STUDIES ON THE STRUCTURE AND DEVELOPMENT OF THE THYMUS IN THE INDIAN RUNNER DUCK

(ANAS PLATYRHYNCHOS DOMESTICUS)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE STRUCTURE AND DEVELOPMENT OF THE THYMUS IN THE INDIAN RUNNER DUCK" is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

C.K. SREEDHARANUNNI.

Mannuthy,
6-10-1976.

CERTIFICATE

Certified that this thesis, entitled "STUDIES ON THE STRUCTURE AND DEVELOPMENT OF THE THYMUS IN THE INDIAN RUNNER DUCK" is a record of research work done independently by Sri. C.K. Sreedharanunni under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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C O N T E N T S

INTRODUCTION

INTRODUCTION

The domestic duck of today is derived through domestication of the wild Mallard, Anas platyrhynchos domesticus (Scott, 1964). At least a dozen breeds are available, of which the Khaki Campbell and Indian Runner are important laying breeds and Aylesbury and Pekin are good table breeds.

Based on Food and Agriculture Organisation Report (1969) the world duck population is 100.2 millions, out of which 64.3 millions in Asia. India has approximately 16 million ducks representing 16% of the domesticated duck population of the world.

Ducks occupy the place of importance after hens for the production of table eggs in the country. They form about 7% of total poultry population of India. Duck raising is profitable as chicken is, provided good management, health cover and proper marketing are ensured, apart from reasonable feed cost. Ducks lay 40-50 more eggs on an average than hens do. During the second year, they lay at about the same rate as during the first year. The size of duck egg is larger than the hen's egg, with higher energy and fat contents. Ducks do not have the problem of cannibalism and agonistic

behaviour is not apparent. Ducks can be housed either in individual or in multiple bird cages, as in the case of chicken.

Because of certain religious taboos and socio-economic conditions, the consumption of duck eggs/meat varies from region to region. At present India produces more than 400 million duck eggs per year, constituting about 5% of total egg production. West Bengal leads in Duck population followed by Assam, Tamil Nadu, Andhra Pradesh, Kerala, Bihar, Orissa, Jammu & Kashmir and Haryana. This gives an idea that coastal states are the habitats of ducks in India.

Duck husbandry is subjected to vagaries of nature and its resources of feeding and demanding migration of flocks.

The health and the disease resistant capacity of the birds are due to the active functioning of the immunopoetic systems of the body in which thymus plays a vital role in the development of immune potency as adaptive immunity as well as in the growth and maturation.

The mechanism of adaptive immunity in birds have two components: one in the bursa dependent system,

ie. the bursa of fibricius with germinal centres and plasma cells in various tissues. This is responsible for humoral immunity. The other is the thymus dependent system ie. the thymus and scattered collections of lymphocytes which are related to cellular immunity. Furthermore, lymphoid tissue can be divided into "central" and "peripheral" tissues, the former are believed to be the primary cites of development of lymphocytes. In birds these are the thymus and bursa as opposed to the thymus alone in mammals.

For many years the role the thymus played in the body was neither appreciated nor understood. Only recently light has been thrown on this matter, and at the moment the investigation of possible functions of the thymus is an exciting area of research, for which the basic knowledge of anatomy of thymus is essential.

According to Dmitrieva (1939) thymus is known to influence growth and development of chicken after hatching. Also it seems to increase bird's resistance to avitaminosis, bacteria and malignant tumors. But there is no indication of a possible endocrine function of the thymus in the embryo. The development of thymus seems to be independent of the influence of other endocrine glands (Venzke, 1952).

Towards the end of incubation thymus is one of the organs of the body in which hemopoietic activity takes place. This continues into the post-embryonic period, probably until thymic regression takes place. Lymphopoiesis is a known thymic activity and occurs principally during fetal and early postnatal life (Leeson & Leeson, 1972).

Thymus is a complex organ composed of a permanent stroma and a regenerating lymphoid tissue extending along the cervical to thoracic region along each jugular vein as far as the thyroid gland (Bachlechner, 1926). The word 'Thymus' is the ancient greek name coined by Theophrastus for a particular plant. And because of the similarity of the shape of the lymphoid organ to the thyme leaf it has been named Thymus (Ham, 1969).

Thymus is the largest endocrine gland in the youngest animal, grows upto puberty and then starts atrophy when the animal becomes mature.

Histology and embryology of thymus of Duck have escaped the notice of various research workers, with the result the literature available on the histology and embryology of thymus is scanty.

The object of the present investigation is to study the gross, microscopic and development of thymus during the embryonic and post-embryonic periods.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Macroscopic study.

Hamilton (1915) stated that the thymus was first visible as a rudiment in 3 day duck embryo. In the embryo of sparrow thymus was visible as a rudiment at 5.1 mm. stage and at 14 mm. stage the embryo had 11 distinct lobes on right side and only 4 on the left (Helgesson, 1913).

Latimer (1924) had shown that the thymus increased in absolute weight as well as in percentage weight upto sexual maturity and then involuted. The thymic changes were seen to be more closely related to age than to body weight. The weight of thymus of day old chicken was found to be 0.101 g or 0.312 per cent of body weight.

According to Bachlechner (1926) the thymus of chicken consisted of three to eight pairs of separate pale red or yellowish irregular lobes of varying size and shape extending along each jugular vein as far as the thyroid gland. Moughan (1938) stated that there were, in all approximately 14 distinct thymic lobes usually 7 on each side of the neck, loosely joined together. No thymic material was found in the thoracic

cavity. Hohn (1947) opined that the thymus of the domestic duck and goose probably resembled that of closely related wild species in which there were typically five pairs of lobes, the most caudal was always the largest.

Greenwood (1930) observed in fowl a definite relationship between the gonad and thymus involution. He carried out a detailed study on the involution of thymus in chicken and found that involution was progressive after 17 weeks of age, so that in males and females between 13 and 19 months of age it was about 2.2 g and 0.6 g respectively. But remnants of all the lobes including the cranial ones, persisted at least upto 16 months of age.

Greenwood and Blyth (1931) observed that thymic tissue was present near and posterior to the thyroid. The distribution of the thymic tissue was variable. It was present under the thyroid capsule penetrating the substance of the thyroid gland external to the thyroid capsule or extending around the posterior lobe of the thyroid and coming to lie between it and the parathyroid glands. Hohn (1956) stated that the location of thymus in duck and goose was the same as that of chicken.

Verdun (1898) observed that in chick embryos the fourth cleft was separated from the pharynx at 164 hours of incubation. Sun (1932) reported that prior to fifteen days of incubation, the thymus of the chick appeared as a group of undifferentiated cell masses held together by mesenchymal tissue.

The thymus gland was derived from the third and fourth pharyngeal pouches (Venzke, 1942; 1952; Schrier and Hamilton, 1952 and Hammond, 1954). Venzke (1952) had carried out a detailed study on the morphogenesis of the thymus of chick embryo and found that the third and fourth visceral pouches sever their connections with the pharynx by 134 hours of incubation. He had also reported that the thymus was first visible in the 5 day old chick embryo.

In a comparison of thymic weight to body weight in chicks, Dmitrieva (1939) found that development of thymus was slow in proportion to the rest of the body from the 10th to the 16th incubation day. After 16th day the thymus increased greatly in weight. At hatching an abrupt decrease in thymic weight in both sexes was noticeable.

Hohn (1956) stated that the location of thymus in duck and goose was the same as that of chicken.

The thymus was one of the largest glands in embryonic and young chicks (Romanoff, 1960).

Deniz (1968) had observed that there were cervical and thoracic lobes in the turkey but only cervical lobes in the goose. With increasing age the goose retained its individual lobes, but in the turkey they gradually united.

Wolfe et al. (1962) described that in White Rock male chicken the maximum total weight (the left and right thymus together) of 15.76 g was reached at 17 weeks of age. He found that in chicken between 4 and 17 weeks the total thymus weight was about 0.5 per cent of body weight and by 23 weeks about 0.16 per cent. Maximum absolute mean thymus weight was 15.76 g at 17 weeks of age.

Payne (1971) reported that thymic tissue sometimes penetrated the thyroid and parathyroid, making complete thymectomy difficult.

Hohn (1956) found that in adult chicken the thymus was markedly enlarged following the breeding season. The enlargement of the thymus in the post-breeding season suggested that this gland performed some functions which

did not involve other parts of lymphatic tissue.

Ham (1969) found that size of the thymus gland varied greatly in relation to age.

Microscopic study.

Thymus gland was derived from the third and fourth pharyngeal pouches (Verdun, 1898), third pharyngeal pouch was the major source.

It was stated by Venzke (1952) that by 19th day of incubation, the chick embryo had typical Hassall's corpuscles having diameter between 30 and 40 microns. He had shown that in the seven day chick embryo the thymus was an epithelial structure, syncytial in character with reticular appearance. The mesenchyme closely invested the thymus and, in places, the cytoplasmic processes of the mesenchymal cells appeared to fuse with the cytoplasm of the epithelial cells. By the end of eleventh day of incubation the blood vessel entered the thymus. In chick embryos by 16 day and 16 hours of incubation, eosinophils appeared in great numbers and by 17th day of incubation thymus was well defined into zones (Romanoff, 1960).

Eosinophils and plasma cells were also present but in few numbers. In the medullary substance the reticular cells had more cytoplasm, thymocytes were few with no mitotic figures (Trautman and Fiebiger, 1957).

Defendi and Metcalf (1964) stated that the thymus was complex organ composed of a permanent stroma and a regenerating lymphoid tissue. The stroma accounts for about ten per cent of organ weight and is composed of a reticulum and epithelial cells; the lymphoid cells accounted for 99.9 per cent of the mytotic activity and were a mixture of thymocytes and transient bone marrow cells.

In the embryo, the lymphoid cells populated bone marrow, which became the major lymphocyte production centre (Marie and Lablond, 1964). They have also described the presence of a space between the epithelial membrane and vessels of medulla known as perivascular space. The epithelial reticular cells which formed the basic frame work to the substance of thymus, could not be seen very clearly.

In chicken thymus each lobe comprised a number of lobules partially separated by connective tissue. The

lobules were clearly devided into an outer zone of densely packed small lymphocytes with scattered reticulum cells, the cortex and an inner (medullary) zone consisting of less densely packed lymphocytes, reticulum cells and islands of epithelial cells (Hassall's corpuscles). Among the medullary lymphocytes large, pale staining nuclei of the epithelial reticulum could be seen. Granulocytes are scattered in the medulla; degenerating masses of these cells occurred, sometimes encysted within Hassall's corpuscle some of which showed degeneration and hyalinization of epithelial cells. Granular argentaffin cells occurred in medulla (Bell & Freeman,1971). Regression of the thymus at the onset of sexual maturity was characterised by loss of the cortex leaving medullary type tissue with few lymphocytes (Warner, 1964).

