Morphologic and Developmental Differences between the Cells of the Chicken's Thymus and Bursa of Fabricius

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The chicken has two distinct gut-associated lymphoid organs, the thymus and the bursa of Fabricius (fig. 1). Although these organs lie at opposite ends of the chicken's gastrointestinal tract, they have certain similarities that have prompted investigators to consider them together.1,2 The lobules of lymphoid tissue that characterize both of these organs are referred to as "lymphoepithelial" because they appear to arise from the epithelial lining of the gut. Microscopically, the lobules of both thymus and bursa consist of a cortex of dark staining lymphocytes and a medulla of large reticular cells (figs. 2A and B). These similarities of the thymus and bursa did not take on real biologic significance, however, until Glick discovered a functional role of the bursa.3 He demonstrated that bursectomy early in life interferes with the chicken's later capacity to form circulating antibody. Neonatal thymectomy, by contrast, does not affect the chicken's capacity to produce circulating antibody, although it may have an effect on the development of cellular hypersensitivity.4,5 The bursa and thymus also appear to have different roles in the development of a virus-induced lymphoma called visceral lymphomatosis. Bursectomy, but not thymectomy, will prevent the development of the malignancy if performed during the first 2 months of life in birds inoculated with oncogenic virus at hatching.6

The data to be presented suggest that the development of the chicken thymus is characterized by early maturation of its lymphoid cell population, occurring before hatching for the most part, whereas the maturation of the lymphoid cells of the bursa begins later, is slower, and continues well beyond hatching. Furthermore, even at its developmental peak, the bursa's lymphocyte population differs from that of the thymus. The stage of differentiation of thymus and bursa cells was characterized by their size—a superficial, but judging from other studies, a reasonable index of differentiation. Auerbach and Ball have demonstrated that the mouse thymocytes change from large to small cells during their maturation and that this size change provides a useful index of differentiation.7,8 Our studies with the
Fig. 1.—Thymus and bursa from a newborn chicken. The thymus (T) consists of several lobes attached to each carotid artery. The bursa (B) is a single outpouching of the dorsal cloacal wall.

Mouse thymus confirmed the observation of Auerbach and Ball and encouraged the application of their technic to the chicken's lymphoid organs. The study of the cells of the chicken's thymus and bursa using the same methodology is particularly interesting, for the mouse thymus, like the chicken bursa, is intimately involved both with immunogenesis and the development of lymphocytic malignancies.

MATERIALS AND METHODS

Fertile White Leghorn chicken eggs were obtained from a local hatchery and incubated at 37 C. in a standard egg incubator. Eight to 12 eggs were removed for histologic studies at daily intervals between the tenth and twenty-first day of incubation. Chickens hatching on the twenty-first day of incubation were fed standard feed and sacrificed at various ages ranging from 1 day to 4 months. The thymus and bursa were removed from each chicken and prepared for both microscopic examination and for analysis of the size distribution of the organ's cell population. Material for microscopic examination was fixed in 10 per cent formalin and stained with hematoxylin-eosin. The method of Auerbach and Ball was used to determine the size distribution of the cells.
Fig. 2A.—Microscopic section through a 4-month-old chicken thymus. The dark staining cortex is composed of small lymphocytes, the medulla of large, pale staining epithelial-stromal cells.

Fig. 2B.—Microscopic section through a 4-month-old chicken bursa. Follicles of lymphoid tissue fill the bursal walls. The follicles consist of a cortex of lymphocytes, a medulla of pale cells: they superficially resemble the thymic lobules.
This consisted of cutting the tissues into small pieces and dispersing them in filtered 0.15 M sodium chloride containing 10 per cent horse serum. The tissue fragments were then agitated for 30 minutes at room temperature with a magnetic stirrer. Periods of agitation up to 2 hours resulted in only a 1 per cent increase over the number of cells released after 30 minutes and therefore the 30-minute period was uniformly used. An aliquot of the cell suspension containing approximately 100,000 cells per ml. was then assayed for cell size with an electronic Coulter counter and particle size distribution plotter. This machine determines the volume of individual cells and automatically records the number of cells of various sizes. The resultant size distributions are illustrated in this paper by plotting the logarithm of the volume in cubic microns on the abscissa against the percentage of cells of a stated volume times the volume on the ordinate. Representative data only are presented as the cell size distributions were very consistent among birds of the same ages.

**Results**

**Thymus**

The thymus cells develop their lymphoid characteristics between 12 and 14 days of incubation and at that time are easily separated from the stromal tissue for cell size analysis. The general cell size can be estimated by microscopic examination of thin organ sections, but is more accurately represented by an analysis of the actual individual cell volumes. Figure 3A illustrates the microscopic appearance of thymic lymphocytes from a 15-day embryo and figure 3B the appearance of thymic cells from a 17-day-old embryo.
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The size distributions of approximately 250,000 cells from 15, 16 and 17 day embryo thymuses are seen in figure 3C. At 15 days, the modal cell volume is 122 μm³. Between the fifteenth and seventeenth days of incubation a progressive and consistent change occurs: the cells become smaller and by the seventeenth day the modal volume is 74 μm³. After 17 days of embryonation, no further change occurs in the thymic cell size. The thymic cell size distribution is the same at 4 months of age as it was prior to hatching. Auerbach has observed this same change in cell size.¹⁴

Bursa of Fabricius

The lymphoid tissue of the bursa first appears as clusters of cells along the endodermal crypts at approximately 14 days of incubation (fig. 4A). The clusters of cells later develop into the lymphoid follicles that characterize the fully developed bursa. The bursal lymphoid tissue retains its intimate association with the gut endoderm unlike the thymic tissue which separates from the gut wall early in its development. Microscopic examination reveals a predominance of large cells, both early in embryonation and throughout the life of the bursa (fig. 4B and C). The agitation procedure which dislodges thymic lymphocytes does not usually release lymphocytes from bursae of chickens younger than 6 weeks of age. In no instance have bursal cells been released from the bursal stroma of embryos, and in only 1 out of 20 attempts were bursal lymphocytes from a newly hatched chicken obtained for analysis by this means. Analysis of these dispersed cell popula-
Fig. 3C.—Size distribution of chicken thymus cells at various times during incubation. The thymus cell population at 15 days of incubation is considerably larger than at 17 days. At 16 days an intermediate size population is present.

