duel et al. (1943) in chickens with different levels of vitamin A speaks well of the ability of liver to store vitamin A. The results obtained by them are presented below to show the critical level of vitamin A supplement at which the storage in liver has dropped down to a remarkable extent.

T A B L E - 3.2

Table showing effects of different levels of vitamin A intake and its content in liver of chickens.(Duel et al.,1943).

Vitamin A supplement per lb. of food in microgram.	Per gm. of liver vitamin A in microgram.	Per gm. of liver vitamin A in I.U.
0	111	326.34
333	200	588.00
666	-	-
4500	-	-
9000	1378	4151.32
18000	2082	6121.08
30000	1396	4104.24
60000	1388	4080.72

From perusal of the above data it is evident that liver vitamin A storage is relatively higher at all level of intake in contrast with the findings of Hill et al. (1961). Quite possibly, the forthcoming explanation would be attributable to such higher storage as the kind of vitamins and the vehicles used in the feed.

Marusich and Bauernfeind (1963) reported the cumulative nature of vitamin A storage in the liver. They also observed a marked relationship to the amount of dietary vitamin A and the

length of period on the diet. Their findings are follows:-

T A B L E - 3.3

Table showing storage of vitamin A in liver in 7 weeks at different level of feeding.(Marusich et al., 1963).

Supplements	I.U. of vitamin A per lb. of feed.	I.U. of Vitamin A consumed in 7 weeks.	I.U. of Vitamin A found in liver.	% of Vitamin A stored in liver.
Dry Vitamin A beadlets.	2500	18,310	5150	27.8
Dry Vitamin A beadlets.	5000	36,200	16910	46.8

In another experiment Marusich et al. (1963) demonstrated the different level of intake of vitamin A and its storage in the liver showing higher level of intake accompanied by increase in storage percentage.

T A B L E - 3.4

Table showing storage of vitamin A in liver in 4 weeks at different level of feeding. (Marusich et al.,1963).

Supplments	I.U.of vitamin A per lb. of feed.	I.U. of Vitamin A consumed in 4 week.	I.U. of Vitamin A found in liver.	% of vitamin A stored in liver.
Dry Vitamin A beadlets.	500	1210	30	2.6
-do-	1000	2380	200	8.3
-do-	2500	5880	1480	25.2
-do-	5000	12060	4320	35.8

Similar observations have been made by a good number of workers. The reports of Harem, Reid and Couch (1955) and Kurnic et al. (1961) are in agreement with the findings of Marusich et al. (1963).

In order to serve the body carotene must be converted to vitamin A. Conversion of carotene to vitamin A in the intestinal wall has been reported in chickens.(Maynard, 1956).

Liver is the large reservoir for storage of vitamin A in animal body. Guilbert and Hart (1934) demonstrated that total storage of carotene and vitamin A in the liver and depot fat of cows getting carotene rich ration all along their life ranged from 67 to 93%. In aged cows it was 3.6 gms. which was nearly 6 times more than those of the younger animals estimated to consist of 0.6 to 0.7 gms. only.

Subsequent upon supply of massive dosage of the vitamin to rats, the adult is capable of storing vitamin A in liver too sufficient to meet its requirement till a centuary. But the fact remains that during this superfluous storage, elemination rate is too rapid to remain in state of massive storage till reaching the state of stability.

The liver vitamin A storage of pullets was studied by Kurnick et al. (1961). They observed marked difference in the storage of vitamin A in the liver owing to difference in the content of the vitamin supplied in the feed per lb. The liver vitamin A storage was found to range from 3.5± 10.45 I.U. of vitamin A per liver when 500 I.U. of vitamin A per lb. of feed was

given, to 336 ± 44 I.U. per liver at 3000 I.U. per lb.

EXPERIMENTAL PROCEDURE

ESTIMATION OF VITAMIN A.

Vitamin A was estimated in the liver and feeds of the poultry according to the method of Oser, Melnick and Pader (1943).

Reagents:

1. Alcoholic Potassium Hydroxide - 0.5 N
2. freshly redistilled Anaesthetic Ether.
3. Aqueous Potassium Hydroxide - 0.5 N
4. Anhydrous Sodium Sulphate.
5. Antimony Trichloride Solution (A.R.) 25%.
6. Phenopthalin.

Estimation of Vitamin A in liver:

One gm Two gms. of fresh liver immediately after sacrificing the bird was accurately weighed on a sensitive balance in a clean dry watch glass. It was then thoroughly triturated in 15 30 ml. of freshly prepared 0.5 N alcoholic potassium hydroxide solution in a glass pestle and mortar. Vitamin A is present in the unsaponifiable portion of the lipid. It was therefore saponified for 30 minutes in a 50 ml. conical flask by refluxing it in a boiling waterbath. The saponified contents in the conical flask was then washed with an equal volume of distilled water and transferred to a Separatory separating funnel. 15 ml. ~~anaesthetic~~ ether was added to the separating gms funnel and after extraction, the aqueous phase was discarded. This procedure was repeated thrice with the same

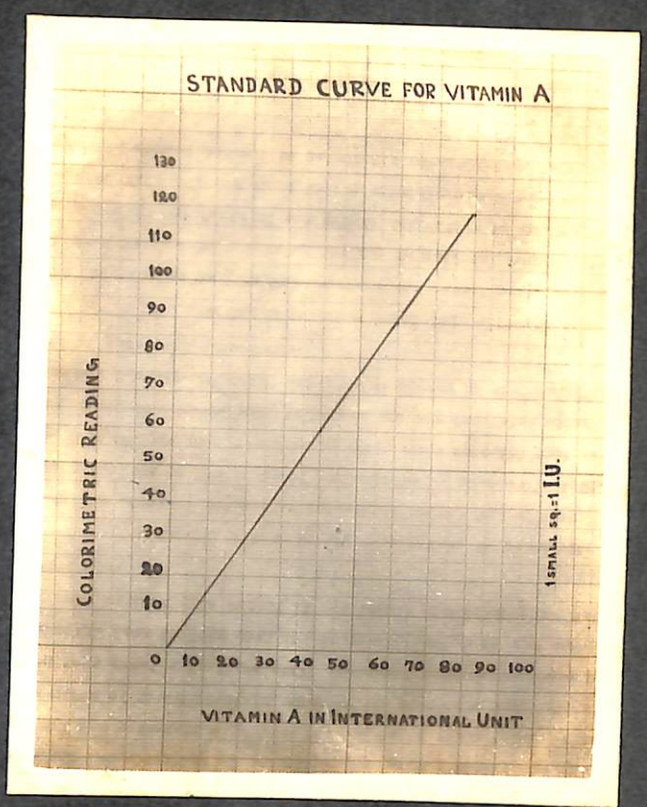
quantity of ether. Then the ether was washed with 50 ml. distilled water and once again with 25 ml. of 0.5 N aqueous potassium hydroxide. This was followed by repeated washing with distilled water till no pink colour developed in the last washing with a few drops of phenopthalin. To ensure complete removal of even the last trace of water, 10 gms. of anhydrous sodium sulphate was added. The ether extract was thus made free from all traces of water and then transferred to a 50 ml. clean dry beaker. It was evaporated to dryness in a waterbath. Immediately after dissolving the residue in 4 ml. chloroform (A.R.) 1 ml. of the dissolved residue was transferred to the cuvette of the photo-electric colorimeter. 9 ml. of 25% Antimony trichloride (A.R.) solution was immediately added to the cuvette containing the extracted vitamin A which developed a blue colour. The reading was taken at 560 millimicron wavelength by using filter no.54. Reading of the unknown was taken by setting the blank at zero.

The concentration of the unknown was calculated from the following formula.

Concentration of unknown = Reading of unknown x Calibration factor

Preparation of standard:

A stock solution of 1 ml. of commercially prepared vitamin A containing 300000 I.U. as vitamin A palmitate was made by diluting it to 30 ml. with analytical grade chloroform. This solution therefore, contained 10000 I.U. per ml. Out of this stock solution 1 ml. was taken in a 100 ml. volumetric flask and volume was made upto 100 ml. with addition of chloroform. Thus each ml. of this solution contained 100 I.U. of vitamin A. In 5 different


STANDARD CURVE FOR VITAMIN A
COLORIMETRIC READING
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80 90 100
VITAMIN A IN INTERNATIONAL UNIT
1 SMALL SQ.=1 I.U.

standard test tubes 0.00, 0.1, 0.2, 0.4, and 0.8 ml. of the workable standard solution were taken and volume of each made 1 ml. with chloroform. Thus each ml. of the standard solution in 5 different test tubes contained 00, 10, 20, 40, and 80 I.U. vitamin A respectively.

Reading was taken with rapid addition of 9 ml. of 25% Antimony trichloride solution by using filter no.54. Concentration of standard was measured in duplicate in a Klette summerson photo-electric colorimeter by setting the blank at zero. Calibration factor for each concentration of standard was determined and their average value was taken. The calibration factor used in this experiment was 0.66. It was determined with the following formula:-

$$\text{Calibration factor} = \frac{\text{Concentration of standard}}{\text{Reading of standard.}}$$

Correction for carotene in Vitamin A estimation:

The determination of the extent to which carotene is responsible for development of blue colour in Carr-Price reaction is essential to know the exact content of Vitamin A in any of the biological sample; because in this reaction, in course of extraction of Vitamin A, carotene is also extracted along with it.

The method suggested by Pett and Lapage (1940) has been followed in the experiment for determination of correction factor.

Procedure:

5 mg. of crystaline beta carotene was accurately weighed on Torsion balance. It was dissolved in 100 ml. analytical grade chloroform in a 100 ml. volumetric flask so as to contain

50 ug. of carotene per ml. To 1 ml. of chloroform containing this solution i.e. 50 ug. beta carotene, 9 ml. of 25% Antimony Trichloride solution was added. Development of blue colour was measured on a photoelectric colorimeter using filter no.42 (blue). Readings were taken in duplicate, the average of which was multiplied by the calibration factor for Vitamin A which gave the value of Vitamin A equivalent for 50 ug. of beta carotene. Thus 1 ug. beta carotene equivalent of Vitamin A value was found out which was 0.72

Carotene estimated in the biological sample was multiplied by the correction factor which gave the value of Vitamin A equivalent of beta-carotene present in the biological sample which was substracted from the amount of Vitamin A estimated. Thus actual amount of Vitamin A content of the sample was determined.

Preparation of standard:

10 mg. of crystalline beta-carotene was accurately weighed on Torsion balance and dissolved in 100 ml. of Petroleum Ether (b.p.40-60^{o}C) in a volumetric flask. Thus 1 ml. of Petroleum Ether contained 100 microgram of beta-carotene. Out of the stock solution of beta-carotene containing 100 ug. per ml. 0.05 ml., 0.1 ml., 0.2 ml., 0.4 ml. were taken in different test tubes and the volume of each tube was made 1 ml. by adding Petroleum Ether. Therefore 1 ml. of each standard contained 5, 10, 20, and 40 ug. of beta-carotene respectively. Calibration factor was determined in duplicate for each concentration. The calibration factor used in the experiment was 0.10.