Ackerman and Knouff (1964) stated that the earliest formation of lymphocytes and lymphocytic precursors could be observed in a relatively simple and uncomplicated situation than the definitive thymus of the chick. The lymphoblasts developed by the gradual proliferation and transformation of "undifferentiated" epithelial cells comprising the primordial thymus. Lymphoblastic transformation began on the 7th day of embryonic development

in the chick and was characterized by increased cytoplasmic and nucleolar basophilia and chromatin condensation. "Undifferentiated" epithelial cells underwent two distinct lines of differentiation between the 7th and 10th days into lymphoblasts and stellate reticular epithelial cells which constituted the organ parenchyma. All stages of lymphocytic maturation might be observed by 10-11 days as the thymus assumed a predominantly lymphocytic character.

Kohnen and Weiss (1964) studied about the thymic corpuscles in the guinea pig and the mouse. They found Hassall's corpuscle with a typical concentric, lamellated pattern. Reticular cells formed layers around central elements of variable appearance. One or more reticular cells or cystic structures often constituted the centre of corpuscle. Reticular cells at the periphery of thymic corpuscles might be contiguous with cells of cyto-reticulum. Intercellular or intracellular cysts were regularly seen and were frequently related to thymic corpuscle. Thymic corpuscles and cysts were having the epithelial nature.

Phagocytic reticular cells were occasionally seen in the medulla of guinea pig thymus (Izard, 1966).

Kostowiecki and Harty (1966) had recorded following

observations in the thymus of cat, dog, calf, guinea pig and man that the reticular fibres of the interlobular thymic septa were condensed on each lobule where they formed a bilaminar basket like frame work. The internal layer was made up of extremely fine fibres while the external layer displayed a coarser structure which accompanied blood vessels into the thymic cortex. In the cortex argyrophil fibres were thin, forming a sparse net work running with the capillaries and larger vessels into the medulla. At the corticomedullary junction the reticular net work was denser due to the presence of many variously sized blood vessels. In the medulla the argyrophil fibres were thicker than those in the cortex and were arranged in net works associated with the intramedullary blood vessels. Several Hassall's corpuscles which developed in different parts of medulla contained reticular fibres passing through their core or wall. Hassall's corpuscles were developed amongst the argyrophil fibres which surrounded the intramedullary blood vessels. These corpuscles might be partially or even completely enclosed by a net work of reticular fibres which were gradually pushed aside. Some mesenchymal reticular cells might incorporated along with Hassall's corpuscle.

Miller (1967) suggested that there were two mechanisms in the development of immune systems by thymus in which one was the stem cells from bone marrow migrated to the thymus, differentiated the thymus lymphocytes from whence they might migrate as immunologically competent cells. Alternatively the thymus could produce a humoral substance capable of inducing competence in primitive stem cells.

Windle (1969) had recorded that the thymic medulla contained more reticular cells and macrophages, but fewer lymphocytes than the cortex and consequently looked lighter in stained sections.

Kathiresan (1970) found that in the echidna thymus some of the lobules showed an aggregation of strongly PAS positive cells in the centre which could be an analage for the formation of Hassall's corpuscles seen relatively in large numbers contained PAS positive amorphous material. Polygonal cells rich with PAS positive granules and lying close to the blood vessels had been identified as the mast cells.

Mandel (1971) found undifferentiated and dividing epithelial cells at postnatal stage in the medulla of mouse thymus in all the animals examined. He had also

recorded the presence of small developing Hassall's corpuscle postnatally.

Baron et al. (1971) has found that from the histological view point no involution of thymus was noticed prior to 8-12 months of age in rabbits.

Payne (1971) had stated that in the chick the microscopic anatomy of thymus was similar to that of mammals.

Connective tissue septa extending from the capsule divided the gland into incomplete compartments. The peripheral part of each lobule heavily infiltrated with lymphocytes was termed the cortex and the more central part of lobule that does not contain so many lymphocytes was termed the medulla. In the medulla the epithelial reticular cells often became grouped concentrically around a central focus to form some keratin which is surrounded by a few rings of flattened epithelial cells. These bodies were termed Hassall's corpuscles (Ham, 1969).

Sugimura (1971) observed myoid cells in the calf's thymus at histological and ultrastructural levels. These cells appeared in the thymic medulla but not in the cortex

and comprised 2.4 per cent of all cells in medulla. The cells were found to be oval, 15-20 micron in size with an oval nucleus.

Islets of epitheloid cells were described in the interstitial tissue with fine granules and vacuoles of varying size within the cell (Schwarz and Neurand, 1972).

Dung (1973) found lipid laden cells in the involuted thymus which was absent in normal thymus. Also numerous Hassall's corpuscles were found in the involuted thymus of lethargic mutant mice.

Gilmore and Bridges (1974) had found that myoid cells were a constant feature in the medulla from 1 day to 84 weeks of age in the thymus of fowl.

Droege et al. (1974) had worked on the developmental changes in the cellular composition of the chicken thymus and found that there were two major cell populations in the young chicken thymus; the existance of a third type was also described. Microscopically all these three appeared to be small lymphocytes. They have stated that adult chicken thymus had practically no cortex, contained mainly one relatively large cell type which might represent the medullary lymphocytes.

It was also noticed that before thymus involution at 16 weeks old, smaller and larger cells were both present.

Wyrzykowski (1974) reported that in the fowl the number of mast cells decreased as the involution progressed but the structure and site remained unchanged. The plasma cells increased, reaching a maximum at 405-570 days. The investigation did not show any correlation between the growth of mast cells and plasma cells during the development of the bird.

Bearman et al. (1975) found that in human thymus the width of perivascular space was proportional to the size of vessel it surrounded, it was wide around the vessels in the septa and at the corticomedullary junction but narrow around the capillaries.

Dourin and Joterean (1975) reported that in the chick embryo the thymic endoderm separated from the pharynx during the 5th day of incubation as a cord of epithelial cells which elongated along the jugular vein. A thin mesenchymal capsule surrounds the endodermal primordium and the mesenchymal cells penetrated it, lobulation and vascularization of the organ occurring

together. Around the 11th day of development lymphoid differentiation of the thymus became evident.

MATERIALS AND METHODS

MATERIALS AND METHODS

Macroscopic study.

The thymus was collected from day old to 180 days old ducklings (Indian Runner Duck) which were reared in the department for the research work. The thymus was collected from a total of 60 ducklings of various age groups in the following interval.

Day old	- 3 nos.	
8 days	- 6 "	
15 days	- 3 "	Weekly interval.
22 days	- 3 "	
30 days	- 5 "	
45 days	- 6 nos.	
60 days	- 6 "	15 days interval.
75 days	- 6 "	
90 days	- 6 "	
120 days	- 6 nos.	
150 days	- 4 "	30 days interval.
180 days	- 6 "	
Total	60 "	

The ducklings were sacrificed after taking the body weight and then a longitudinal incision was made from cervical to thoracic portion. The number of pairs of lobes present was also noted. Thymic lobes were collected

and preserved in normal saline. The length and breadth of the anterior, middle and posterior lobes were measured separately by using vernier calipers. The weight of each lobe and the total weight of all the lobes were also recorded. Then the thymus tissue was fixed in the Bouin's fluid.

The data obtained was analysed statistically (Snedecor,& Cochran, 1967) to determine the following:

1. Relation between age and thymic weight.
2. Relation between body weight and thymic weight.
3. Pattern of thymic growth curve.
4. Whether the age or body weight has greater bearing on the thymic weight.
5. Variation in the length, breadth and weight of the anterior, middle and posterior lobes.
6. The percentage of thymus weight to the body weight at different ages.

Microscopic study.

The same material collected for macroscopic study was used for microscopic study during post-incubation period.

The tissues were fixed in Bouin's fluid. Those lobes

larger in size were cut to 2-3 mm. thickness before fixation. The fixed tissues were washed in running water, dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections of five microns thickness were cut and Harris hematoxylin and Eosin (Regressive method) method was employed for routine staining.

Reticular fibres were demonstrated by silver impregnation using James's method (Disbrey and Rack, 1967). Mallory's Triple connective tissue stain was used for the demonstration of collagen fibres (Humason, 1967).

The diameter of Hassall's bodies and the size of lobules taken at random by using an ocular micrometer standardized against a stage micrometer. The average measurements were based on twenty counts per age group. Only those Hassall's corpuscles which showed a well demarkated concentric lamellated appearance were measured. For determining the size of lobules the width between the two adjacent trabeculae were measured.

The values obtained were statistically analysed to determine whether there was any significant correlation between the diameter of Hassall's corpuscles and age of the bird as per Snedecor and Cochran (1967).

Duck embryos at third, eighth, fifteenth and twenty second day of incubation were collected. They were fixed in Helly's fluid and embedded in paraffin. Serial sections were taken from the whole embryos of 3rd and 8th day of incubation. Serial sectioning of cervico-thoracic region was made from the embryos of 15 and 22 day of incubation. Sections were taken at 7 microns thickness and were stained by Harris hematoxylin and Eosin.

RESULTS

Macroscopic study.

Details of the body weight and thymus weight of ducklings at different age groups were recorded in Table 1. The thymus weight increased with increase of age upto 180 days.

The thymus gland consisted of separate lobes with pale white or yellowish white in colour. The lobes were paired extending along the jugular vein as far as the thyroid (Fig. 1) no thymic tissue was found penetrated to the thyroid gland. The anterior lobes were located at the middle of the neck, arranged in a row on either side. The thymus was seen in the lower half of the cervical region and no thymic tissue was found to be extended into the thoracic area.

Variations were noticed in the size and shape of different lobes and also in the number of pairs. The anteriormost lobe was elongated whereas the posteriormost lobe was irregular in shape with a twist at the caudal end. The number of thymus lobes in different age groups were given in Table 2. The posteriormost lobe was the largest in length, breadth and weight (Tables 3, 4, 5, and Chart I, II, III). With increasing age the size and the twist of

the posteriormost lobe were found to be increased. The posteriormost lobe was always the largest.

The analysis of variance of the weights of the three lobes - anterior, middle and posterior - had been given in Table 6. It was found that there was significant difference between the weights of the three lobes. A pair-wise comparison between the weights of anterior and middle lobes showed no significant difference between them ($t = 0.39$), but significant difference was observed between the weights of anterior and posterior lobes ($t = 3.15$, $P < 0.01$) as also the weights of middle and posterior lobes ($t = 2.77$, $P < 0.01$).

