

**STUDIES ON THE METABOLIC ACTIVITY OF THE
REPRODUCTIVE SYSTEM OF CHICKEN**

BY

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THESIS

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requirements for the degree

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DECLARATION

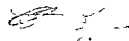
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GENERAL INTRODUCTION

CHAPTER I GENERAL INTRODUCTION

The processes of egg formation and laying are complex mechanisms involving the co-ordinated activity of many enzymes and hormones. The intrinsic potentiality of production is controlled to a great extent by the genetic make-up, but extrinsic factors like managerial practices can modify the productive ability. It is well known that in the fowl, the development of the ovary and oviduct are controlled by the gonadotrophins and sex hormones. Full secretory activity of the avian oviduct may be controlled by a combination of sex hormones (Gades and Brown, 1965; Brown, 1966). Ribonucleic acid and deoxyribonucleic acid production and alkaline phosphatase activity have also been found to be under the control of sex steroids (Chakravarti and Saha, 1965).

Plasma activity of enzymes like the phosphatases has been shown to exhibit wide fluctuations depending on the physiological status of the bird (Bell, 1971).

The yolk subunits are formed in the liver, transported to the ovary and then transferred through the follicular membrane to the oocyte. Many of the other components of the egg are synthesized in the oviduct itself. An assessment of the activity of some of the enzymes present in these

organs might throw light on the specific role of the sources, if any, in the functioning of these organs.

The relationship between sex steroids and the qualitative and quantitative distribution of enzymes in the reproductive tract has not been studied in detail. Such a study is expected to throw light on the various biochemical mechanisms associated with the formation of an egg. It was further thought worthwhile to examine whether administration of sex steroids could bring about marked alterations in enzyme pattern even in the absence of active egg formation.

In the present investigation an attempt has been made to work out the relationship between exogenous sex steroids and the enzyme pattern in the plasma, ovary and oviduct in two breeds of fowls viz., the White Leghorn which is considered as a layer strain and the White Plymouth Rock which is a meat type of bird. The enzyme pattern was also studied in normal birds of different age groups. The histochemical localization of some of the enzymes in the reproductive tract has also been attempted.

Studies on some of the enzymes in the blood plasma and reproductive organs of White Leghorn and White Plymouth Rock breeds of fowls.

CONCLUSIONS

1. The present study has shown that the rate of albumin synthesis in the oviduct is controlled by oestrogen. However, the precise mechanism of action of oestrogen has not been fully elucidated. There are evidences to show that in hens oestrogens stimulate the production of ribose nucleic acid (RNA) and many enzymes and finally the secretion of protein by the oviduct (Gorsal, 1964; Gorski *et al.*, 1965; Larsson, 1965; Barker *et al.*, 1966).

A qualitative and quantitative assessment of the enzymes in the reproductive tract of females might help to indicate the various biochemical mechanisms connected with the formation of the egg.

REFERENCES

The deposition of albumin around foreign materials, introduced into the lumen of the fowl oviduct was noticed as early as 1904 by Jacobsonoff. This suggested the possibility for the existence of a mechanical stimulus for the synthesis of albumin in the oviduct. But, Larsson and Cord (1961) stated that oestrogenic stimulus alone

was inadequate for the secretion of proteins. The secretory mechanism was considered to be much more complex and independent of a direct mechanical stimulus caused by the presence of a foreign ovum (Anderson and Laker, 1940).

However, Wentworth (1960) supported the possibility of the mechanical stimulus as the cause for albumen secretion in the oviduct.

Evidence for the role of oviduct in the synthesis of egg protein was provided by Chakravarti and Radu (1963) who found high levels of free amino acids in the magnum of actively laying hens. Schraer and Schraer (1965) found a comparatively lower protein level in the magnum of the laying chicken than in other regions of the oviduct.

Parsoner and Card (1941) noticed that calcification proceeded normally even after resection of 60% of the infundibulum in fowls. However, Wilson and Johnston (1963) believed that the formation of calcification in the infundibulum was related to the activity of certain enzymes secreted by this region.

The oviducts contained about 30 nucleotides and carbonyl groups and one of them were thought to be involved in glycoprotein synthesis (Graham et al., 1965).

In the formation of the eggshell in the distal region of the oviduct, various workers have described the importance and mechanism of the process (Allison and Houghton, 1933; Cornish, 1941; Tutovain and Mitchell, 1965).

Gutowska *et al.* (1943) expressed the view that phosphatases played only an insignificant role in the formation of egg-shell by the uterus. However, a gradual rise in the plasma alkaline phosphatase activity which might be due to increased osteoblastic processes to replace bone lost in egg-shell formation was noticed by Bell (1960). Chakravorti and Sadhu (1964) reported the presence of alkaline phosphatase both in the oviduct epithelium and gland cells and found a higher level in the hen than in pigeons. Brown and Sadana (1962) found increased alkaline phosphatase activity in the uterus than in any other region of the oviduct, the activity being highest when there was an egg in the uterus.

Further, it is stated by Brown and Sadana (1962) that the respiration rate of magnum was the lowest whenever an egg was located in this region. Conversely, respiratory rate was the highest when no ovum was present in the oviduct or whenever the ova were retained in the ovary ready for release.

It was indicated that the level of energy metabolism in magnum was greater during the stage of accumulation of secretory protein in the tissue than during its actual secretion (Brown and Sadana, 1962).

The respiration rate of the uterus was found to

be greater than the rate observed with oestrogen-stimulated immature oviducal tissue (Brown and Badman, 1962).

The levels of alkaline and acid phosphatases during the various stages of the laying cycle were studied by Taylor and Williams (1964). They found that the level of acid phosphatase was minimal when egg-shell formation was not in progress in the oviduct. However, it rose rapidly during egg-shell calcification; the maximum being found at the time of oviposition. This was followed by a precipitous fall in its concentration.

It has been found that the isthmus region of the oviduct played a significant role in the movement of calcium even though the exact mechanism and the cellular constituents involved could not be defined (Schraer and Schraer, 1965). These workers noticed an ubiquitous distribution of zinc throughout the oviduct, which, if taken to be indicative of the presence of zinc-containing enzymes, carbonic anhydrase, suggested that the enzyme was not restricted in its localisation to the uterine region of the oviduct alone. Carbonic anhydrase is involved in the production of carbonates from metabolic carbon-di-oxide for egg-shell formation (Hodges and Lorcher, 1967). However, a correlation between carbonic anhydrase concentration and egg-shell strength could not be demonstrated by Heald *et al.* (1963).

Among the enzymes involved in the synthesis of

avian egg components the studies pertaining to glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were probably first conducted by Cornelius et al. (1959). McDaniel and Chute (1961) working on the transaminase activities in tissues and plasma confirmed the earlier finding of Cornelius et al. (1959) of the presence of GOT and GPT in both tissues and plasma. They could show only very feeble GPT activity in the plasma of chicken.

Anderson and Rosenberg (1976) studied the variations in the oviducal content of adenosine triphosphatase (ATPase) with the ovulatory cycle in hens. Rosenberg et al. (1977) characterized the extra cellular ATPase found in the oviducal secretions.

MATERIALS AND METHODS

One hundred numbers each of White Leghorn (WL) and White Plymouth Rock (WR) one-day-old clinically healthy chicks were selected at random from the Kerala Agricultural Unive city Poultry farm. They were reared under standard farm conditions. The birds were given feed and water ad libitum. Up to the age of 3 months they were given standard chick mash and thereafter adult mash (vide Appendix I).

Blood samples were collected for enzyme analyses from one-to two-months-old, two-to three-months-old and five-to six-months-old (10 numbers under each category) birds

by cardiac puncture, using heparin as the anticoagulant. Immediately after collection, blood was centrifuged (2100 x g; 30 min) in a refrigerated centrifuge (0°C). The separated plasma was diluted 1 : 20 with double distilled water (DDW) except for glucose-6-phosphatase (G-6-Pase) determination where citrate buffer was used instead of DDW; and stored at 4°C. The birds were then sacrificed by decapitation and the ovaries and oviducts were carefully dissected out. The adhering tissues were trimmed and secretions were wiped off. Small pieces of tissues of approximately equal weights from the ovary, infundibulum, magnum, isthmus, uterus and vagina were collected from adult birds. But from chicks less than three months of age, ovary and the entire oviduct were collected. They were weighed accurately, cut into small pieces and homogenized in an ice-bath using Potter Lichenizer tissue homogenizer, as described by Bergmeyer (1953). The final volume of the homogenized material was made upto 20 ml with DDW in all cases except for G-6-Pase where citrate buffer was used instead. The tissue homogenates were filtered through double layers of muslin. The filtrates were collected and stored at 4°C.

The plasma and the filtrates of tissue homogenates were used for the assay (vide Appendix II to VI) of the following enzymes.

Alkaline and acid phosphatases were determined by the method of Bodansky (1933). Glutamate oxaloacetate trans-

malate and glutamate pyruvate transaminase were determined by the colorimetric method using 2,4-dinitrophenylhydrazine (Loomeyer, 1965).

The method given by Bergmeyer (1965) was adopted for estimating G-6-Pase activity.

The methods adopted for statistical analysis of the data were those given in Snedecor and Cochran (1967).

Comparisons were made between the enzyme contents of identical tissues in the two breeds of fowls. To test the equality of two means in these cases, Student's 't' was used when two variances were identical and Cochran's 't' where variances were unequal.

RESULTS

The enzyme content in the WL and WFR breeds of one-to two-months, two-to three-months and five-to six-months-old birds are given in Tables 2.1, 2.2 and 2.3 respectively.

Alkaline phosphatase in the blood plasma of one-to two-months-old (40.74 Bu/ml), two-to three-months-old (24.16 Bu/ml) and five-to six-months-old (12.58 Bu/ml) WL birds did not reveal any difference due to breed, as compared to one-to two-months-old (27.32 Bu/ml) two-to three-months-old (50.05 Bu/ml) and five-to six-months-old (38.61 Bu/ml) WFR birds.

In the ovarian tissue, alkaline phosphatase was significantly higher ($P < 0.01$) in the WL one-to two-months-old chick (4.75 Bu/mg) compared to the WFR one-to two-months-

old chicks (1.39 Bu/mg). The ovarian tissue of two-to three-months-old WL (2.52 Bu/mg) and five-to six-months-old WL (1.39 Bu/mg) did not reveal any significant breed difference when compared with those of two-to three-months-old (1.52 Bu/mg) and five-to six-months-old (1.34 Bu/mg) WR birds.

The oviducal content of alkaline phosphatase also revealed no breed difference between WL one-to two-months-old chicks (12.33 Bu/mg) two-to three-months-old pullets (0.67 Bu/mg) and five-to six-months layers (0.22 Bu/mg) in comparison with the WR one-to two-months-old chicks (3.55 Bu/mg) two-to three-months-old pullets (0.55 Bu/mg) and five-to six-months-old layers (0.39 Bu/mg).

In between the segments, Isthmibulum, magnum, isthmus, uterus and vagina of the mature oviduct in WL and WR, there was no significant breed difference in the alkaline phosphatase content.

Acid phosphatase could not be detected in the blood plasma of one-to two-months-old chicks and five-to six-months-old layers. The two-to three-months-old pullets in WL breed recorded 2.05 Bu/ml while that in WR breed it was 2.53 Bu/ml. There was no significant difference between these two values.

The ovarian acid phosphatase concentration did not reveal any breed influence; the WL and WR one-to two-months-old chicks recording 6.24 and 2.60 Bu/mg respectively, the

TABLE 2.1

Concentration of the different enzymes in the plasma, ovary and oviduct of one-to two-months-old WL and WPR chicks. (Values are mean \pm SD)

Tissue	AEP (BU)		ACP (BU)		GOT (RU)		GPT (U/G)		G-6-PATK (U)	
	WL(10)	WPR(10)	WL(10)	WPR(10)	WL(10)	WPR(10)	WL(10)	WPR(10)	WL(10)	WPR(10)
Plasma	40.74 \pm 10.05	27.32 \pm 4.42	Nil	Nil	146.00 \pm 15.79	126.00 \pm 13.68	20.00 \pm 6.67	Nil	21.10 \pm 1.37	9.57 \pm 0.93
Ovary	4.75 \pm 0.71	1.99 \pm 0.22	6.24 \pm 1.81	2.69 \pm 0.30	13.37 \pm 2.37	7.42 \pm 1.20	4.53 \pm 1.45	0.54 \pm 0.09	1121.18 \pm 292.33	331.34 \pm 37.11
Oviduct	12.33 \pm 3.09	3.55 \pm 0.53	16.13 \pm 6.29	10.59 \pm 2.94	20.25 \pm 2.53	15.50 \pm 3.41	7.57 \pm 2.13	1.69 \pm 0.48	1158.28 \pm 294.24	2148.43 \pm 565.68

Values in parenthesis indicate the number of birds

** Significant at 1% level

BU : Bodansky units/ml of plasma or μ g of tissue

RU : Racker units/ml

U : Stroblewski units

U : Units/ml

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TABLE 2.2

Concentration of enzymes in the plasma, ovary and oviduct of two-to three-months-old WL and WR pullets (Values are mean \pm SE)

Tissue	ALP (BU)		ACP (BU)		GOT (RU)		GPT (MU)		G-C-PLS ₂ (U)	
	WL(10)	WR(10)	WL (10)	WR(10)	WL(10)	WR(10)	WL(10)	WR(10)	WL(10)	WR(10)
Plasma	24.16 \pm 6.45	50.05 \pm 14.24	2.35 \pm 1.27	2.51 \pm 1.00	466.00 \pm 65.14	595.00 \pm 73.40	95.00 \pm 42.56	9.00 \pm 3.70	9.66 \pm 1.04	13.36 \pm 1.78
Ovary	2.52 \pm 0.60	1.52 \pm 0.23	2.67 \pm 0.62	3.08 \pm 0.45	5.00 \pm 1.07	0.25 \pm 2.66	0.25 \pm 0.03	3.19 \pm 0.97	105.66 \pm 15.96	353.31 \pm 37.15
Oviduct	0.67 \pm 0.37	0.55 \pm 0.15	2.39 \pm 0.60	3.53 \pm 0.63	4.78 \pm 0.96	15.30 \pm 7.21	0.24 \pm 0.09	3.22 \pm 3.00	130.91 \pm 26.50	695.67 \pm 152.00

Figures in parenthesis indicate the number of birds

** Significant at 1% level

BU : Bodansky unit/ml of plasma or mg of tissue

RU : Racker unit/

MU : Wroblewski unit

U : Unit/

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TABLE 2.3

Concentration of different enzymes in the plasma, ovary and oviducal regions in W and WPR fowls
(Values are mean \pm S.E.)

Tissue	ALP (AU)		MGAT (BU)		GOT (RU)		G.T (AU)		G-6-PASE (U)	
	W(10)	WPR(10)	WL(10)	WR(10)	WL(10)	WPR(10)	WL(10)	WR(10)	WL(10)	WPR(10)
Plasma	12.50 \pm 0.77	38.61 \pm 10.22	Nil	Nil	142.00 \pm 16.15	122.00 \pm 15.71	22.00 \pm 14.13	Nil	19.71 \pm 1.01	4.10 \pm 1.19
Ovary	1.39 \pm 0.17	1.34 \pm 0.45	1.83 \pm 0.45	1.73 \pm 0.53	2.30 \pm 0.37	1.69 \pm 0.33	0.09 \pm 0.02	Nil	62.60 \pm 22.52	28.70 \pm 6.02
Infundibulum	0.23 \pm 0.05	0.50 \pm 0.15	2.63 \pm 1.00	2.99 \pm 0.02	3.91 \pm 0.61	1.73 \pm 0.23	0.07 \pm 0.02	Nil	65.11 \pm 17.63	23.21 \pm 4.14
Magnus	0.22 \pm 0.05	0.37 \pm 0.09	1.35 \pm 0.33	4.40 \pm 0.93	3.29 \pm 0.56	2.03 \pm 0.28	0.15 \pm 0.05	0.02	40.76 \pm 19.64	30.74 \pm 8.87
Isthmus	0.27 \pm 0.09	0.87 \pm 0.37	2.16 \pm 0.39	4.02 \pm 0.75	5.52 \pm 0.99	2.59 \pm 0.38	0.19 \pm 0.03	0.12	43.45 \pm 16.12	46.18 \pm 10.50

TABLE 2.3 (Continued)

Tissue	ASP (B ₁)		ACP (B ₁)		GOT (R ₀)		GPT (R ₀)		G-6-PDase (U)	
	ML(10)	WR(10)	ML(10)	WR(10)	ML(10)	WR(10)	ML(10)	WR(10)	ML(10)	WR(10)
Uterus	0.13±	0.34±	1.80±	3.10±	3.55±	1.69±	0.13±	0.11	23.13±	35.70±
	0.95	0.10	0.45	0.75	0.73	0.32	0.37		7.91	8.46
Vagina	0.22±	0.24±	0.52±	1.43±	2.45±	1.21±	0.04±	0.11	31.27±	48.19±
	0.11	0.08	0.79	0.23	0.44	0.26	0.02		17.69	11.77
Oviduct	0.22±	0.39±	1.69±	3.34±	3.58±	2.03±	0.09±	0.11	15.73±	25.22±
	0.02	0.09	0.33	0.69	0.56	0.30	0.03		12.31	8.52

Figures in parentheses indicate the number of birds

** Significant at 1% level

BU : Bodanby units / ml of plasma or mg of tissue

RU : Racker units / " "

GU : Grobstein units/ " "

U : Units / " "

two-to three-months-old pullets having 2.67 and 3.09 Bu/mg and five-to six-months-old layers of WL and WR birds showing 1.93 and 1.79 Bu/mg respectively.