Estimation of carotene in liver:

Carotene like Vitamin A is destroyed by oxidation and

the process is accelerated especially at high temperature. Application of heat or use of alkali in the extraction process is also responsible for inaccuracy in determination of carotene in biological materials. Of course heat without oxygen has a minor effect.

Reagents:

1. 1:1 Acetone and Petroleum Ether (b.p. 40-60°C).
2. Hydroquinone.
3. Glass wool.
4. Anhydrous sodium sulphate.
5. Bone meal.

The method of Bacharach (1950) was followed for determination of carotene in the biological material with a little modification as suggested by Majumdar and Gupta (1960).

Processing of bonemeal for use as a chromatogram:

The bonemeal was carefully sifted to remove any adhering material. It was then thoroughly washed with hot water and dried in a hot air oven. After boiling it in absolute alcohol under reflex for four hours it was again dried in hot air oven. Then sufficient quantity of Petroleum Ether was added to it and it was left overnight in the refrigerator. It was finally dried in hot air oven. By this processing the bonemeal was purified as it was essential to remove, any fat and pigment present in the bonemeal, for use as chromatrogram.

Procedure:

Two gram of liver was accurately weighed on a sensitive balance in a clean dry water glass. It was transferred to a glass

pestle and mortar and 25 ml. of 1:1 Acetone and Petroleum Ether (b.p. 40-60°C) was added to it. It was thoroughly triturated and blended with a flattend glass rod and for complete homogenization it was run in a waring blendor. The whole homogenised biological material was extracted thrice with addition of 25 ml. of 1:1 Acetone and Petroleum Ether. The extract was then filtered through glass wool to remove the suspended materials. The glass wool was finally washed with 10 ml. of 1:1 Petroleum Ether and Acetone mixture. Thus last trace of carotene on the glass wool was removed with the filterate. Then it was thoroughly washed with distilled water to enable removal of any trace of acetone. As acetone is miscible with water, four washing with distilled water was sufficient for its removal. In a clean beaker the residual Petroleum Ether was collected and 10 gm. of Anhydrous sodium sulphate was added to it to absorb the last trace of water in it. As bonemeal absorbs xanthophylls and other non-carotenoid pigments, 7 gms. of the purified bonemeal was added to it and was shaken well. The pure carotene thus obtained was allowed to stand for half an hour and then it was filtered through a whatman No.40 filter paper.

The volume of the pure carotene extract was made constant at 10 ml. before taking the final reading in the photo-electric colorimeter. By using filter no.42 the reading was taken at 440 millimicron wave length. The reading of the unknown was taken against a blank set at zero.

Calculation:

The concentration of unknown was calculated from the

following formula:-

Concentration of unknown = Reading of unknown x Calibration Factor.

Where Calibration Factor = $\frac{\text{Concentration of standard}}{\text{Reading of standard.}}$

Estimation of Carotene in feeds:

The procedure adopted for estimation of carotene in feeds was same as discussed earlier for the liver. The feed sample obtained from the general stock of the Central Poultry Farm, Patna was powdered in a glass pestle and mortar. 2 gms. of this finely grinded feed was accurately weighed on a sensitive balance and carotene was estimated as per previous procedure for liver, but the feed was extracted with 1:1 Petroleum Ether 4 times instead of thrice as in the liver.

VITAMIN A AND CAROTENE IN BLOOD

Vitamin A is not found free in blood. It is always in association with blood protein as Vitamin A-Protein-Complex.

In a study on the carotene and Vitamin A content of blood serum in chickens at different level of dietary Vitamin A Duel et.al. (1943) observed marked decrease in the carotene level of plasma following administration of massive doses of Vitamin A. From the results of Duel et.al.(1943) showing high concentration of Vitamin A it is evident that Vitamin A content of plasma does not follow any definite pattern. Moreover it shows that blood serum tend to resist any marked change in Vitamin A concentration during higher level of intake.

T A B L E - 3.5

Table showing carotene and Vitamin A content of blood serum in chickens at different dietary level of Vitamin A.

(Duel et.al. 1943)

Vitamin A supplement in microgram per lb. of food.	Carotene in microgram per 100 cc. serum.	Vitamin A in microgram per 100 cc. serum.
0	143.2	139
333	218.0	166
9000	111.4	134
18000	105.8	174
30000	62.6	133
60000	37.0	207

But the findings of Taylor et.al.(1947) are not in fair agreement with the results of Duel et.al.(1943) as presented in the Table 3.5 above. Individual variation in the Vitamin A content of blood serum of laying hens receiving 3070 I.U. pro-vitamin A per lb. of feed was reported by the former. The level of Vitamin A in the plasma is primarily a function of body storage. Marked depletion in the body reserve is reflected when the average plasma Vitamin A value drops down to 75 I.U. per 100 ml. Average value below 30 I.U. speaks of the total depletion thereby indicating serious effects.(Taylor et.al.1947)

Squibb (1961) reported 41.25 microgram Vitamin A content per 100 ml. in serum of White Leghorn hens maintained at 3000 I.U. of Vitamin A per lb. of feed. He also observed

individual variations in the Vitamin A content of the serum.

Almquist (1952) pointed out that plasma Vitamin A in chickens and turkeys bear a linear relationship to the log of concentration of Vitamin A in the liver. He also stated that as regards poultry nutrition, concentration of Vitamin A in the plasma is a fair index of Vitamin A. His views find support in a similar observations made by Taylor et.al.(1947), Squibb(1961) and Barter (1963).

With respect to utilization of Vitamin A by chickens Castano et.al.(1951) stated that the amount of Vitamin A present in the blood is related to Vitamin A intake.

Dolge ... et.al.(1953) reported a correlation between carotene intake and plasma carotene of calves.

Plack et.al.(1966) reported a higher level of retinal per 100 ml. in laying hens as compared to immature pullets and mature cockerel.

A lot of factors have been reported to effect the concentration of Vitamin A and carotene in the blood like plant pigments and hormones.

Estimation of Vitamin A in blood:

5 ml. serum was collected from the clotted blood obtained after sacrificing the bird as described in the general procedure. In order to break up the"Vitamin A Protein Complex" in the blood addition of alcohol or saponification is essentially required.

Vitamin A was estimated in serum according to the

method of Dann and Evelyn (1938).

Reagents:

1. Absolute alcohol.
2. Petroleum Ether (b.p. 40-60°C).
3. Antimony Trichloride (A.R.) 25%.
4. Chloroform (A.R.).

Procedure:

Estimation of Vitamin A in blood was carried out by taking 5 ml. of serum in a 25 ml. capacity centrifuge tube. To this was added 5 ml. of absolute alcohol and was slowly shaken while adding it. Then 10 ml. of Petroleum Ether (b.p. 40-60°C) was added. The centrifuge tube was stoppered well and shaken for 1 minute at 1500 rpm. Then 5 ml. of supernatant layer of Petroleum Ether was pipetted out in a cuvette and was evaporated to dryness in a waterbath. It was dissolved in 1 ml. analytical grade chloroform. Reading was taken on photoelectric chlorimeter using filter no.54 after adding 9 ml. of 25% antimony trichloride solution to develop the blue colour within 4 seconds of addition of antimony solution.

Reading of unknown was taken against a blank set at zero. The Vitamin A content of the serum was calculated from the following formula.

Concentration of unknown = Reading of unknown x Calibration factor

Generally various experimental works seem to have been expressed in different units of Vitamin A by different workers. As such, for the shake of comparison International Unit, the

recognized standard prevalent, seems to be a better measure and all the different units converted into it are as follows:-

One International Unit of Vitamin A is estimated to be equivalent to

= 1 U.S.P. Unit.

= 0.7 Sherman Munsell Unit.

= 0.34 microgram of crystalline Vitamin A acetate.

= 0.30 microgram of Vitamin A alcohol.

60 I.U. of Vitamin A = 1 Blue Unit.

RESULTS AND DISCUSSION

L I V E R:

The results obtained in the experiment carried out are presented in Tables 3.6, 3.7 and 3.8.

T A B L E - 3.6

Table showing Vitamin A content per gm.liver in I.U.

Layers.	Non-layers.	Pullets.
392.04	210.48	524.68
485.20	246.99	431.93
590.33	443.62	551.86
299.82	392.22	471.15
425.55	210.33	-
490.76	231.06	-
392.15	204.16	-
525.26	-	-
458.66	-	-
Av.with S.E. 455.30±25.72	276.98 ± 37.13	494.90±26.86

Over all average with S.E.403.27 ± 26.65.

T A B L E - 3.7

Table showing analysis of variance of Vitamin A content.

Source of variation.	Degree of freedom	S. S.	M. S.	F.
Between group.	2	172019.94	86009.97	12.24**
Within group.	18	126464.06	7025.78	-
Total :-	20	298484.00		

** Highly significant.

T A B L E - 3.8

Table showing the mean difference of Vitamin A content per gm. liver in I.U. 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%
Layer vrs.non-layers.	178.32	118.86	86.77
Layer vrs. Pullets.	39.60	151.30	110.45
Non-layers vrs.Pullets.	217.92	153.89	112.34

** - Significant at 1% level.

The Table - 3.6 shows that the Vitamin A content of liver in the laying, non-laying groups and the pullets approaching sexual maturity is 455.30 ± 25.72, 276.98 ± 37.13 and 494.90 ± 26.86 respectively. From perusal of the table it is clearly seen that the liver Vitamin A value is significantly

higher both in the laying and pullets. The difference in Vitamin A content of the liver between the laying and non-laying birds was significant at 1% level. Such significant difference was also noticed between the pullets and the non-layers. However, with respect to Vitamin A value in the laying birds and the pullets the trend in the latter appeared to dominate over the former. Moreover the potential storage capacity in the two groups other than the non-layers also appeared to be more.

The above observation leads to an enevitable conclusion that the high level of liver Vitamin A in the laying and the pullets over the non-laying birds might be subject to such contributory factors as would arise out of the difference in the level of different hormones in the body, because all the birds were allowed a free choice of food intake without any prejudicial treatment with respect to quantity of food supply to any of them. At same level of feed supply and maintained in identical environment under same managerial condition of feeding, watering and housing the outstanding difference in the storage of liver Vitamin A in the birds indicates changes in the physiological state of the 3 groups under study.

The process of living enevitably requires adaptation to environmental physiological and pathological situation and consequantly alteration in endocrines are continuous. Dumm and Ralli (1961).

Hechter (1955) pointed out the response of body to the changed metabolic situation induced by the hormones. Increase or decrease in the release of hormones will be reflected in cellular

metabolism and will affect the cellular requirement for certain vitamins and other essential nutrients (Dumm et al., 1961). It is clear that during increased physiological activity of laying eggs and growing stage, intensive metabolism is involved. The liver and fatty tissue tend most readily to store estrogen.

Camp et al. (1957) observed that estrogenized chickens fed on low protein diet gained 13 to 16% more weight than those receiving high protein level. Similar observation have also been reported in turkeys by Miner et al. (1959).

It shows that during the period approaching sexual maturity, the pullets might have had, under the extreme influence of high level of estrogen, more food intake called upon by increased demand while growing. The maximum rather better efficiency of utilization and conversion under active role of thyroid as such might have resulted in more storage of vitamin A available as carotene as well as preformed vitamin A present in the feed stuff.