Using analysis of variance technique a comparison between the length of the three lobes - anterior, middle and posterior - was made (Table 7). It was found that there was significant difference between the length of the three lobes. A pair-wise comparison between the length of anterior and middle lobes showed no significant difference between them ($t = 0.03$), but significant difference was observed between the length of middle and posterior lobes ($t = 7.23$, $P < 0.01$) and the anterior and posterior lobes ($t = 7.26$, $P < 0.01$).

The analysis of variance of breadth of the three

lobes - anterior, middle and posterior - has been given in Table 8. It was found that there was no significant difference between the breadth of the three lobes.

The percentage of the thymus weight to body weight at different age groups was illustrated in Graph No. I. The mean thymus weight, mean body weight and percentage of thymus weight to body weight at different ages is given in Table 9. The mean weight of the thymus of day old duckling was found to be 28.3± 1.7 mg. (0.03 g). The percentage of thymus weight to body weight in day old duckling was found to be 0.08 and in 180 days old duckling it was 0.24. The maximum percentage was obtained from 15 days old duckling and it was 0.43.

The correlation between age and thymus weight was positive and was found to be equal to $r = +0.674$. This was found to be significantly different from zero with $P < 0.01$ ($t = 8.99$). The correlation between body weight and thymus weight was found to be equal to $r = +0.630$. This was found to be significantly different from zero with $P < 0.01$ ($t = 7.89$). The relation between age and thymus weight was recorded in Graph No. II. The graph indicated that a linear function did not completely explain the relation between the two.

The relation between thymus weight and body weight (Graph No. III) was indicated that a linear function did not completely explain the relation between the two. The correlation between age and body weight was found to be 0.36 indicating positive correlation. This was found to be significantly different from zero with P $<$ 0.01 (t=3.429).

Correlation between age and thymus weight after eleminating body weight was found to be 0.619 (t = 5.954) and the correlation between body weight and thymus weight after eleminating the effect of age was found to be 0.560 (t = 5.100). In both cases this partial correlations were found to be significantly different from zero (P $<$ 0.01). On examining the equality of these correlations with the help of "Fisher's" z transformation it was observed that there was no significant difference between the two (u = 0.482). This showed that both were having equal influence on thymus weight. The mean weight of thymus at 180 days was found to be 2890.8 $\pm$ 600.9 (varies from 1200 to 4895 mg.).

Microscopic study.

The capsule of thymus was formed of a thin layer of fibrous tissue (Fig. 2) which was surrounded by areolar connective tissue. From the capsule thin septa penetrated

into the substance and divided the lobe into incomplete lobules of varying sizes. The stroma was formed of reticular fibres (Fig. 3). The outer zone was darkly stained and inner zone was lightly stained (Fig. 4). The cortex, the outerzone was densely packed with lymphocytes of all sizes small, medium and large. The trabeculae extended perpendicularly from the capsule through the cortex to the corticomedullary junction. The blood vessels were running along the trabeculae (Fig. 5).

The lymphocytes occupied the space of cytoreticular meshwork and largely obscured the reticular network. The region of the medulla was lightly stained and less compact. Among the medullary lymphocytes large pale-staining nuclei of the epithelial reticulum could be seen. Their nuclei contained distinct nucleoli. The reticular cells were prominent in the medulla. Medulla contained Hassall's bodies ranging in diameter from 19.8 microns to 115.5 microns at various ages. Each Hassall's corpuscle was a nest of epitheloid cells arranged like a layered ball (Fig. 6). The component cells of thymic corpuscles were acidophilic and this corpuscles were in direct continuity with the nearby cytoreticulum. The central cells were larger and formed a core to the total

mass. They were surrounded by flattened cells arranged concentrically. There was much hyalinization and degeneration especially at the centre. It was noticed that these Hassall's bodies contained reticular fibres. These fibres were incorporated between the concentric bodies. On silver impregnation staining along with the reticular fibres the concentric ring of Hassall's corpuscles were also taking the positive colour.

Arteries entered along the medullary core and distributed largely to the cortex. Capillaries had a thick basement membrane.

In day old ducklings the capsule was very thin, formed of loose connective tissue whereas in eight day old ducklings the capsule was slightly thicker than the day old. The capsule was formed of dense layer of collagenous fibres containing blood vessels which were surrounded by areolar connective tissue. The cortical septa contained blood vessels.

In 15 day old ducklings longitudinal sections of the lobe showed an axial strand of medullary tissue forming the main medulla and it forms a continuous central axis or core within each lobe. This central medullary core sent a bud like lateral offshoot into each lobule. Each lateral

bud of medullary tissue was surrounded by a cap of cortical tissue. Capsule was similar to the eight day old duckling.

In 30 day old ducklings many of the Hassall's bodies found to be extended to the adjacent cytoreticulum in a cord like fashion. This cord was acidophilic containing cells in the cytoreticular frame work. This mass of tissue appeared to coalesce with the adjacent Hassall's corpuscle (Fig. 7).

In 90 day old ducklings irregular cord like Hassall's corpuscles were seen. Hassall's corpuscles with a length of 580.8 microns was found.

On statistical analysis it was found that the diameter of Hassall's bodies were positively correlated with age, $r = 0.41$ $(t = 2.55)$ $P < 0.05$. As age advances the diameter of Hassall's bodies also increased.

The age-wise mean diameter of Hassall's bodies was given in Table 10 and relation between age and Hassall's diameter was represented in Graph IV. The age-wise mean lobule size was given in Table 11 and relation between age and lobule size is given in the Graph V.

The primordium of thymus was visible at the third day of incubation. It appeared as a hollow cellular primordium

(Fig. 8, 9) which began to elongate from the 3rd pharyngeal pouch. Fourth pouch also appeared to contribute in the formation of thymus. The mass of epithelial cells elongated to form an epithelial cord extending along the jugular vein (Fig. 9). The thymus at eight day of incubation was an epithelial structure appearing as an elongated mass of protoplasm and syncytial in character (Fig. 10).

In the embryo of 15 days old the thymic tissue had become lobulated and showed accummulations of lymphocytes in the mesenchymal tissue (Fig. 11, 12). This was noted in the middle and lower portions of the neck, under the skin above the cartilage mould of developing cervical vertibrae. The thymic tissue contained small and large lymphocytes (Fig. 13). The epithelial cells had light staining cytoplasm and their nuclei were oval with very little chromatin material. From the surrounding mesenchyme capillaries filled with red blood corpuscles were seen entering between the lobules. Free red blood corpuscles were seen inside the lobules.

In the 22 day embryo the lobules of the gland had a densely packed periphery, whereas the central regions were looser. Capsule and interlobular septa with blood vessels were visible. The vascular supply was greatest in the central

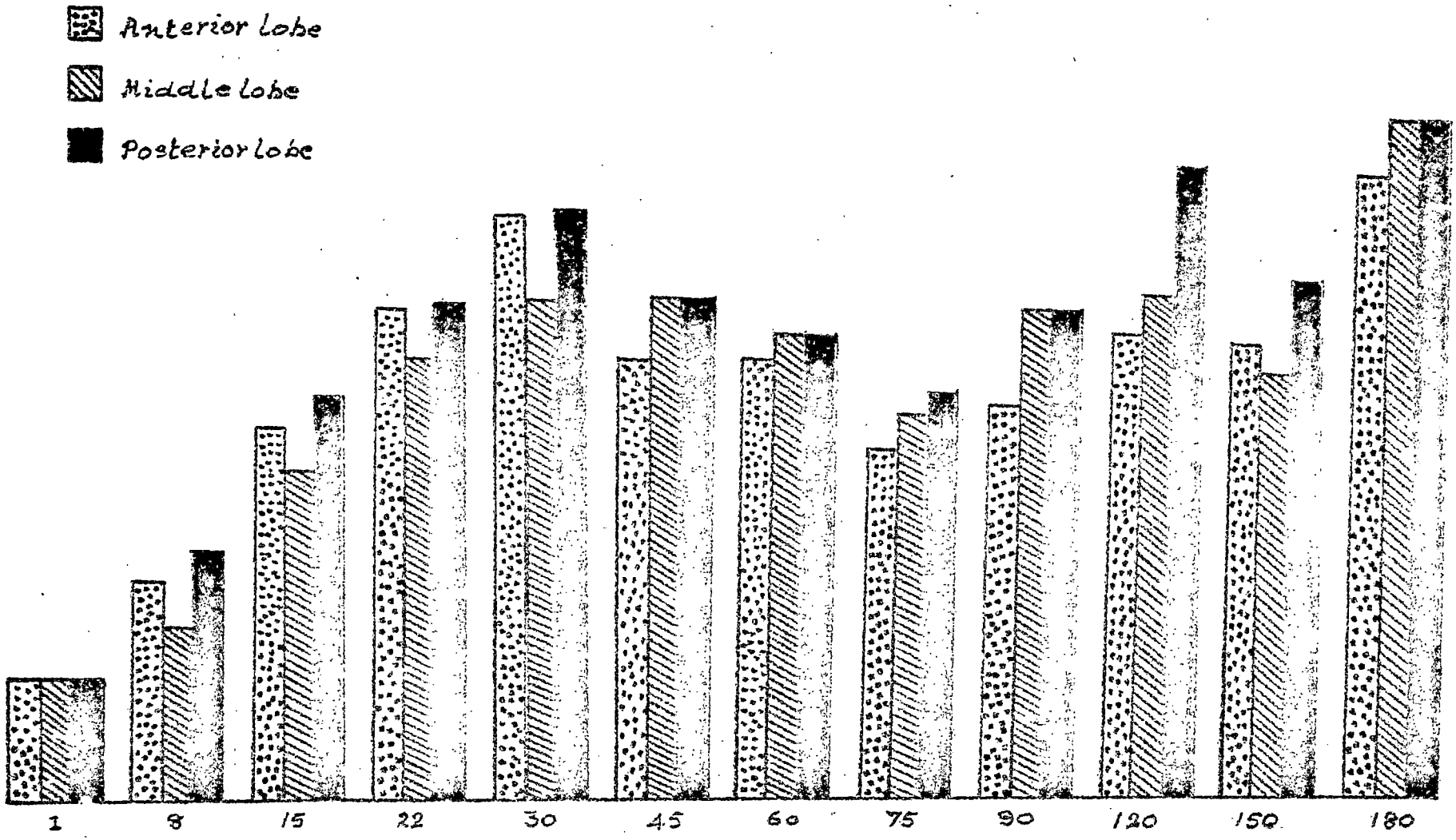
portion of the lobule. The typical axial strand of medulla extending the full length of the lobe was clearly seen (Fig. 14, 15, 16). Thus there were distinct cortical and medullary zones. Epithelial cells in the medulla occurred singly or in groups of two or three. These epithelial clumps were perhaps the beginning of Hassall's corpuscles. All types of lymphocytes were found in the cortex and were scattered in the meshes of the reticulum of the medulla.

