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HISTOLOGICAL AND QUANTITATIVE STUDIES ON THE POSTNATAL GROWTH OF THE DUCK SPLEEN*

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The postnatal growth of the duck spleen was studied histologically, and the relative development of the splenic constituents was estimated by means of microscopic point-counting planimetry in the spleen from the day of hatching up to 22 weeks of age. On the day of hatching, the red pulp showed a relatively high value because of the scant development of white pulp elements; small areas of periarterial and perivenous lymphoid tissues were present with almost no periellipsoidal lymphoid tissue. Remarkable development of the periellipsoidal lymphoid tissue, however, was ascertained from 3 weeks onward, and this was estimated to be a principal element of the white pulp throughout its postnatal life. India ink particles injected into the blood stream were deposited within the periellipsoidal lymphoid tissues. Germinal centers were firstly noted in the 7 week-old spleen and appeared in the periarterial lymphoid tissue more frequently than in the perivenous lymphoid tissue.

These results suggest that the development of the white pulp is completed within several weeks of posthatching life, and that there is a close relationship between the development of the white pulp elements and the immune system in the duck spleen.

INTRODUCTION

The cytological process occurring in the postnatal development of the splenic white pulp of birds is affected by two different cell systems of thymic and bursal origin, respectively. In the chicken, it has been believed that the periarterial lymphoid tissue (PALT) and the perivenous lymphoid tissue (PVLT), which consist mainly of small lymphocytes, belong to the thymus-dependent system (JANKOVIĆ & ISAKOVIĆ, 1964; HOSHI & MORI, 1973; DEKRUYFF et al., 1975), whereas the periellipsoidal lymphoid tissue (PELT), consisting mainly of medium or large-sized lymphocytes, germinal centers and cells of plasma cell series, belong to the bursa-dependent system (COOPER et al., 1966; HOSHI, 1972; DEKRUYFF et al., 1975).

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In recent experiments with thymectomized-bursectomized ducks, SUGIMURA & HASHIMOTO suggested the existence of a thymus- and bursa-independent population of lymphoid cells in the spleen¹⁹⁾ and the lymph node²¹⁾. In the ducks, therefore, it was designated that the PALT belongs to the thymic system, and that the PELT, and probably the germinal centers belong to the bursal system. The PVLТ and the existence of the plasma cell series were, on the other hand, independent of the two cellular systems. These cellular elements of the spleen differ not only in their cytology, but also in respect of their distribution and close relation to the splenic vascular systems (FUKUTA et al., 1969).

Since the white pulp elements of the duck spleen differ from those of the chick spleen regarding the thymus- and/or bursa-dependency, it is important to clarify the postnatal growth of the splenic constituents of the duck spleen. In the present study, the authors focused their attention on the quantitative changes of splenic constituents taking place in the spleen during postnatal growth, and the immune system of the spleen as compared to the chick spleen.

MATERIALS AND METHODS

White Pekin ducks in good health aged hatching day, 1, 3, 5, 7, 9, 11, 13, 17 and 22 weeks were used in this study. The spleens were removed from the ducks immediately after death, and their weight was recorded. Blocks 3~5 mm thick, cut from the plane of the largest cross section of the spleen, were fixed in Carnoy's or Bouin's fixative, and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin (HE) or methyl green-pyronine. Microscopic point-counting planimetry (WEIBEL, 1969) was employed in an area of $5 \times 10^6 \mu\text{m}^2$ for a relative estimation of each splenic element at a 100 fold magnification. This method allows the separate estimation of quantitative changes occurring among the thymus-dependent, bursa-dependent and other possible types of lymphoid tissues, and takes into consideration that the splenic distribution of these elements is non-uniform. The weight of each splenic element was calculated by multiplying the splenic absolute weight by the relative value, shown in a percentage, which was obtained by microscopic point-counting planimetry.

To avoid a possible difference between the relative estimation of PELT by means of point-counting planimetry and the actual number of lymphoid cells belonging to this area during the few weeks of posthatching life, the number of periellipsoidal lymphoid cells per exact cross-sectioned ellipsoid in the spleens of each age group was estimated so as to follow the real development of this element. The trabecular area, including the capsule, was estimated at the same time with that of the white pulp, and the remaining areas were estimated to be red pulp. The number of germinal centers per section and the number of plasma cells in an area of $1.25 \times 10^6 \mu\text{m}^2$ of the red pulp were also estimated. To make clear the permeability of the sheathed capillaries, India

ink was injected into the blood stream of some ducks.