Dennenberg et al. (1966) observed a highly significant concentration of retinal and retionol esters in liver of pregnant rats as compared to the pseudopregnant females. Also appearance of decidual cells were noticed.

As such the interpretation of the above observation can not be ruled out to imply that the pullets and birds laying eggs had a higher concentration of vitamin A in their liver owing to the influence of the hormone governing phenomenon they were passing through. Various reports are available to show that blood lipids of the laying female are considerably higher than in non-laying

hens and males and most of the surplus fat is deposited in the egg to form the yolk.

Hill et al. (1958) reported that increased fat production following administration of estrogen is due primarily to increased energy consumption and to a less extent to peripheral fat synthesis at the expense of the protein.

The rate of absorption of carotene is not only controlled by thyroxine and thiouracil but it has also been found that digestibility of this pigment is a function of the thyroid secretion. It has been demonstrated by Chanda et al. (1951) that carotene digestibility was higher during the administration of thyroxine and lower during thiouracil feeding.

More efficiently carotene conversion to vitamin A has been demonstrated in hyperthyroid rat than in the animal possessing a normal thyroid function (Goodwin, 1948). It has been confirmed that vitamin A deposition in the liver after feeding of carotene was decreased following thiouracil treatment to rats and increased with thyroid supply. Similar observations have been made by Johnson and Baumann (1947).

T A B L E - 3.9

Table showing vitamin A content in I.U. in the feed stuff made available to three grapps of W.L.H. birds.

Vitamin A in I.U. in 120 gm. mash.	Vitamin A in I.U. supplemented in the feed.	Total vitamin A in I.U. available to the birds.
1284.12	960.00	2244.12

Although there are reports in disagreement with the above observation but the possibility based on experimental findings is in conformity with those bearing upon the fact that the layers and maturity pullets might have higher demand of thyroxine to cope with situation of growth and egg production. Subsequently the major part of carotene available to the birds from the feed might have been absorbed more efficiently and converted effectively to vitamin A and hence more deposition in the liver. A converse of it is as such reflected in the non-laying group. Our this presumption finds support with the views expressed by Barnes (1951) who reported that vitamin A is primarily transported in the form of carotene and this substance is converted to free vitamin A by the fetal liver.

In the present investigation, the point does not deviate from the fact that the maximum intake of carotene from the feed stuff must maximally have been transported in this form and stored in the liver by the layers and pullets.

Shellenberger et al. (1960) made observation on the effect of vitamin A level of diet on feed conversion and utilization of energy by growing chickens. They found higher liver and serum vitamin A in laying birds than the broilers at all level of intakes, which is true of our results with a marginal fluctuation in pullet over the laying ones.

At three levels of vitamin A, 2000, 4000 and 8000 units per lb. of ration fed to 19 and 9 months old birds after a depletion period of six weeks, no effect on egg production and feed

conversion was observed. But the liver vitamin A storage was found to be 5 times that of start with 8000 units and it tended and continued to decline till level of 2000 units supply was reached (Halloran, 1960).

This observation is in fair agreement with the results obtained. The difference in the effect particularly on feed conversion incorporated in the discussion may be accountable for the difference in the form of vitamins and vehicle used in the ration. Various workers have pointed out that digestibility co-efficient of carotene varies with the variation in the vehicle used with it.

Kemmerer and Frap (1938) reported that coefficient of digestibility was 51 when carotene was given in oil to rats and 22 when it was fed as dehydrated alfalfa without added fat. They also found that digestibility of beta carotene in rats varied with the amount fed and vehicle used. When it was fed to rats in dehydrated alfalfa at level 1, 10.5 and 20 parts per million the digestibility coefficient were 43, 22 and 18 to 23 respectively.

Vitamin A occurs in lipids portion of animal origin and has been grouped in fat soluble vitamins. Liver is particularly responsible for synthesis of blood lipids. Quite possibly the larger value of vitamin A storage in the liver of laying and pullet might have been the outcome of the role of estrogen anticipated to be much higher in these two groups.

The two birds of the non-laying group severely affected with liver disease were found to have lowest vitamin A content of all the groups. This decline in the storage of vitamin A in their

liver may conclusively be corroborated with the observation on inflammatory and degenerative disease of heart. An indirect support can be derived from the reference of Kalbe et al. (1966) who reported that in heart diseases the blood vitamin A is normal but reduced in liver as the liver capacity to store vitamin A is also disturbed.

BLOOD SERUM:

The results of vitamin A concentration of serum also estimated in the three groups of birds are recorded in the Tables 3.10, 3.11 and 3.12.

T A B L E - 3.10

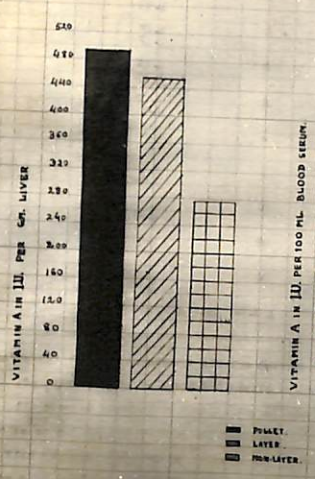
Table showing vitamin A content of Blood Serum per 100 ml. in I.U.

Layer	Non-layer	Pullet.
462.00	330.00	462.00
396.00	396.00	382.80
330.00	448.80	330.00
396.00	330.00	396.00
424.40	264.00	330.00
369.60	343.20	375.70
290.04	277.20	-
296.00	-	-
264.00	-	-
330.00	-	-
Average with S.E. 365.80±26.69	341.31±24.34	379.42±20.00

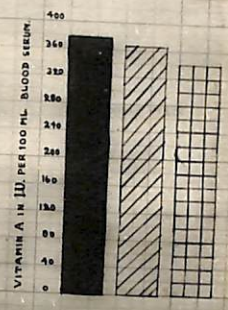
Over all average with S.E. 361.90± 12.24.

BAR DIAGRAM SHOWING

VITAMIN A CONTENT PER GM. LIVER IN THREE DIFFERENT EXPERIMENTAL GROUPS OF BIRDS



VITAMIN A CONTENT PER 100 ML. OF BLOOD SERRUM IN THREEDIFFERENT EXPERIMENTAL GROUPS OF BIRDS



T A B L E - 3.11

Table showing analysis of variance of vitamin A content in I.U. in Blood Serum.

Source of variation	Degree of freedom.	S. E.	M. S.	F.
Between groups.	2	4959.89	2479.94	0.70 N.S.
Within group.	20	71000.78	3550.04	
Total:-	22	75960.67		

T A B L E - 3.12

Table showing the mean difference of vitamin A content in I.U. in Blood Serum with critical difference at 1 and 5% level.

	Mean difference	C.D. at 1%	C.D. at 5%
Layers Vs. Non-layers.	24.49 N.S.	84.50	61.68
Layers Vs. Pullets.	13.62 N.S.	95.29	69.56
Non-layers Vs. Pullets.	38.11	84.53	64.63

Non-significant at both the levels.

From perusal of the table, vitamin A concentration found in three groups of birds shows non-significant differences. The values are 365.80 ± 26.69, 341.31 ± 24.34 and 379.42 ± 20.00 in layer, non-layer and pullet respectively.

The concentration in the serum of non-layer was not expected to come almost parallel to the other two groups but the result is beyond any shade of doubt for almost an equal value. It is obvious from the findings of Jones et al. (1955) that carotene intake of cows directly affected blood plasma carotene, and milk fat and vitamin A, but not blood vitamin A. Liver carotene and blood plasma carotene reflected the intake but were not related to the content in blood or milk.

The above observation confirms the result in the present investigation as to why the non-significant differences in the layers and the non-layers. Moreover, it appears that plasma tends to resist any change in vitamin A concentration despite wide variation in the amount of vitamin A stored in the liver.

In the framework of such observation is the copious discharge of opinions and the views expressed by Lewis et al.(1942) do support to it.

The work of Krause (1949), to some extent, also favours the views of other investigators.

It has been demonstrated by Duel et al. (1943) that while vitamin A content of plasma was 166 and 174 microgram per

100 ml. of plasma, the vitamin A content per gm. liver was 200 and 2082 microgram respectively.

The fluctuating differences marked in the concentration of liver and serum vitaminA either with respect to individual or within group of the birds in this experiment is in fair agreement with the above report. Therefore it may not be suggestive to conclude that from vitamin A content of plasma, assessment of concentration in the liver can be made.

Apparently, the serum vitamin A content of pullet at sexual maturity was relatively higher than both the layers and the non-layer groups.

But reports are available to show that appearance of significant amount of retinal in the plasma of maturing pullets coincided with the hypertrophy of oviduct, with the onset of laying eggs and with the increase in concentration of plasma lipids (Plack et al., 1966).

The above observation bears substance in coinciding with the concentration of vitamin A in the pullets which were at the proximity of intense phase of sexual maturity under the command of estrogen.

According to Common et al. (1947-1948) estrogen increases plasma riboflavin, vitamin A, liver fat and liver protein in the chicken. This holds true in the present investigation as well so far the vitamin A concentration in the blood of the two groups of birds having high level of estrogen is concerned.

C H A P T E R - VI.

Liver Function Test

SECTION-I.

Icterus Index.

ICTERUS INDEX

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

Icterus Indéx measures the intensity of yellow colour of the serum. The colour is chiefly due to the presence of haemobilirubin or cholebilirubin. The intensity of yellow colour of serum is compared with a standard potassium dichromate solution. The colour intensity is not directly proportional to the amount of bilirubin present. Cholebilirubin in serum gives a deeper colour than the corresponding amount of haemobilirubin. As such icterus index is not always in fair agreement with the quantitative bilirubin determination.

In human beings icterus index has a definite value in recognition of latent jaundice. It is also a valuable parameter as an aid to detecting anaemia and liver damage.

Apart from its importance as a clinical aid in medico pathological conditions, the justification for study of icterus index lies behind the importance of pigmentation attached to egg yolk, vent, beak and toes by the poultry breeders. In marking score to differentiate a good and bad layer, the intensity of pigmentation as well as its rate of disappearance serves as an index to them.

Jull (1951) has described significance and various factors responsible for pigmentation in different breeds of poultry. Presence of fat or lipochrome pigment in epidermis of all American

breeds and some others, gives rise to yellow shank colour whereas melanic pigment accounts for a black shank. In English breeds both the pigments are absent from epidermis and dermis and hence a white colour shank. Presence of lipochrome pigment in the epidermis and melanic pigment in the dermis is said to be the cause of a green shank.

The pigment has been impressed upon to the extent that it can very well speak of the reproductive efficiency of the hen. The size and texture of the comb and wattle can serve a practical purpose for determining whether or not a hen is in laying condition. In female having yellow shank it is possible to estimate the approximate time in egg production by seeing the shade of the shank colour. The yolk obtain very little amount of carotenoid pigment from the tissue of the laying hen. The lipochrome present is diverted from the feed to the yolk as egg production increases instead of going to the shank. This factor in relation to culling flocks is important.