The chicken thymus develops from foregut endoderm and becomes a lymphoid organ by 14 days of embryonation. The earliest lymphocytes are large cells, but by 17 days of embryonation, a further differentiation has occurred to smaller cells. The small cell population present at 17 days of incubation characterizes the thymic cell size for at least the first 4 months of the chicken's life. The bursa, by contrast, develops fully only after hatching and its lymphocytes remain large for the duration of the bursa's existence. Two distinct differences are thus demonstrated between the lymphoid cells of the thymus and bursa, the embryologic time of development of the cells and their size. The size of the lymphocytes is one indication of their stage of differentiation, the larger cells being less differentiated than the smaller cells. The failure of the bursal lymphocytes to dislodge easily from the stromal elements may also be a manifestation of a developmental immaturity. Ackerman has demonstrated that desmosomes connect the bursal epithelial cells, both with one another and with immature forms of lympho-
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Fig. 4A.—Fourteen-day embryo bursa. Clusters of cells along endodermal lining of bursa (arrows) denote first anlage of lymphoid follicles.

Fig. 4B.—One-day-old bursa. The large lymphocytes cannot be dislodged from the stromal elements by gentle shaking as can the thymic lymphocytes.
Fig. 4C.—Bursa from a 4-month-old chicken. The lymphoid follicles remain in intimate association with the gut epithelium. Large lymphocytes predominate.

Fig. 5A.—Three-month-old thymus. Small lymphocytes predominate.
Fig. 5B.—Three-month-old bursa. Large lymphocytes predominate.

Fig. 5C.—Size distribution of thymus and bursa cells from a 3-month-old chicken. The bursa contains a population of larger cells whereas the thymus cells are relatively small.
cytes. Although desmosomes are not seen on more mature bursal lymphocytes, these cells may adhere to one another until still later in their differentiation and thus not freely suspend themselves in the fluid media used for size analysis. Cell size and adhesiveness are thus two useful parameters that reflect differentiation.

The morphologic and developmental differences between the chicken’s thymus and bursa may have been obvious to the many investigators who have studied these organs, but these differences now deserve special emphasis for they may be clues to understanding the apparent functional differences between the thymus and bursa disclosed by the ablation experiments. If the thymus has passed through its major developmental stages prior to hatching time as the data suggest, it may also have exerted its major immunologic function prior to that time. It would be difficult, therefore, to detect a functional abnormality by performing a thymectomy after hatching. The prevention of visceral lymphomatosis by bursectomy, but not by thymectomy, might also be explained by these differences. Considerable evidence suggests that cells are susceptible to oncogenic viruses only during a critical stage in their differentiation. The bursa may contain the susceptible lymphoid cells, whereas the thymic lymphocytes, being more mature by the time of hatching, are no longer susceptible to a virus injected during the newborn period. If the chicken were infected with lymphoma-inducing virus early in embryonation, the thymic cells might be involved and then neonatal bursectomy alone would not prevent the disease.

The functional differences between thymus and bursa may thus be accounted for by the observed differences in the embryologic time of development and the degree of maturation of the lymphocyte population of the two organs.

An alternative explanation for the apparent functional differences of thymus and bursa might be that the cells of the two organs are basically different. Thymic lymphocytes may be differentiating into one type of cell, bursal lymphocytes into another. Until better methods are devised to study these cells and their progeny, this possibility will remain.

SUMMARY

Apparent functional differences between the chicken’s thymus and bursa, two lymphoepithelial organs considered similar in many ways, have prompted an evaluation of the morphologic and developmental differences between the cells of these two organs. Thymic lymphocytes are well developed prior to the time of hatching, whereas bursal lymphocytes remain large and possibly relatively immature for the duration of the bursa’s existence. These differences may provide a reasonable explanation for the functional differences observed between the bursa and thymus.

SUMMARIO IN INTERLINGUA

Apparente differentias functional inter le thymo e le bursa del gallina—le quales es duo organos lymphoepithelial considerate como simile in numerose
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respectos—ha inspire un evalutation del differentias inter le cellulas del duo organos con respecto a lor morphologia e lor desenvolvimento. Le lymphocytos del thymo es ben desenvollpate ante le tempore del exition ab le ovo, durante que le lymphocytos del bursa remane grande e forsan relativemente immatur durante le intege periodo del existentia de ille structura. Il pare probable que iste differentias suffice a explicar le differentias functional inter le bursa e le thymo.

ADDENDUM

Since completion of this manuscript, Cooper et al.\textsuperscript{19} have defined further the two lymphoid cell populations in the chicken, one bursa-dependent and the other thymus-dependent. The bursa-dependent system produces the immunoglobulins and is represented morphologically by the larger, slightly pyroninophilic lymphocytes, as seen in the germinal centers, and by plasma cells. The small lymphocytes of the white pulp are the typical morphologic expression of the thymus-dependent system in the chicken. This is the effector system in "cellular" immunity, and apparently also the recognition-information component of specific antibody production.

REFERENCES

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