RESULTS

1 Gravimetric change of the spleen

The weight of the spleen and the percentages of the body weight for all age groups

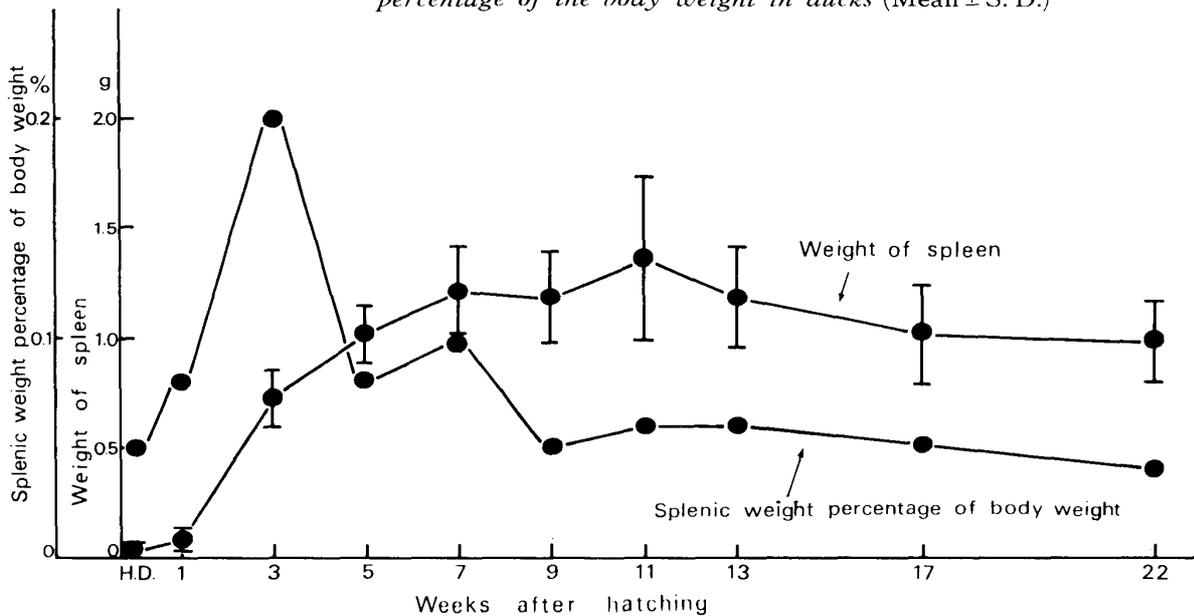
TABLE 1 *Postnatal growth of the spleen and the splenic weight percentage of the body weight in ducks*

| AGE IN WEEKS | BODY WEIGHT | SPLEEN | SPLENIC WEIGHT PERCENTAGE OF THE BODY WEIGHT |
|--------------|-------------------------|---------------------------|--|
| H. D.*(8) | 59.6 ± 4.5 ^g | 0.03 ± 0.004 ^g | 0.05 |
| 1 (5) | 103.8 ± 26.9 | 0.08 ± 0.05 | 0.08 |
| 3 (5) | 359.0 ± 64.7 | 0.73 ± 0.13 | 0.20 |
| 5 (8) | 1335.0 ± 117.8 | 1.01 ± 0.14 | 0.08 |
| 7 (7) | 1260.0 ± 238.0 | 1.21 ± 0.20 | 0.10 |
| 9 (7) | 2194.0 ± 263.1 | 1.18 ± 0.21 | 0.05 |
| 11 (6) | 2300.0 ± 291.2 | 1.36 ± 0.38 | 0.06 |
| 13 (5) | 2066.0 ± 366.0 | 1.18 ± 0.23 | 0.06 |
| 17 (6) | 2150.0 ± 273.3 | 1.01 ± 0.23 | 0.05 |
| 22 (4) | 2315.0 ± 179.4 | 0.98 ± 0.18 | 0.04 |

Mean Weight (g) ± Standard deviation

*: Hatching day, (): Number of ducks

TEXT FIGURE 1 *Postnatal growth of the spleen and the splenic weight percentage of the body weight in ducks (Mean ± S. D.)*



are shown in table 1 and text figure 1. Through postnatal life, the spleen, as a whole, varied less in weight than central lymphoid organs, such as the thymus and the bursa of Fabricius (HASHIMOTO & SUGIMURA⁶, 1976). During the first several weeks of post-hatching life, the spleen grew rapidly, and at the 3rd week, its weight as a percentage of the body weight reached its maximum, while the absolute weight showed a successive increase up to the 11th week in spite of a constant decrease of its relative value to the body weight. Beyond this age group, the spleen appeared to reach a stable weight, although a slight decrease in weight was observed from the 13th week of age.

2 Histology of the splenic elements

The splenic constituents were divided into three elements: the white pulp (WP); the red pulp (RP); and the trabecular tissue (TR). The white pulp was subdivided into four elements: the periarterial lymphoid tissue (PALT); perivenous lymphoid tissue (PVLТ); periellipsoidal lymphoid tissue (PELT); and the germinal center (GC).

1) PALT and PVLТ: Both lymphoid tissues consisted mainly of small lymphocytes neighbouring the arterioles or the veins and were distinguished from each other by the accompanying blood vessels, though the two elements were almost similar in their cytology. The PALT were localized in close relation to the central arteries, whereas the PVLТ were seen in close proximity to the collecting or trabecular veins (figs. 1 & 2). In aged ducks, germinal centers were occasionally found in these areas (figs. 11 & 12).