Moreover, estrogen has a marked influence over blood lipids of which liver is the principal organ for synthesis. Any functional disorder of liver due to various factors, may therefore, indirectly interfere with reproduction. From this view point also it may serve as a clue to reflect on the productive and reproductive efficiency of the poultry.

The literatures available seem to be virgin in either of the above utility of icterus index in poultry. Various techniques for determination of icterus index in blood serum, mostly

converging on the same point of a match against a standard dichromate solution either visual or colorimetric, have been developed. Serum or plasma is diluted with physiological saline solution until it matches in colour of a 1 in 10,000 solution of potassium dichromate. The dilution factor is termed "Icterus Index" (Menlengracht cited by Wootton, 1964).

Bromosulphalein a dye colourless in acid solution but coloured in alkaline solution is also made use of in determination of icterus index.

In this study, the visual colorimeter technique to determine the icterus index has been followed owing to applicability of its simple procedure in existing facilities. The method adopted is according to Gradwohl (1963).

REAGENTS :

(1) Potassium Dichromate standard solution.

(a) Potassium Dichromate (A.R.) .. 50 mg.

(b) Sulfuric acid. ... .. 0.2 ml.

(c) Distilled water. ... .. 500 ml.

50 mg. Potassium dichromate was dissolved in 400 ml. water, 0.2 ml. acid was added and then the remainder distilled water in a dark coloured bottle. It was stoppered and kept in a dark place.

This standard is equivalent to 1 icterus unit.

(2) Physiological saline solution .. 0.9%

PROCEDURE :

1 ml. serum was taken in a clean test tube and 10 ml. standard potassium dichromate solution in another tube. To the test 0.9% physiological saline solution was added and compared until the colour was lighter than the standard. The dilution was recorded and the reading was taken on photoelectric colorimeter against a water blank set at zero using blue filter no. 42.

CALCULATION :

$$\text{Icterus index} = \frac{\text{Reading of standard}}{\text{Reading of serum}} \times \text{Dilution.}$$

INTERPRETATION :

The icterus index is denoted in units the value of which reflects the physiological or pathological state of the subject. In human beings the normal range of icterus index is from 4 to 6. An elevation from 7 to 15 is marked in latent jaundice in which icterus index parallels the serum bilirubin. Icterus index is not specific for bilirubin but is influenced by factors like, lipimea, carotenaemia which give a false elevation of icterus index confusing with true increase of bilirubin.

RESULTS AND DISCUSSION

The results of icterus index of laying, non-laying and pullets are presented in Table 4.1 and 4.2.

T A B L E - 4.1

Table showing Icterus Index of three groups of W. L. H. birds.

	Layers	Non-layers	Pullets.
	4.1	6.8	4.7
	5.6	4.5	5.5
	5.7	7.0	4.2
	5.7	6.1	4.4
	3.2	3.5	5.8
	2.2	5.4	6.2
	12.3	-	-
	4.4	-	-
	13.5	-	-
Average with S.E.	6.30±1.31	5.55±1.75	5.13±0.33

T A B L E - 4.2

Table showing analysis of variance of Icterus Index.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	5.25	2.62	0.345 N.S.
Within group.	18	136.52	7.57	
Total:-	20	141.77		

The icterus index value was 6.30 ± 1.31, 5.55 ± 1.75 and 5.13 ± 0.33 in the laying, non-laying hens and pullets respectively. The differences were found non-significant.

However, from perusal of the table it is evident that there is a wide range of variation within the laying group ranging from 2.2 to 13.5 icterus index units. The low value might be on account of more efficient and regular passage of the pigments to the yolk. Although no record about the laying efficiency of the birds were made available, but it would not be irrelevent to assume that this may be a good guide to throw light on the reproductive efficiency. The higher value, at the extreme end of all the groups, on the other hand, may be due to individual variation. The probability that the birds might have had sufficient storage of fat or lipochrome pigment during the laying period under influence of estrogen but the same could not have been passed to the yolk as efficiently as in others under duress of various deterimental health factors.

The range of such a variation is worth comparable to the other two groups consisting of the pullets and the non-laying birds in which the trend of pronounced individual variation was not marked. This seems to be related to the physiological activities with respect to the source of excretion of the carotenoid pigments available from the feed. In both these two groups, the excretory source of the pigment is not channelized as in the laying birds. It is therefore, assumed that whatever little fluctuation is observed, would very well apply to the natural pheno-

-menon of variability from individual to individual.

Further investigation may throw more light on it.

*

SECTION-II

Serum Bilirubin.

B I L I R U B I N

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

Mann (1941) stated that "liver is a dynamic organ and the character of its function and the capacity for any particular function vary greatly within relatively short period of time".

This statement, of course, is true of liver, the vital organ of the body which is credited with the secretion of bile, and is of paramount importance in most of the metabolic activity in relation to food stuff.

Liver is a store house for vitamin A in the body. Its major activities in respect of utilization of food, storage of food stuff and their manufacture as well as regulation stand unparallel to other organ. Storage of fat and carbohydrate as glycogen in relatively large amount, storage of protein in hepatic tissue, deamination of amino acid and concersion of nitrogenous protein to urea by the hepatic cells are accountable to the function of liver.

Moreover, liver is site of origin for plasma protein and phospholipids (Ranney and Chaikoff, 1951). Total plasma lipid is higher in laying hens than the non-layers or the male or the immature birds. It has been amply demonstrated that the rise in plasma lipids with the onset of sexual maturity is caused by the secretion of estrogen by the maturing ovary. But Heald and Badman (1963) have shown that the quantities of these components

decrease markedly when laying commenses. Compbell (1957 b) reported that estrogen tends to protect the liver from damage produced by a sub lethal dose of hepatotoxic alkaliod. Campbell (1957 c) also reported that in a chronically damaged liver estrogen tends to speed up its regeneration.

Apart from its interelation with estrogen, liver is also the site of several other physiological substances such as fibrinogen, and prothrombin, the essential factor for coagulation of blood.

Liver also makes some substance innocuous by conjugation on one hand, and protects the body, acting as detoxicant, from harmful substances by destruction.

Mann (1937) concluded that hepatic function may be altered either directly or indirectly by many of the hormones. He stated that hepatic activity can be changed by hormone by

(a) an effect on the rate of body mechanism;

(b) an effect on the intrinsic mechanism of liver either from storage or manufacture of food and

(c) by an effect on the amount of available food material reaching the liver.

Anaesthesia also has an important effect on liver function. According to Ojers, Holmes and Dragstedt (1941) histamin content of the liver of dog is reduced during anaphylatic shock as the liver is greatly conjested and possesses blood pressure lowering properties. Evidences show that much of the histamin during anaphylactic shock in dog comes from liver.

Histamin has been described as being enigma to pharmacologist and physiologist. Histamin has been proposed as being important in the induction of labour blastocyte implantation, uterine decidual development and as an indicator of estrogen. In addition histamin has been implicated as the responsible agent in pregnancy toxemia and shock and as a possible factor in uterine defence mechanism (Wrenn et al., 1963).

Danowski (1962) summarised that plasma may contain small traces of triiodothyronine and perhaps other derivatives in addition to thyroxine. The circulating thyroxine is converted to triiodothyronine and degraded further thereafter and excreted in bile as a glucuronide or sulfoconjugate.

It had been shown in rat by Myant (1957) that there was not only a increased rate of biliary excretion of thyroxine but also an increase in the bile plasma concentration ratio. This increase in biliary extraction of plasma thyroxine was found to be correlated with a saturation of the more potent thyroxine binding protein in the plasma. Triiodothyronine had a similar effect on increasing radiothyroxine excretion but was less effective than thyroxine and triiodothyronine itself was more rapidly excreted than thyroxine in the bile. At lower throxine doses an increase thyroxine conjugation with glucuronic acid rose to the level of increase in thyroxine excretion but with very high doses the mechanism was saturated and most of the thyroxine was excreted in un-conjugated form. The increased liver plasma thyroxine ratio occured with increasing doses.

Newcomer (1957) observed that in the chickens in contrast to other animals thyroxine and triiodthyronine have equal biological activity. Similar views has been expressed by Shellabarger (1956).

It is worth remembering that there is disassociation of function of the liver so that injury may impair one function to a greater extent than others. Normal physiological condition may impair some of the hepatic function such as a fat diet may produce an increase in fat content of the liver and a decrease in some hepatic function.

There may be hepatic injury with restoration of hepatic tissue without apparent loss of hepatic function. Only a small amount of normal hepatic tissue may maintain normal hepatic activities under protection from functional stress.

Therefore the result of liver function test may vary in relation to tissues. Even a slight variation, like change in diet and length of fast preceding the test, may alter the result of the test showing normal function in one case and impairment of the function in another.

These features reflect on the functional aspect of liver. Study of the functional activity of liver in relation to circulation and flow of blood and circulation in relation to hepatic restoration, either from medio surgical or physiological view point, is of great interest.

The present investigation was taken up to study the

potential role of liver under various physiological activities in W. L. H. laying and non-laying hens ans sexually maturing pullets.

Numerous tests have been recommended according to the specific function of the liver involved and injury to the liver (Varley, 1963 and Gradwohl, 1963).

These tests dependent upon specific function are based on -

(1) Abnormalities of pigment metabolism:

(a) Serum bilirubin and van den Bergh reaction.

(b) Urine bilirubin.

(c) Urine and faecal urobilinogen.

(d) Urine coproporphyrin.

(2) Liver's part in carbohydrate metabolism:

(a) Glactose Tolerance test.

(b) Levulose Tolerance test.

(3) Changes in plasma protein:

(a) Determination of plasma protein, albumen, globulin, fibrinogen.

(b) Flocculation test, thymol turbidity test, thymol flocculation test. Serum colloidal Gold test, Cephalin Cholestrol flocculation test, Zinc sulphate test, Wettmann test, Takata-Ara-test, Formol gel test.

(4) Abnormalities of lipids:

(a) Serum cholestrol determination,

(b) Determination of faecal fats.

(5) Detoxicating function of the liver:

(6) Excretion of injected substances by the liver:

(a) Bromosulphalein test.

(b) Bilirubin excretion test.

SERUM BILIRUBIN TEST:

In the present study of liver function, serum bilirubin test was taken up on the line suggested by Lathe and Ruthven(1958) a modification of Malloy-Evelyn (1937).

This test is based on the abnormalities of pigment metabolism indicating the functional state of liver. The quality and quantity of the diet has pronounced influence on the body pigmentation which affect the quality of the carcass. Carotenoid pigment influences the colour of the egg yolk.

Strick (1954) reported that an error in feeding (protein deficiency) cause brown pigmentation of fat and appearance of faecal smell in pork carcass. He suggested that such ration as protein deficient (milo) supply incomplete or poorly absorbed protein and that liver damage, insufficient to cause jaundice or visible cirrhosis, is to blame for the effect of colour and odour. Richter and Oslage (1956) reported that pigs on low protein had a pale coloured meat of poor quality and meat gave up its water more readily than that of the other group.

Kik (1962) reported that dark chicken meat had a

slightly higher nutritive value than light. He concluded in his studies on nutritive value of chicken meat and its value in supplementary rice protein to rats that, a protein supplied by chicken meat and rice gave better growth than either at the same protein level.