In the oviducts of WL and WR breeds of fowls there was no significant difference in acid phosphatase content either in the one-to two-months age group (16.13 Bu/mg and 10.99 Bu/mg respectively) two-to three-months age group (2.09 Bu/mg and 3.59 Bu/mg respectively) or in the five-to six-months-old birds (1.69 Bu/mg and 3.04 Bu/mg respectively).

As in the case of alkaline phosphatase the acid phosphatase also did not reveal any variation in the concentration among the different oviducal segments in the five-to six-months-old layers of both WL and WR breeds.

Blood plasma content of GOT in the one-to two-months-old WL chicks (146 Ru/ml) did not show any significant difference from that of the concentration in the WR one-to two-months-old chicks (126 Ru/ml). In the two-to three-months-old group the plasma content of the enzyme in WL breed of pullets was 466 Ru/ml while that in the WR it was 595 Ru/ml. There was no significant difference between these two values. Similarly in the five-to six-months-old layers also no breed difference in the plasma GOT content could be seen in WL (142 Ru/ml) or in the WR (122 Ru/ml).

In the ovaries of WL one-to two-months-old chicks the GOT content (13.37 Ru/mg) continued to show no signifi-

and variation from that in the WFR chicks (7.42 Ru/mg). Similar results were obtained in two-to three-months-old WL (5.00 Ru/mg) and WFR pullets (8.25 Ru/mg) without any indication for any breed difference in the enzyme contents. In the layers of both the breeds the GOT content continued to show no influence due to breed, the WL having 2.56 Ru/mg and the WFR 1.69 Ru/mg of ovarian tissue. However, among the different segments of the oviducts the infundibular region in the WL layers revealed significantly higher ($P < 0.01$) GOT content (3.91 Ru/mg) compared to the WFR layers' infundibular (1.73 Ru/mg).

GPT activity could not be detected in the blood plasma in one-to two-months-old WFR chicks and in the blood plasma, ovary, entire oviduct and except for magnum and isthmus of five-to six-months-old WFR layers. In spite of this no significant difference due to breed could be detected either in the blood plasma, ovary or in the entire oviduct. However, among the oviducal segments significantly higher GPT activity could be noticed in the WL infundibulum (0.07 u/mg).

G-6-Pase content in the blood plasma of one-to two-months-old WL chicks (21.10 U/ml) was significantly higher ($P < 0.01$) than that in the WFR plasma (9.57 U/ml). But no significant difference of the enzyme level could be seen in the plasma of two-to three-months-old pullets which showed the values of 3.66 U/ml in WL and 13.36 U/ml in WFR

pullets. However, in the case of five-to six-months-old layers, significantly higher ($P < 0.01$) G-6-Phase content was noticed in WL (10.71 U/ml) compared to the WR plasma content of 4.10 U/ml.

Significantly higher ($P < 0.01$) content of G-6-Phase was recorded in the ovaries of one-to two-months-old WL chicks (1121.18 U/mg) compared to the WR ovary (351.34 U/mg). But no such significant difference could be seen manifested either between the two-to three-months-old WL pullets (103.66 U/mg) and WR pullets (150.31 U/mg) or between five-to six-months-old WL layers ovaries, 62.60 U/mg and WR layers ovaries (28.70 U/mg).

In the entire evident significant breed difference in the G-6-Phase content could be seen only in the two-to three-months-old (Table 2.2) WR pullets (635.67 U/mg) compared to that in the WL pullets (180.91 U/mg).

No evidential segment in the five-to-six-months-old layers (Table 2.3) showed any significant difference in their G-6-Phase content between the WL and WR breeds of chicks.

DISCUSSION

In the one-to two-months-old chicks belonging to both WL and WR breeds all the enzymes studied, except acid phosphatase, were present in the blood and reproductive organs. But in the WR chicks, GPT was also absent in the plasma.

Ovarian alkaline phosphatase content was significantly higher ($P < 0.01$) in the WL compared to WFF. The significance of this finding is not evident. Gilbert (1967) considered steroidogenesis as the only activity of the ovary of immature birds. The ovarian sensitivity to gonadotrophins becomes evident only from maturity.

The total absence of acid phosphatase from the blood plasma of healthy chicks in the present investigation may be due to the non-release of the enzyme from lysosomes. The enzyme which is lysosomal in location is rarely released from healthy, normal cells. This is released only when the cells are damaged, destroyed or diseased.

Acid phosphatase was noticed in the different regions of the reproductive organs. The absence of any significant concentration difference between the regions of the reproductive organs may indicate the non-involvement of this enzyme in any specific oviducal activity. The presence of the enzyme in detectable amounts in the reproductive organs may be the result of release of the enzyme from the lysosomes due to cell destruction as a result of tissue homogenization done for assaying the enzymes.

Except for the absence of GPT in plasma of WFR chicks (Table 2.1), there was no significant difference in the concentration of either GOT or GPT between plasma and reproductive organs among the two breeds, one of which is a high egg producing strain. The enzymes may be associated

only with the routine transamination reactions normal to the cells and may not have any correlation with the level of productive capacity.

There is an age-dependent decrease in the plasma content of GPT (Cassler, 1963). The W R chicks as they grow may be progressively losing GPT activity in the plasma while, WL chicks may be retaining the GPT activity which could be due to peculiarity of the strain.

In the two-to three-months-old pullets (Table 2.2) none of the enzymes studied showed any significant difference between the breeds. This may indicate that the basal cellular reactions catalyzed by the enzymes, functioned without much difference in the reproductive organs of the two breeds. However, G-6-Pase in the two-to three-months-old WFR oviduct was higher in concentration. The significance of this observation needs further study to elucidate the exact mechanism associated with this.

In the five-to six-months-old WL and WFR layers alkaline and acid phosphatases showed no significant breed differences (Table 2.3). No acid phosphatase activity could be detected in the blood plasma.

GOT and GPT concentrations were higher in the infundibulum in the WL fowls compared to that in WFR layers.

Eventhough chalaza formation has been assigned as the function of infundibulum, the degree of its involvement has not yet been fully ascertained. It is known that the chalaza becomes apparent only as the ovum appears at the caudal levels of magnum. The formation of chalaza is completed only in the isthmus and uterus.

It could be seen from the investigation that GPT was present in the immature birds and UL layers. No biological reason can be attributed for the absence of the enzyme in WPR. This quantitative variation could only be attributed as a breed characteristic. However, the age-dependent decrease in GPT activity was more pronounced in the WPR fowls. Further, the WPR fowls showed GPT activity only in the magnum and isthmus regions, both of which are highly secretory tissues.

GPT was seen widely distributed throughout the oviducal regions in the UL and WPR breeds of layers. This may indicate the widespread activity of the transaminase along oviducal regions.

A significantly high value of G-6-Phase was obtained in the plasma of UL layers. The reproductive organs of both Wb and WPR layers showed no breed differences in the enzyme content. The higher G-6-Phase concentration in the blood plasma may reflect only a higher activity in other parts of the body. However, the distribution of the enzyme throughout

the reproductive organs in WL and WFR fowls without any breed difference may indicate their functional significance in the cellular metabolic activity associated with the general processes of egg-laying.

Alkaline and acid phosphatases, GOT, GPT and G-6-Pase were present throughout the reproductive organs, except for GPT in the ovary, the infundibulum, the uterus and the vagina in the adult WFR fowls. Except for the higher contents of GOT and GPT in the infundibulum of the WL fowls, as compared to the WFR fowls, no other difference could be observed in the enzyme contents, of the reproductive organs between the two breeds.

Effect of administration of exogenous sex hormones on the development of the oviduct in immature chicks.

CHAPTER III

EFFECT OF ADMINISTRATION OF EXOGENOUS OXY HORMONES ON THE DEVELOPMENT OF THE OVIDUCT IN STIMULATED CHICKS

In the hen, only the left ovary and the left oviduct are functional. In a sexually healthy and active mature fowl the oviduct is about 90 cm long, tortuous and runs from the ovary to the cloaca.

It is well known that the development of the oviduct, as a whole, is under the control of oestrogens. They induce differentiation of epithelium and accelerate the synthesis of proteins (Brant and Malbandov, 1956). Progesterone, apparently, induces the synthesis of avidin, the effect being dependent upon previous sensitization of the oviduct by oestrogens.

The immature chicks' oviduct responds dramatically to administered oestrogen. The precise mechanism of action of oestrogen in the stimulation of growth of the oestrogen-sensitive tissue such as the uterus has not yet been fully resolved. There is good evidence to show that there is an early stimulation of RNA and protein synthesis. Oestrogen has been reported to increase the levels of many enzymes in the uterus (Barker *et al.*, 1956). Oestrogen stimulates oviduct growth in day-old and older chicks. Formation of secretory granules within the oviducal glands and release of an albumen-like material into the lumen were reported following administration of oestrogen and progester-

rone or oestrogen and androgen combinations.

Synergistic effect of progesterone when injected with oestrogen on the oviduct weight of pullets has been recorded. An antagonistic action of progesterone on oviductal growth, when stilbestrol was administered, has also been reported. These conflicting results could be attributed to variations in the dose level of the hormones.

REVIEW OF LITERATURE

Cowson et al. (1947) and Bolton (1955) showed that the administration of gonadal steroid hormones to sexually immature chicks resulted in morphological development and growth of the oviduct similar to that occurring in adults. Jackson and Brown (1956) demonstrated that following oestrogen treatment there occurred an increased oviductal retention of nitrogen and an increase in the size of the oviduct. However, the ovary remained quiescent. Brown and Jackson (1956) recorded higher protein content in the magnum, higher fat content in the infundibulum and uterus and higher levels of deoxy pentose nucleic acid (DNA) in functional oviduct of hens compared to those observed in oestrogen-stimulated oviducts. No difference in the amino acid composition of protein was noticed between the two groups. Barker et al. (1966) noticed an oestrogen induced protein synthesis. O'Malley et al. (1968) investigated the effects of oestrogen stimulation on the rate of protein synthesis in the immature chicks' oviduct. They found that for the synthe-

sis of new proteins, new mRNA might be forming to direct the phenomenon in the oviduct. Wingman et al. (1967) noticed that oestrogen led to a general increase in DNA, RNA and tRNA in the oviduct. Formation of new species of mRNA was also observed by the above workers. Similar findings were reported by Oka and Schicks (1969).

McGuire and O'Malley (1969) were of the opinion that progesterone acted synergistically with oestrogen and that this action occurred through the mediation of RNA and DNA polymerase activities. Contrary to the general belief that testosterone and progesterone act synergistically with oestrogen, Oka and Schicks (1969) stated that progesterone was antagonistic in action to oestrogen.

Herrick and Adams (1951) found that following administration of high levels of progesterone (20-40 mg/bird/week), a total inhibition of egg production occurred, this being followed in 10-14 days by a rapid resuit. According to Adams (1955) a direct antagonistic action may be operating between the naturally secreted androgen, progesterone and oestrogens.

Kohler et al. (1968) based on immunofluorescent and autoradiographic studies showed that albumen and avidin were produced by specifically different cells of the oviduct in chicken. The mechanisms of selective induction of synthesis of albumen by oestrogen and that of avidin by progesterone were due to specific stimulation of different target cells by the two hormones. Hawkins et al. (1969) and Kerezias (1969)

stated that the action of a hormone is dependent on its target organs and that little is known about the target organs in the fowl oviducts.

Mason (1952) showed that the activities of progesterone and oestrogens on the weight gain of the oviduct depended on the relative doses of the two hormones. Adame (1955) suggested that the action of the hormones, both synergistic and antagonistic, depended on their relative dose levels. Fox (1955) reported that changes in size and weight of the oviduct under the influence of oestrogen and progesterone were dose dependent and that progesterone alone was ineffective in increasing the weight of the oviduct. Further, low levels of progesterone caused oviducal growth in the presence of high levels of stilbestrol but not at low levels of oestrogens. Breneman (1956) considered that mere progesterone was ineffective in bringing about any morphological development of oviduct. He considered that a combination of progesterone with oestrogen and androgen was more effective in inducing the oviducal development. Brandt and Nalbandov (1956) showed that oestrogen-androgen combination produced much more pronounced development of the oviduct compared to oestrogen-progesterone combination. However, they also have given evidence to show that a synergistic effect between oestrogen and progesterone could be produced by adjusting the dose levels of the two hormones. They were of opinion that the oviducal response to oestrogen in

combination with either testosterone or progesterone was dependent, primarily, on the relative doses of the two hormones administered simultaneously.

An increase in the plasma alkaline phosphatase activity following administration of oestradiol, stilbestrol, testosterone and corticoids was observed by Tanabe and Wilcox (1961) and Brown and Jackson (1961). The latter workers further considered that progesterone acted counter to oestrogen in this respect. It is known that both oestrogen and androgen were required for medullary bone development in the fowl. Chakravarti and Jadhav (1965) stated that for the development of the full secretory activity in the oviduct, either progesterone or testosterone along with oestrogen was required. The observations of Oades and Brown (1965) were in conformity with this finding.

Stokes and Cunningham (1976) showed that progesterone stimulated luteinising hormone and this in turn stimulated the secretion of progesterone.

MATERIALS AND METHODS

One-day-old, clinically healthy, female White Leghorn (WL) and White Plymouth Rock (WPR) chicks, 100 numbers under each category, were selected at random, from the Kerala Agricultural University Poultry farm. The birds were maintained under standard farm conditions on standard chick mash (Appendix I) and water given *ad libitum*.

The effects of administration of exogenous sex hormones were studied in 21-days-old sexually immature WL and W2 chicks. The chicks were randomly divided into five groups. The chicks in Group I received stilbestrol dipropionate (SDP) 1 mg/day/bird in 0.25 ml olive oil. In Group II, the chicks were given 1 mg SDP in 0.25 ml olive oil and 1 mg testosterone propionate (TP) in 0.25 ml olive oil/day/bird. The chicks in Group III received 1 mg SDP in 0.25 ml olive oil and 1 mg progesterone (PG) in 0.25 ml olive oil/bird/day. The birds in Group IV and V served as controls, the former receiving 0.25 ml and the latter receiving 0.50 ml of olive oil/bird/day. The preparations were injected into the pectoral muscles in the morning, once a day, for 10 days.

Blood samples were collected by cardiac puncture, using heparin as the anticoagulant from the experimental and control chicks, 24 h after the last injection of the hormone for analyses of enzymes.

Immediately after collection, the blood samples were centrifuged (2100 x g; 30 min) in a Kemi refrigerated centrifuge (0°C). The separated plasma was siphoned off. It was diluted 1 : 20 with ice-cold double distilled water (DDW) and kept under refrigerator temperature until enzyme estimation.

After collection of blood, the birds were killed by decapitation and the viscera was exposed. The ovary and oviduct were carefully dissected out, using ice-cold pair of scissors and forceps. The adhering tissues were trimmed off.

The blood and other body fluids adhering to the tissues were removed by placing the tissues within the folds of a filter paper. The ovaries, the different regions of the oviduct, in the case of experimental chicks and the entire oviduct in the case of control chicks were weighed separately. The tissues were cut into small pieces using ice-cold pair of scissors and homogenized in an ice-bath using a Potter-Elvehjem tissue homogenizer as described by Bergmeyer (1965) and diluted in each case to 20 ml with ED₂. The tissue homogenates were then filtered through double layers of muslin. The collected filtrates were stored in ice-bath for estimation of enzymes.

