THE ROLE of the thymus in the development of immunologic capacity in mammals has become fairly clearly established in recent years. The relation between the thymus and immunologic phenomena, moreover, appears to hold for vertebrates in general with the exception of some lower cyclostomata. In birds, however, two organs—the thymus and the bursa of Fabricius—have both been implicated in the development of immune competence; it has been suggested, although not clearly established, that the two organs perform two distinct functions, the thymus being responsible for cell-bound immunity such as the homograft reaction and the bursa being involved in the development of the ability to produce humoral antibodies.

Studies by Ball have demonstrated that the differentiation of mouse embryonic thymus rudiments can be described in quantitative terms by the use of a Coulter Counter and Cell Analyzer. This method takes advantage of the fact that the mature small lymphocyte is considerably smaller in volume than the large lymphocytes and lymphoblast cells from which they originate. Recently this method was applied by Peterson and Good to briefly describe chicken thymus and bursa development, but their utilization of cells pooled from different thymic lobes and their inability to obtain embryonic bursal cell suspensions rather limit the usefulness of that study.

In the present investigation the methods of Ball, refined by the development of critical technics of numerical analysis and appropriately modified for use with chicken embryonic material, have been applied to study the sequence of developmental events leading to chicken bursa and thymus morphogenesis. Preliminary results concerning the effects of 19-nortestosterone on bursa and thymus development are also presented.

MATERIALS AND METHODS

Methods used in this study are essentially those developed in our laboratory for study of mouse lymphoid systems. White leghorn chicks and fertilized chick eggs were obtained from a local supplier. Incubation of eggs was carried out at 37.5 C. Thymic lobes were dissected from the surrounding connective tissue, cut into small fragments, transferred to 10 x 75 mm. test tubes containing 2 ml. of a solution of 10 per cent horse serum in saline and agitated for 1 minute by means of a vortex mixer. Bursal cells were obtained by gently squeezing large fragments of bursal tissue in horse serum-saline solution followed by further...
dissociation using fine-bore orally controlled pipettes. The resultant suspensions were subsequently diluted and analyzed on a model B Coulter Counter set at amplitude 1, aperture current 1, gain 100, with an aperture opening of 100 μ. These settings were calibrated by the use of two sizes of pollen grains and two sizes of latex particles, as well as lymphocyte and red blood cell standards, to yield a unit volume of 3.5 cubic microns per division and 14 cubic microns per window. Raw data obtained from the plotter were analyzed by use of an IBM 1604 computer programmed to determine mean cell volume, mean volume of the major cell component and the normal curve best describing the data. All data were converted to per cent of total cell count to permit comparison, and the modal cell volume was also recorded. Suspensions were kept sufficiently dilute to alleviate the necessity for correcting raw data for coincidence, and diluting solutions were filtered to prevent significant background count.

Representative tissues were fixed in Bouin's solution, sectioned at 5–7 μ thickness and stained with eosin and hematoxylin.

19-nortestosterone solution was prepared by dissolving 1.27 g of hormone in 200 ml of corn oil. Injection into the yolk sac of chick eggs was performed as described by Meyer et al.

**RESULTS**

The lymphocyte population of the chick thymus undergoes characteristic changes between days 12 and 18 of development (Table 1). Thymus suspensions from days 12 and 13 indicate a wide range of large cells, but the method of preparation leads to sufficient debris and fragmentation of cells to render size distribution plots difficult to analyze. Separate analysis of the seven lobes of a single thymus anlage indicates that the development of the anterior lobes precedes that of more posterior ones. Thus, on day 12, lobe 1 (anterior) has a modal cell size of 105μ³, lobe 6 has a modal cell size of 133μ³, and lobe 7 has not yet developed to the point of yielding a recognizable cell peak. A major cell component can be readily detected at day 14, at which time the modal cell volume is 98μ³. In the next 4 days (the period of development described by Peterson and Good) a progressive reduction in cell size occurs so that at day 18 the modal cell volume has shifted to 63μ³. The overall shape of cell size distribution curves (Fig. 1) changes predictably during this period, reflecting transition from an immature to a mature cell population. While the modal cell volume is shifting from 98μ³ to 63μ³, a corresponding alteration of the mean cell volume from 133μ³ to 90μ³ is observed. The normal curve of best fit, centered around the modal cell size, becomes progressively narrowed as the percentage of cells close to the modal point increases.