Tuma et al. (1963) observed the variation in physical and chemical character of Longismus dorsi muscle of Herford cattle and found that muscles were darker in older animals.

Similarly Miller et al. (1966) reported less free amino acid in light meat than dark meat of chickens of same strain, age and sex.

Disappearance of pigmentation from vent, beeck etc. differentiates a good and bad layers.

Therefore the liver function test on pigment metabolism is of great interest in study of reproductive activities in birds.

PRINCIPLES :

Two forms of serum bilirubin have been reported

(a) conjugated which is mostly with glucuronic acid and

(b) unconjugated which is free bilirubin.

The heme portion of haemoglobin is converted by the reticulo endothetial system to biliverdin-globin following opening of the porphyrin ring and then to bilirubin. The amount of bilirubin in human blood is normally very small which ranges from 0.1 to 0.5 mg./100 ml. of serum (Thorpe). Figure in excess indicates

regurgitation of bilirubin that has been acted upon by the liver cell (Gradwohl, 1963).

Bilirubin from reticulo-endothenal system and that of bile markedly differ from each other as is evident in van-den Bergh test which consist in coupling the pigment with diazo solution (P-sulpho-phenyl-diazonin chloride) to form a red violet solution. This noticeable difference in behaviour of bilirubin from the two different sources lies in the immediate (direct) reaction of bilirubin of bile and a strong (in-direct) reaction of normal serum after treatment with alcohol (Thorpe). Both conjugated and unconjugated bilirubin give purple azo-bilirubin with diazotized sulphanilic acid. Conjugated bilirubin reacts in aqueous solution (direct) whereas unconjugated bilirubin requires an accelerator or solubilizer such as alcohol and hence called indirect(Wootton, 1964).

The indirect reaction as with van-den Bergh test owes its explanation to the fact that normal serum contains free bilirubin, which requires ethanol to keep it in solution at the acid reaction of the diazo-solution in contrast to bilirubin in bile which is present as diglucuronide, is very soluble in acid reaction.

Any physiological or pathological hinderance to the excretion of the pigment owing to injury to the polygonal cells of the liver causes abnormal rise in bilirubin content and results in diffusion through capillary giving a characteristic yellow appearance to the skin and mucous surface (Thorpe).

REAGENTS :

(1) Sulphanilic acid solution .. 1%

(2) Hydrochloric acid solution .. 0.2 N

(3) Sodium Nitrite solution. .. 0.5%

(4) Methanol (A. R.).

(5) Bilirubin standard.

PROCEDURE :

0.2 ml. of serum was taken in a first tube and diluted with 5.4 ml. of distilled water and on mixing well, 2.8 ml. was pipetted out in a second tube for use as a blank. 0.7 ml. of freshly prepared diazo reagent consisting of a mixture of 10 ml. of 1% solution of sulphanilic acid prepared in 0.2 N Hcl and 0.3 ml. of 0.5% sodium nitrite solution was added to the test. To the blank 0.7 ml. of sulphanilic acid solution was added. After mixing, both the tubes were allowed to stand for 5 minutes.

Reading was taken in a photoelectric colorimeter using a green filter which gave the amount of conjugated bilirubin.

In order to obtain the total bilirubin 3.5 ml. of methanol was added to each tube permitting all the bilirubin to react with the diazo reagents. Reading was taken after allowing to stand for 5 minutes.

PREPARATION OF STANDARD :

10 mg. of crystalline bilirubin was accurately weighed

and dissolved in 100 ml. analytical grade chloroform in a volumetric flask. Therefore 1 ml. of chloroform contained 0.1 mg. of standard bilirubin.

0.2 ml. of bilirubin standard was taken in a test tube and 3.5 ml. of methanol was added to it. Then 0.7 ml. of freshly prepared diazo-reagent was added and after thorough mixing 2.6 ml. of distilled water was added to it.

After 5 minutes the reading was taken against a water blank.

CALCULATION :

Conjugated Bilirubin.

$$\text{Mg. conjugated bilirubin/100 ml.} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 10 \times 1.05.$$

As conjugated bilirubin has a lower extinction in water, the factor 1.05 was used.

CALCULATION :

Total Bilirubin.

$$\text{Total Bilirubin} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 20.$$

RESULTS AND DISCUSSION

The results of bilirubin content of blood serum of the birds under present investigation have been presented in Tables 4.3, 4.4, 4.5 and 4.6.

T A B L E - 4.3

Table showing Conjugated Bilirubin in mg./100 ml. in Blood Serum.

Layers	Non-layers	Pullets
0.29	0.26	0.35
0.28	0.87	0.28
0.58	0.30	0.34
0.12	0.65	0.42
0.12	0.63	0.38
0.15	0.20	0.49
0.26	0.16	-
0.17	-	-
Average with S.E. 0.25±0.17	0.44±0.103	0.38±0.028

T A B L E - 4.4

Table showing analysis of variance of Conjugated Bilirubin.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	0.1452	0.0726	2.03 N.S.
Within group.	18	0.6408	0.0356	
Total:-	20	0.7860		

T A B L E - 4.5

Table showing Total Bilirubin in mg./100 ml. in Blood Serum.

Layers	Non-layers	Pullets.
0.55	0.77	0.80
0.76	1.10	0.56
0.66	0.33	0.60
0.32	0.90	0.63
0.55	1.06	0.65
0.29	0.66	0.62
0.50	0.30	-
0.66	-	-
Average with S.E. 0.53±0.020	0.73±0.12	0.64±0.03

T A B L E - 4.6

Table showing analysis of variance of Total Bilirubin.

Source of variance	Degree of freedom	S. S.	M. S.	F.
Between groups.	2	0.1510	0.755	1.52 N.S.
Within group.	18	0.8928	0.496	
Total:-	20	1.0438		

The total bilirubin content in mg./100 ml. was 0.53 ± 0.020, 0.73 ± 0.12, 0.64 ± 0.03 in laying, non-laying and pullets respectively. Similarly the conjugated bilirubin in same order of the groups was 0.25 ± 0.17, 0.44 ± 0.103 and 0.38 ± 0.028 mg./ 100 ml. serum. Subject to statistical tests, both the total and the conjugated bilirubin were found non-significant. Search of literature bears a vacant look on this aspect. However, the value are within the normal range in comparision with the human in which it ranges from 0.5 to 1 mg./ 100 ml. Value exceeding, are construed upon for abnormal condition of liver.

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C H A P T E R - VII.

Study on Organic Weight.

STUDY OF THE ORGANIC WEIGHTS

INTRODUCTORY

That anterior pituitary is the main orchestra of endocrine organs in the body has been duly recognized. So is the importance of thyroid which controls metabolism, development and growth and regulates various function of the body. And adrenal too which commands the maintenance of physiological balance and various other metabolic processes, can not be ignored.

With growing significance of the domestic fowl for its biological food value, it had been a tool in the hand of the scientific investigator for use as a parameter in the field of experimental biology. The recent past stands witness to the glory of nutritive value and food for research on poultry - the turkey meat with the astronauts during their rendezvous to materialize the dream of a walk on celetial body. The genetic make up of the fowl differentiates its physiology from that of the mammals. As such for scientific development of poultry, equipping oneself, with a thorough knowledge of endocrines and their interrelationship with reproductive organs, is essential. As cyclic changes in the reproductive processes are governed by these hormones secreted by these glands, changes occuring in the glands due to various factors seem worth to be studied.

In the present investigation an attempt has been made for a comparative study of the above glands in different stages of the domestic fowl.

Studies on pituitary by various workers indicate that sex difference is not attributable to the variation in the gland weight. But of course, changes in the weight has been marked with changes in the size of the gland (Payne, 1946).

According to Breneman (1950) the male gland is heavier upto 120 days. Oakberg (1951) stated that there was no difference in the gland weight of the normal male and female W. L. H. before 120 days but male gland was heavier at this age (Nakajo and Imai, 1961).

T A B L E - 5.1

Table showing pituitary weight in mg. of W.L.H. hens in laying and non-laying conditions.

Breed	Reproductive stage.	Anterior lobe	Caudal lobe	Cephalic lobe
W.L.H.	Non-laying.	8.4	2.8	5.3
W.L.H.	Laying.	9.0	3.0	5.4

Likewise thyroid has attracted the attention of various workers. In proportion to total body weight, it has been found that thyroid of domestic fowl does not vary markedly with increasing age. Of course its absolute weight increases with the age. Growth rate of thyroid is almost constant from hatching until about 50 to 60 days after hatching, the rate is then accelerated until between

100 and 120 days. At this stage pronounced slowing occurs (Breneman, 1954).

In domestic fowl the thyroid weight in per cent of body weight has been reported to be 0.004 to 0.077 (Schultze and Turner, 1945).

Shaklee and Knox (1956) reported that the correlation between thyroid weight and body weight of 4 week old New Hampshire chick was + 0.59 and linear regression co-efficient was 9.85 mg. of thyroid per 100 gm. body weight.

Besides various other factors such as sex, diet, climatic condition, hypophysectomy affect the size of the thyroid. Iodine which is essential for biosynthesis of thyroid hormone affects the size markedly as is evident from the report of various workers. Feeding 10 to 14.5 per cent fats have been found to have reduced the size of thyroid (March and Biely, 1957).

Restricted caloric intake does not influence the thyriod weight even though metabolic rate is lowered (Mellen Hill and Duke, 1954), as is the thyroid secretion rate (Premchandra, 1962).

Available reports show that administration of goitrogenic substances have pronounced effect on enlargement of thyroid size. Sadhu (1948) and Blaxter et al. (1949) studied the physiological mechanism of experimental goitrogenesis and reported enlargement of thyroid with inhibitory effect in thyroxine secretion.

Conner and Shaffner (1954) also reported a great hyper-

-trophy in thyroid in such combination as thiouracil and thyroprotein. Statistically significant smaller thyroid of birds under high environmental temperature compared to those under lower temperature have been reported by Huston and Caronon (1962).

Nestor and Jaap (1963) reported significant differences in thyroid weight in day old chickens despite maintaining uniformity in respect of identical condition in incubation of different hatches.

Herbert and Brunson (1957) observed that feeding 0.15 to 0.2% thiouracil in combination with 12 - 15 mg. diethyl stilboestrol pellet implant upto the age of 12 weeks improved carcass quality of the chickens.

Evans et al. (1960) found that daily injection of 1 ug. L-thyroxine/ 100 gm. body weight to thyroidectomized female rats for 96 days could resume the ovarian weight to normal.

Depending upon age, sex, stress and various other influencing factors, considerable variations have been reported by many workers. Breneman (1954) studied the adrenal weight of W.L.H. chickens from 2 - 140 days age and found that as early as 30 days of age, the adrenal weight in male was heavier than that of the female.

According to Crile and Quiring (1940) proportion to body weight chickens weighing 40 to 50 gms. were found to have approximately twice as large adrenal as chickens weighing 300 gms.

According to Sauer and Latimer (1931) no difference in

the right or left adrenal in chickens was observed. Riddle (1923) claimed that although male pigeon adrenal was slightly heavier than the non-laying females but female adrenal enlarged preceding and during ovulation. The size following ovulation was marked with regression.