Chart - I

Length of anterior, middle and posterior lobes of thymus

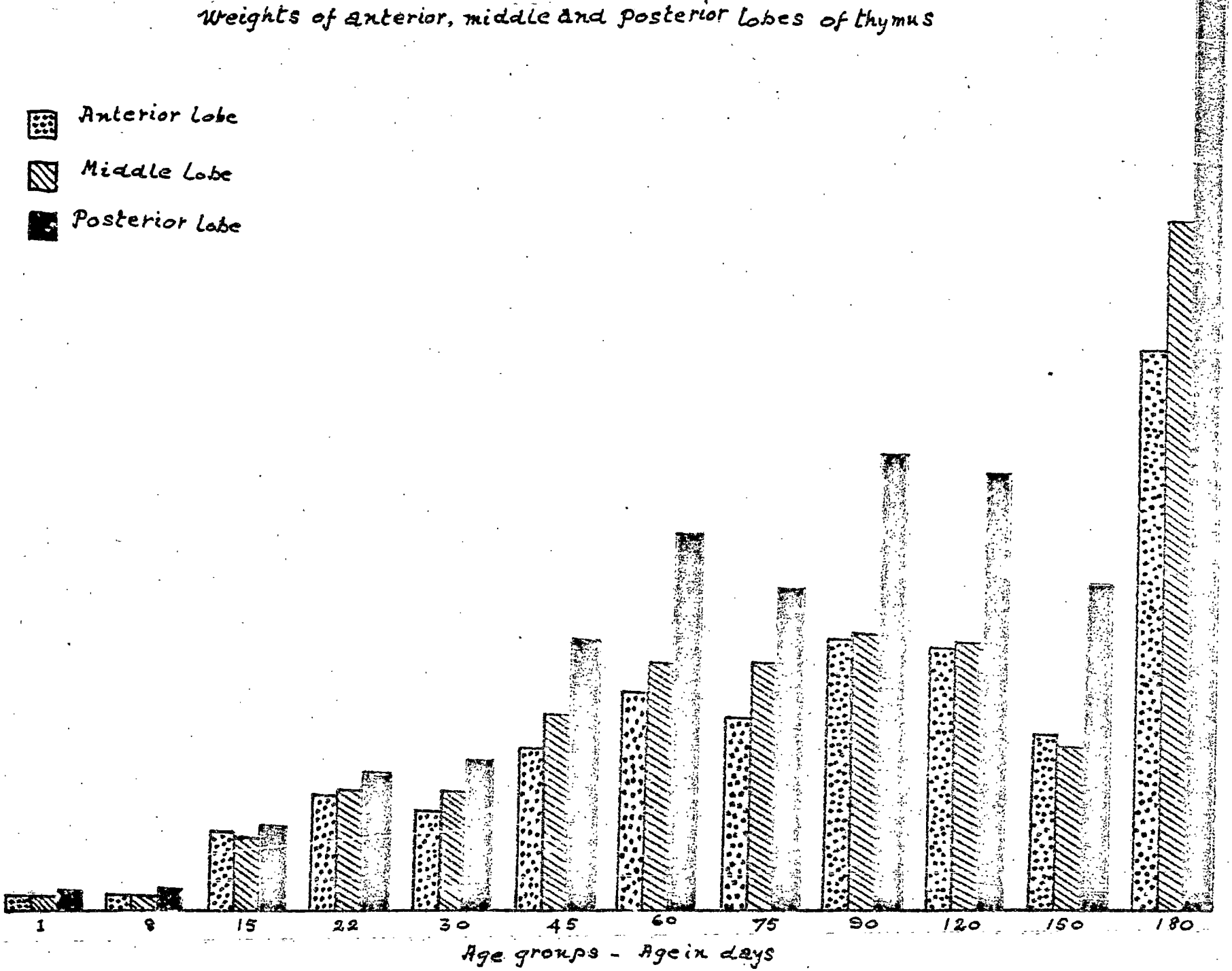
Chart - II

Breadth of anterior, middle and posterior lobes of thymus



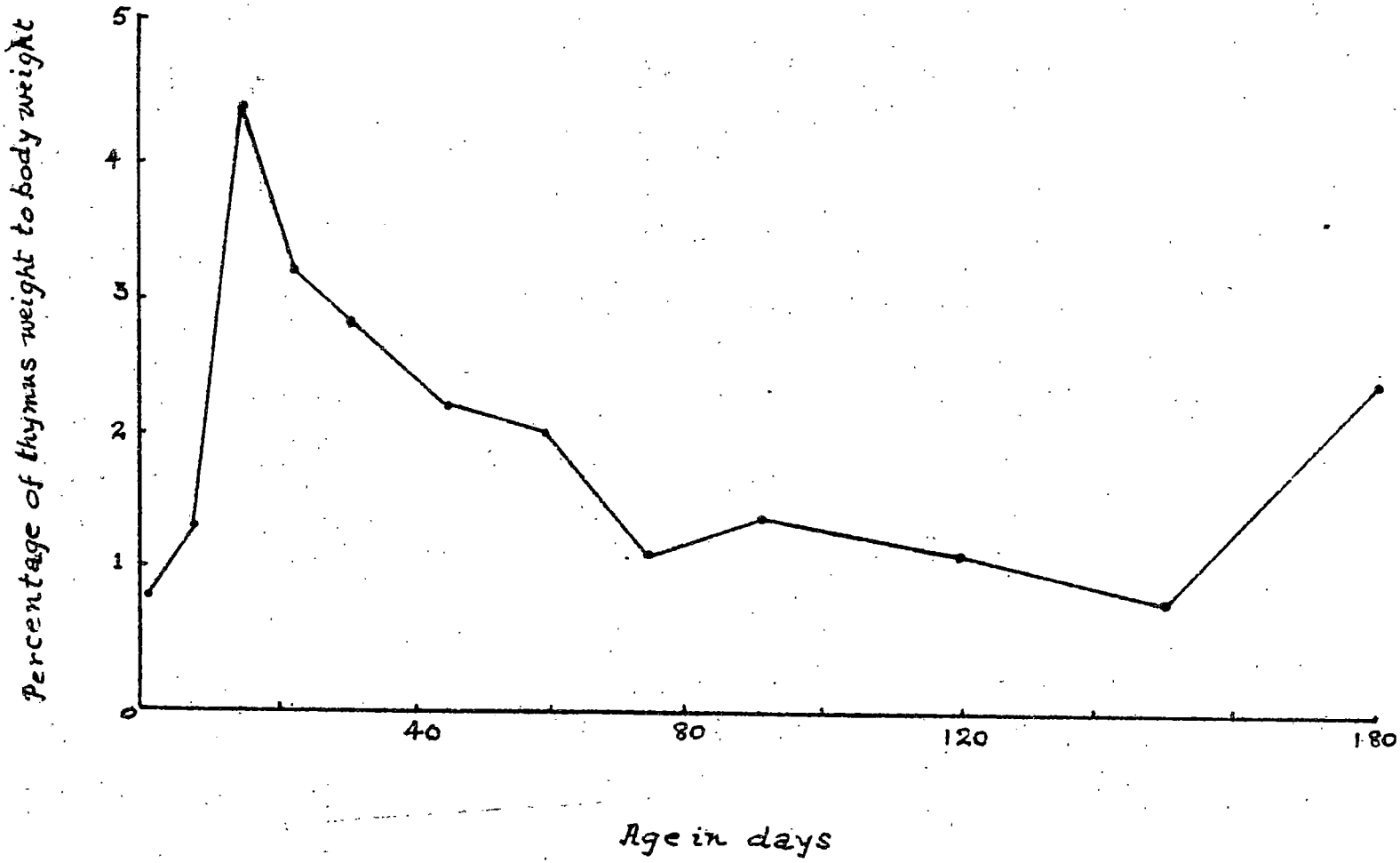
Age groups - Age in days

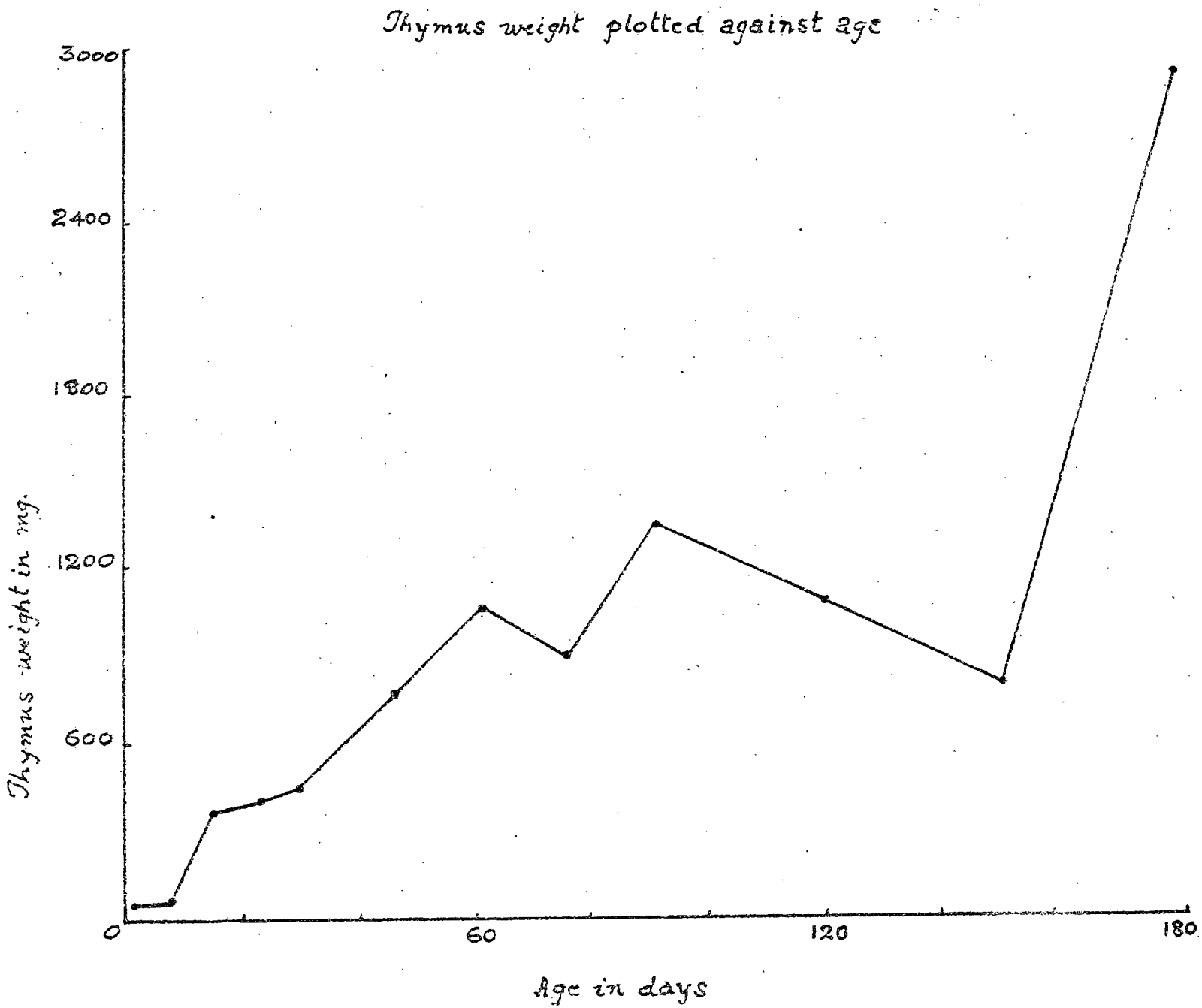
Weights of anterior, middle and posterior lobes of thymus