Enzymatic assays were carried out in the plasma, in the filtrates from the homogenates of the ovary, the entire oviduct from the control chicks, the different regions of the oviduct in the experimental chicks and the pooled samples of the filtrates from the different parts of the oviduct of the experimental chicks.

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were determined using the colorimetric method with 2,4-dinitrophenyl hydrazine (Appendix II,III) as given by Bergmeyer (1965).

Method of Lodansky (1953) was followed in the determination of alkaline (ALP) and acid (ACP) phosphatases (Appendix IV,V).

Estimation of glucose-6-phosphatase (G-6-Phase) was

carried out by the method (Appendix VI) given by Bergmeyer (1965).

Statistical analysis of the data was carried out by the methods given by Snedecor and Cochran (1967).

Enzyme contents were compared:

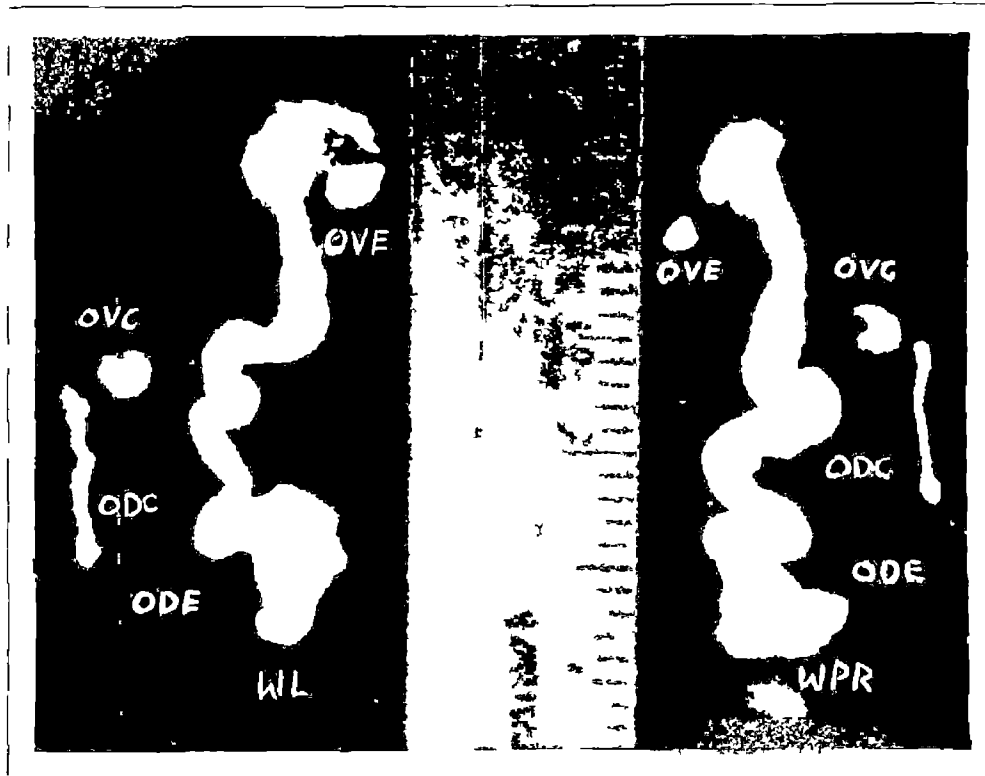
- 1) between identical tissues in the two breeds of chicks under each treatment, and
- 2) between identical tissues within the breed under each treatment.

Student's 't' was used to test the equality of two means when the variances were identical and Cochran's 't' when the variances were unequal.

RESULTS

The effects of administration of SD₂ on the weight and development of the ovary and oviduct of immature W₂ and W₁R chicks are given in Table 3.1 and Fig.III.1. The effects of administration of combinations of SD₂-T₂ and D₂-T₂ on the weight-gain and morphological development of oviduct in immature W₂ and W₁R chicks are given in Tables 3.2, 3.3 and Fig.III.2 and III.3. The results obtained on the weight-gain of the ovary and oviduct in the two breeds of chicks under the influence of the different hormone-treatments have been consolidated and presented in Table 3.4.

Administration of the hormone resulted in pronounced increase in the size and weight of the oviduct in both the breeds studied compared to their controls. However, the



ovaries failed to reveal any such alterations. The oviduct in the experimental groups was enlarged in size. The component segments like the infundibulum, the magnum, the isthmus, the uterus and the vagina, ^{in/c/c} well differentiated.

Significant ($P < 0.01$) increase in the weight of the oviducts in the experimental chicks could be noticed compared to the controls. There was no significant difference in weight between the entire oviducts of the experimental groups or of the different regions of the oviduct between the two breeds of chicks (Table 3.1). Similar were the results obtained when D-E and SDP-SG combinations of hormones were given (Tables 3.2 and 3.3). The results obtained on the weight-gain of the ovary and oviduct in the two breeds of chicks under the influence of the different hormone-treatments have been consolidated and presented in Table 3.4.

Table 3.5 shows the concentrations of GPT and GPT in the blood plasma, ovary and in the different regions of the oviduct of chicks developed under the influence of administration of SDP compared to the control chicks. No significant difference could be noticed in the concentration of the enzymes studied, either within the same breed or between the two breeds. Both the enzymes could be detected in the two breeds of chicks. However GPT was absent from the isthmus and uterus of the oviduct of WBR chicks given SDP. The G-T activities noticed in the plasma and tissues were feeble and highly variable.

TABLE 3.1

Effect of administration of ODP on the weight (g) of the ovary and different regions of the oviduct in WL and WR chicks (Values are mean \pm S.E.)

Tissue	WHITE LEGHORN		WHITE RHODE ISLAND REDS	
	Experimental (15)	Control (14)	Experimental (8)	Control (9)
Ovary	0.066 \pm 0.006	0.064 \pm 0.012	0.095 \pm 0.006	0.084 \pm 0.010
Infundibulum	0.193 \pm 0.017		0.295 \pm 0.039	
Magnum	0.023 \pm 0.040	Could not be distinguished	1.363 \pm 0.159	Could not be distinguished.
Isthmus	0.421 \pm 0.024		0.528 \pm 0.040	
Uterus	1.299 \pm 0.057		1.875 \pm 0.158	
Vagina	0.631 \pm 0.052		0.796 \pm 0.030	
Oviduct	3.367 \pm 0.155*	0.048 \pm 0.010	4.056 \pm 0.398*	0.052 \pm 0.009

Figures in parentheses indicate the number of birds

** Significant at 1% level within the breed

TABLE 3.2

Effect of administration of UDP and TP on the weight (g) of the ovary and different regions of the oviduct in WL and W.R chicks (Values are mean \pm S.E.)

Tissue	WHITE BREASTED		WATERBURY	
	Experimental (10)	Control (10)	Experimental (10)	Control (10)
Ovary	0.072 \pm 0.003	0.056 \pm 0.010	0.330 \pm 0.006	0.303 \pm 0.010
Infundibulum	0.198 \pm 0.025		0.213 \pm 0.027	
Magnum	1.062 \pm 0.192	Could not be distinguished	1.165 \pm 0.195	Could not be distinguished
Isthmus	0.449 \pm 0.036		0.472 \pm 0.033	
Uterus	1.530 \pm 0.163		1.727 \pm 0.221	
Vagina	0.629 \pm 0.063		0.710 \pm 0.076	
Oviduct	3.368 \pm 0.451 ^{**}	0.354 \pm 0.010	4.234 \pm 0.535 ^{**}	0.371 \pm 0.010

Figures in parenthesis indicate the number of birds

** Significant at 1% level within the breed

TABLE 3.3

Effect of administration of SDI and ZG on the weight (g) of the ovary and different regions of the oviduct in WB and WPR chicks (Values are mean \pm S.E.)

Tissue	WHITE LEGHORN		WHITE PLYMOUTH ROCK	
	Experimental (10)	Control (10)	Experimental (10)	Control (10)
Ovary	0.369 \pm 0.007	0.244 \pm 0.010	0.379 \pm 0.005	0.343 \pm 0.010
Infundibulum	0.178 \pm 0.019		0.139 \pm 0.031	
Magnum	0.731 \pm 0.083	Could not be distinguished	1.230 \pm 0.196	Could not be distinguished
Isthmus	0.456 \pm 0.030		0.491 \pm 0.037	
Uterus	1.401 \pm 0.224		1.785 \pm 0.218	
Vagina	0.625 \pm 0.073		0.725 \pm 0.069	
Oviduct	0.361 \pm 0.074**	0.059 \pm 0.010	4.429 \pm 0.493**	0.370 \pm 0.010

Figures in parenthesis indicate the number of birds

** Significant at 1% level within the breed

TABLE 3.4

Consolidated statement of the data on the influence of administration of TD, SDF-TV and SDF-IG on the weight-gain of the reproductive organs in ♀ and ♂R chicks (Values are mean \pm SE)

Tissue	WHITE LEGHORN			WHITE PLYMOUTH ROCK		
	SD	SDF-TV	SDF-IG	SD	SDF-TV	SDF-IG
Ovary	0.066 \pm 0.006	0.072 \pm 0.008	0.069 \pm 0.007	0.085 \pm 0.006	0.030 \pm 0.000	0.079 \pm 0.005
Infundibulum	0.193 \pm 0.017	0.198 \pm 0.025	0.170 \pm 0.019	0.295 \pm 0.039	0.210 \pm 0.027	0.199 \pm 0.031
Magnum	0.825 \pm 0.048	1.062 \pm 0.192	0.701 \pm 0.083	1.363 \pm 0.199	1.165 \pm 0.195	1.250 \pm 0.196
Isthmus	0.421 \pm 0.024	0.449 \pm 0.036	0.456 \pm 0.030	0.528 \pm 0.040	0.472 \pm 0.033	0.491 \pm 0.037
Uterus	1.299 \pm 0.057	1.530 \pm 0.163	1.401 \pm 0.224	1.375 \pm 0.158	1.727 \pm 0.221	1.735 \pm 0.218
Vagina	0.631 \pm 0.052	0.629 \pm 0.063	0.625 \pm 0.073	0.796 \pm 0.080	0.710 \pm 0.070	0.725 \pm 0.069
Ovi duct	3.367 \pm 0.155	3.368 \pm 0.451	3.361 \pm 0.374	4.856 \pm 0.393	4.287 \pm 0.505	4.429 \pm 0.493

TABLE 3.5

Effect of administration of CDE on the levels of GOT and GPT in the ovary, oviduct and plasma of WL and WER chicks (Values are mean \pm SE)

Tissue	GOT (IU)				GPT (IU)			
	EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL	
	WL (15)	WER (8)	WL (14)	WER (9)	WL (13)	WER (8)	WL (15)	WER (8)
Ovary	5.65 \pm 1.73	19.90 \pm 8.72	10.61 \pm 4.69	8.13 \pm 4.03	0.92 \pm 0.48	4.31 \pm 3.59	1.17 \pm 0.71	0.07 \pm 0.07
Infundi- bulum	3.58 \pm 0.54	2.37 \pm 0.75	0.23 \pm 0.11	0.33 \pm 0.30
Meguum	2.01 \pm 0.27	1.64 \pm 0.56	0.08 \pm 0.02	0.01 \pm 0.03
Isthmus	2.24 \pm 0.35	1.71 \pm 0.56	0.19 \pm 0.08	0.11

Table 3.6 indicates the results of the estimations of alkaline and acid phosphatases in the blood plasma and reproductive organs under the influence of SDP administration. No significant difference in concentration of the two enzymes was noticed either within the same breed or between the two breeds studied.

The effects of administration of a combination of SDP and TP were also studied in the two breeds of WL and WBR immature female chicks. The administration of the hormones resulted in pronounced growth of the oviducts in chicks of both the breeds (Fig. III.9). The oviduct in the control chicks of both the breeds remained ill-developed. In the experimental chicks the oviducts were markedly developed with all the constituent regions well demarcated and distinct. The morphological development of the oviducts in the two breeds receiving the hormones simulated those that received SDP alone. The combination of SDP and TP could not, however, induce any morphological change in the ovaries, which remained ill-developed as in the case of control chicks.

Table 3.7 presents the data for the different enzymes studied in the plasma, ovary and oviduct of both treated and control chicks.

Compared to the control groups, birds which were given exogenous SDP and TP showed marked difference in the concentrations of alkaline and acid phosphatases and GOT

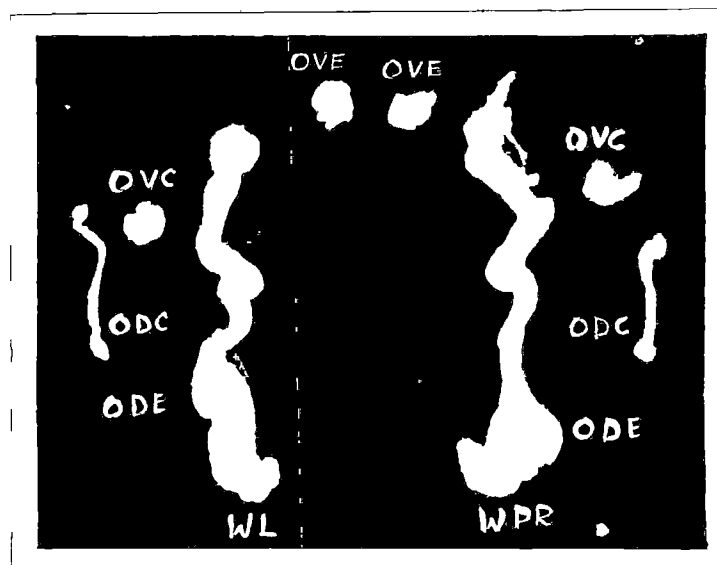


TABLE 3.6

Effect of administration of GDP on the levels of ALP and ACP in the plasma and reproductive organs of WL and WFR chicks (Values are mean \pm SE)

Tissue	ALP (BU)				ACP (BU)			
	Experimental		Control		Experimental		Control	
	WL (3)	WFR (5)	WL (3)	WFR (5)	WL (3)	WFR (5)	WL (3)	WFR (5)
Ovary	22.73 \pm	163.13 \pm	58.26 \pm	375.24 \pm	79.51 \pm	277.75 \pm	71.14 \pm	61.38 \pm
	5.03	54.06	13.73	46.57	20.07	166.55	17.10	34.94
Infundibulum	33.67 \pm	23.43 \pm	157.46 \pm	103.75 \pm
	15.94	9.92	11.18	43.44
Magnum	37.20 \pm	8.65 \pm	166.91 \pm	72.21 \pm
	5.51	2.51	27.50	14.16
Isthmus	23.33 \pm	2.12 \pm	138.23 \pm	108.24 \pm
	7.27	2.12	16.15	21.76

TABLE 3.6 (Continued)

Tissue	AHP (BU)				AOP (BU)			
	Experimental		Control		Experimental		Control	
	WL (3)	WR (5)	WL (8)	WR (5)	WL (9)	WR (5)	WL (3)	WR (5)
Uterus	17.60 ±	9.74 ±	54.27 ±	20.35 ±
	3.79	2.65	16.85	11.48
Vagina	29.63 ±	23.99 ±	63.53 ±	87.63 ±
	8.06	9.31	20.23	44.03
Oviduct	17.79 ±	9.55 ±	11.24 ±	..	68.75 ±	60.21 ±	22.53 ±	..
	4.02	2.14	7.39	Nil	15.80	16.34	10.02	Nil
Plasma	13.32 ±	10.76 ±	3.75 ±	78.62 ±	29.22 ±	64.55 ±	0.39 ±	40.55 ±
	3.04	3.36	1.14	53.09	6.09	29.45	0.19	36.44

Figures in parentheses indicate the number of birds

BU : Bodgusay units per ml of plasma or per mg of tissue

in the reproductive organs. Compared to the control group, the experimental WL chicks showed significantly higher ($P < 0.01$) contents of acid phosphatase and G.T in their ovaries, and acid phosphatase, GGT and G.T in their oviducts (Table 3.7). But the pattern of distribution of enzymes in the MR chicks was different; the experimental group showing significantly lower ($P < 0.01$) levels of G.T in the plasma and alkaline and acid phosphatases and GGT in their oviducts ($P < 0.01$ in all cases). Again, a comparison between the control groups showed that the MR breed had higher content of plasma G.T and oviducal alkaline phosphatase ($P < 0.01$), than those in WL. However, a comparison of the results of enzyme contents in the oviducal segments between the two experimental groups (Table 3.8) showed that the MR chicks had higher content of acid phosphatase in the isthmus and glucose-6-phosphatase ($P < 0.01$ in both cases) in the vaginal region compared to WL chicks.