2) PELT: This area was the most voluminous in all of the white pulp elements. In the 3 week-old and older ducks, it was composed of several layers of medium or large-sized lymphocytes aggregated as cellular sheaths around the ellipsoidal tissue of the sheathed capillaries. No small lymphocytes were found in this area, however (fig. 3). When the India ink was injected into the blood stream, many ink particles were noted firstly within the ellipsoidal tissue, and then showed a tendency to move into the PELT to pass gradually through into the neighbouring red pulp (fig. 9).

3) GC: Splenic distribution of germinal centers occurred in close relation to the vessels exclusively within the PALT (fig. 11) or PVLТ (fig. 12). They consisted of a variable population of pyroninophilic lymphoid cells and reticular cells suggestive of macrophages. The germinal centers in the PALT were clearly encapsulated with thin reticular fibers, while the ones in the PVLТ were occasionally lacking in them.

4) RP: Among the reticular frameworks of this area, the cells in the plasma cell line were localized together with the blood cells (fig. 4). This line of cells were frequently detectable near the collecting or the trabecular veins, and occasionally in the venous lumen.

5) TR: The duck spleen included less developed trabecular tissue. Since large veins were located within them (fig. 2), these venous lumens were labeled a trabecular area in histoplanimetry.

3 Quantitative changes of splenic elements

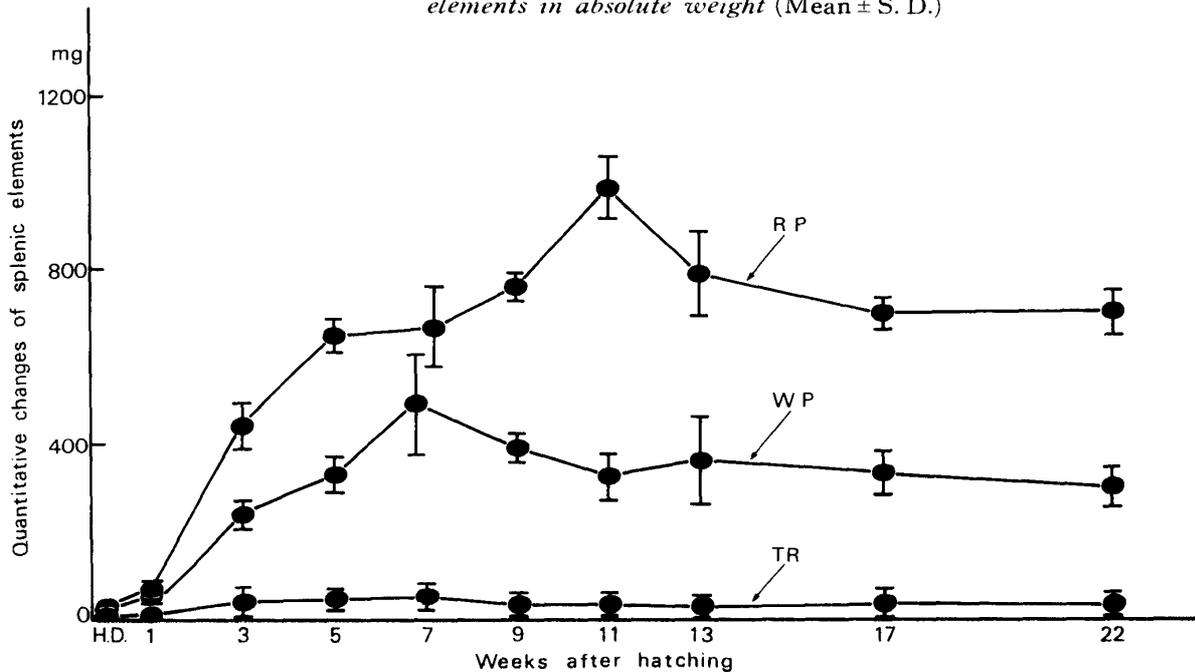
The postnatal changes of each splenic element are shown as a percentage in table 2, and as a weight in text figures 2 and 3.