Graph - I

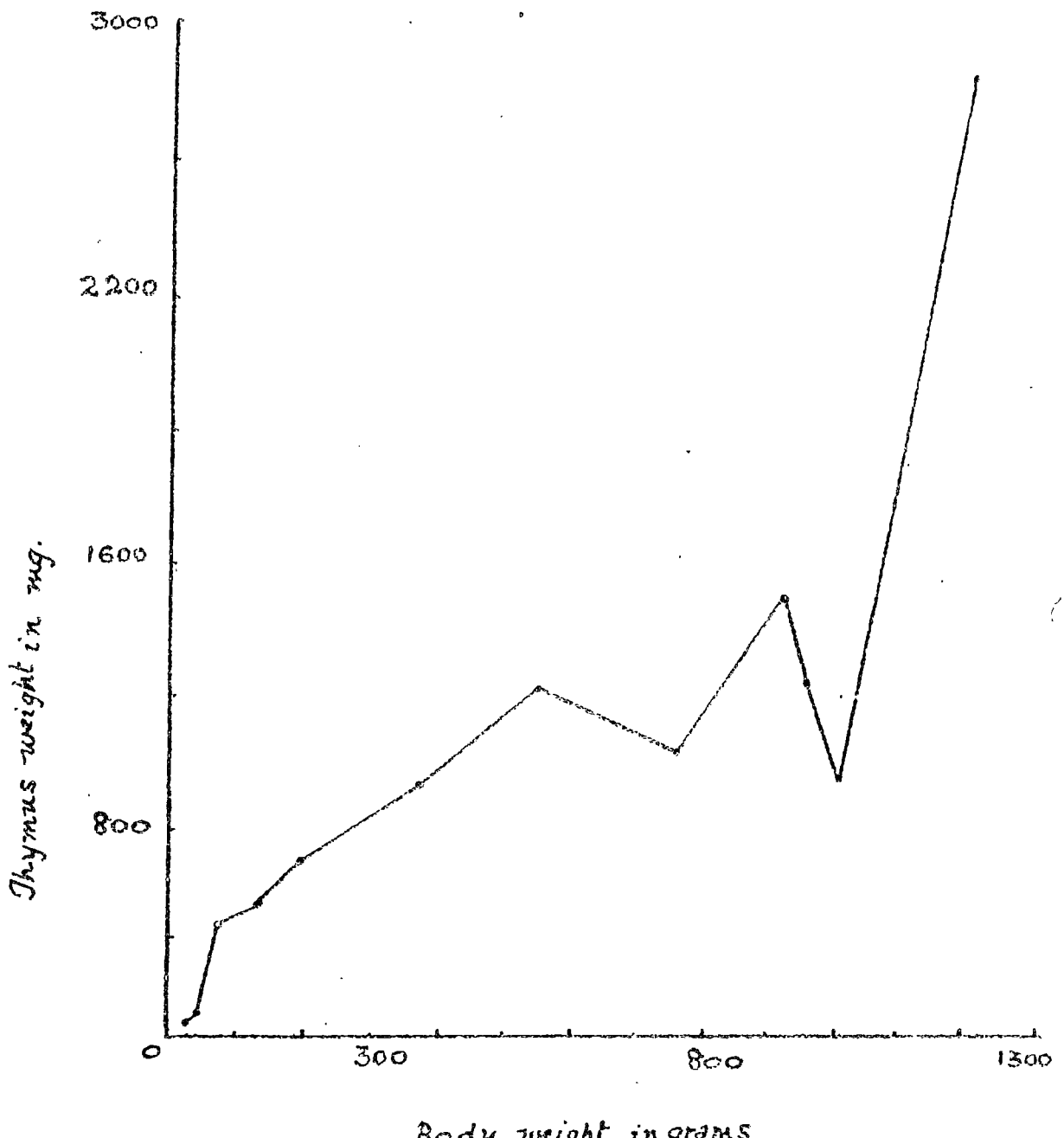
Percentage of thymus weight to body weight plotted against age




Thymus weight plotted against age
3000
2400
1800
1200
600
0
Thymus weight in mg.
60
120
180
Age in days