The administration of another combination of hormones, SDF with progesterone (G) resulted in growth and enlargement of the immature oviduct in both the breeds (Fig. 4.3). Here again, the ovaries remained without any response, the organ evincing no change from the control. The oviducts in the control chicks in both the breeds were under-developed, thread-like and could not be differentiated into component parts. But, the oviducts in the experimental group of chicks were well-developed and component parts could

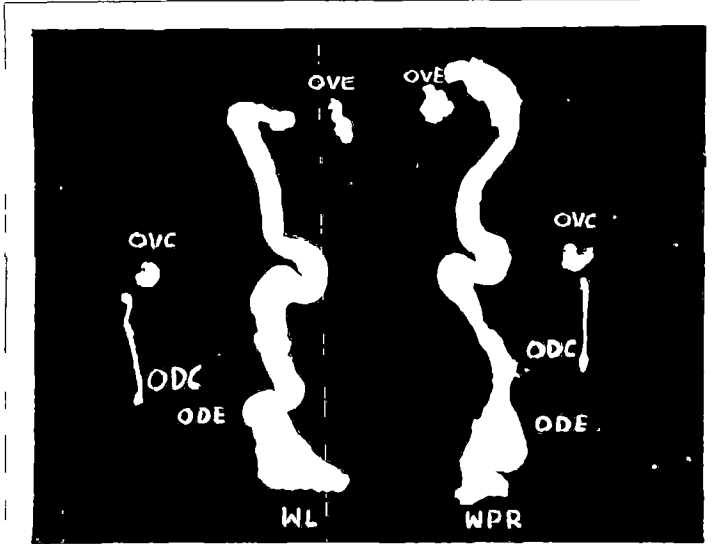


TABLE 5.7

Enzyme content of plasma, ovary and oviduct of W and NR chicks under the influence of SLD and T₂ (Values are Mean \pm SE)

Enzyme	Tissue	WHITE LEGHORN		WHITE LIZZARD ROCK	
		Experimental (10)	Control (10)	Experimental (10)	Control (10)
AAU (BU)	Plasma	11.67 \pm 5.45	11.69 \pm 1.24	24.14 \pm 2.93	17.35 \pm 5.55
	Ovary	0.77 \pm 0.13	2.25 \pm 1.64	3.11 \pm 3.92	3.12 \pm 0.57
	Oviduct	0.55 \pm 0.09	0.53 \pm 0.20	0.56 \pm 0.17	2.96 \pm 0.63 ^{###}
ACP (BU)	Plasma	Nil	Nil	Nil	Nil
	Ovary	1.03 \pm 0.06 ⁺⁺	Nil	7.79 \pm 4.15	1.39 \pm 0.46
	Oviduct	1.20 \pm 0.21 ⁺⁺	Nil	2.37 \pm 0.34 ^{bc}	2.79 \pm 3.61 ^{##}
GOT (RU)	Plasma	116.00 \pm 19.28	170.00 \pm 31.73	104.00 \pm 24.37	196.00 \pm 15.43
	Ovary	4.39 \pm 1.12	5.57 \pm 1.26	6.03 \pm 2.34	4.67 \pm 0.88
	Oviduct	2.90 \pm 0.49 ⁺⁺	Nil	1.88 \pm 0.24	6.19 \pm 1.26 ^{##}

TABLE 3.7 (Continued)

Enzyme	Tissue	WHITE L CHICK		WHITE PUFFIN CHICK	
		Experimental (10)	Control (10)	Experimental (10)	Control (10)
G-7 (WU)	Plasma	Nil	Nil	Nil	6.00 ± 5.89 ^{§§}
	Ovary	1.42 ± 0.15 ⁺⁺	Nil	1.53 ± 0.32	1.41 ± 0.71
	Oviduct	0.39 ± 0.04 ⁺⁺	Nil	0.32 ± 0.07	0.53 ± 1.33
G-6-PLANE (U)	Plasma	0.49 ± 0.14	0.24 ± 0.04	0.84 ± 0.35	0.69 ± 0.14
	Ovary	14.63 ± 5.07	Nil	24.95 ± 7.55	23.97 ± 25.39
	Oviduct	5.97 ± 0.48	Nil	3.86 ± 0.85	20.70 ± 5.77

The figures in parenthesis indicate the number of birds

++ Significant at 1% level between experimental and control groups in W

§§ Significant at 1% level between experimental and control groups in P

** Significant at 1% level between experimental groups among WL and WLP

Significant at 1% level between control groups among WL and WLP

N.B. Values given for oviduct in the experimental groups are from the pooled sample prepared by mixing the filtered homogenates of the different regions of the oviduct of treated chicks

be easily identified.

The morphological development of the oviduct in the chicks treated with SDF and PG was similar to those receiving SDF alone or SDF in combination with TP. Similarly, as in the two previous experimental studies, the ovaries continued to be non-responsive and remained ill-developed.

Tables 3.9 to 3.12 give the data for different enzymes in the plasma, the ovary and the oviduct in the WL and WR chicks given a combination of SDF and PG.

The WR experimental chicks recorded a significantly higher ($P < 0.01$) content of GOT in the oviduct compared to the control chicks (Table 3.9).

No GOT activity could be detected either in the plasma, or in the reproductive organs of WL and WR experimental chicks.

In the case of alkaline phosphatase (Table 3.10) WL control chicks showed a significantly higher ($P < 0.01$) content of the enzyme in the plasma. No other significant difference could be noticed in the enzyme content of other regions of the reproductive organs.

Significantly higher ($P < 0.01$) levels of acid phosphatase (Table 3.11) were observed in the ovaries of WL controls compared to those in WL experimental groups as well as WR control groups.

TABLE 3.9

Effect of administration of a combination of SDP and ES on the level of GSE (IU) in the ovary, oviduct and plasma of WL and WPR chicks (Values are mean \pm SD)

Tissue	WHITE LEGHORN		WHITE PLYMOUTH ROCK	
	Control (10)	Experimental (10)	Experimental (10)	Control (10)
Ovary	3.03 \pm 0.78	1.54 \pm 0.15	1.81 \pm 0.31	1.69 \pm 0.07
Infundibulum	..	1.11 \pm 0.18	0.87 \pm 0.17	..
Magnus	..	0.81 \pm 0.12	1.11 \pm 0.08	..
Isthmus	..	0.74 \pm 0.17	1.00 \pm 0.16	..
Uterus	..	0.50 \pm 0.07	0.60 \pm 0.12	..
Vagina	..	0.98 \pm 0.31	0.50 \pm 0.09	..
Oviduct	1.74 \pm 0.51	1.08 \pm 0.41	0.85 \pm 0.09 ^{**}	..
Plasma	160.00 \pm 20.66	143.33 \pm 20.28	93.33 \pm 13.33	86.67 \pm 6.67

Figures in parenthesis indicate the number of birds

IU : Radio Units/ml of plasma or per mg of tissue

** : Significant at 1% level within the breed (between the control and experimental)

No significant difference in the concentration of G.D-Pase either within the breed or in between the two breeds of chicks could be noticed (Table 3.12).

The results obtained from the estimation of enzymes in the blood plasma and reproductive organs in the two breeds of chicks under the influence of the different hormone-treatments have been consolidated and presented in Table 3.13.

Significantly higher ($P < 0.05$) content of alkaline and acid phosphatases could be detected in the WL chicks treated with SDP alone compared to those given SD³ and SP or SDP and PG.

In the case of GOT and GPT, the WL chicks receiving SDP alone revealed a significantly higher ($P < 0.05$) content of GOT in the vagina and GPT in the infundibulum and plasma compared to those receiving SDP-SP combination of hormone. But, in WL chicks receiving SDP alone, GPT in the ovary and infundibulum and GPT and GOT in the vagina, the isthmus and the uterus recorded significantly higher levels compared to the WL chicks receiving SDP-PG combination.

The plasma TPT level was also higher in WL chicks receiving SDP alone compared to those receiving combination of SDP and PG.

In the WL chicks receiving SDP alone the levels of alkaline and acid phosphatases were significantly higher

TABLE 3.12

Effect of administration of a combination of SDP and PG on the level of glucose-6-phosphatase (U) in the ovary, oviduct and plasma of WL and WR chicks

(Values are mean \pm SE)

Tissue	WHITE LEGHORN		WHITE PLYMOUTH ROCK	
	Control (10)	Experimental (10)	Experimental (10)	Control (10)
Ovary	165.20 \pm 39.87	67.03 \pm 5.70	53.90 \pm 12.31	54.09 \pm 2.08
Infundibulum	..	59.88 \pm 14.23	44.98 \pm 25.32	..
Magnum	..	18.45 \pm 5.38	25.71 \pm 6.89	..
Isthmus	..	22.88 \pm 11.58	15.47 \pm 3.70	..
Uterus	..	13.06 \pm 4.06	11.25 \pm 5.00	..
Vagina	..	14.07 \pm 6.20	10.18 \pm 2.30	..
Oviduct	119.77 \pm 24.52	19.68 \pm 5.84	15.40 \pm 0.03	52.79 \pm 11.39
Plasma	7.09 \pm 0.83	6.11 \pm 1.32	5.82 \pm 0.79	4.61 \pm 0.36

Figures in parenthesis indicate the number of birds

U : Units/ml of plasma or per mg of tissue

TABLE 3.13

Comparative effects of administration of SDP-TP or SDP-PG combinations of hormones with respect to administration of SDP alone on the enzyme pattern in the plasma and reproductive organs in WL and WPR chicks (Values are mean \pm SE)

Enzyme	Tissue	SDP		SDP-TP		SD-PG	
		WL (8)	WPR (5)	WL (10)	WPR (10)	WL (10)	WPR (10)
	Ovary	22.78 ^{**} \pm	168.18 ^{**} \pm	0.77 \pm	3.11 \pm	0.12 \pm	0.10 \pm
		5.88 ^{**}	54.06 [*]	0.13	0.92	0.04	0.02
ALP (BU)	Infundibulum	80.67 ^{**} \pm	23.43 \pm	0.48 \pm	0.47 \pm	0.21 \pm	0.22 \pm
		15.94 [*]	9.92	0.06	0.15	0.08	0.09
	Diaphragm	37.25 ^{**} \pm	8.65 ^{**} \pm	0.27 \pm	0.58 \pm	0.05 \pm	0.02 \pm
		5.61 [*]	2.52 [*]	0.05	0.23	0.01	0.02
	Isthmus	23.38 \pm	2.12 \pm	1.12 \pm	0.37 \pm	0.04 \pm	0.02 \pm
		7.27	2.12	0.40	0.16	0.00	0.00

Figures in parenthesis indicate the number of birds
 BU : Bodansky units/ml of plasma or per mg of tissue



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TABLE 3.13 (Continued)

Enzyme	Tissue	LD ₅		LD ₅ -TR ^a		LD ₅ -L	
		WU (8)	WAR (5)	AL (10)	WAR (10)	WU (10)	WAR (10)
ALP (BU)	Uterus	17.69 ^{***} ±	9.74 ^{**} ±	0.40 ±	0.37 ±	0.09 ±	0.07 ±
		3.79 ^{**}	2.65 ^b	0.02	0.07	0.00	0.03
	Vagina	35.68 ±	23.99 ±	0.25 ±	0.48 ±	0.02 ±	0.03 ±
		8.86	9.31	0.00	0.15	0.00	0.00
	Oviduct	17.70 ^{***} ±	9.55 ^{**} ±	0.55 ±	0.56 ±	0.15 ±	0.08 ±
		4.02 ^b	2.14 ^a	0.09	0.17	0.06	0.02
	Plasma	13.32 ±	10.76 ±	11.67 ±	24.14 ±	31.20 ^b ±	41.60 ^{***} ±
		3.94	3.36	5.45	2.93	2.92	3.46

Figures in parenthesis indicate the number of birds
 BU : Bodansky units/ml of plasma or per mg of tissue

TABLE 3.13 (Continued)

Enzyme	Tissue	SDP		GMP-TP		GMP	
		ML (8)	KPR (5)	ML (10)	KPR (10)	ML (10)	KPR (10)
ADP (50)	Ovary	79.54 ^{**} ±	277.75 ±	1.03 ±	7.79 ±	0.16 ±	0.23 ±
		2.97 [†]	166.53	0.06	4.15	0.02	0.05
	Infundibulum	157.46 ^{**} ±	108.75 ±	1.40 ±	1.40 ±	0.26 ±	0.22 ±
		11.18 [†]	43.44	0.19	1.73	0.03	0.02
	Sagnum	166.91 ^{**} ±	72.21 ^{**} ±	0.84 ±	1.21 ±	0.49 ±	0.94 ±
		27.59 [†]	14.16 ^{**}	0.19	0.39	0.36	0.46
Tethnus	103.23 ^{**} ±	108.34 ±	0.53 ±	1.86 ±	0.22 ±	0.50 ±	
	16.15 [†]	21.76 [†]	0.07	0.37	0.07	0.20	

Figures in parentheses indicate the number of birds
 BU : micromoles units/ml of plasma or per mg of tissue

Table 3.13 (continued)

Enzyme	Tissue	CDR		SDC-PP		CDL-0	
		WL (8)	WPR (5)	WL (10)	WPR (10)	WL (10)	WPR (10)
ACD (33)	Ovary	54.27 ^{**}	30.35 ±	1.47 ±	2.73 ±	0.19 ±	0.37 ±
		16.85 [*]	11.48	0.21	0.47	0.05	0.13
	Vagina	68.53 ^{**}	87.60 ±	0.34 ±	1.19 ±	0.10 ±	0.24 ±
		20.23 [*]	44.03	0.05	0.28	0.10	0.06
	Oviduct	68.75 ^{**}	60.21 ^{**}	1.20 ±	2.37 ±	0.29 ±	0.47 ±
		15.80 [*]	16.34 [*]	0.21	0.34	0.15	0.18
	Plasma	29.22 ^{**}	64.55 ±	Nil	Nil	Nil	Nil
		6.09 [*]	29.45				

Figures in parenthesis indicate the number of birds
 BU : Bodo 67y units/ml of plasma or per mg of tissue

Table 3.13 (continued)

Enzyme	Tissue	SDP		SDI-T ₁		JL-WG	
		no. (8)	Wt (8)	no. (10)	Wt (10)	no. (10)	Wt (10)
	Ovary	5.65 ^{##} ± 1.73	19.90 ± 8.72	4.09 ± 1.12	8.03 ± 2.34	1.10 ± 0.15	1.81 ± 0.31
	Infundi- bulus	3.58 ^{##} ± 0.54	2.37 ± 0.75	2.17 ± 0.41	2.54 ± 0.56	1.11 ± 0.10	0.87 ± 0.17
GGT (IU)	Magnum	2.01 ^{##} ± 0.27	1.84 ± 0.56	2.76 ± 0.80	1.64 ± 0.19	0.82 ± 0.12	1.11 ± 0.13
	Isthmus	2.34 ^{##} ± 0.35	1.74 ± 0.56	3.06 ± 0.26	2.29 ± 0.54	3.84 ± 0.17	1.00 ± 0.16

Figures in parenthesis indicate the number of birds

IU: molar units /ml plasma or per mg tissue

TABLE 3.13 (continued)

Enzyme	Tissue	SDP		SDP-TF		D. 20	
		ML (8)	WtH (8)	ML (10)	WtH (10)	ML (10)	WtH (10)
	Uterus	1.48 ⁺ ±	0.94 ±	3.27 ±	2.21 ±	6.50 ±	3.60 ±
		0.22	0.22	2.37	0.22 ⁺	0.07	0.12
	Vagina	2.12 ±	1.40 ±	1.08 ±	1.32 ±	0.90 ±	0.50 ±
		0.45 ⁺	0.43	0.11	0.35	0.01	0.09
GPT (IU)	Oviduct	1.55 ±	0.99 ±	2.00 ±	1.88 ±	1.00 ±	0.85 ±
		0.17	0.32	0.49 ⁺	0.24	0.14	0.09
	Lamina	117.30 ±	247.50 ⁺⁺ ±	116.00 ±	104.00 ±	140.00 ±	90.30 ±
		13.99	60.47 ⁺	19.28	24.37	23.2	13.33