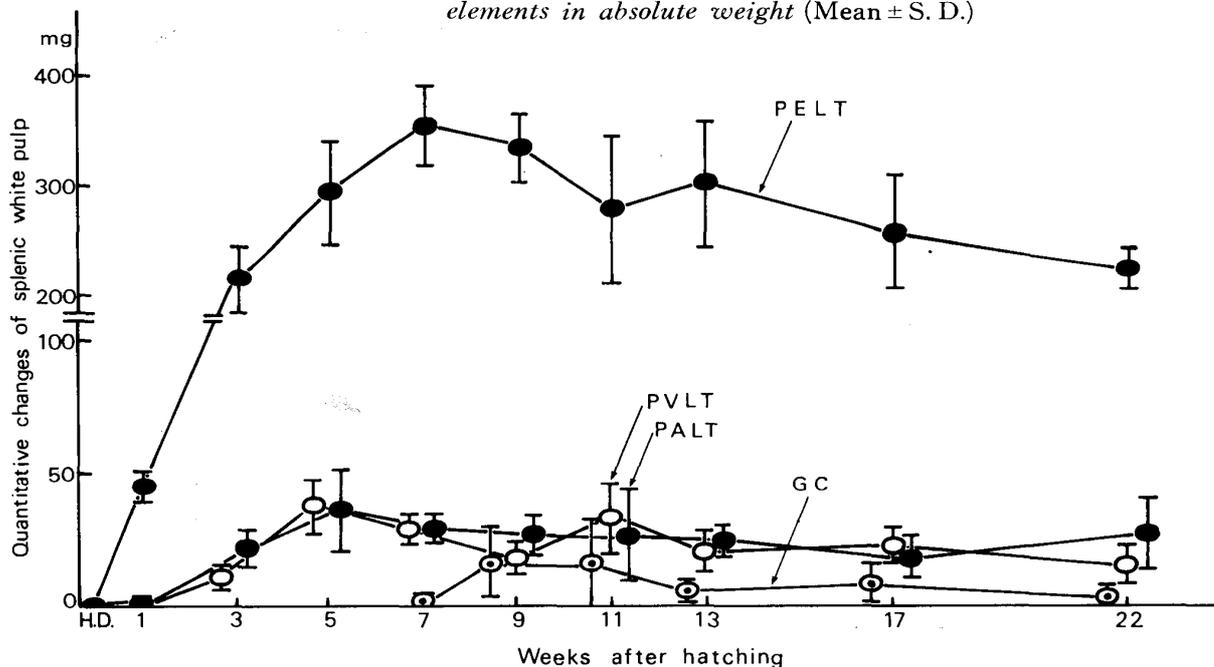
TABLE 2 Postnatal quantitative changes of duck splenic elements

| AGE IN WEEKS | PALT | PVLT | PELT | GC | RP | TR |
|--------------|---------------|---------------|---------------|-------------|----------------|---------------|
| H. D.* | 1.22 ± 0.50 % | 1.01 ± 0.34 % | 7.78 ± 3.40 % | 0 % | 86.33 ± 2.74 % | 3.66 ± 1.70 % |
| 1 | 1.02 ± 0.25 | 1.46 ± 0.59 | 35.90 ± 4.50 | 0 | 57.22 ± 3.70 | 4.40 ± 1.20 |
| 3 | 3.05 ± 0.93 | 1.49 ± 0.59 | 30.06 ± 4.76 | 0 | 60.24 ± 7.99 | 5.16 ± 2.20 |
| 5 | 3.67 ± 1.65 | 3.81 ± 1.08 | 29.63 ± 4.64 | 0 | 58.68 ± 3.91 | 4.20 ± 1.87 |
| 7 | 2.40 ± 0.46 | 2.35 ± 0.53 | 29.40 ± 3.05 | 0.04 ± 0.06 | 52.11 ± 10.20 | 3.70 ± 1.60 |
| 9 | 2.25 ± 0.81 | 1.54 ± 0.67 | 28.40 ± 2.64 | 1.42 ± 1.14 | 63.93 ± 1.89 | 2.46 ± 1.12 |
| 11 | 1.99 ± 2.02 | 2.45 ± 1.05 | 20.05 ± 5.05 | 1.20 ± 1.26 | 71.50 ± 5.74 | 2.34 ± 0.90 |
| 13 | 2.04 ± 0.50 | 1.74 ± 0.72 | 25.85 ± 4.90 | 0.48 ± 0.25 | 67.78 ± 9.44 | 2.10 ± 1.27 |
| 17 | 1.82 ± 0.94 | 2.22 ± 0.67 | 25.24 ± 4.94 | 0.79 ± 0.68 | 67.05 ± 3.46 | 2.88 ± 1.94 |
| 22 | 2.68 ± 1.50 | 1.54 ± 0.59 | 22.25 ± 2.09 | 0.30 ± 0.52 | 70.35 ± 5.29 | 2.88 ± 2.62 |

*: Hatching day, PALT: periarterial lymphoid tissue, PVLT: perivenous lymphoid tissue, PELT: periellipsoidal lymphoid tissue, GC: germinal center, RP: red pulp, TR: trabecula

TEXT FIGURE 2 Postnatal quantitative changes of duck splenic elements in absolute weight (Mean ± S. D.)



TEXT FIGURE 3 *Postnatal quantitative changes of white pulp elements in absolute weight (Mean \pm S. D.)*

1) Changes of splenic elements: As shown in text figure 2, the WP showed active growth for several posthatching weeks and reached its maximum at the 7th week. Beyond this week, in spite of a slight decrease at age 7th to 11th week, the WP reached the plateau level. This may continue throughout the life. The RP also grew rapidly, and its successive growth was maintained up to the 11th week. A decrease of RP occurred in quantity from this week onward, but the weight of this splenic element was maintained grossly in duplication of the WP at almost every week. On the other hand, the TR was seen as a considerably smaller splenic element.