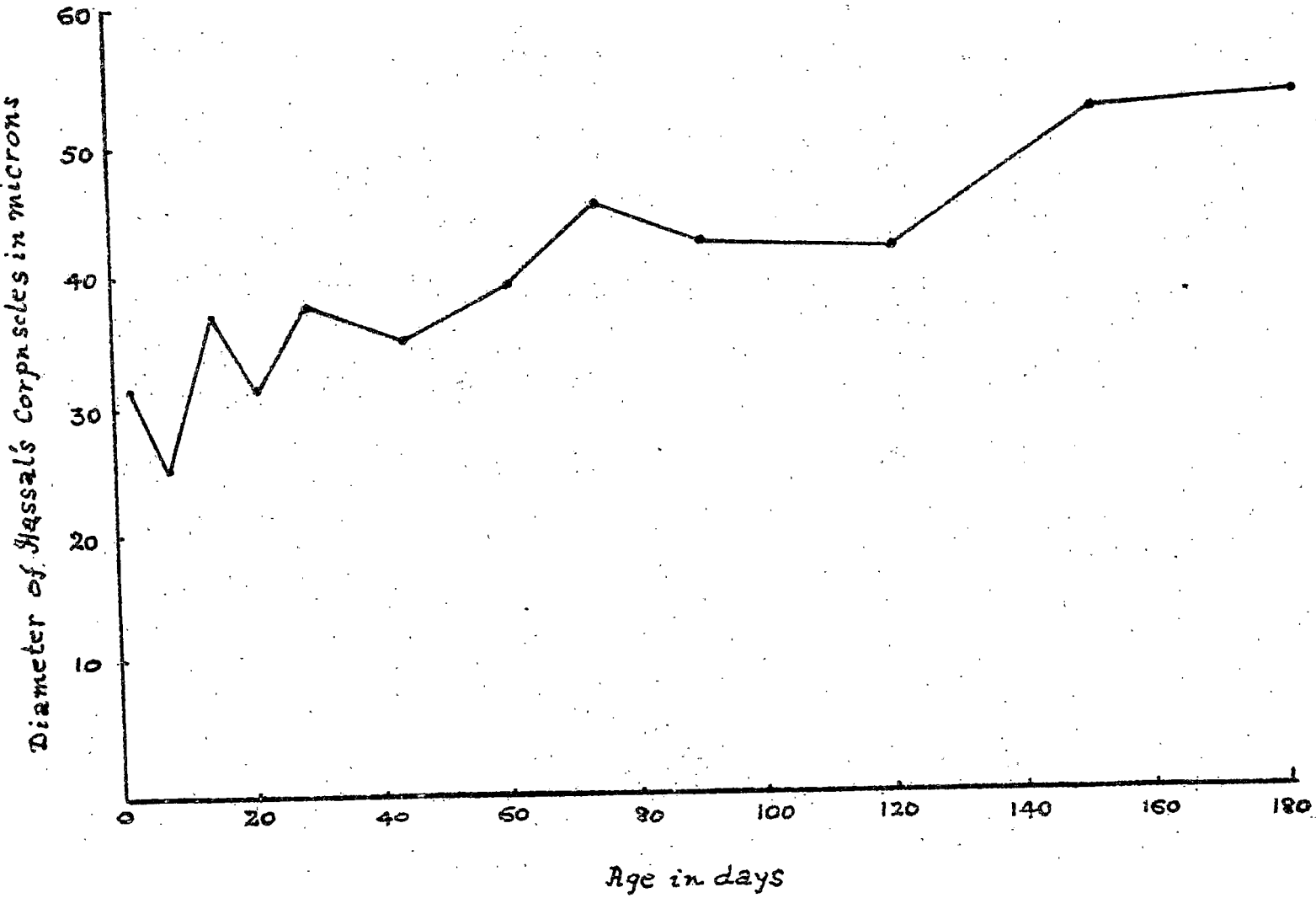
Graph · III

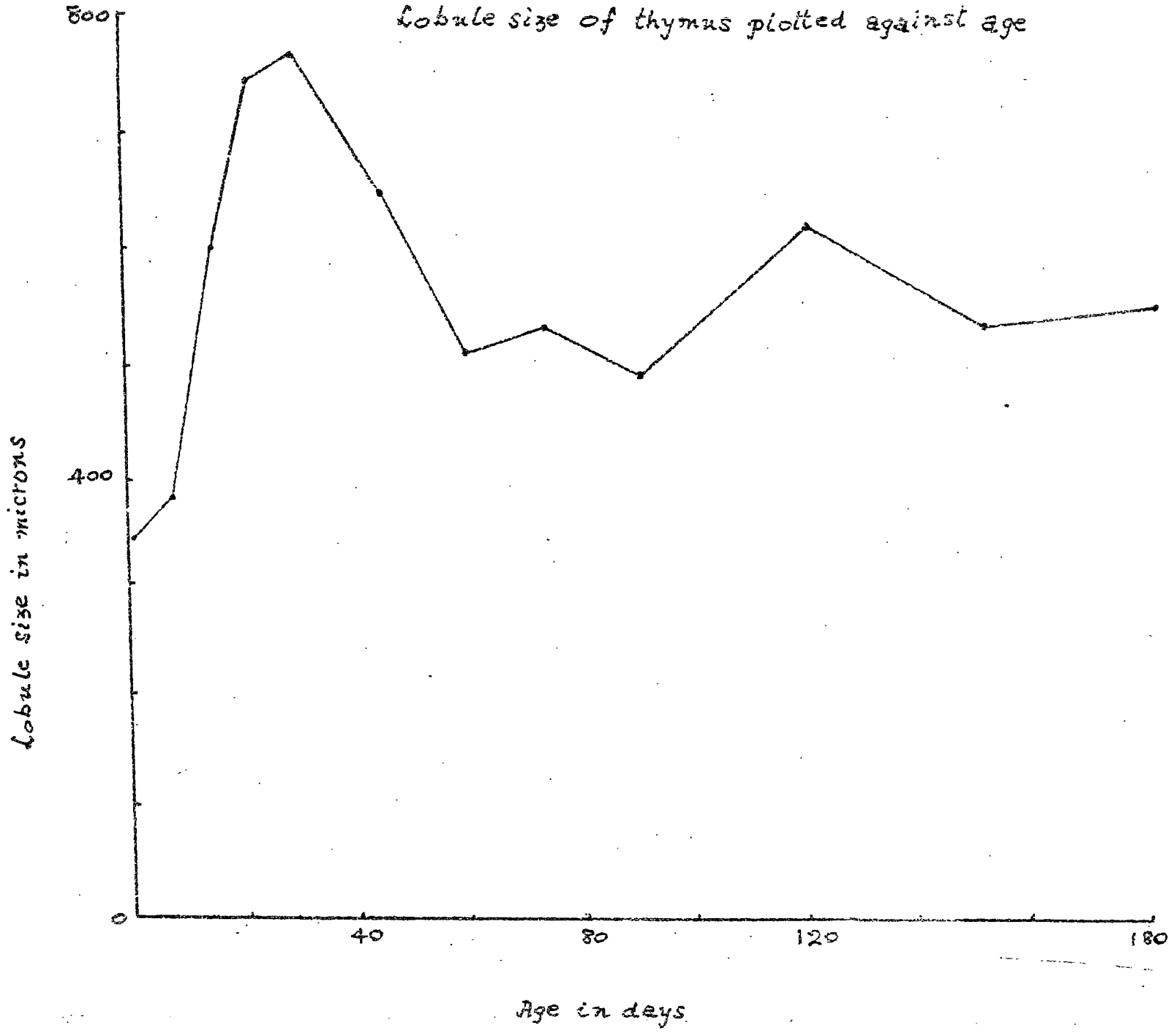
Thymus weight plotted against body weight



Graph - IV

Diameter of Hassall's Corpuscles plotted against age




Lobule size of thymus plotted against age
800
400
0
Lobule size in microns
40
80
120
180
Age in days

DISCUSSION

DISCUSSION

Macroscopic study.

The thymus gland in all the ducks examined consisted of pale, white or yellowish white lobes arranged in chains along the jugular vein as far as the thyroid. Similar observations were made by Bachlechner (1926) and Moughan (1938) in chicken.

In the present study the thymus was seen in the cervical region and no thymic tissue was found in the thoracic cavity. Moughan (1938) had observed that there was no thymic tissue in thoracic cavity of chicken whereas Deniz (1968) had reported that there were cervical and thoracic lobes in the turkey but only cervical lobes in the goose.

The present study showed that the thymus lobes were separate throughout and no fusion of lobes was seen with the advancement of age. Deniz (1968) reported that with increasing age in the turkey the thymic lobes gradually united, but this did not happen in goose.

Here it is observed that there was no penetration of thyroid by the thymus as against Payne (1971) who reported that in chicken thymic tissue sometimes penetrated the thyroid and parathyroid.

In the present study 31 ducks showed consistently 5 pairs of lobes whereas the rest 29 had lobes varying from 3 to 7 on both sides. Hohn (1947) opined that the thymus of duck and goose were having typically 5 pairs of lobes.

The present observation is in agreement with the findings of Hohn (1947) in that the caudal most lobe was always the largest.

The average weight of thymus of day old duckling was found to be 28.3 ± 1.7 mg or 0.0283 g or 0.08% as against 0.101 g or 0.312 per cent of body weight in chicken recorded by Latimer (1924).

In the present study it was seen that total weight of thymus was 4.895 g (average 2.891 g) in ducks of 180 days old, whereas Wolfe et al. (1962) found that in chicken of 120 days old the maximum total thymus weight was 15.76 g.

In the present study it is found that both age and body weight has got equal influence on thymic weight as against the observation of Greenwood (1930) who found that the thymic changes were more closely related to age than to body weight in chicken. The size of thymus gland varied greatly with age (Tables 3, 4 and Chart I & II).

No regression of thymus was found upto 180 days in ducks but Hohn (1956) reported recrudescence of thymus in adult chicken.

Microscopic study.

In the present study it was found that the thymus is invested with a capsule of fibrous tissue surrounded by areolar connective tissue. From the capsule thin septa penetrate into the substance and divide the lobe into incomplete lobules. The stroma was formed of cytoreticular tissue. The cortex was formed of dense lymphoid tissue and the medulla was less densely packed with lymphocytes. The characteristic Hassall's bodies were seen in the medulla. This is in agreement with the findings of Payne (1971) who stated that in the chick the microscopic anatomy of thymus was similar to that of mammals.

In the present study blood vessels were seen running along the trabeculae. Similar observations were made by Kostowiecki and Harty (1966) in the thymus of cat, dog, calf, guinea pig and man in which blood vessels were seen accompanied to the thyomic cortex.

Droege et al. (1974) stated that adult chicken thymus

has practically no cortex, contained mainly one type relatively large cell, which may represent the medullary lymphocytes. But in this study it was found that there was an outer zone densely packed with lymphocytes of all sizes - small, medium and large - the cortex and a lightly stained inner zone, the medulla with less number of cells.

Sugimura (1971) observed myoid cells in calf's thymus medulla, which were round to oval 15-20 micron in size with an oval nucleus. But in the present study such cells were not seen.

The Hassall's bodies were seen to be composed of epitheloid cells arranged like a layered ball. The component cells are acidophilic and this corpuscles were in direct continuity with the nearby cytoreticulum. The central cells were larger and formed a core to the total mass. They were surrounded by flattened cells arranged concentrically. There was much hyalinization and degeneration especially at the centre. Similar observations were made by Ham (1969) which is as follows. In the medulla the epithelial reticular cells become grouped concentrically around a central focus to form some keratin which is surrounded by a few rings of flattened epithelial cells known as Hassall's corpuscle with a typical concentric lamellated pattern. Reticular cells form layers around

central elements of variable appearance. And the reticular cells at the periphery of thymic corpuscle may be continuous with cells of cytoreticulum. It was noted that the reticular fibres are incorporated in the concentric structure of Hassall's corpuscle, which was seen to take a positive stain with silver impregnation. This supports the view of Kistoweicki and Harty (1966) who stated that Hassall's corpuscles which developed in different parts of medulla contain reticular fibrils passing through their core or wall.

Venzke (1952) stated that in chick embryo by 19th day of incubation the Hassall's corpuscles have a diameter of 30-40 microns. But in the present study in day old ducklings the Hassall's corpuscle's diameter ranged from 19.8 to 39.6 microns. And in 180 days old ducklings the Hassall's diameter ranged from 26.4-105.6 microns. This was in agreement with Lesson and Lesson (1972) who stated that the Hassall's corpuscle diameter varied from 20 to more than 100 microns. Almost same statement is given by Windle (1969) that Hassall's diameter varies from 30-100 microns. The relation of the diameter of Hassall's corpuscles to age was represented graphically in Graph No. IV and the age-wise mean Hassall's corpuscles diameter is given in Table 10.

The lobule size varied from 283.3-416.7 microns in day

old duckling. And in 180 days old ducklings it was 333.3-999.9 microns. But regarding the size of lobules in domestic animals Trautmann and Fiebiger (1957) stated that it would vary with an average of 5 mm. to 13 mm. The lobule size when plotted against age was represented in the graph. Age-wise mean size of lobule was given in Table 11.

In the present study many of the Hassall's corpuscles are seen to extend into the adjacent cytoreticulum in a cord like fashion. This acidophilic cord appended to coalesce with the adjacent Hassall's corpuscle. They were not the intracellular or intercellular cysts seen regularly in guinea pig and mouse thymus which were related to thymic corpuscle and which were having the epithelial nature as Hassall's corpuscle (Kohnen and Weiss, 1964).

The present study reveals that the thymic primordium makes its appearance by the 3rd day of incubation. This observation is in agreement with the earlier report of Hamilton (1913) whereas Venzke (1952) reported that in the chicken it appeared only by the 5th day of incubation. The lobulation of thymic tissue with accummulation of lymphocytes have been noticed by the 15th day of incubation, and the distinct cortical and medullary zones are apparent by the 22nd day of incubation in the present study. Venzke (1932) has reported that lobulation was distinct

by 9½ days of incubation in the chicken and the distinctness of the cortical and medullary zones could be demonstrated by 15th day of incubation.

From the above findings it is seen that eventhough the thymic primordium in duck embryo appears as early as the 3rd day of incubation it takes a longer period for its development in comparison to the chicken embryo. This may be attributed to the longer incubation period of duck embryo.

S U M M A R Y

SUMMARY

A detailed study on the structure and development of thymus in the Indian Runner Duck during embryonic and post-embryonic period was carried out.

The experimental birds numbering 60 varied in age from day old to 180 days and were divided into 12 groups. Embryonic studies were conducted in embryos of 3rd, 8th, 15th and 22nd day of incubation.

The thymus of duck was confined to the lower half of the cervical region lying in 3 to 7 pairs of lobes with an average of 5 pairs. The weight of thymus increased with the increase in age upto 180 days.

The posteriormost lobe was found to be the largest.

A significant difference was observed between the weights of anterior and posterior lobes ($t = 3.15$, $P < 0.01$) and middle and posterior lobes ($t = 2.77$, $P < 0.01$). A pair-wise comparison between the length of anterior and posterior lobes ($t = 7.26$, $P < 0.01$) and middle and posterior lobes (7.23, $P < 0.01$) showed significant differences.

Histological structure of the thymus was similar to that in mammals. The diameter of Hassall's corpuscle was positively correlated with age, $r = 0.41$ ($t = 2.55$, $P < 0.05$).

The thymic primordium appeared by the 3rd day of incubation and by 15th day lobulation of thymus with accummulation of lymphocytes was evident. The division of lobules into cortex and medulla was noticed by the 22nd day of incubation. Hassall's bodies appeared by this time.

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APPENDIX

TABLES

Table 1. Body weight and thymus weight of experimental ducklings at different ages.

S.No.	Age in days	Body weight in g.	Thymus weight in mg.
1	2	3	4
1	1	38.000	30
2	1	30.000	25
3	1	32.000	30
4	8	42.000	40
5	8	30.000	30
6	8	30.720	28
7	8	31.000	55
8	8	29.050	55
9	8	40.000	48
10	15	84.000	362
11	15	75.000	330
12	15	85.000	360
13	22	142.000	570
14	22	118.000	220
15	22	135.000	420
16	30	140.000	370
17	30	290.000	570
18	30	233.500	620
19	30	118.000	600
20	38	590.000	3300
21	45	318.000	720
22	45	180.000	320
23	45	469.000	1060
24	45	350.000	850
25	45	390.000	800
26	45	400.000	800
27	60	525.000	2550
28	60	520.000	350
29	60	480.000	700
30	60	469.000	1060

(contd.)