Figures in parenthesis indicate the number of birds

RU = micro units/ml plasma or per mg tissue

TABLE 3.13 (continued)

Species	Tissue	EDF		CBK-2		Cobalt	
		µg (13)	µgR (8)	µg (10)	µgR (10)	µg (10)	µgR (10)
G E (55)	Ovary	0.92 ±	4.32 ±	1.42 ±	1.53 ±	nil	nil
		0.48	3.50	0.13	0.32
	Infundibulum	0.23 ±	0.05 ±	0.71 ±	0.49 ±
		0.11	0.03	0.12	0.14 ^h
	Sagittae	0.03 ^h ±	0.01 ±	0.19 ±	0.16 ±
		0.02	0.009	0.03 ^h	0.03 ^h
Isthmus	0.19 ±		0.17 ±	0.20 ±	
	0.03	nil	0.03	0.03 ^h	

Figures in parenthesis indicate the number of birds

µg = micrograms/ml of plasma or per mg of tissue

TABLE 3.13 (continued)

Enzyme	Tissue	SDP		SDP-TP		SDP-IG	
		WL (13)	WPR (8)	WL (10)	WPR (10)	WL (10)	WPR (10)
G6P (WU)	Uterus	0.02 [±]		0.19 ±	0.20 ±	Nil	Nil
		0.505	Nil	0.00	0.05 [±]
	Vagina	0.20 ±	0.02 ±	0.26 ±	0.16 ±
		0.16	0.02	0.04	0.08 [±]
	Oviduct	0.10 ±	0.04 ±	0.39 ±	0.32 ±
		0.08	0.02	0.04 [±]	0.07
	Alaska	27.69 [±]	7.50 ±	Nil	Nil
		6.22 [±]	7.50		

Figures in parenthesis indicate the number of birds

WU = Wroblewski units/ml of plasma or per mg of tissue

TABLE 3.13 (continued)

Enzyme	Tissue	SD ₁ -TP		SD ₁ -G	
		WL (10)	WER (10)	WL (10)	WER (10)
	Ovary	14.68 ± 5.07	24.95 ± 7.53	67.93 ± 11.70	53.90 ± 12.31
	Infundibulum	17.87 ± 13.35	6.49 ± 2.33	53.83 ± 14.83	44.98 ± 25.32
	Magnum	5.36 ± 3.58	2.10 ± 0.57	18.45 ± 9.30	25.71 ± 6.89
C-6-ase (U)	Isthmus	2.19 ± 0.53	3.84 ± 0.96	22.83 ± 11.93	15.47 ± 3.70
	Uterus	4.50 ± 1.46	2.24 ± 0.67	13.06 ± 4.00	11.25 ± 5.00
	Vagina	1.44 ± 0.30	3.40 ± 0.59	14.07 ± 6.23	10.18 ± 2.30
	Oviduct	5.97 ± 3.48	3.86 ± 0.95	19.63 ± 10.34	15.40 ± 3.03
	Plasma	0.45 ± 0.14	0.34 ± 0.11	6.11 ± 1.52	5.32 ± 0.79

Figures in parenthesis indicate the number of birds

U : Units/ml of plasma or per mg of tissue

* : Comparisons made between SD₁ with SD₁-TP combination in the SD₁ breeds, WL and WER significant at 5% level

** : Comparisons made between SD₁ with SD₁-G combination in the SD₁ breed significant at 5% level

§ : Comparisons made between SD₁-TP combination with SD₁-G combination in the WER breed significant at 5% level

§ : Comparisons made between SD₁-TP combination with SD₁-G combination in the WER breed significant at 5% level

in the magnum and the oviduct compared to those receiving ODP-EP or ODP-EG combinations. Further, alkaline phosphatase in the left ovary and uterus, and acid phosphatase in the isthmus also recorded higher ($P < 0.05$) contents in the WPR chicks receiving ODP compared to those receiving the two different combinations of hormones. In the blood plasma of WPR chicks receiving EP the GGT content was also seen higher ($P < 0.05$) than those receiving EP-EP or EP-EG.

In the chicks receiving ODP and EP, GGT and GPT contents in their oviducts and GPT content in their magnum were significantly higher than those receiving ODP alone.

In WPR chicks treated with ODP-EP, uterine GGT content and GPT concentration in the infundibulum, magnum, isthmus, uterus, vagina and oviduct was higher ($P < 0.05$) as compared to those receiving ODP alone.

In both WL and WR chicks given a combination of ODP and EG only the plasma alkaline phosphatase was seen higher ($P < 0.05$) compared to those receiving ODP.

DISCUSSION

Gonadotrophins are present in the chick embryopituitaries from the 13th day of incubation, but their activity varied with the age of the bird; being only about 1/14th potent at the age of 40 days compared to those at 3 to 4 months of age (Sturkie, 1955). The birds studied in the present investigation were 21-days-old. The very

low potency of gonadotrophins present at this stage may explain the absence of any morphological development of the ovary and therefore of the oviducts in these immature control chicks.

Gilbert (1967) stated that the secretion of follicle stimulating hormone could be controlled by a feedback mechanism from the ovary involving oestrogen and progesterone and that administration of oestrogen to intact chicken decreased the gonadotrophin output. The exogenous oestrogen (JDP) administered in the present study might have exerted a negative feedback mechanism over the already inadequate level of gonadotrophins released at this age of the chicks. This might be the reason why the ovaries were found not influenced morphologically in the three treatments.

Common (1943) and Bolton (1955) reported growth responses in the oviducal tissues under the influence of gonadal steroids. The morphological development of the oviduct can therefore be attributed to the synthetic oestrogen, JDP, in the present investigation. Evidences to show that oestrogens enter the oviducal tissue are provided by Hawkins *et al.* (1969). An increase in the uptake of osine acids and water by oviducts of chicken treated with oestrogen has been demonstrated by Oka and Gehinke (1969). Further, oestrogen administration has been shown to cause increased retention of nitrogen by the oviduct (Jackson and Crown, 1950). The pronounced morphological development noticed in the

present investigation can be attributed to an increased protein synthesis under the influence of the synthetic oestrogen administered. Hormones exert their effect on the target cell through the oxidation of adenosine 3, 5-mono-phosphate (cyclic ATP). On binding with the specific receptors present in the membrane covering the target cells, the hormone activates the enzyme, adeny cyclase. This in turn results in the production of cyclic ATP from cytoplasmic ATP. This cyclic ATP initiates cellular mechanisms.

Terenius (1969) found that the entry of oestrogen into oviducal tissue was independent of the presence of other sex steroids like testosterone or progesterone. Grant and Halbanov (1952) state that oestrogen was mainly associated with the morphological development of the oviduct. The identical development of the immature chicken oviduct in the two breeds studied in the present investigation, under the influence of D₂ alone or under D₂ in combination with either D₃ or D₆, may also point to a similar inference. The study revealed the primary function of oestrogen in the morphological development of the oviduct. The presence or absence of other sex steroids failed to interfere, influence or modify the main function of oestrogens.

No significant difference could be noticed either within the same breed or between the two breeds of W₁ and W₂ chicks, in the concentration of GGT and GPT in the plasma and the female reproductive organs developed under the influ-

ence of GGT. Oestrogen, when given alone, failed to induce full glandular development and secretory activity of the oviduct (Brant and Halbanov, 1956; Oades and Brown, 1965; Brown, 1966). Taylor *et al.* (1970) stated that the formation of egg in the hen's oviduct was associated with decreased plasma levels of essential amino acids and an increased concentration of non-essential amino acids. The transamination reactions associated with the synthesis of protein might necessitate an elevation in the transaminase activities. In the present investigation, following administration of exogenous oestrogen, no significant elevation in GOT and GPT activity was noticed. Egg formation was not present in these immature birds treated with exogenous oestrogen. This might explain the absence of any significant elevation in GOT.

GPT activity noticed in the plasma and tissues was feeble and highly variable. Common and Mok (1953) could measure only GOT activity in plasma and tissues of chicks but not GPT activity. Madanlal and Gupta (1961) could detect only very feeble GPT activity in the plasma. They could not detect any breed difference in this respect. Goswami (1963) reported an age-dependent decrease in the plasma concentration of GPT in pullets.

There are conflicting reports regarding synergism and antagonism existing between oestrogen and androgen or oestrogen and progesterone. It is generally considered that testosterone acted synergistically with oestrogen in

oviductal growth (Brown, 1966; Gilbert, 1967), where as progesterone was antagonistic to oestrogen (Oka and Sebaste, 1969). Oades and Brown (1965) and Brown (1966) considered that for the development of the full secretory potential of the oviduct, it required either progesterone or testosterone along with oestrogen. Similar findings have been reported in relation to DNA, RNA and alkaline phosphatase activity by Chakravorti and Sathu (1969).

However, Yu and Marquardt (1975) while supporting a synergistic effect between oestrogen and androgen, stressed the importance of the relative doses of the two hormones. According to them, the degree of cellular hyperplasia and cellular hypertrophy depended on the ratio of the two hormones. Only cellular hypertrophy was observed with very high dosage of androgen in combination with oestrogen but not an increase in cell number. Jackson *et al.* (1971) studied the effect of a combination of oestrogen and testosterone in GOT and GPT contents in the oviduct of birds. They reported a significantly lower enzyme contents in the group of chicks receiving the combined hormonal treatment compared to their own control group of chicks and those receiving testosterone alone.

In the present investigation, the administration of GDP and TP increased oviductal concentration of GOT in the WL experimental chicks and decreased the concentration in the WH experimental group (Table 3.4). There was no trend

difference in the enzyme content between the various oviducal segments (Table 3.9). ODP and TP administration resulted in a significantly higher content of the enzyme in the entire oviduct in the WZ and in the uterus of the WBR chicks (Table 3.10). The addition of TP to ODP did not produce a marked enhancement in the enzyme response. Hence, the results obtained do not support a synergistic action between the two hormones at the dose level tried.

In the case of ODP, administration of a combination of ODP and TP increased ovarian and oviducal enzyme contents in the WZ experimental chicks (Table 3.4). In the WBR chicks, the plasma enzyme content was higher in the control compared to the experimental group. The oviducal segments did not reveal any significant breed difference in their ODP content (Table 3.5). The infundibulum, magnum and the entire oviduct showed a higher content of ODP in the WZ chicks treated with ODP-TP combination while this treatment had a depressing effect on the enzyme content in the blood plasma. Compared to the WBR chicks receiving ODP alone, those receiving ODP and TP, recorded a higher content of ODP in all the oviducal segments and in the entire oviduct.

Among the enzymes studied, ODP alone recorded a higher content in the oviducal segments as a result of administration of a combination of hormones (ODP and TP) compared to the administration of ODP alone. This is noticed, more especially, in the WBR breed of chicks and not so

markedly in WL chicks.

Gonadotropins are mainly associated with the morphological development of the oviduct (Brazat and Falbardov, 1952). Bell (1971) stated that high alkaline phosphatase activity could be expected in tissues and cells with a high turnover or in cells with high anabolic rate. DDP administration in itself did not show any influence due to breed on the alkaline phosphatase content in WL and WPR chicks (Table 3.3). But the DDP-TP combination seems to depress the concentration of alkaline phosphatase in the entire oviduct in the WPR chicks (Table 3.4). Breed did not seem to influence the TP content in the oviducal segments either (Table 3.5). But within the same breed, in both WL and WPR chicks, administration of DDP and TP together depressed the enzyme content (Table 3.10). This effect was evident in the ovary, magnum, uterus and the entire oviduct in both the breeds. This is in addition to depressed alkaline phosphatase content in the infundibulum segment of the WL breed of chicks.

The significantly higher content of alkaline phosphatase in chicks receiving only DDP may be due to the effect of DDP itself and that the TP might have exerted the effect either on the hormone by itself or in the dose given under the present conditions, as an antagonist to DDP. The increased cellular development in the oviducts under the influence of DDP and their augmented cellular activity may explain the

higher alkaline and acid phosphatase content in chicks receiving SDP alone in the present investigation. The reduced phosphatase concentration in the group receiving SDP and TP combination might indicate an antagonistic action of TP with SDP.

Acid phosphatase content of all the tissues investigated in the WL chicks showed a depression in enzyme content as a result of the administration of SDP-TP combination; but in the WFR breed, only the magnum, isthmus, and the entire oviduct revealed such an effect (Table 3.10).

The concentration of acid phosphatase following SDP administration did not reveal any breed difference. In WFR chicks, subsequent to the administration of SDP-TP combination, the control chicks showed a higher content than the experimental group. The WFR experimental chicks revealed a higher concentration of the enzyme compared to the WL chicks in experimental group. The latter chicks showed higher ovarian and oviducal acid phosphatase, compared to the control (Table 3.4). The higher levels of acid phosphatase in the entire oviduct and isthmus of WFR chicks compared to WL chicks are suggestive of a breed difference.

Synergistic action between oestrogen and progesterone has been reported by Mason (1952) and Grant and Halbandov (1956). However, antagonistic action between oestrogen and progesterone has also been reported by other workers in this respect (Adams, 1955; Brown and Eadsman, 1961;

Wu and Schinke, 1969). This antagonism was reported to be dependent on the relative dose levels between the two hormones; lesser doses of progest rone alone being stimulatory (Dax, 1955; Imeson, 1956; Gilbert, 1967; Wilson and Sharp, 1976).

No breed differences could be detected in the enzymes studied in the WL and WR chicks under the influence of OEP-PE combination. OEP activity could also not be detected in the plasma or reproductive organs in WL and WR chicks receiving OEP-PE. Further, in chicks receiving OEP-PE, excepting for a higher plasma content of alkaline phosphatase, in both WL and WR breeds, alkaline and acid phosphatases in all other tissues were significantly lower compared to chicks receiving OEP alone.

Significantly higher ($P < 0.05$) content of OEP was seen in WL chicks receiving OEP alone in their ovary, infundibulum, magnum, isthmus and uterus and in the case of WR chicks, in the blood plasma.

OEP activity could not be demonstrated in tissues studied after administration of OEP-PE combination in both the breeds of chicks (Table 3,10). This is indicative of the existence of a possible antagonism between oestrogen and progesterone.

An age dependent decrease in the level of OEP has been reported by Habega and Chedokec (1974).

The S-G-Pase levels in ovary, infundibulum, magnum

and plasma were significantly higher in WL chicks receiving SDP-PG combination. However, in the NBR chicks, the enzyme was more in the magnum, isthmus, vagina and plasma.

Administration of SDP resulted in profound morphological development of the immature fowl oviduct. The development of the oviduct under the influence of SDP in combination with PG or PG was found to be in no way different from that produced as a result of administration of SDP alone. Administration of PG or PG in combination with SDP appeared to induce an antagonistic effect on the enzyme level in the reproductive organs as compared to that produced by administration of SDP alone.

Histochemical localization of enzymes in the reproductive organs of White Leghorn and White Plymouth Rock hens and female immature chicks treated with synthetic sex hormones.

CHAPTER IV

ALCOHOLIC DEHYDROGENASE ACTIVITY OF THE OVIDUCT, UTERUS, AND VAGINA OF CHICKEN AND THE EFFECT OF SYNTHETIC SEX HORMONES ON THE ACTIVITY OF THESE ENZYMES IN THE REPRODUCTIVE ORGANS OF CHICKEN

The histological and histochemical changes of the female reproductive organs of chicken and those of immature chicks developed under the influence of synthetic sex hormones have received only scant attention in the past. Such studies on the precise location and activity of different enzymes in the reproductive organs of chicken are likely to enlighten the location of various physiological activities. Egg-formation, being a complex process involving secretion and incorporation of numerous substances secreted by the oviduct into the egg, is likely to be associated with high enzyme activities in the reproductive organs. Hence, it was thought worthwhile to investigate the localization and distribution of two of the more commonly distributed enzymes viz., alkaline and acid phosphatase in the reproductive organs of chicken both in the case of adult hens and those developed under the influence of synthetic hormones.