2) Changes of WP elements

(1) PALT and PVL: On hatching day, both lymphoid elements were already noted but were small in quantity (figs. 5 & 6). At the first week, it appeared that the PVL area microscopically predominated over that of the PALT. From the 3rd week of posthatching life, the relative values tended to increase, showing slight variations as shown in table 2. At the 5th week the PALT and the PVL reached their maximum mean of percentages. Both elements, thereafter, showed a tendency to decrease in spite of some variations. These results indicate that the total area of the PALT and the PVL is less than 10% in the duck spleen, and occupies less than 1/5 of the PELT.

(2) PELT: Lymphoid cells in this element was scarcely found in sections on the hatching day, but ellipsoidal tissues were already noted (fig. 7). At the first week, several number of lymphoid cells were found in this area (fig. 8). A marked increase

TABLE 3 *Frequency of periellipsoidal lymphoid cells, plasma cells and germinal centers in the postnatal duck spleen*

| AGE IN WEEKS | PERIELLIPSOIDAL ² LYMPHOID CELLS | PLASMA CELLS ³ | GERMINAL CENTERS ⁴ | |
|--------------------|---|---------------------------|-------------------------------|----------------------------------|
| | | | in PALT | in PVLТ |
| H. D. ¹ | 2.1 ± 1.5 ⁵ | 12.4 ± 9.1 | 0 | 0 |
| 1 | 56.6 ± 9.2 | 26.8 ± 11.1 | 0 | 0 |
| 3 | 110.0 ± 21.7 | 49.7 ± 9.0 | 0 | 0 |
| 5 | 125.0 ± 10.7 | 124.8 ± 62.8 | 0 | 0 |
| 7 | 129.0 ± 12.0 | 107.8 ± 37.9 | 1.8 ± 2.4 (0- 7) | 0.2 ± 0.4 (0- 1) ⁶ |
| 9 | 137.0 ± 21.2 | 118.4 ± 29.1 | 17.6 ± 12.0 (1-34) | 4.4 ± 4.8 (0-13) |
| 11 | 142.0 ± 18.1 | 193.4 ± 52.3 | 30.7 ± 28.3 (2-76) | 2.8 ± 1.3 (1- 5) |
| 13 | 123.0 ± 13.9 | 117.0 ± 8.5 | 14.2 ± 13.8 (0-37) | 0.8 ± 1.2 (0- 3) |
| 17 | 113.0 ± 10.5 | 85.4 ± 13.4 | 21.6 ± 17.7 (2-43) | 1.0 ± 0.6 (0- 2) |
| 22 | 105.8 ± 14.3 | 162.3 ± 40.6 | 11.8 ± 20.4 (0-47) | 0.5 ± 0.9 (0- 2) |

1: Hatching day

2: Mean number in a crossly-sectioned PELT

3: Mean number estimated in $1.25 \times 10^5 \mu\text{m}^2$ of red pulp

4: Mean number per cross section

5: Mean number ± Standard deviation

6: Range of occurrence

of lymphoid cells was observed from the first week onward. These aggregations of lymphoid cells grew rapidly and made up the periellipsoidal sheaths, which consisted of 3~5 layers of cells from the 3rd week of age (figs. 9 & 10). As shown in table 3 and text figure 3, the active increase of periellipsoidal lymphoid cells terminated at the 5th to 7th week, and in quantitative respects, it seemed to become set in a grown up period. A slight decrease in the number of lymphoid cells was observed from the 13th week, and this was in accordance with the postnatal change of the splenic weight.

(3) GC: The initial germinal center formation was noted at the 7th week and showed a tendency to increase in number per section, although it was accompanied by a considerable variation. The germinal center could not always be found in the spleen of every age group from the 7th week onward, but it was noteworthy that the frequency of the germinal centers in the PALT was more than ten times that in the PVLТ.

(4) Plasma cells: On the hatching day, a considerable number of plasma cells was seen in the red pulp area in spite of almost no periellipsoidal lymphoid cells. The number of plasma cells, as well as the periellipsoidal lymphoid cells, showed a clear tendency

to increase with age; however, a possible difference was noted in their increasing pattern; it was relatively slow in the plasma cells, while the periellipsoidal lymphoid cells showed a remarkable increase from the first week onward, as shown in table 3. From the 5th week of age, the number of plasma cells reached its plateau level and tended to develop along with a slight tendency to decrease in number at the 13th and 17th week.

DISCUSSION

The present result shows that the postnatal changes of duck spleen weight basically coincide with that of the chicken (NORTON & WOLFE, 1949), and that they grow parallel with the growth of body weight in spite of the occurrence of age involution in the thymus and the bursa of Fabricius from the 9 week onward (HASHIMOTO & SUGIMURA⁹, 1976).