1	2	3	4
31	60	780.000	950
32	60	375.000	560
33	75	450.000	500
34	75	500.000	510
35	75	900.000	1250
36	75	950.000	920
37	75	850.000	800
38	75	850.000	1200
39	90	1080.000	1620
40	90	650.000	650
41	90	1050.000	1810
42	90	750.000	1800
43	90	1250.000	925
44	90	650.000	1090
45	120	1050.000	1870.
46	120	800.000	850
47	120	950.000	750
48	120	1000.000	980
49	120	900.000	750
50	120	950.000	1170
51	150	1100.000	750
52	150	900.000	470
53	150	1000.000	510
54	150	1000.000	1340
55	180	1250.000	2000
56	180	1100.000	3040
57	180	1300.000	4340
58	180	1150.000	4895
59	180	1250.000	1870
60	180	1200.000	1200

(concl.)

Table 2. Details showing the number of lobes of thymus in Indian Runner ducks from day old to 180 days.

S.No.	Age in days	Number of lobes		S.No.	Age in days	Number of lobes	
		Right	Left			Right	Left
1	1	3	3	31	60	4	4
2	1	4	4	32	60	5	6
3	1	5	5	33	75	5	5
4	8	5	5	34	75	5	5
5	8	4	4	35	75	4	4
6	8	4	4	36	75	4	5
7	8	5	5	37	75	3	4
8	8	4	4	38	75	4	5
9	8	4	4	39	90	5	5
10	15	4	4	40	90	5	5
11	15	5	5	41	90	5	5
12	15	5	5	42	90	5	5
13	22	5	5	43	90	5	5
14	22	4	4	44	90	5	5
15	22	5	5	45	120	4	4
16	30	4	4	46	120	5	5
17	30	5	5	47	120	5	5
18	30	7	5	48	120	4	4
19	30	5	5	49	120	4	4
20	30	6	6	50	120	5	5
21	45	5	5	51	150	5	5
22	45	6	5	52	150	5	5
23	45	5	5	53	150	4	4
24	45	5	5	54	150	5	5
25	45	5	5	55	180	4	4
26	45	5	5	56	180	4	4
27	60	5	5	57	180	4	4
28	60	5	5	58	180	4	4
29	60	5	5	59	180	4	4
30	60	5	5	60	180	4	4

Table 3. Average length of anterior, middle and posterior thymic lobes of ducklings at different ages.

S.No.	Age in days (No. of birds)	Group No.	Mean length in mm.		
			Anterior	Middle	Posteri
1	1 (3)	I	3.0	2.0	3.0
2	8 (6)	II	3.8	3.0	4.0
3	15 (3)	III	5.3	5.3	7.3
4	22 (3)	IV	6.6	6.0	11.0
5	30 (5)	V	10.8	8.2	14.4
6	45 (6)	VI	8.6	8.3	18.8
7	60 (6)	VII	7.3	8.1	15.6
8	75 (6)	VIII	5.5	8.3	13.3
9	90 (6)	IX	10.3	8.5	15.6
10	120 (6)	X	12.5	11.0	17.5
11	150 (4)	XI	7.5	7.2	12.7
12	180 (6)	XII	11.8	15.3	26.6

Table 4. Average breadth of anterior, middle and posterior thymic lobes of ducklings at different ages.

S.No.	Age in days (No. of birds)	Group No.	Mean breadth in mm.		
			Anterior	Middle	Posterior
1	1 (3)	I	1.0	1.0	1.0
2	8 (6)	II	1.8	1.3	2.0
3	15 (3)	III	3.0	2.6	3.3
4	22 (3)	IV	4.0	3.6	4.0
5	30 (5)	V	4.8	4.2	4.8
6	45 (6)	VI	3.6	4.1	4.1
7	60 (6)	VII	3.5	3.8	3.8
8	75 (6)	VIII	2.8	2.1	3.3
9	90 (6)	IX	3.1	4.0	4.0
10	120 (6)	X	3.8	4.1	5.1
11	150 (4)	XI	3.7	3.5	4.2
12	180 (6)	XII	5.0	5.5	5.5

Table 5. Average weight of anterior, middle and posterior thymic lobes of ducklings at different ages.

S.No.	Age in days (No. of birds)	Group No.	Mean weight in mg.		
			Anterior	Middle	Posterior
1	1 (3)	I	5.0	5.0	6.3
2	8 (6)	II	5.3	5.0	9.0
3	15 (3)	III	43.3	40.7	48.3
4	22 (3)	IV	66.7	68.3	76.7
5	30 (5)	V	60.0	67.5	90.0
6	45 (6)	VI	93.3	106.7	155.0
7	60 (6)	VII	131.7	143.3	218.3
8	75 (6)	VIII	109.2	137.5	185.8
9	90 (6)	IX	160.0	159.2	263.3
10	120 (6)	X	152.5	156.7	255.0
11	150 (4)	XI	105.0	96.2	187.5
12	180 (6)	XII	320.0	394.2	750.0

Table 6. Analysis of variance of breadth of anterior, middle and posterior thymic lobes during development.

Source	df	SS	MSS	F
Between lobes	2	4.0	2.0	1.8 NS
Error	177	182.3	1.1	
Total	179	186.3		

Inference: There is no significant difference in breadth between anterior, middle and posterior lobes.

Table 7. Analysis of variance of length of anterior, middle and posterior thymic lobes during development.

Source	df	SS	MSS	F
Between lobes	2	1,777.8	888.9	35.05**
Error	177	4,488.5	25.4	
Total	179	6,266.3		

Inference: There is high significant difference in length between anterior, middle and posterior lobes.

Table 8. Analysis of variance of weights of anterior, middle and posterior thymic lobes during development.

Source	df	SS	MSS	F
Between lobes	2	3,59,885.8	1,79,942.9	5.91**
Error	177	53,88,993.0	30,446.3	
Total	179	57,48,878.8		

Inference: There is significant difference in weight between anterior, middle and posterior lobes.

Table. 9. Mean body weight, mean thymus weight and percentage of thymus weight to body weight at different ages.

S.No.	Age in days (No. of birds)	Group No.	Mean body weight in g.	Mean thymus weight in mg.	Percentage of thymus weight to body weight
1	1 (3)	I	33.333	28.3	0.08
2	8 (6)	II	33.795	42.7	0.13
3	15 (3)	III	81.333	350.7	0.43
4	22 (3)	IV	131.667	403.3	0.31
5	30 (5)	V	195.550	540.0	0.28
6	45 (6)	VI	351.167	758.3	0.22
7	60 (6)	VII	524.833	1028.3	0.20
8	75 (6)	VIII	750.000	863.3	0.12
9	90 (6)	IX	910.000	1315.8	0.14
10	120 (6)	X	941.667	1061.7	0.11
11	150 (4)	XI	1000.000	767.5	0.08
12	180 (6)	XII	1208.300	2890.8	0.24

Table 10. Age-wise mean Hassall's corpuscles diameter in microns.

S.No.	Age in days	Group No.	Hassall's corpuscles diameter (in microns)
1	1	I	31.4
2	8	II	24.8
3	15	III	36.3
4	22	IV	31.3
5	30	V	37.9
6	45	VI	35.1
7	60	VII	39.7
8	75	VIII	45.1
9	90	IX	42.0
10	120	X	42.1
11	150	XI	52.8
12	180	XII	53.0

Table 11. Age-wise mean lobule size in microns.

S.No.	Age in days	Group No.	Lobule size in microns
1	1	I	336.1
2	8	II	370.8
3	15	III	589.6
4	22	IV	737.8
5	30	V	756.2
6	45	VI	639.9
7	60	VII	499.9
8	75	VIII	527.8
9	90	IX	479.3
10	120	X	613.3
11	150	XI	519.4
12	180	XII	538.9

F I G U R E S

Fig. 1. Thymus, showing lobes, lying along the course of the jugular vein.

Fig. 2. Section of thymus showing capsule. H & E. stain; x 100.

Fig. 3. Section of thymus showing reticular frame work. Silver impregnation; x 100.

Fig. 1

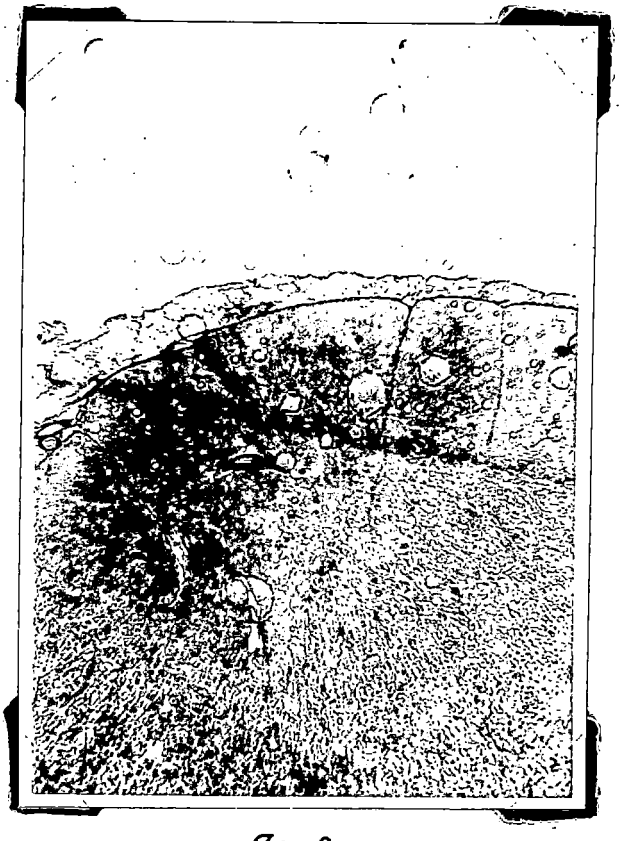

Fig. 2

Fig. 3

Fig. 4. Section of thymus showing outer cortex and inner medulla. Note Hassall's corpuscles in the medulla.
H & E. stain; x 100.

Fig. 5. Section of thymus showing trabeculae containing blood vessels.
Mallory's tripple connective stain; x 400.

Fig. 6. Section of medulla of thymus showing Hassall's corpuscles
H & E. stain; x 400.