Although there are few reports to show relation between adrenal size and reproductive cycle, but it has been found that circulating estrogen or androgen in the sexes under study, different condition produce effect on the size of the gland (Breneman, 1954, Corner and Shaffner, 1954).

There are a lot of other factors which govern the weight and size of adrenal. Depriving laying hens of vitamin D in diet increased adrenal weight from 105 to 220 mg. probably as a result of secretion of ACTH (Urist, 1959).

Similar effects resulting from vitamin B deficiency in pigeon and chickens have been reported by Beznak (1923). Enlargement in adrenal size owing to fasting has been observed (Sure, 1938).

Miller and Riddle (1942) reported that formaldehyde, ACTH and other anterior pituitary hormones bring about increase in adrenal size of pigeon, whereas cortical hormones of adrenal as cortisone, corticosterone and hydrocortisone have depressing effect on adrenal weight of chickens.

Avian leucosis in the chickens have been found to cause hypertrophy and hyperplasia of the adrenal and thyroid and change in the adrenal mainly in the internal tissue has been

observed (Arvy and Gabe, 1951).

The effect of hypophysectomy and lack of ACTH upon adrenal vary. Brown et al. (1958 a) reported a modern decrease in adrenal weight after hypophysectomy which is in conformity with the report of only a significantly slight decrease in adrenal weight of cockrels 25 to 28 days after hypophysectomy reflecting no change in adrenal ascorbic acid and or corticosteroid (Newcomer, 1959).

There appears to be difference of opinion as regards the effect of hypothyroidism on adrenal hypertrophy.

Various reports available show that adrenal activity is associated with growth and development. Corticoid injection into chickens embryo has been found to produce reducing effect on it as reported by Karnofsky, Stock and Rhoads (1950). Significantly depressing effect on egg production following administration of cortisone acetate (3 mg. per lb. body weight) in chickens have been reported by Kudzia and Champion (1963).

L I V E R :

No specific mention of the weight of avian liver under normal physiological condition other than experimental or pathological ones seems to appear in the literature.

However, Clavert (1944) cited by Sturkie (1965) reported that the weight of liver increased by 80 per cent in pigeon depending upon the dose of oestrogen administered. He ascribed the

increase mainly due to increase in number and size of the liver cells.

Common et al. (1950) reported that thyroid feeding increased the liver weight and also increased total protein in chickens.

Increase in relative liver weight with diethylstilboestrol implantation in White Cornish chickens was reported by Ischinoe and Ishijima (1961).

Snedecor and King (1964) found no difference in liver weight of both radiothyroidectomized and controlled chickens though the liver were found to be enlarged in thyroidectomized birds when calculated on the basis of percentage body weight.

Freedland (1965) reported that thyroxine injection and feeding of iodinated casein to rats decreased the liver weight.

Ray et al. (1965) observed increased liver weight when expressed as percentage of final body weight of the rats treated with massive doses of vitamin A against the control.

The report on the gall bladder of avian with respect to its weight, is lacking.

EXPERIMENTAL PROCEDURE:

All the birds were first weighed in a spring balance. Immediately after they were sacrificed, the endocrine organs were taken out one by one, extraneous tissue removed, dehydrated on moisten filter paper and weighed to the nearest accuracy on torsion

balance. The weight of the liver, soon after its removal, was taken along with the gall bladder on a separate sensitive balance. The weight of the gall bladder was then taken separately.

In order to avoid the chance of error on account of differences in the weight of the bird varying from individual to individual the weights were expressed in percentage except liver and gall bladder which have been expressed in terms of kilogram body weight.

RESULTS AND DISCUSSION

WEIGHTS OF ENDOCRINES:

The results obtained in the present investigation are presented in Tables 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 5.10 and 5.11.

T A B L E - 5.2

Table showing weight of pituitary in mg./100 gm. body weight in layers.

Body weight	Weight/100 gm. body weight.
2250	0.80
2000	0.60
2250	0.49
1750	1.02
2000	0.66
2000	0.75
Total:-	4.31
Average with S.E.	0.72 ± 0.07

T A B L E - 5.3

Table showing weight of pituitary in mg./ 100 gm. body weight in non-layer.

Body weight.	Pituitary weight/100 gm. body weight.
1500	0.53
2000	0.45
2000	0.65
1250	0.40
1750	0.80
1500	0.73
Average with S.E.	0.59±0.06

T A B L E - 5.4

Table showing weight of pituitary in mg./ 100 gm. body weight in pullets.

Body weight	Pituitary weight/100 gm. body weight.
1500	0.60
1250	0.88
1250	1.28
1250	0.88
Average with S.E.	0.91±0.14

T A B L E - 5.5

Table showing analysis of variance of pituitary.

Source of variation	Degree of freedom	S. S.	M. S.	F.	
Between groups.	2	0.2407	0.1203	2.94	N.S.
Within group.	13	0.5308	0.0408		
Total:-	15	0.7715			

T A B L E - 5.6

Table showing non-layer thyroid weight in mg./100 gm. body weight.

Body weight.	Right	Left	Average.
1500	2.06	2.93	2.49
2000	1.20	1.80	1.50
2000	5.05	3.80	4.42
1250	2.48	1.84	2.16
1750	2.51	3.54	3.02
1500	3.33	2.80	3.06
Average with S.E.	2.77±0.53	2.78±0.34	2.77±0.41

T A B L E - 5.7

Table showing layer thyroid weight in mg./100 gm. body weight.

Body weight.	Right.	Left.	Average.
2250	3.33	5.78	4.55
2000	3.00	3.30	3.15
2250	1.42	1.86	1.64
1750	3.31	2.74	3.02
2000	2.65	2.90	2.77
2000	2.30	2.20	2.25
Average with S.E.	2.67±0.29	3.13±0.55	2.89±0.40

T A B L E - 5.8

Table showing pullet thyroid weight in mg./ 100 gm. body weight.

Body weight.	Right.	Left.	Average
1500	1.20	1.93	1.56
1250	2.80	2.16	2.48
1250	2.80	1.92	2.36
1250	2.64	2.08	2.36
Average with S.E.	2.36±0.38	2.02±0.05	2.19±0.21

T A B L E - 5.9

Table showing analysis of variance of thyroid.

Source of variation	Degree of freedom	S. S.	M. S.	F.
Between groups.	2	1.2956	0.6478	0.81 N. S.
Within group.	13	10.2976	0.7921	
Total:-	15	11.5932		

T A B L E - 5.10

Table showing adrenal weight in mg./100 gm. body weight.

Layers.		Non-layers		Pullets.	
R.	L.	R.	L.	R.	L.
4.40	3.70	4.60	4.26	4.60	4.23
3.50	5.75	4.25	3.50	7.04	6.72
2.26	1.40	3.45	1.85	5.28	6.12
5.60	9.54	6.80	5.40	5.20	5.48
4.90	6.75	6.92	8.65	-	-
3.25	3.60	6.06	3.20	-	-
Average with S.E.	3.97±0.49	5.12±1.16	5.34±1.86 4.48±4.48	5.53±0.52	5.64±0.53

T A B L E - 5.11

Table showing analysis of variance of adrenal weight taken as mean of both the right and left gland.

Source of variation	Degree of freedom	S. S.	M. S.	F.
Between groups.	2	3.4437	1.7218	1 N.S.
Within group.	29	100.9521	3.4811	
Total:-	31	104.3958		

The analysis of variance of thyroid and adrenal gland consists of the mean weight of both the right and left glands. Average with S.E. for gland of each side, the right and left has also been presented.

Tables show that there are non-significant differences in weight of the endocrines studied. The non-significant difference may be subject to non-influence of estrogen on the anterior pituitary. The result is supported by the findings of Kar (1947).

Similarly, the differences in the weight of thyroid were found non-significant. The result is in perfect agreement with the findings of Kumaran and Turner (1949 b) who reported that estrogen did not influence the weight of thyroid which was expected to be high in the laying hens and pullets. Also, the weight of adrenal did not differ significantly. There are differences in

opinion as regards the weight of adrenal. Miller and Riddle (1939), Kumaran and Turner (1949 a) reported increase in adrenal weight due to estrogen whereas Kar (1947 b) stated that increase in adrenal weight might not be due to the effect of estrogen. The observation of the latter is in perfect agreement with the result in the present investigation.

Very few reports are available to throw light on the weight of right and left gland of thyroid and adrenal. It is apparent from the table that there is no marked difference in the right and left gland when compared with the respect gland of the other group or in between the same group. This is in conformity with the report of Sauer and Latimer (1931) who reported that there was no difference in the size of right and left adrenal in White Leghorn chickens.

LIVER AND GALL BLADDER:

The results have been shown in the Tables 5.12, 5.13, 5.14 and 5.15.

T A B L E - 5.12.

Table showing liver weight per kg. body weight.

Layers.	Non-layers	Pullets.
23.25	19.80	12.90
11.00	15.55	13.45
17.14	19.29	17.44

Contd...

Layers.	Non-layers	Pullets.
19.58	15.00	-
18.44	30.20	-
2.56	16.86	-
17.14	19.77	-
12.07	-	-
Average with S.E. 15.15±3.08	19.49±5.13	14.59±2.67

T A B L E - 5.13

Table showing analysis of variance of liver weight.

Source of variation	Degree of freedom	S. S.	M. S.	F.
Between groups.	2	81.8110	40.9055	1.32 N.S.
Within group.	15	464.4092	30.9606	
Total:-	17	546.2202		

T A B L E - 5.14

Table showing weight of gall bladder (as in situ) in gm. per kilogram body weight.

Layers.	Non-layers.	Pullets.
0.39	0.51	0.26
0.39	0.31	0.26
0.71	0.66	1.17
0.81	0.69	-
0.52	1.46	-
0.80	0.37	-
1.36	0.41	-
Average with S.E. 0.71±0.34	0.63±0.07	0.56±0.12

T A B L E - 5.15

Table showing analysis of variance of gall bladder.

Source of variation	Degree of freedom.	S. S.	M. S.	F.
Between groups.	2	0.0417	0.0208	N. S.
Within group.	14	38.9159	2.9797	
Total:-	16	38.9576		

The weight of liver and gall bladder studied separately in each group when subjected to statistical test, were found non-significant. The average weight with S. E. of the liver was 15.15 ± 3.08, 19.49 ± 5.13 and 14.59 ± 2.67 gm./Kg. in layer, non-layer and pullets respectively. Similarly the weight of gall bladder in the respective group above was 0.71 ± 0.34, 0.63 ± 0.7 and 0.56 ± 0.12 gm. per kg.

The non-significant difference found both in the weight of liver and gall bladder is quite in agreement with the report of May et al. (1946) who stated that thyroid administration did not influence the effect as long as food intake was same for both control and experimental birds. In the present investigation too, all the 3 groups of birds were maintained on the same level of intake of food stuff. But thyroid activity in the laying and pullets was anticipated to be high as is apparent from the results already discussed in preceding Chapers.

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C H A P T E R - VIII.

Microscopic features of Endocrine.

STUDY ON MICROSCOPIC FEATURES OF SOME ENDOCRINES.