A nodular aggregation of lymphocytes in the circumscribing areas of the central arteries or small veins of the duck spleen has already noted on the hatching day, and this is clearly earlier than that discovered in the chick spleen, where the initial appearance of the PALT was found as groups of small lymphocytes associated with arterioles 24 hours after hatching (HOSHI, 1972). This developmental difference of the lymphoid tissues may be responsible for the longer incubation period of ducks -28 days for embryonic life.

The PALT and the PVLТ have been referred to as the thymus-dependent element of white pulp in chick spleen (NAGY & FEHÉR, 1972; HOSHI & MORI, 1973), while the duck PALT has been designated as thymus-dependent, and the PVLТ as thymus- and bursa-independent, respectively (SUGIMURA & HASHIMOTO¹⁹, 1976). The present result of histometry shows almost no differences in their development curves during posthatching life; however, a remarkably high number of germinal centers in the PALT, morphological diversity between the two types of germinal centers, and the possible need for T-lymphocytes in the germinal center formation, as well as the chick spleen (HOSHI & MORI, 1973; TOIVANEN & TOIVANEN, 1977), suggest that the thymus-dependent number of lymphocytes is much less in the PVLТ than in the PALT. The PVLТ should be designated as a proper lymphoid element of the duck spleen throughout its life, but the actual function of this lymphoid tissue remains unclear at the present time, and further studies on its origin and immunological functions are needed.

In the chick spleen it has been demonstrated that the cells of PELТ and the plasma cell series belong to the clear bursa-dependent system, as well as to the germinal centers (COOPER et al., 1966), while in the spleen of bursectomized ducks, there are no PELТ, but an almost normal number of plasma cell series (SUGIMURA & HASHIMOTO²⁰, 1976). Despite the suggestion by ANDERSON (1973) that cellular transformation takes place from the lymphoid cells in the PELТ to the plasma cells, a discrepancy of the developmental curves of both cellular populations and the appearance of plasma cells earlier than the

lymphoid cells in the PELT show the tenuous developmental relationship between them. The true origin of the plasma cell series of duck spleen must be ascertained by further ontogenic studies.

In past studies with splenectomized chickens (WOLFE et al., 1950; WOLFE & LINK, 1961; ROSENQUIST & WOLFE, 1962) where there was a delayed antibody response and a later peak titer, and where death occurred because of severe inability to combat an infection by *Plasmodium lophurae* (LONGENECKER et al., 1966), it was believed that a greater number of immunologically competent cells were present in the spleen. Antibody synthesis and distribution of immunoglobulin-containing cells were demonstrated in the chick spleen by immunohistochemical methods (WHITE et al., 1967; FRENCH et al., 1969; KINCADE & COOPER, 1971). In the duck spleen as well, after injection of horseradish peroxidase (HRP) with adjuvant, it was demonstrated that the plasma cells and the large lymphoid cells in the germinal centers were anti-HRP antibody-producing cells (HASHIMOTO & SUGIMURA⁸⁾, 1976). It has been reported, however, that the ability of splenic cellular populations to antibody production may vary with the age of the birds, and that birds younger than 4~5 weeks have been found to be less effective producers of antibodies than adult ones of the same species (NORTON et al., 1950; RODAK et al., 1969). This poor and variable response in the juvenile birds may be explained by the slower appearance and smaller quantity of PELT from hatching day to the 3rd posthatching week, although the present result on the PELT seemed to appear somewhat earlier than in the chicken. Concerning the antigen localization of this area, WHITE et al. (1970) demonstrated the presence of antigenic materials at the periphery of the chick ellipsoids. The detection of India ink particles injected into the blood vessel only within the ellipsoidal tissue and the PELT suggests a possibility that the periellipsoidal lymphoid cells of the duck spleen also show characteristic antigen susceptibility in the course of their immunological response in spleens. Therefore, it seems likely that a poor or nil response to antigenic stimulations in young birds may be due to a complete or almost complete absence of periellipsoidal lymphoid cells. This may be the same in the bursectomized ducks which show a complete absence of periellipsoidal lymphoid cells simultaneously in spite of a normal population of plasma cells in the red pulp (HASHIMOTO & SUGIMURA⁷⁾, 1976; SUGIMURA & HASHIMOTO²⁰⁾ 1976).

From the quantitative and functional results found, the PELT is considered to be a principal element of the white pulp as well as of the immune response in duck spleen. Further studies are necessary to ascertain the actual function of the element.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 The PALT in the spleen of a 13 week-old duck
The PALT, consisting mainly of small lymphocytes, can be seen around and close to the central arteries (CA).
Hematoxylin-Eosin × 250
- Fig. 2 The PVLТ in the spleen of a 13 week-old duck
The PVLТ packed with numerous small lymphocytes localized in the periphery of the trabecular vein (TR. V).
Hematoxylin-Eosin × 250
- Fig. 3 The PELT in the spleen of a 7 week-old duck
Well developed PELT, which are composed of several layers of medium or large-sized lymphocytes, are clearly noted around the ellipsoids.
Hematoxylin-Eosin × 500
- Fig. 4 Plasma cells in the spleen of an 11 week-old duck
Numerous pyroninophilic cells suggestive mainly of plasma cell series are found in the red pulp area.
Methyl green-Pyronine × 500