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Gutowski *et al.* (1949) could find no differences in the concentration or in the distribution of alkaline phosphatase among the oviductal segments, uterus and vagina,

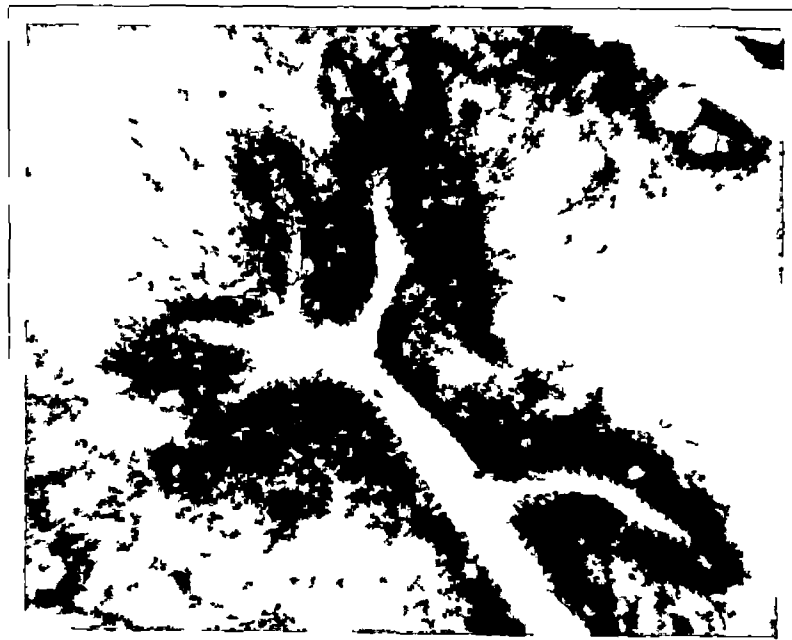




Fig. IV.7 White Plymouth Rock hen
Eschans x 150
Alkaline phosphatase activity

Fig. IV.8 White Plymouth Rock hen
Vagina x 150
Alkaline phosphatase activity

Fig. IV.9 White Leghorn hen
Intestine x 150
Acid phosphatase activity





of DDP, DDP-EP and DDP-PS administrations also showed the distribution and localization of alkaline and acid phosphatases (Fig. IV.14 to IV.25) similar to those noticed in the adult laying ♀ and ♂ hens.

In VL chicks treated with DDP the secretory epithelia in all the oviducal segments and ovarian follicles and stroma in the ovary revealed localization of alkaline phosphatase (Fig. IV.14 to IV.17). The intensity of reaction for alkaline phosphatase in WER chicks under the influence of DDP was similar to that in VL chicks.

The staining reaction denoting localization of alkaline phosphatase in the ovary and oviducal segments in VL and WER chicks developed under the influence of a combination of DDP and EP (Fig. IV.18 to IV.20) seemed to show a slight non-significant reduction in its intensity.

The activity of alkaline phosphatase in the female reproductive organs of immature VL chicks developed under the influence of DDP and PS also showed (Fig. IV.21 to IV.24) a decrease in intensity of the reaction compared to those receiving DDP alone. WER chicks, treated identically also exhibited similar results.

Acid phosphatase activity in the reproductive organs of VL chicks developed under the influence of DDP (Fig. IV.25 and IV.26) was more or less similar to that of alkaline phosphatase. Similar observations were made in WER chicks treated with DDP alone or with a combination of

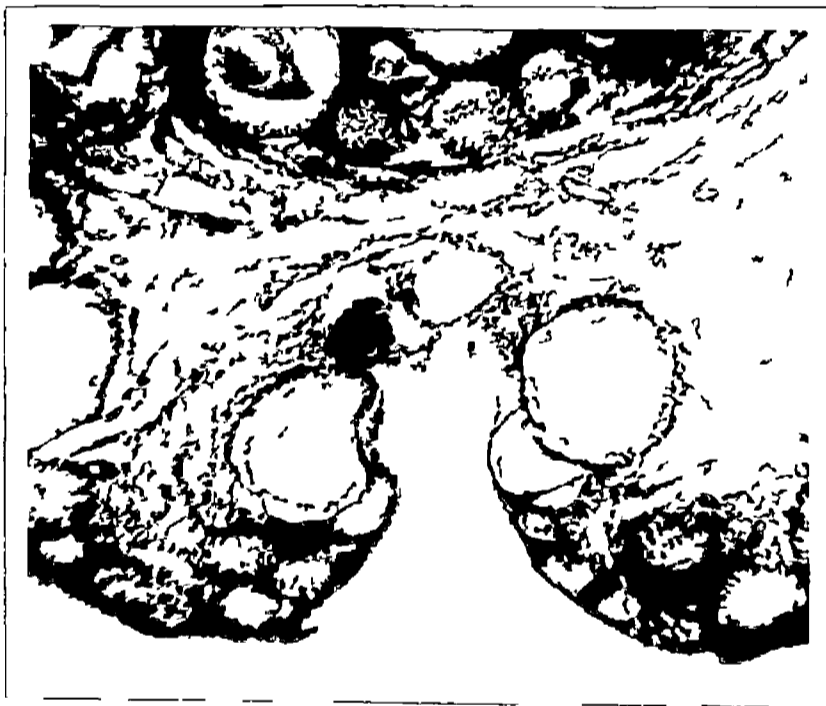


Fig. IV.16 White Leghorn chick
(stilbestrol diacrylate)
Ovary x 150
Alkaline phosphatase activity

Fig. IV.17 White Leghorn chick
(stilbestrol diacrylate)
Vagina x 150
Alkaline phosphatase activity

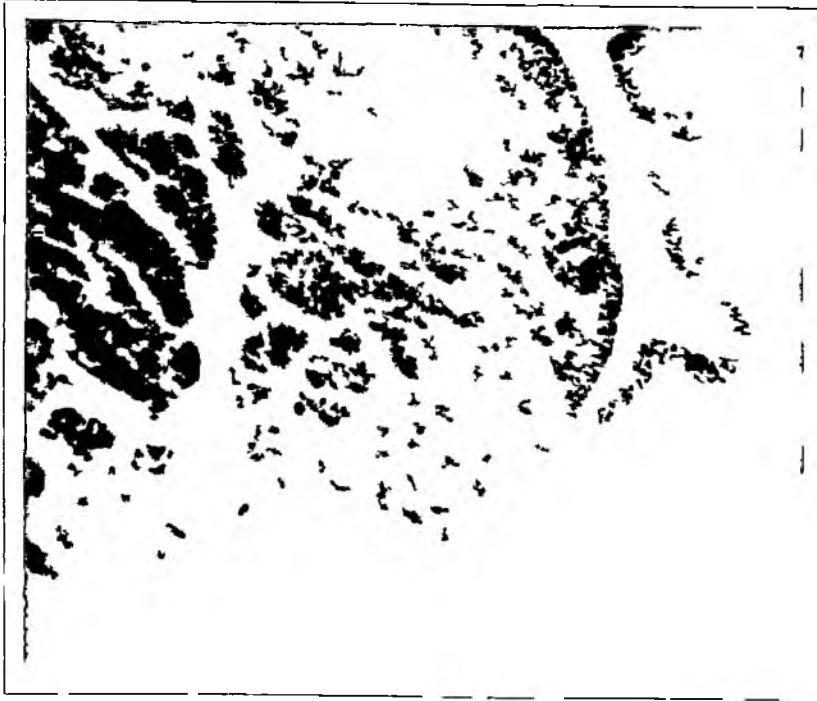
Fig. IV.18 White Leghorn chick
(stilbestrol diacrylate and
testosterone propionate)
Ovary x 150
Alkaline phosphatase activity



Fig. IV. 25 White Leghorn chick
(Niltbestrol dipropionate)
Ovary x 150
Acid phosphatase activity

Fig. IV. 26 White Leghorn chick
(Niltbestrol dipropionate)
Isthmus x 150
Acid phosphatase activity







hormones, UDP and TP or UDP and PG.

DISCUSSION

Distribution of alkaline phosphatase in the oviducal segments was shown to be uniform throughout oviducal segments by Gutowska *et al.* (1943). But, Chakravorti and Sathu (1961) noticed a stronger alkaline phosphatase activity in the vaginal segment of chicken oviduct compared to the other segments. In the present study, the ovary and the different regions of the oviduct of fowls revealed the histochemical localization of alkaline and acid phosphatases with no marked difference in the distribution of enzymes among the different segments except for the vagina where a comparatively stronger reaction was detected.

According to Chakravorti and Sathu (1961), the infundibulum and isthmus are not active sites for protein synthesis, as was evidenced by milder reaction for enzyme activities. But, the intense enzyme activity observed in the vagina cannot be explained on the basis of protein secretory activity as this region of the avian oviduct is not directly involved in any secretory activity and serves merely as a passage for the discharge of the egg which is fully formed when it reaches the vagina. The intense reaction of alkaline phosphatase in this region requires further elucidate the exact mechanism involved herein.

The results of the present study are at variance with those reported by Brown and Dedman (1962). According to these workers, the uterine tissue showed a higher alkaline phosphatase activity compared to other segments. This was stated to be more evident whenever an egg was located in the uterus.

While acid phosphatase activity was considered to be negligible in the oviduct of hen (Chakravorti and Saha, 1961), the results of the present investigation revealed that the reaction was not insignificant.

Histochemical localization of alkaline and acid phosphatases followed; administration of DHP alone or DHP in combination with either TP or PQ in 8W and 10W chicks showed slightly more intense reaction for the enzymes in those chicks receiving DHP alone compared to the other two groups. This finding is at variance with the observation of Brant and Halbandow (1956) on the synergistic action between oestrogen, androgen and progesterone. However, this is in agreement with that reported by Adams (1955) who stated that an antagonistic action exists between androgen, progesterone and oestrogen. Oka and Gehlke (1969) also provided evidences for the antagonistic effects between oestrogen and progesterone. The milder staining reactions for the enzymes studied in the present investigation in chicks receiving TP or PQ in combination with DHP as compared to those receiving DHP alone

may be due to this antagonistic action between the hormones.

Histochemical localization of alkaline and acid phosphatases revealed that the enzymes were localized in the follicular epithelium and stroma in ovary and all along the secretory epithelium in the different regions of the oviduct. The epithelial lining cells in the vagina in WL and WPR hens showed stronger activity in the case of both the enzymes studied. The staining reactions for alkaline and acid phosphatases in reproductive organs of chicks receiving TP or PG in combination with SDP, were milder as compared to those receiving SDP alone which is suggestive of a probable antagonistic action between TP or PG when administered along with SDP.

GENERAL DISCUSSION

CHAPTER V

GENERAL DISCUSSION

Sexual maturity is accompanied by pronounced morphological and functional development of the reproductive organs in chicken. Attempts have been made in the past to focus the importance of hormones in the morphological development and of enzymes in the functional development of the avian oviduct. A majority of these investigations has been carried out on chickens and a few on pigeons. It is well known that the development of the oviduct is controlled by oestrogens. Evidences indicate that there is increased production of ribonucleic acid, deoxyribonucleic acid, many enzymes and proteins in the oviduct (Gorski, 1964; Gorski *et al.*, 1965; Barker *et al.*, 1966) as a result of sex hormone stimulation.

A qualitative and quantitative evaluation of enzymes in the reproductive organs of female chicken was expected to enlighten the various biochemical mechanisms associated with the formation of an egg. In the present investigation, a quantitative assay of some of the enzymes in the ovary and oviduct has been carried out in two breeds of fowls of different age groups including adult laying fowls. The enzyme pattern in chicks given exogenous sex hormones was also studied. An attempt has

been made for the histochemical localization of alkaline and acid phosphatases in the various regions of the female reproductive tract.

All the enzymes studied were present in the blood plasma of White Leghorn and White Plymouth Rock breeds of fowls without any significant breed difference except for acid phosphatase in the one-to two-months-old White Leghorn chicks and adult White Leghorn fowls, and for glutamate pyruvate transaminase in the one-to two-months-old White Plymouth Rock chicks and adult White Plymouth Rock fowls.

The similar pattern of plasma enzyme distribution in the two breeds may be due to the fact that both White Leghorn and White Plymouth Rock fowls are descendants of the same ancestral fowl, Gallus gallus. It is only selective breeding that gave rise to the two breeds viz., White Leghorn and White Plymouth Rock, the former developed for better egg production and the latter for greater efficiency in meat production. The genetic set up being the same, it is reasonable to believe that the enzyme pattern in the blood serum may also be similar. Sinnerman et al. (1965) noticed characteristic pattern of serum enzyme distribution for each species of animal. According to them, the distribution of serum enzymes, as in the case of other serum proteins, is controlled genetically. This points towards

similar pattern of enzyme levels in the blood of closely allied strains or species under the same order of animals and birds inspite of selective and intense breeding for different economic purposes.

The concentration of various enzymes in the ovarian tissue revealed no difference due to breed except for higher contents of alkaline phosphatase and glucose-6-phosphatase in the one-to two-months-old White Leghorn chicks. The ovarian tissue in the immature chick is inactive and dormant. Even in the adult birds, the ovarian contribution towards the formation of an egg is negligible. The egg yolk is formed in the liver, transported by the blood and at the ovarian level only a transfer of the transported fat is all that is effected. Gilbert (1967) considered steroidogenesis as the only activity of the ovary in immature birds. The ovarian sensitivity to gonadotrophins becomes evident only from maturity. The absence of any significant difference in the concentration of various enzymes may indicate that they do not have any particular role to play in relation to follicular development. Steroidogenesis is perhaps the most important function assigned to this organ in the immature and mature state.

When the oviduct was considered as a whole, there was no significant difference in the concentration of the enzymes between the one-to two-months-, two-to three-months-,

and five-to six-months-old fowls except for the higher level of glucose-5-phosphatase in the two-to three-months-old White Plymouth Rock pullets compared to those of White Leghorn group of birds. The absence of any significant difference between the breeds on the enzyme contents in the entire oviduct may be due to the genetic influence which controls the enzyme pattern. The higher content of glucose-5-phosphatase in the two-to three months-old White Plymouth Rock pullets may be only due to individual variations.

Among the various oviducal compartments in the adult laying fowls, the infundibulum may be one of the segments that does not contribute much to the formation of the avian egg. The lining cells produce mainly mucin which provides lubrication for the transfer of the ovum released from the ovary. The enzymes present in the infundibulum in this study might be those that catalyze the routine cellular metabolic activities. The higher glutamate oxaloacetate transaminase and glutamate pyruvate transaminase contents in the infundibulum of White Leghorn fowls may be a breed peculiarity. However, the formation of chalaza has been considered as an infundibular function, even though the extent to which it is involved has not been fully ascertained. In the infundibulum the ovum released from the ovary remains only for a short period of about 15 min. This may be too short a period for any enzyme action to manifest leading

to any discernible change in the secretory activity of the infundibulum. Even though the formation of chalaza has been considered to occur in the infundibulum, it has been shown (Barmeester and Card, 1941) that chalaza formation could proceed normally even after resection of 80% of the infundibular tissue. Further, it has been shown that the chalazae become apparent only by the time the ovum reaches the terminal portion of the magnum and that the ovum, during its descent through the long coiled canal of the magnum, might have undergone rotation in its own axis resulting in the formation of visible chalaza from the mechanical coiling effect.

The magnum is considered as the part mainly associated with synthesis and secretion of proteins in the avian oviduct (Schraer and Schraer, 1965). But the different enzymes in this region failed to reveal any marked difference in their concentrations in both the breeds investigated. This may be due to either (1) absence of active secretory activity in this region at the time of the investigation because of the absence of an egg being located in the magnum or (2) due to the low level of activity of the cellular enzymes after the completion of synthesis and secretion of proteins associated with the formation of an egg, or (3) it may be the manifestation of an after-effect of events like ovulation, calcium secretion or

oviposition which might be taking place in the other regions of the reproductive tract at the time when the studies were carried out. Controversy exists regarding the distribution of enzymes in the avian oviduct. Chakravarti and Sahu (1961) reported the presence of alkaline phosphatase in the epithelial gland cells in the magnum of fowls. However, Aitken (1971) identified the enzyme only in the vacuolar endothelium. Brown and Badger (1962) noticed only moderate alkaline phosphatase activity in the magnum with fluctuations in the concentration of the enzyme reaching a peak near the time of ovulation.

In the isthmus region of the oviduct in adult fowls, the egg remains for a period of about 1 h and the egg-shell-membrane is secreted here. The egg-shell formation starts in the isthmus. In an egg-laying cycle, the actual secretory activity taking place in the isthmus is only for a period of 1 h and that too, at the time when a newly arrived egg is getting located in this region. Since the egg-shell-membrane is mainly composed of water, protein and carbohydrates, the enzymes associated with the synthesis and transfer of such materials are likely to be present in large amounts in this region. The reason why there was no significant change in the concentration of the enzymes may be due to the absence of an active secretory process in the region of isthmus at the time of the investigation.