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

The assumption, that hormonal regulation by the pituitary gland is involved in the process of laying, is supported by the findings of Perk et al. (1957). In various phases of life in women presence of basophil cells have been reported. The hypophysis of the non-pregnant animal at the time of full sexual activity has been reported to show similar characterestic. Early pregnancy near term and during the immediate post partum period, the aspect of hypophysis are similar to those in the period of sexual activity without pregnancy (Cipani, 1961).

Sexual maturity and laying condition in poultry marked with increase in physiological activity is related to the secretory activity of the gland as in mammals. Decrease or increase in the level of hormones is subject to various factors. Changes in the cell during such physiological phenomenon, are certainly apt to resemble with the changes during reproductive processes in poultry.

As most of the glands are interrelated, their independent role is influenced by the part played by the other endocrines. As such change in one is bound to reflect on the other. Thus thyroid and adrenal are caught sight of. The change in cellular structure, therefore, inspires to know the out in detail from within. The justification behind the study of microscopic feature

of the gland portrayed in the thesis, is as such, wrapped in an attempt to uncover the distinguishing features.

Studies were made under light microscope only and hence under existing facilities, cytological or ultramicroscopic structure are not covered up.

Payne (1955) gave his double seal on his previous report with his further observation that in laying hens the basophils are large numerous and dominate the picture. A most interesting feature that he observed was the presence of acidophils granules in the gonadotropic secreting basophils of laying hens and absence in the non-laying hens. The chromatin net work in basophil were found practically fading away and the nucleoli, usually one and some times two, stand prominently.

As regards the acidophils the chromation net work is clearly visible and nucleoli barely distinguishable from the chromatic particles.

Payne (1961) reported large masses formed by the increase in size and fusion of the secretary vesicles in castrate and ovariectomized and old fowls. He also stated a relationship to ovulation following observation on a typical type of cells concluding that a gonadotroph may synthesize more than one kind of hormone found in laying hens only.

Under the control of hypophysis there exists important relationship between the thyroid on one hand, and the adrenal and sex glands dn the other (Trautmann, 1957). Description of thyroid of fowl finds place in various text books,(Bradley and

Grahame, 1950, Sturkie, 1965).

Bradley and Grahame (1950) have described the fowl's thyroid as oval bodies surrounded by compact capsule having round oval or medium follicles. They have also described the cell structures with respect to their secretory activities. Singh (1967) has elucidated the histology and histochemistry of thyroid gland of female and male fowl of various ages in details pointing out the presence of three capsules - the outer one, called capsula serosa, the middle adiposa and inner capsula fibrosa. A vivid picture of the structure of fowl's thyroid has been given by him describing various shape and size of the follicles lined by squamous, cuboidal or columnar cells varying in their size according to functional activities. Features distinguishing active and in active follicles have been pointed with the report of large and oval follicles in aged birds, high cuboidal or columnar lining in active and squamous epithelium in the inactive folliclers.

Copenhaver (1964) has given a general feature of the follicles indicating that in highly activated glands the colloid not only is reduced in amount but has in usual preparation vacuoles throughout giving it a network or stringy appearance. He has also mentioned the presence of large number of vacuoles, absorption vacuoles specially in human being in activated gland.

Trautmann and Fiebiger (1960) pointed out that structure of thyroid changes with advancement of age, time of the year and during pregnancy. Turner (1961) pointed out that when thyroid is inactive, there is tendency for the coolid to accumulate in

the follicles, for the epithelium to become low cuboidal or squamous; when it is over active, the colloid stores are depleted and epithelium becomes columner and plicated. But these features may vary depending upon the state and nature of influences.

Adrenal gland of fowl has been discribed by various workers. Bradley and Grahame (1950), Sisson and Grossman (1953) and Sturkie (1965) gave an account of the gland as paired oval shaped bodies enclosed by fibro elastic capsule with no zonal demarcation into cortex and medulla, which is characteristically marked into separate zones in mammalian.

Singh (1967) described the adrenal cortex of the young and adult fowls and observed vacuolated cytoplasm of most of the cells in young birds as compared to adults.

Chromaffin arrangement on the periphery in the form of dense granules were noted. Bradley and Grahame (1950) discribed the medullary cells as polyhedral.

Singh (1967) observed increase in medullary tissue with increase in age. He also observed the presence of chromaffine granules. The chromaffinenetwork was found diffusely distributed with a prominent nucleolus in it. He concluded that nuclei of medullary cells can be distinguished from the nuclei of cortical cells by their smaller size and denser staining. Large binucleated cells were occasionally seen.

Shepherd and West (1951) cited by Chaudhury and Sadhu (1962) demonstrated that noradrenalinepredominated the adrenal of domestic fowl even at the adult stage.

Trautmann and Fiebiger (1957) described that the chromaffine medullary cells stained yellow or brown on chromic acid fixation and that chromaffin cells were polygonal in shape,

Wood (1963) observed special pattern of distribution of the two types of medullary cells in cat, chicken, mice and other domestic animals differing from each other in respect of pattern.

Pellegrini and Pellegrini (1966) studied details of cattle adrenal medulla during pregnancy and found difference in respect of shape and size of the cells in different period of gestation.

Hillarp and Hokfelt (1954) observed the presence of nor-adrenaline cells complexes in the adrenal medulla in cat and rat under different condition of secretory activity.

Knoff and Hartman (1951) made a microscopic study of the adrenal gland of the brown pelican. They found that chromaffine tissue was proportionately lesser than any other avian adrenal.

MATERIALS AND METHODS

SOURCE:

The present investigation included observation on the anterior pituitary, thyroid and adrenal of laying, non-laying and pullets obtained from Central Poultry Farm, Patna.

FIXATION:

The glands were taken out from the birds killed, and

were put into 10% formalin solution in which they were kept for at least two days for fixation. Treatment for chromaffine tissue was done separately.

DEHYDRATION:

The tissues, after getting rid of the fixative were then passed through three jars of dehydration as follows :-

(1) Jar I - Containing half N-butyl alcohol and 95% of ethyl alcohol - 4 hours.
(2) Jar II - Containing N-butyl alcohol - 4 hours.
(3) Jar III - Containing N-butyl alcohol - 16 hours.

CLEARING AND INFILTRATION:

After dehydration the glands were made to pass through 4 jars of this process which were kept inside the paraffin oven at 57°C for infiltration of the paraffin.

(1) Jar I - Containing half N-butyl alcohol and half paraffin 57°C - 2 hours.
(2) Jar II - Containing 57°C paraffin - 2 hours.
(3) Jar III - Containing 57°C paraffin - 2 hours.
(4) Jar IV - Containing 57°C paraffin - 18 hours.

EMBEDDING:

The tissues were embedded in paraffin and blocks were made.

SECTIONING:

Sections were mostly cut at 5 microns but a few at 7 microns.

The following stains were used in this work :-

(1) Haemotoxylin and counter stains with ethyl eosin were used as a routine method of staining (Culling, 1957).

REAGENTS REQUIRED:

(1) Haemotoxylin	-	6 gm.
(2) Absolute alcohol.	-	300 ml.
(3) Distilled water.	-	300 ml.
(4) Glycerol.	-	300 ml.
(5) Glacial acetic acid	-	30 ml.
(6) Potassium alum.	-	In excess.

PREPARATION:

1 gm. haemotoxylin was accurately weighed and accordingly 50 ml. absolute alcohol in proportion was added to dissolve it. Then an equal quantity of distilled water and glycerol and 30 ml. glacial acetic acid were added one by one. Finally potassium alum in excess was added and the content in a loosely stoppered bottle was placed on the window sill for ripening.

(2) Periodic Acid Schiff technique was used for glycogen only (de Tomasi, 1936).

REAGENT:

(1) Basic fuchsin. - 1 gm.

(2) N Hcl. - 20 ml.

(3) Potassium metabisulphite - 1 gm.

(4) Activated charcoal. 2 gm.

PREPARATION:

1 gm. basic fuchsin was accurately weighed and dissolved in 200 ml. boiling distilled water in a bottle. It was shaken for 5 minutes and cooled exactly to 50°C. After filtering it, 20 ml. N Hcl. was added to the filterate. Before adding potassium metabisulphite the filterate was cooled to 25°C by keeping it on ice. After adding potassium metabisulphite the content in the bottle was kept in a dark place for 24 hours. Then 2 gm. activated charcoal was added to it and shaken for 1 minute. It was filtered and then kept in refrigerator.

Before use in staining procedure it was allowed to reach the room temperature.

(3) Mallory's stains were used for acidiphils and basophil cells (Culling, 1957).

REAGENTS:

(1) Anilin blue - Orange G. Mixture.

(i) Anilin blue - 0.5 gm.

(ii) Orange G. - 2.0 gm.

(iii) Phosphotungustic Acid - 1.0 gm.

PREPARATION:

100 mg. anilin blue, 400 mg. orange G. and 200 mg.

phosphotungustic acid were accurately weighed and each dissolved separately in 20 ml. distilled water and then mixed together.

(4) Wood's (1963) stain for adrenaline and non-adrenaline cells.

REAGENTS FOR FIXATIVES:

(1) Potassium Dichromate solution	-	2.5%
(2) Sodium sulphate (anhydrous)	-	1%
(3) Acetate buffer at pH 4.1	-	M/5
(4) Formaldehyde solution.	-	10%

PREPARATION:

This new fixative consists of a solution of 2.5% potassium dichromate and 1% sodium sulphate anhydrous in an M/5 acetate buffer at a pH. of 4.1.

At the time of fixation a 10% formal dehyde solution was added in a ratio of one part of formal dehyde of ten parts of the dichromate solution. The pH of the resulting fixative was then carefully adjusted to 4.0 - 4.2.

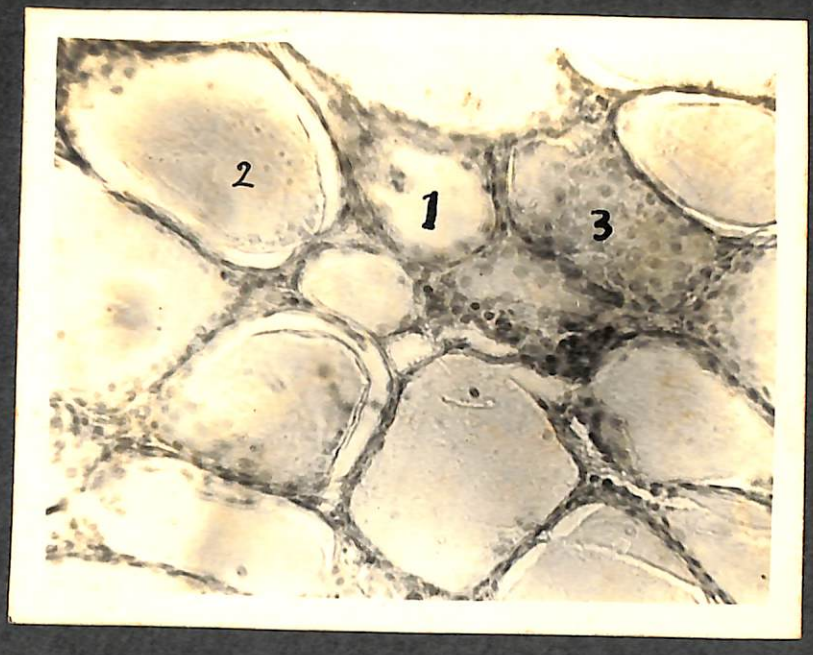
The composition of Wood Stain was as follows :-

1% aqueous eosin yellow.