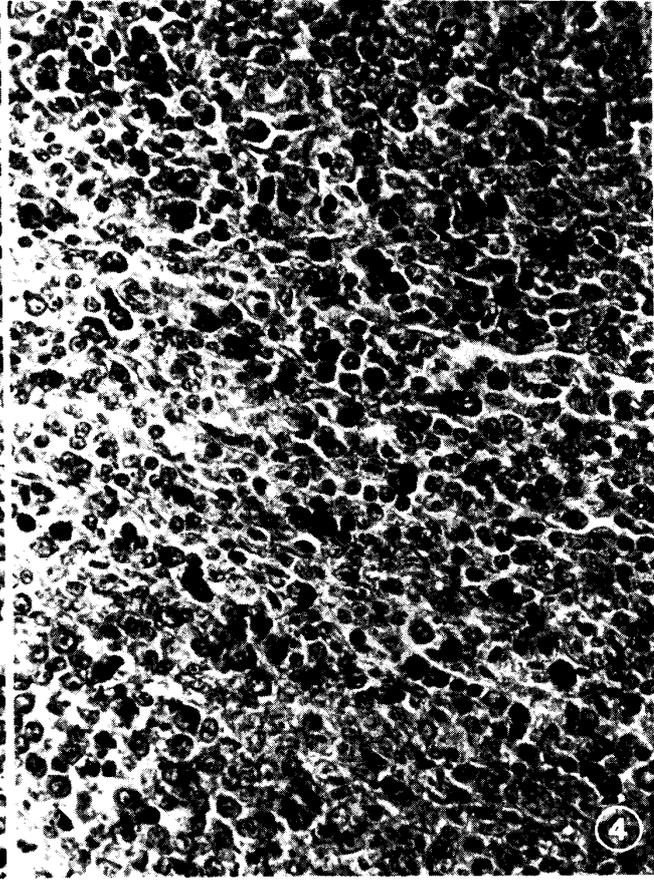
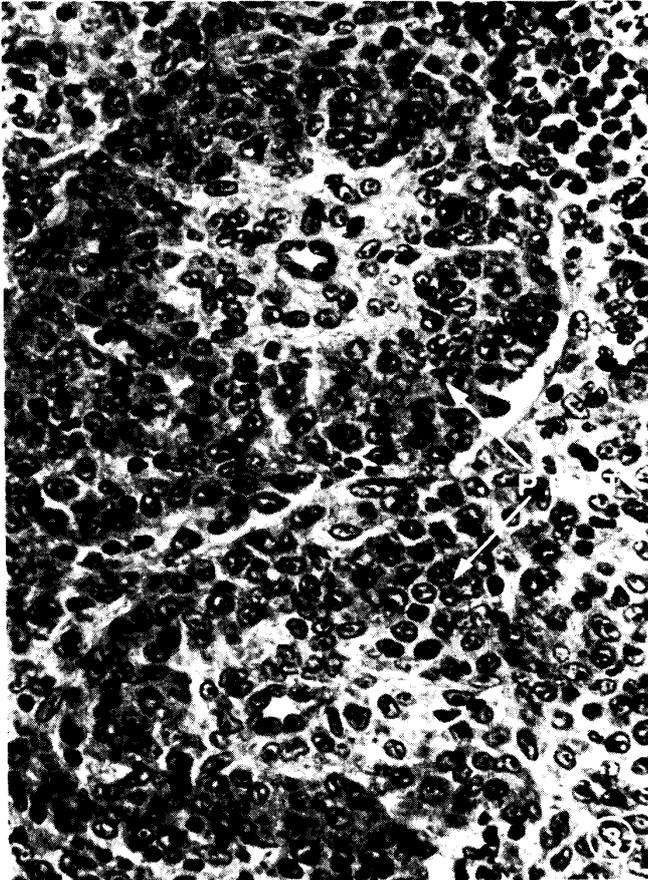


PLATE II

- Fig. 5 Early PALT in the spleen of a day-old duck
Cellular aggregation of small lymphocytes suggestive of PALT is clearly found in the circumscribing region of the central artery.
Hematoxylin-Eosin × 500
- Fig. 6 Early PVLТ in the spleen of a day-old duck
In close relation to the trabecular vein, a small mass of lymphoid cells can be seen; this is clearly discernible as an PVLТ.
Hematoxylin-Eosin × 500
- Fig. 7 Early ellipsoid in the spleen of a day-old duck
An ellipsoidal tissue is clearly noted around a sheathed capillary, but almost no lymphoid cells can be seen in the peripheral area at this time.
Hematoxylin-Eosin × 500
- Fig. 8 Early PELТ in the spleen of a week-old duck
A few slightly pyroninophilic lymphoid cells are localized around the ellipsoidal tissue.
Methyl green-Pyronine × 500