The enzymes studied in the uterine segment showed no significant differences in their concentrations. Egg-shell formation occurs in the uterus and the egg remains here for a comparatively long period of about 20h. So it is quite unlikely that variations in the concentration of any one or all of the enzymes studied should go undetected. But, in this investigation, almost all the enzymes studied did not show any noticeable variation. The probable reason for this can be the absence of an egg in the uterus undergoing active shell-formation in all the birds studied. There is controversy regarding the importance of phosphatases in the process of egg-shell calcification. Chakraverti and Sadhu (1961) observed alkaline phosphatase activity throughout the oviducal epithelium of hens, while Brown and Balsam (1962) considered that alkaline phosphatase was higher in concentration in the uterus than in any other part of the oviduct. Aitken (1971) by histochemical studies showed the presence of the enzyme in the vascular endothelium. He failed to show any significant variation in the concentration at different stages of the laying cycle.

The vagina is short and U-shaped with no apparent role to play in the formation of the egg. It serves more as a passage for the fully formed egg. The absence of any significant difference in the concentration of enzymes in this region may be due to the insignificant contribution

made by this region of the oviduct in the formation of the egg.

The results of the study involving the administration of stilbestrol dipropionate alone, and stilbestrol dipropionate in combination with either testosterone propionate or progesterone revealed that mainly the synthetic oestrogen, stilbestrol dipropionate, was associated with the morphological development of the immature chicken oviduct. Further, this development was not influenced or modified by the presence of either testosterone propionate or progesterone at the dose levels employed in the present study. Studies conducted to determine the role of the sex hormones on the functional development of the oviduct in both White Leghorn and White Plymouth Rock birds showed that chicks receiving stilbestrol dipropionate alone evinced significantly higher enzyme response in their oviducal tissue compared to those receiving the combinations of sex hormones. This is indicative of an apparent antagonism existing between stilbestrol dipropionate and testosterone propionate on the functional development of the immature chick oviduct at the dose level employed. A similar effect was evident with the combination of stilbestrol dipropionate with progesterone also. However, stilbestrol dipropionate-testosterone propionate combination resulted in synergistic action in few instances.

tion indicated total estrogen. Further, it appeared from
 increases. Estrogenic diploids and progesterone con-
 tained in this study, advised against in only some
 diploids and progesterone diploids, in the dose levels
 in general. The effects of the sex hormones, estrogenic
 could not influence the concentration of the ovarian enzymes
 logical development of the ovary and that broad differences
 total diploids is entirely responsible for the work-
 The results of the study indicated that estrogenic

found that progesterone and estrogenic in action to
 action of DNA and RNA-polymerase. Oka and Gellera (1963)
 static in effect, the effects being mediated through the
 were of the view that progesterone and estrogen were syn-
 androgen and progesterone. Kozlitz and O'Halley (1968)
 may be existing between the naturally secreted estrogens,
 Adams (1955) was of the opinion that a direct estrogenic
 higher levels of progesterone (30-40 mg per bird/week).
 of egg production followed by a wait in 10-14 days with
 Harter and Adams (1951) reported total estrogen

total.
 increase in DNA, RNA and RNA in the output of immature
 administration and Dlugosz et al. (1963) noticed a general
 of new proteins, new RNA are formed as a result of hormone
 estrogen administration and found that for the synthesis
 O'Halley et al. (1968) studied the effects of

the results that even in the presence of sex steroids the factor that essentially induces alterations in the enzyme activity might be the presence of an egg in the concerned region and not merely the availability of sex steroids at the dose levels employed.

**STUDIES ON THE METABOLIC ACTIVITY OF THE
REPRODUCTIVE SYSTEM OF CHICKEN**

BY

M. G. RAMAKRISHNA PILLAI

ABSTRACT OF A THESIS

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ABSTRACT

Information on the specific role of enzymes in controlling the various biochemical events leading to formation of an egg in the avian oviduct is scanty. Hence, it was considered worthwhile to investigate the enzyme pattern in the plasma and in the reproductive organs in White Leghorn and White Plymouth Rock breeds of fowls, and to study the tissue localization of acid and alkaline phosphatases by histochemical techniques. The influence of various exogenous sex hormones on the development and enzyme pattern of the female reproductive organs was also studied.

The blood plasma and tissue homogenates of the reproductive organs were assayed for alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and glucose-6-phosphatase.

Many of the enzymes studied were present in the plasma and tissue homogenates without any significant breed difference. Acid phosphatase activity could not be detected in the blood plasma of one-to two-months-old White Leghorn and White Plymouth Rock birds. In the White Plymouth Rock chicks, plasma glutamate pyruvate transaminase activity also could not be detected. Alkaline phosphatase activity was higher in the ovary of White Leghorn breed of fowls.

In the two-to three-months-old pullets, White Plymouth Rock birds showed higher concentration of glucose-5-phosphatase in their oviducts.

Five-to six-months-old fowls of both the breeds did not show any plasma acid phosphatase activity. But in the infundibulum, activities of both the transaminases were higher in the White Leghorn fowls. Glutamate pyruvate transaminase activity in White Plymouth Rock oviduct was confined to the segment and isthmus.

While there was no significant difference in the morphological development of the oviduct in immature chicks under the influence of different combinations of stilbestrol dipropionate and testosterone propionate or stilbestrol dipropionate and progesterone compared to chicks on stilbestrol dipropionate alone, the White Leghorn chicks receiving stilbestrol dipropionate and testosterone propionate showed higher ovarian levels of acid phosphatase and glutamate pyruvate transaminase. Oviducal contents of acid phosphatase, glutamate oxaloacetate and glutamate pyruvate transaminases were also high. In the White Plymouth Rock chicks, on the other hand, lower concentration of plasma glutamate pyruvate transaminase and lower levels of oviducal alkaline phosphatase, acid phosphatase and glutamate oxaloacetate transaminase were evident. In between the two experimental groups, the White Plymouth Rock chicks

fowls. However, the intensity of the staining reaction for the enzyme in the reproductive organs of chicks developed under the influence of stilbestrol dipropionate was more compared to that of the other two experimental groups.

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APPENDIX

APPENDIX I

COMPOSITION OF POULTRY FEED

Ingredients	Adult each	Chick each
Maize	25.00 kg	40.00 kg
Ground nut oil cake	15.00 kg	20.00 kg
Singhly oil cake	5.00 kg	5.00 kg
Fish meal	10.00 kg	10.00 kg
Rice polish	20.00 kg	22.50 kg
Wheat bran	20.50 kg	..
Salt	0.50 kg	0.50 kg
Mindif	2.00 kg	2.00 kg
Shell meal	2.00 kg	..
Rotinix	25 g	25 g
Aureofa	..	125 g
Coccidiostat	..	50 g

APPENDIX II

GLUTAMATE OXALOACETATE TRANSAMINASE (GOT)

Colorimetric determination with 2,4-dinitrophenylhydrazine (Bergmeyer, 1955).

The containers used in the preparation of reagents were sterilized to prevent growth of micro-organisms.

Reagents and solutions

1. Substrate buffer solution (0.1M phosphate buffer, pH 7.4, 0.1M alpha aspartate; 2×10^{-3} M alpha oxoglutarate).

Dissolved in less than 100 ml of DDW 1.50 g K_2HPO_4 , 0.20 g Mg_2PO_4 , 0.033 g sodium alpha oxoglutarate and 1.32 g alpha aspartic acid. The pH of the solution was adjusted to 7.4 with 0.4N sodium hydroxide solution and then made upto 100 ml with DDW.

2. Ketone reagent (10^{-3} M 2,4 - dinitrophenyl hydrazine).

20 mg of 2,4 - dinitrophenylhydrazine was dissolved in 1 N hydrochloric acid and made upto 100 ml.

3. Sodium hydroxide (0.4N).

16 g of sodium hydroxide was dissolved in DDW and made upto 1000 ml.

4. Sodium pyruvate

22 mg of sodium pyruvate was dissolved in DDW and made upto 100 ml.

Procedure

Pipetted into the test tubes labelled as experimental and control the following in the proportions shown:

Experimental	Control
1 ml of substrate buffer solution	1 ml of substrate buffer solution
0.2 ml plasma or filtrate from tissue homogenates	..
Mixed by inversion and incubated for 60 min and then added	Not incubated and added
1 ml ketone reagent	1 ml ketone reagent
..	and 0.2 ml plasma or filtrate from tissue homogenates

The reaction mixtures were allowed to stand for 20 min at room temperature. The contents of the tube were mixed and then added 10 ml of 0.4 N NaOH solution. After 5 min read the optical density of the experimental tube against the control in a Spectronic 20 set at 546 millimicrons.

Standard curve and table of values

Pipetted successively into six test tubes sodium pyruvate and buffer substrate in the following proportions.

Test tube No.	Sodium pyruvate (ml)	Buffer substrate (ml)
1	0.05	1.00
2	0.05	0.95
3	0.10	0.90
4	0.15	0.85
5	0.20	0.80
6	0.25	0.75

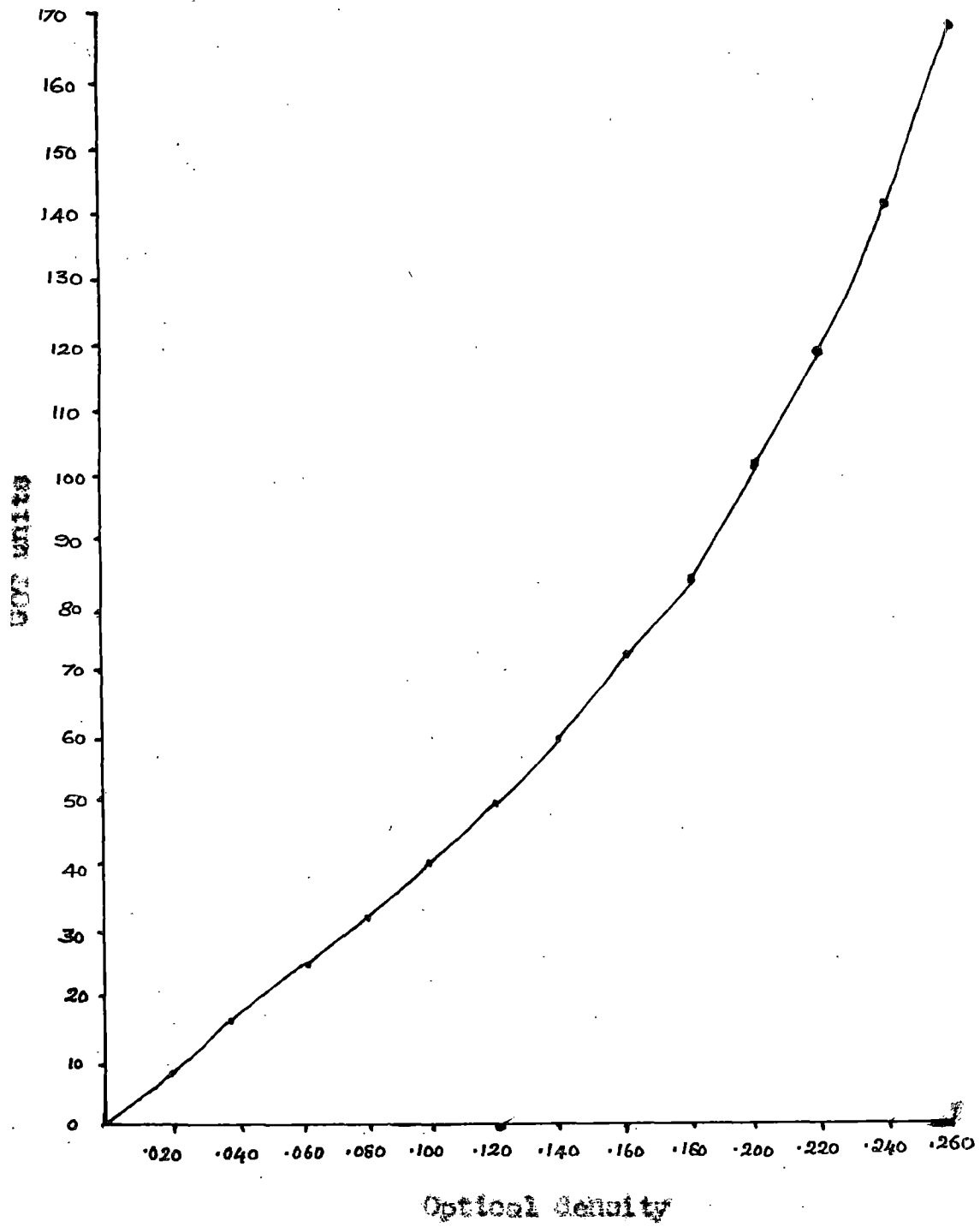
Pipetted into each tube 0.2 ml MDH, 1.0 ml ketone reagent, mixed and allowed to stand for 20 min at room temperature. To each tube was then added 10 ml of 0.4N NaOH. Mixed and read the optical densities after 5 min. The optical densities were plotted against the ordinate and OD units in the abscissa.

The following relationship could be obtained by comparing this data with spectrophotometric method (using MDH as the indicator enzyme).

Test tube No.2 =	21 000 units/ml serum		
.. No.3 =	42
.. No.4 =	64
.. No.5 =	97
.. No.6 =	140

By direct comparison of the two methods the following was constructed for measurements at 546 millimicrons (Bergmeyer, 1955).

CHARACTERISTICS OF OPTICAL
SENSITIVITY OF PHOTOGRAPHIC FILM



Optical density	Units	Optical density	Units
0.020	8	0.160	71
0.040	16	0.180	85
0.060	24	0.200	100
0.080	32	0.220	119
0.100	40	0.240	140
0.120	49	0.250	167
0.140	59		

Calculations

The units of optical density were directly read off from the standard curve prepared from measurements at 545 millimicrons from the above table.

APPENDIX III

GLUTAMATE PYRUVATE TRANSAMINASE (GPT)

Colorimetric determination with 2,4-dinitrophenylhydrazine (Bergmeyer, 1965).

The containers intended for use in the preparation of reagents were sterilized to prevent the growth of micro-organisms.

Reagents and solutions

1. Substrate buffer solution (0.1 M phosphate, pH 7.4, 0.2 M DL-alanine, 2×10^{-3} M alpha oxoglutaric acid)

Dissolved 1.50 g K_2HPO_4 , 0.20g KH_2PO_4 , 0.030 g alpha oxoglutaric acid and 1.78 g DL alanine in DDW and made upto 100 ml.

2. Ketone reagent (10^{-3} M 2,4-dinitrophenylhydrazine)

Dissolved 20 mg of 2,4-dinitrophenylhydrazine in 1 N hydrochloric acid and made upto 100 ml.

3. Sodium hydroxide (0.4N)

Dissolved 16 g of NaOH in DDW and made upto 1000 ml.

4. Sodium pyruvate (2×10^{-3} M)

Dissolved 22 mg of sodium pyruvate in DDW and made upto 100 ml.

Procedure

Proceeded with the preparation of the experi-

experimental and blank tubes as detailed out below.