0.5% aqueous anilin blue.

M/5 acetic buffer at pH 4.

The above constituents were mixed in a 1:1:1 ratio and the pH of the resulting solution was carefully adjusted to 4.0. This stain was used for the study of adreno-medullary catecholamines.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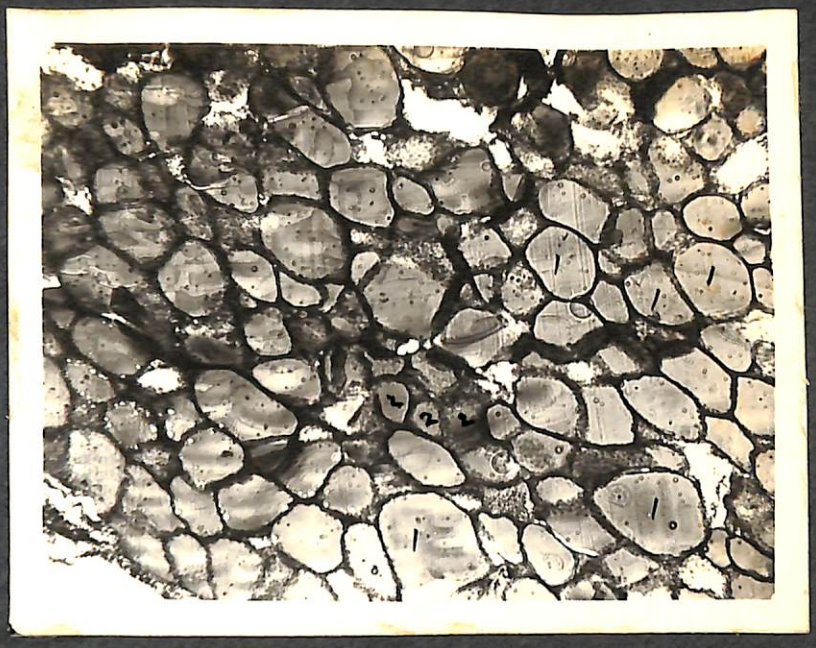
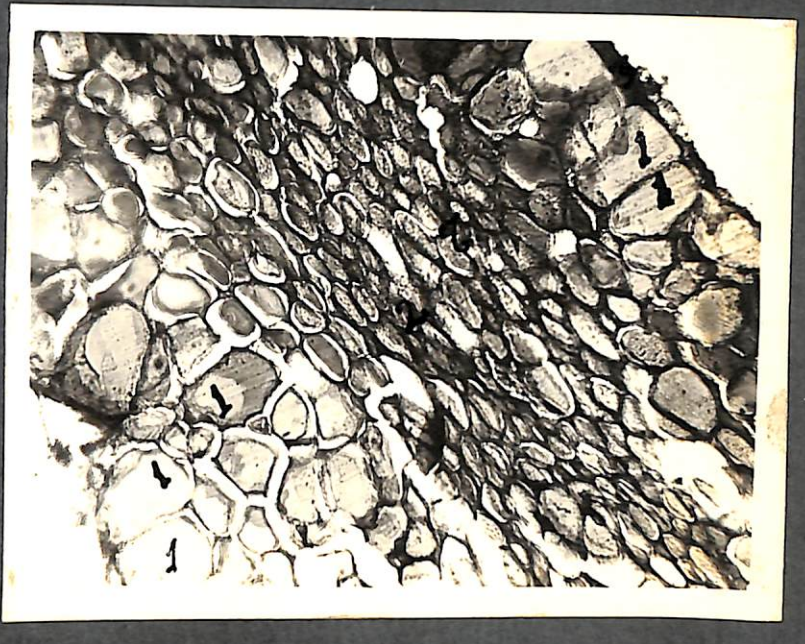
RESULTS AND DISCUSSION

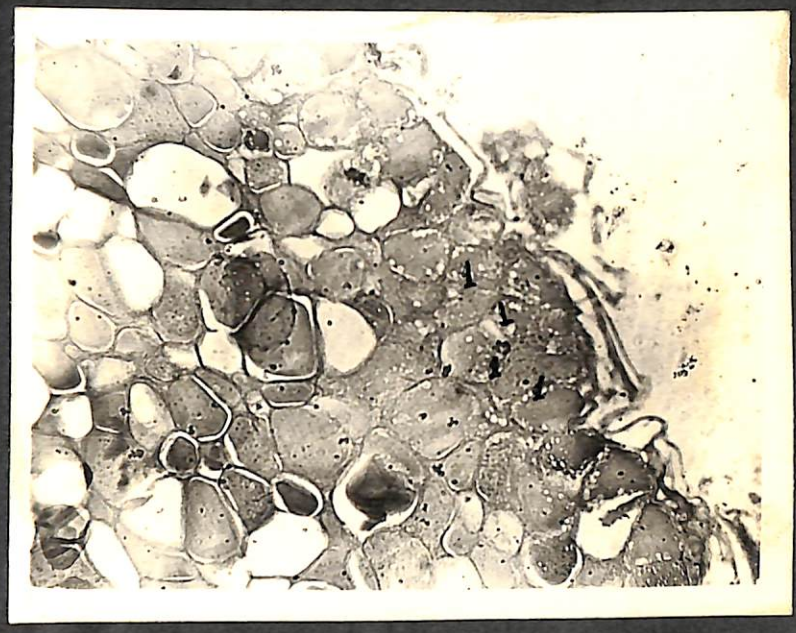
The thyroid glands of fowl have been observed under light microscope for histological studies with a particular attention to notice the important differences in laying and non-laying hens. Histologically, pullets thyroid was observed to differ little from the layers.

The thyroid of fowl was found to be composed of follicles with a little amount of connective tissue. Variation in the pattern of colloidal material of the follicles were observed. Those with acidophilic collid were found to be larger in size than the basophic ones. The former had flattened follicular cells whereas the latter consisted of cuboidal cells lining the follicles. The smaller follicles with basophilic colloid and cuboidal cells were seen in the active follicles, on the other hand, the larger follicles with acidophilic colloid and flattened cells were seen in the inactive follicles. According to Copenhaver (1964) in activated glands the colloid is predominantly basophilic, and in inactive glands it is acidophilic. The acidophilic and basophilic pattern of the inactive and active follicles with more explicit contrast were observed in Mallory's stained preparations. The inactive follicles under this stain showed reddish yellow colloid in contrast to the deep blue colloid in the active ones. Vacuoles in large number were also seen in layer with fast green stain.

In laying birds the active follicles were seen in large number than in the non-laying. Some of the layers presented two







distinct zones comprising active and inactive follicles. The large inactive follicles were seen more in the periphery of the gland and the active ones in the centre. In the non-layers the active follicles were appreciably less in number and scattered throughout the gland. The inactive follicles in non-layers were seen aggregated. The characteristic feature observed in the laying birds was the presence of two contrast colour in the same follicles with Mallory's stain. Peripheral part of the colloid was seen to be of purpluish blue colour and the central part in the same follicle appeared pinkish red in colour. The secretion blebs were also observed in some of the tall cuboidal cells of the follicles which indicated the gland in phase of active secretion. The vacuoles in colloidal mass in layers were more than the non-layers follicles which were filled with almost homogenous mass of colloid. According to Kopenhaver (1964) these vacuoles are most numerous in activated gland. Some authors have considered these vacuoles to be due to resorption and shrinkage whereas Singh (1967) considered them to be mere artifact.

The distinct features in size, shape and staining reactions observed in the follicles reveal that the thyroid gland in the layers are more active than those of the non-laying hens.

The hypophysis of the fowl similarly presented noticeable differences in laying and non-laying condition of the birds. The histological features observed in that of the pullets, were however, approaching to those of the layers.

The anterior lobe of the hypophysis of the non-laying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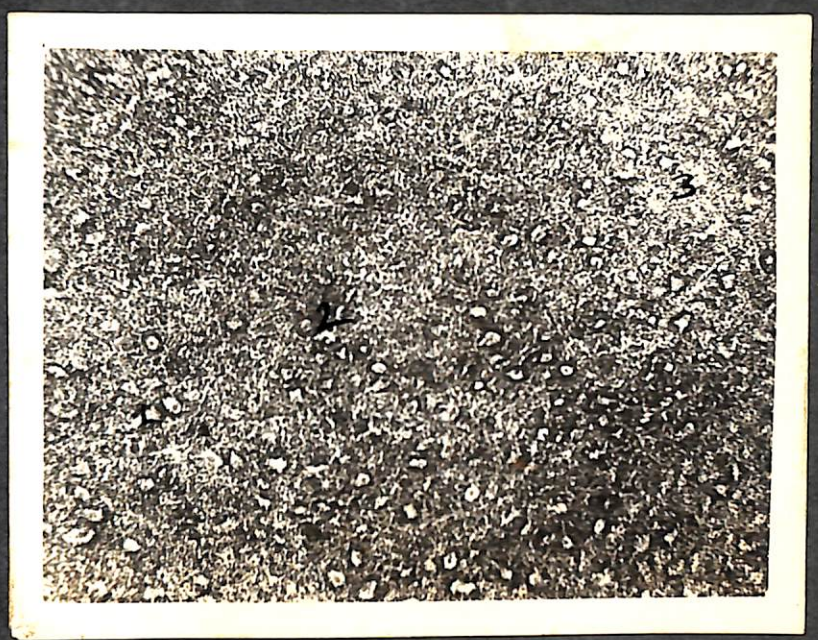
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birds presented two distinct zones i.e. cephalic and caudal. The former was seen to have vesicles mostly made up of basophils whereas the latter comprised of vesicles mostly made up of acidophils. The vesicles were seen larger in size and number in layers than those in the non-layers. The vesicles were filled with oxyphilic substances. With Haemotoxylin and eosin stain and Mallory stain it was observed that the basophilic cells dominate the picture in laying hens. However, in one particular laying hen the hypophysis showed large number of acidophils with a rim of basophils in the periphery of the gland indicating broody cells described by Payne (1946). As such presence of such cells in W.L.H. can not be ruled out. The layers did not show characteristic zonal distinction into cephalic and caudal zone of the anterior pituitary which was seen in the non-laying birds.

These apparent features suggest that the the changes occuring in the gland are according to the different physiological stage of activities as seen in the laying and non-laying birds.

In the present study the cortical and medullary tissues were found intermingled in all the three groups of birds (Sturkie, 1965 and Singh, 1967). It was observed that the cortical tissues were more than the medullary tissues. The cortical cords had mostly spherical cells with foamy cytoplasm whereas the medullary cords had polyhedral cells with intense staining. Bradley and Grahame (1950) have also described such differences.

A clear distinction was observed in the adrenaline and nor-adrenaline cells. The adrenaline cells had brownish grey