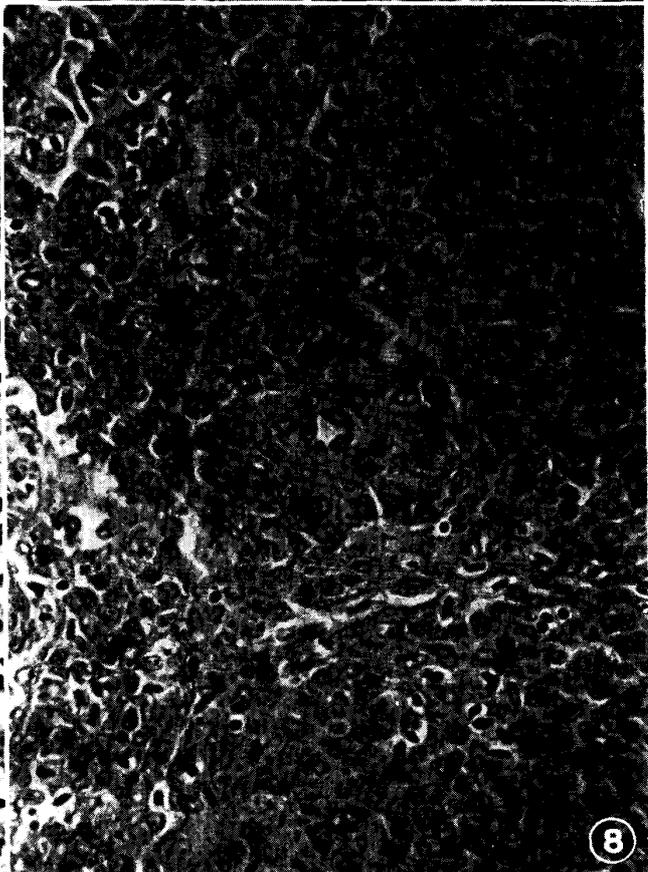
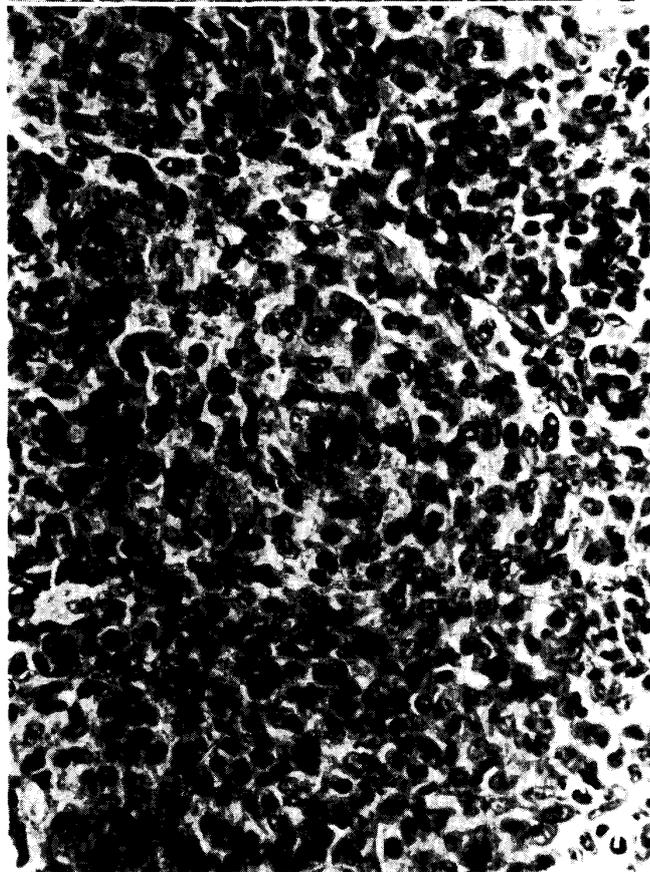


PLATE III

- Fig. 9 The PELT in the spleen of a 3 week-old duck
Numerous ink particles are found in the ellipsoid and periel-
lipsoidal lymphoid tissue area, and some are detectable among
the cells in the red pulp.
Hematoxylin-Eosin × 500
- Fig. 10 The PELT in the spleen of a 7 week-old duck
Completely developed PELT encapsulates the ellipsoidal tissue,
which is accompanied by a vertically sectioned sheathed capillary.
Hematoxylin-Eosin × 500
- Fig. 11 The germinal center occurred in the PALT of the spleen of a
17 week-old duck
A germinal center is seen in close relation to the central arteries.
This type of germinal center is usually circumscribed with thin
layers of reticular fiber, and lymphoid cells in variable size and
macrophages, including tingible body-like substances, are found
in the germinal center region.
Hematoxylin-Eosin × 500
- Fig. 12 The germinal center occurred in the spleen of the PVLV of a
9 week-old duck
Large lymphoid populations of cells are noted along the collec-
ting or trabecular vein. In many case, this type of germinal
center has no capsule around it, and there are few in number.
Hematoxylin-Eosin × 250