Pipetted into test tubes labelled

Experimental	Blank
1 ml substrate buffer solution	1 ml substrate buffer solution
0.2 ml plasma or filtrate from tissue homogenate	
Mixed by inversion Incubated for exactly 30 min and added	Not incubated, added
1 ml ketone reagent	1 ml ketone reagent
..	0.2 ml plasma or filtrate from tissue homogenate

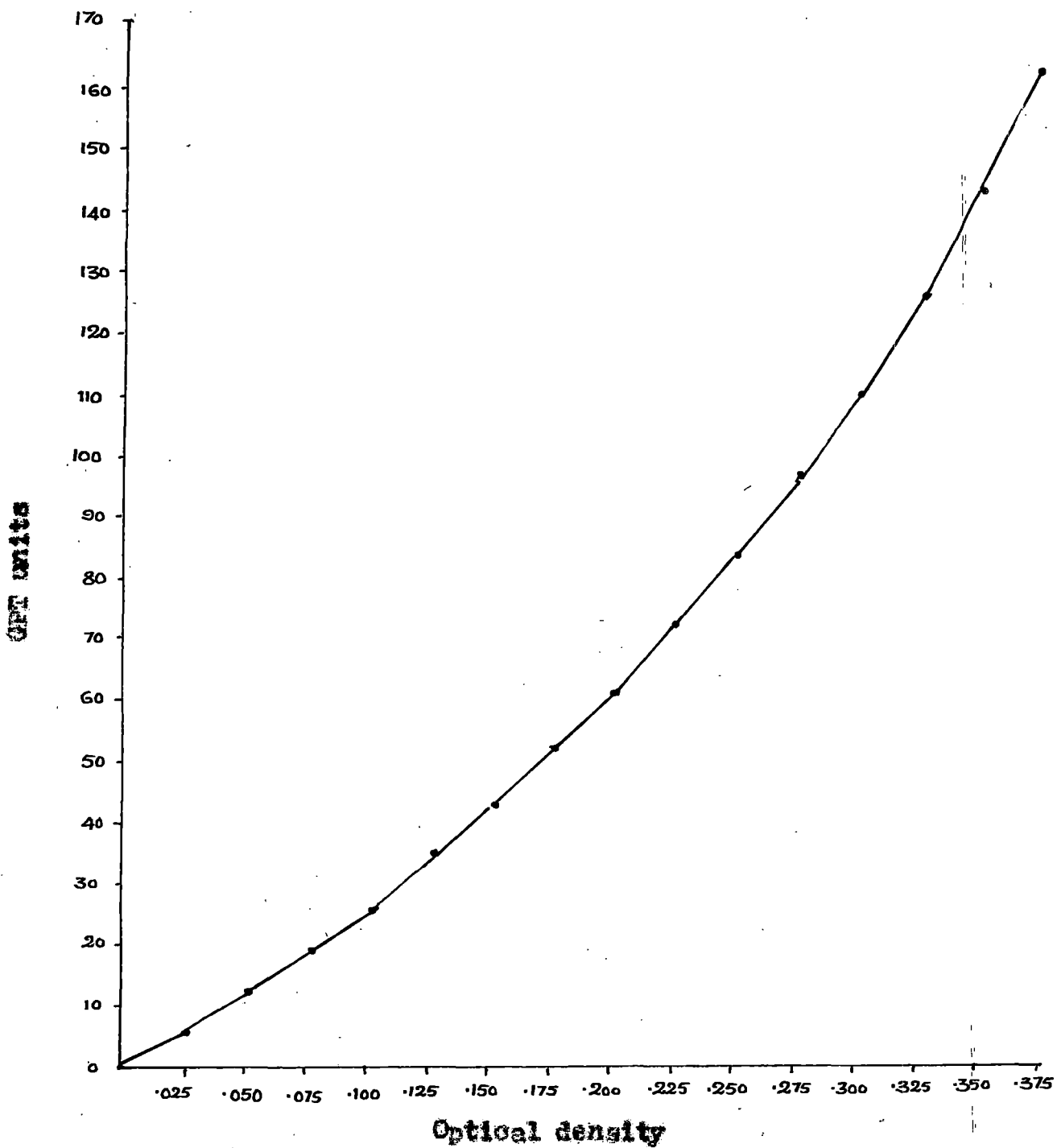
The solutions were allowed to stand for 20 min at room temperature. Added to the experimental and blank tubes 10 ml of 0.4 N NaOH solution, mixed and after 5 min poured in cuvettes and read the optical density against the blank in a Spectronic 20 set at 546 millimicrons.

Standard curve and table of values

Pipetted successively into test tubes

Test tube No.	Sodium pyruvate (ml)	Buffer substrate (ml)
1	0.0	1.0
2	0.1	0.9
3	0.2	0.8
4	0.3	0.7
5	0.4	0.6
6	0.5	0.5

CALIBRATION CURVE
GLUTAMATE PYRUVATE TRANSAMINASE



Into each of the above tubes pipetted

0.2 ml DDW and 1 ml ketone reagent, mixed and allowed to stand for 20 min at room temperature followed by 10 ml of 0.4N NaOH solution. Mixed again and after 5 min read the optical density against tube No. 1. Plotted the optical densities (ordinate) against the GPT units (abscissa). The following relationship was found by direct comparison with spectrophotometric method with ICB as indicator enzyme (Ferguener, 1965).

Test tube No. 2	27 GPT units/ml plasma		
.. No. 3	37
.. No. 4	55
.. No. 5	137
.. No. 6	205

By direct comparison of the two methods the following table was constructed for measurements at 546 millimicrons.

Optical density	GPT units/ml plasma	Optical density	GPT units/ml plasma
0.025	5	0.225	71
0.050	11	0.250	82
0.075	19	0.275	95
0.100	25	0.300	109
0.125	34	0.325	123
0.150	42	0.350	141
0.175	51	0.375	160
0.200	60		

Calculation

The units were read off corresponding to the measured optical density from the standard curve.

APPENDIX IV

ALKALINE PHOSPHATASE (ALP)

Method of Zedansky (1953)

Reagents and solutions

1. Alkaline phosphatase substrate

Introduced successively into a 100 ml volumetric flask 3 ml petroleum ether (BP 20-40°C) about 80 ml DDW, 0.5 g sodium beta glycerophosphate, 0.424 g of sodium diethyl barbiturate and DDW to volume. Expelled the contents into a glass-stoppered bottle containing an inch of petroleum ether and kept in a refrigerator.

2. Trichloroacetic acid

30 g of trichloroacetic acid was dissolved in DDW and made up to 100 ml.

3. Standard phosphate solution

Dissolved exactly 0.351 g of pure dry monopotassium phosphate in DDW and transferred quantitatively to a litre volumetric flask. Added 10 ml of 10 N sulphuric acid, diluted to the mark with DDW and mixed. This solution contained 0.4 mg phosphorus / 5 ml and is stable indefinitely.

6.25 ml of the above stock phosphate standard solution was placed in a 100 ml volumetric flask, added 16.7 ml of 30% trichloroacetic acid, diluted to 100 ml with DDW and mixed. This solution contained 0.04 mg phosphorus in 5 ml

5% trichloroacetic acid,

4. Molybdate solution

Dissolved 25 g of reagent grade ammonium molybdate in 200 ml of DDW. In a litre volumetric flask placed 300 ml 10 N sulphuric acid and added the molybdate solution to it. Diluted to the mark with washings from the molybdate solution and mixed.

5. Amino-naphthol sulphonic acid

Placed 195 ml of 15% sodium bisulphite solution in a glass stoppered cylinder. Added 0.5 g of 1,2,4-amino-naphthol sulphonic acid followed by 5 ml of 20% sodium sulphite solution, stoppered and mixed well. Transferred the solution to a brown glass bottle and stored in the cold.

Procedure

Incubated sample

Measured 3 ml of alkaline phosphatase buffer substrate into a glass stoppered cylinder and placed in an incubator set at 37°C until the fluid attained the incubator temperature. Added 1 ml of plasma/tissue homogenate, mixed, noted the time and incubated exactly for 1 h. Afterwards removed, cooled in ice water for 10 min and added 2 ml of 5% trichloroacetic acid. Mixed well and let stand for 5 min and then filtered through a low-ash filter paper.

Control sample

Near the end of the time for completion of the

incubated sample measured 9 ml of substrate solution into a glass stoppered cylinder and added 2 ml of 30% trichloroacetic acid. While mixing added 1 ml of plasma/tissue homogenate, stoppered, mixed well and filtered as in the incubated sample.

When both the filtrates were ready, transferred 9 ml each of incubated and control samples to test tubes graduated at 10 ml. In a third similar tube placed 9 ml of standard phosphate solution containing 0.04 ml of phosphorus.

In a fourth (blank tube) placed 9 ml of 5% trichloroacetic acid.

When all the tubes were ready added to each 1 ml of molybdate solution and mixed. Added 0.4 ml of amino-naphthol-sulphonic acid reagent to each diluted immediately to 10 ml and mixed. Allowed 5 min for colour development.

Calculation

Read the colour developed in a photometer set at wave length of 660 millimicrons.

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times 0.04 \times 100 \times \frac{1}{2} \text{ mg of inorganic phosphorus / 100 ml of plasma/tissue homogenate.}$$

APPENDIX V

ACID PHOSPHATASE (ACP)

Method of Rodansky (1933)

The principle and procedure was exactly the same as the one used for the estimation of alkaline phosphatase except that a buffered acid phosphate substrate was used for incubation and in the control sample.

Reagents and solutions

Acid phosphate substrate

This was identical to that of alkaline phosphatase buffer described above except that sufficient acetic acid was incorporated to bring the pH to 5.

Calculation

Calculation of the results was the same as that for alkaline phosphatase, the units of acid phosphatase activity being defined as equivalent to the liberation of 1 mg percent of inorganic phosphorous during 1 h at pH 5 (expressed as Rodansky units).

APPENDIX VI

GLUCOSE-6-PHOSPHATASE (G-6-PASE)

Method given by Bergmeyer (1965)

Reagents and solutions

1. Citrate buffer (0.1M; pH 6.5)

Dissolved 2.101 g of citric acid in 50 to 75 ml DDW adjusted the pH to 6.5 with 30% (w/v) sodium hydroxide and diluted to 100 ml with DDW.

2. Glucose-6-phosphate (G-6-P)

Suspended 417 mg of glucose-6-phosphate, Barium salt, $7H_2O$ in 2-3 ml of DDW. Dissolved by addition of the minimum of 1N HCl. Added 114 mg Na_2SO_4 and mixed thoroughly. It was centrifuged and the precipitate of barium sulphate was discarded. To ensure complete precipitation, a drop of Na_2SO_4 was added carefully to see whether there was any precipitate formed. Adjusted the pH to 6.5 with 30% (w/v) NaOH and diluted to 10 ml with DDW.

3. Trichloroacetic acid 10% (w/v)

Dissolved 10 g of trichloroacetic acid in DDW and made up to 100 ml.

4. Ammonium molybdate ($Ca 2 \times 10^{-3}M$)

Dissolved 2.5 g ammonium molybdate in 500 ml DDW, carefully added 14 ml concentrated sulphuric acid to 200 ml

DDW, carefully added 14 ml concentrated sulphuric acid to 200 ml DDW and poured this diluted acid to the molybdate solution. It was made upto 1 litre with DDW.

5. Reducing agent (Ca $4.2 \times 10^{-2} M$ 1-guino-2-naphthol-4-sulphonic acid, Ca $0.56 M SO_3^{2-}$)

Dissolved 5.7 g of $NaHSO_3$ and 0.2 g of Na_2SO_3 in 50 ml DDW. Dissolved 0.1 g of 1-guino-2-naphthol-4-sulphonic acid in this mixture and diluted to 1000 ml with DDW.

6. Phosphate standard solution

Dissolved 69 mg of KH_2PO_4 in DDW, added 10 ml concentrated sulphuric acid and diluted to 1000 ml with DDW. The solution was distributed in several test tubes, each containing the amount required for a day's experiment and stored in the frozen state.

Procedure

Experimental material

The material for activation (tissue or plasma) was chilled in an ice bath. Homogenised a weighed sample of tissue with a definite volume of buffer solution in a Potter-Elvehjem tissue homogeniser and then filtered through double layers of muslin cloth.

Enzyme action

Two test tubes were placed in a water bath at $37^\circ C$, one containing G-6-P solution and the other containing

buffer solution. For each sample a tissue control and for each series a reagent control were also prepared.

Pipetted into centrifuge tubes

Experimental	Control 1	Control 2
0.1 ml filtered homogenate	0.1 ml filtered homogenate	0.1 ml buffer solution

Placed all the tubes in a water bath (37°C) and after 5 min added

Experimental	Control 1	Control 2
0.1 ml G-6-P solution at 37°C	0.1 ml buffer solution at 37°C	0.1 ml G-6-P solution at 37°C

and noted the time of each addition. Incubated the test tubes for exactly 15 min at 37°C and then added 2 ml 10% trichloroacetic acid to each tube, centrifuged and the supernatants were used for phosphate determination.

Phosphate determination

The phosphate content of the supernatant was determined colorimetrically by the method of Fiske and Subbarow (1925).

Pipetted into test tubes

Experimental and control	Standard
5 ml molybdate solution	5 ml molybdate solution
1 ml supernatant	1 ml phosphate standard solution

When all the tubes were prepared, added 1 ml of reducing agent to each tube and noted the time. Allowed sufficient time in between the addition of the reducing agent, so that colorimetric measurement of each tube could be made at the same length of time after addition.

Allowed each tube to stand at room temperature for 15 min and then read the optical density.

Calculation

$$\frac{E_{\text{ex}} - E_{\text{ct}}}{E_{\text{st}}} \times (P) \times 2.2 = \text{micromoles of phosphate liberated in the reaction}$$

where,

E_{ex} = Optical density of the experimental tube

E_{ct} = " " control tube 1

E_{st} = " " standard tube

(P) = micromoles phosphate in the standard tube (0.5 micromoles)

2.2 = volume of the enzymatic reaction mixture after addition of trichloroacetic acid

To convert to micromoles phosphate/min/g of tissue,

multiplied by $\frac{1000}{15 \times \text{weight of tissue in mg}}$

where, 15 = period in min of the enzymatic reaction;

1000 = conversion factor for mg to g.

APPENDIX VII

ALKALINE PHOSPHATASE
(Histochemical localisation)

Calcium phosphate method of Gomori (1952) as given by Drury and Wallington (1967).

Principle

From the substrate, glycerophosphate, phosphate ions are liberated at pH 9.0 by the alkaline phosphatase. The phosphates are precipitated as calcium phosphate. The calcium is substituted by cobalt and is seen as opaque cobalt sulphide. Control sections using BSW in place of substrate are taken through the technique to eliminate false positive reactions given by calcium already present in the tissue.

Fixation

Cold formalin was used for frozen section of tissues from the reproductive organs of adult laying fowls belonging to WL and WPR breeds and the reproductive organs of three weeks old chicks belonging to WL and WPR breeds, developed under the influence of BHP, BHP-EP and BHP-TP combination of hormones.

Sections

Sections were cut at 10 microns in a freezing microtome using carbon-di-oxide for freezing the tissues after cold formalin fixation.

Solutions

Solution A

Dissolved in DDW 6.1 g sodium barbital, 1.2 g calcium chloride, 0.5 g magnesium sulphate and made upto 1000 ml. It was stored in refrigerator.

Solution B

One percent solution of sodium beta glycerophosphate in DDW and stored in refrigerator.

The working substrate solution was prepared by mixing 50 ml solution A and 50 ml solution B and adjusting the pH to 9.

Technique

Incubated the sections in the working substrate solution at 37°C for 3 h. Washed for 3 min in DDW and placed the sections in 2% aqueous cobalt nitrate solution for 5 min. Again washed in 3 changes of DDW for a total period of 3 min. Transferred the sections to 1% solution of yellow ammonium sulphide for 1 min. Washed for 3 min in running tap water. Counterstained with 0.1 percent safranin in 0.1% acetic acid for 2 min. Dehydrated by passing through alcohol and cleared in xylene. Mounted in EMI mountant.

Results

The sites of enzyme activity were seen dark brown.

APPENDIX VIII

ACID PHOSPHATASE

(Histochemical localization)

Lead nitrate method of Goswami (1950) as given by Deury and Wallington (1957).

Principle

In an incubating medium of organic phosphate containing a lead salt, the enzyme acts on the substrate. The phosphate formed produces lead phosphate as it is formed which is subsequently converted to lead sulphide by ammonium sulphide.

Fixation

Cold formalin was used for fixation of tissues obtained from the normal female reproductive organs of hens and those developed after the administration of SDP, SDP-TP and SDP-PC combinations in WL and WFR breeds of chicken.

Sections

Sections were cut at 10 micron thickness in a freezing microtome using carbon-di-oxide for freezing the tissues after cold formalin fixation.

Solutions

Substrate solution:- Added 1.5 g sodium beta glycerophosphate and 0.7 g lead nitrate to 500 ml of 0.05 M

acetate buffer (pH 5) and incubated at 37°C for 24 h. It was then filtered and the filtrate was stored in refrigerator.

Technique

Incubated the sections in the substrate solution at 37°C for 16 h. After washing briefly with DDW, it was transferred to 1% fresh solution of yellow ammonium sulphide for 1 min. Washed and counterstained with 1% aqueous eosin for 5 min. Washed in tap water, DDW and finally mounted in glycerin jelly.

Result

Sites of enzyme activity were showing black deposits of lead sulphide.