Fig. 4

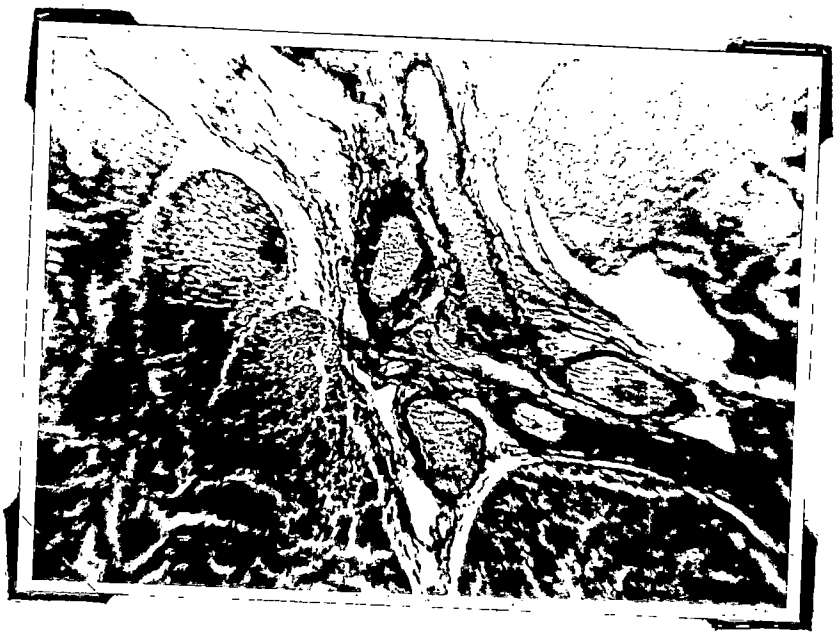

Fig. 5

Fig. 6

Fig. 7. Section of thymus showing large Hassall's corpuscle. Note coalescence of corpuscles. H & E. stain; x 100.

Fig. 8. Three day old embryo showing thymic primordium. H & E. stain; x 100.

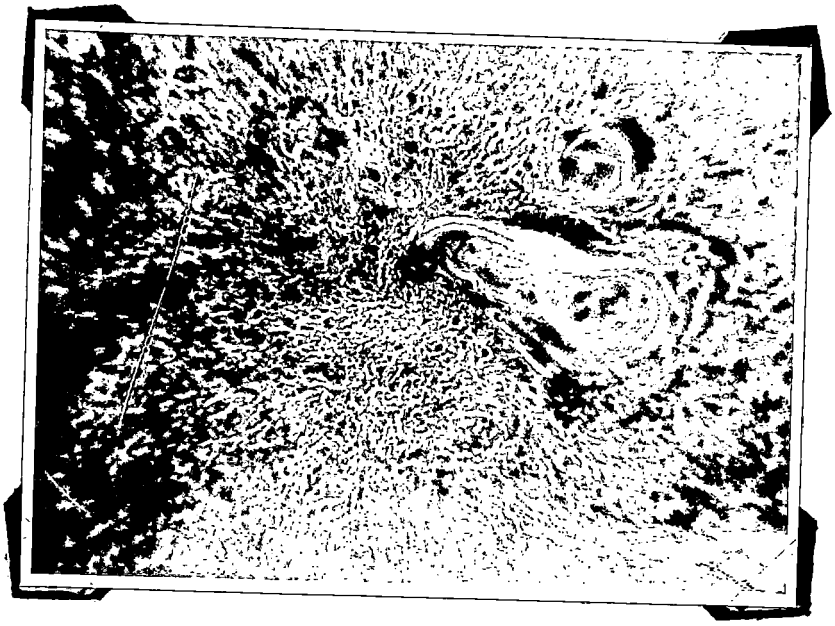

Fig. 7

Fig. 8

Fig. 9. Three day old embryo showing thymic primordium. H & E. stain; x 630.

Fig. 10. Section of 8 day old embryo showing the thymic primordium extending along the jugular vein. H & E. stain; x 630.

Fig. 9

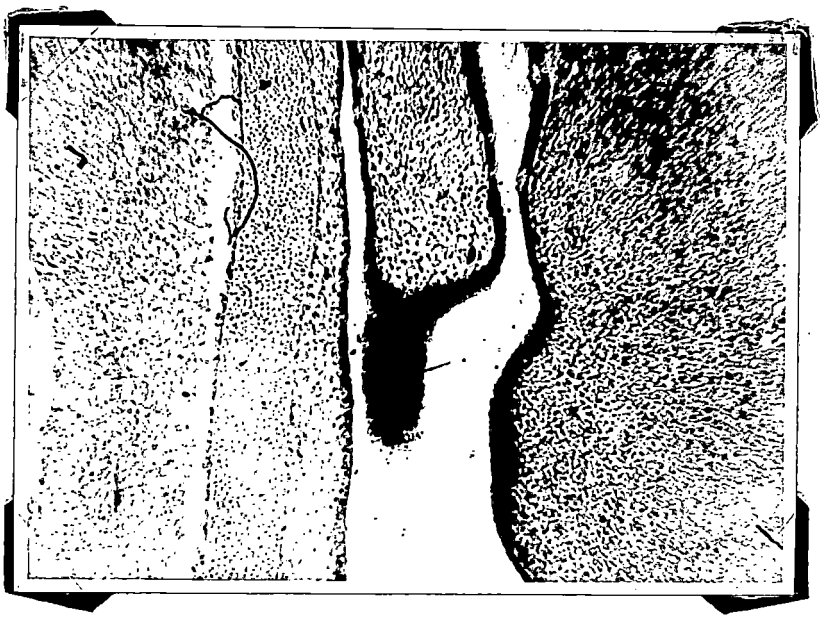

Fig. 10

Fig. 11. Section of 15 day old embryo showing lobulated thymic tissue infiltrated with lymphocytes. H & E. stain; x 100.

Fig. 12. Section of thymus at 15th day of incubation showing lobulation. H & E. stain; x 100.

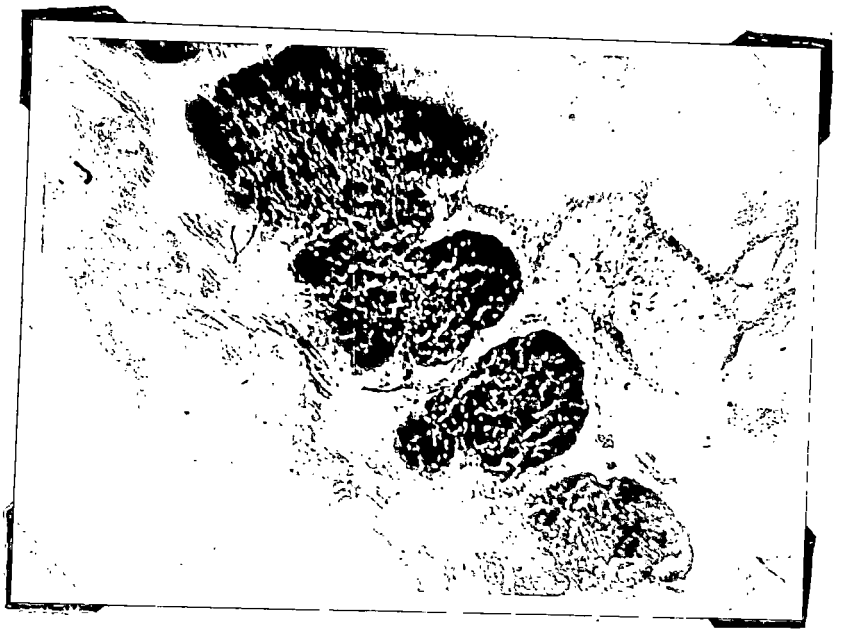

Fig. 11

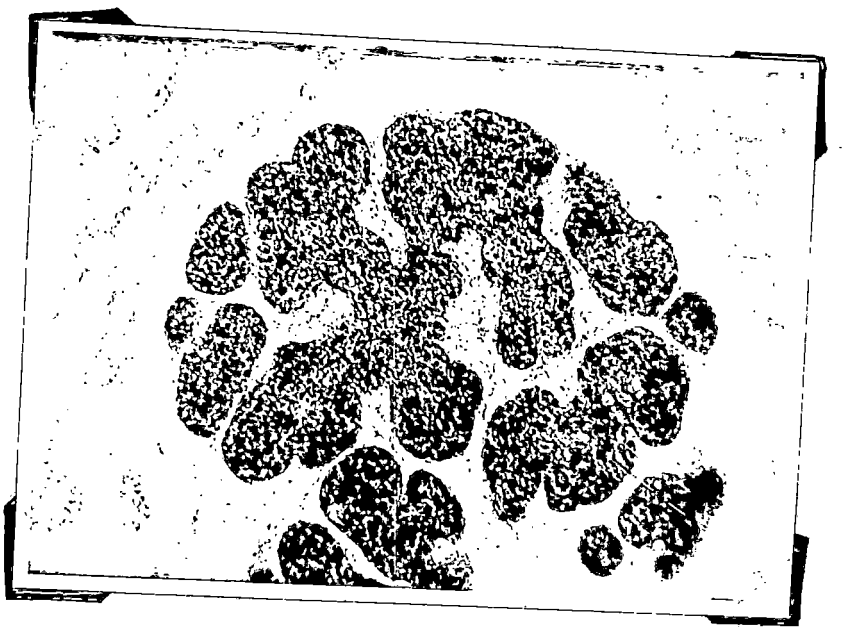

Fig. 12

Fig. 13. Section of thymus at 15th day of incubation showing accummulation of lymphocytes.
H & E. stain; x 630.

Fig. 14. Section of thymus at 22nd day of incubation showing axial strand of medulla and cortex separated by septa.
H & E. stain; x 100.

Fig. 13

Fig. 14

Fig. 15. Section of thymus at 22nd day of incubation showing cortex and medulla.
H & E. stain; x 100.

Fig. 16. Section of thymus at 22nd day of incubation showing cortex and medulla.
H & E. stain; x 100.

Fig. 15

17002

Fig. 16

A B S T R A C T

STUDIES ON
THE STRUCTURE AND DEVELOPMENT OF THE THYMUS IN THE INDIAN RUNNER DUCK

By
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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF VETERINARY SCIENCE

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ABSTRACT

A study on the structure and development of thymus in the Indian Runner Duck during embryonic and post-embryonic period was conducted. Thymus from 60 birds ranging in age from day old to 180 days were collected for the study and embryonic studies were carried out in embryos of third, eighth, 15th and 22nd days of incubation. There were five pairs of lobes, on an average lying in the lower half of the neck. The weight of the thymus increased with age upto 180 days and the posteriormost lobe was the largest. There was a significant difference between the weights of anterior and posterior and middle and posterior lobes. Histological structure was more or less similar to that in mammals. The diameter of Hassall's corpuscle was found to be positively correlated with age. The thymic primordium appeared by the third day of incubation. Lobulation was evident by the 15th day and by 22nd day of incubation cortex and medulla were discriminated.