The pattern of lymphoid cell change in the bursa also involves a reduction in cell volume during maturation (Table 1), the major shift occurring between the eighteenth day of embryonic development and a few days posthatching. Bursal cell suspensions from 16- and 17-day embryos indicate a wide range of large cells, but, as is the case for 12- to 13-day thymus suspensions, debris and fragmentation make quantitative analysis difficult. A major cell component can be detected at 18 days with a modal cell volume of 133μ³. During the next 3 weeks a progressive shift to smaller cells with a modal volume of 98μ³ is observed (Fig. 2). In this period, the mean cell volume shifts from 170μ³ to 100μ³, and the normal curve of best fit again narrows as the size of the modal peak increases in proportion to the total cell population.
CHICK THYMUS AND BURSA DEVELOPMENT

Table 1.—Change in Cell Size Distribution during Development of Chick Thymus and Bursa of Fabricius

<table>
<thead>
<tr>
<th>Age</th>
<th>Thymus</th>
<th>Bursa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Modal Peak</td>
<td>Modal Volume</td>
</tr>
<tr>
<td>10 to 11-day embryo</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>12 to 13-day embryo</td>
<td>10–8†</td>
<td>133–105†</td>
</tr>
<tr>
<td>14-day embryo</td>
<td>7.5</td>
<td>98</td>
</tr>
<tr>
<td>15-day embryo</td>
<td>7</td>
<td>91</td>
</tr>
<tr>
<td>16-day embryo</td>
<td>6.5</td>
<td>84</td>
</tr>
<tr>
<td>17-day embryo</td>
<td>5.5</td>
<td>70</td>
</tr>
<tr>
<td>18-day embryo</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>19-day embryo</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>3-week chick</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>6-week chick</td>
<td>7–7.5</td>
<td>91–98</td>
</tr>
</tbody>
</table>

*No clear modal cell population.
†Variable results (cf. text).
‡Lobes 2–5.

Histologic preparations from stages corresponding to those analyzed on the Coulter Counter were carefully examined. The histologic appearance shows qualitative changes wholly consistent with the quantitative data obtained with analysis of size distribution plots.

To test the usefulness of the assay procedure for experimental analysis, chick embryos were injected with varying amounts of 19-nortestosterone on days 7, 9 and 11 (Table 2). On day 19 the thymus and bursa were removed, examined by gross inspection, and analyzed on the Coulter Counter. As can be seen from Table 2, injection of small amounts of 19-nortestosterone were effective in producing some bursa-deficient embryos without apparent effect on thymus differentiation. On the other hand, doses sufficient to produce a high percentage of bursa-deficient embryos also affected the rate of thymus development.

For purposes of comparison, plots of mature chick bursa and thymus suspensions are shown in Figure 3, and plots of mature mouse thymus and lymph node cell suspension are shown in Figure 4. As expected from comparison of chicken and mouse cells in other systems, mouse thymus cells were found to be considerably larger than equivalent chick thymus cells. It is of special interest, however, that aside from species differences the size relationship between mature bursal and thymic cells in the chicken parallels that seen between mature lymph node and thymic cells in the mouse.

DISCUSSION

The methods developed by Ball7 for mouse thymus analysis have proved to be useful for critical description of morphogenetic events as well as events associated with regeneration and pathogenesis. The present studies demonstrate that analysis of chick thymus and bursa differentiation by these methods is feasible and can yield specific information on the effects of experimental treatment.
The effects of 19-nortestosterone on thymus development may serve as a specific example. Much confusion concerning the relative functions of bursa and thymus in the development of immunological capacities has resulted from the fact that 19-nortestosterone (or testosterone propionate) has been used by different investigators with different breeds of chickens injected at different times in varying amounts, the treatment being intended to produce "chemical bursectomy." Clearly, in such experiments critical information on thymus effects is needed, for the assumption that hormone treatment is specifically directed at producing bursal abnormalities, essential as it is for interpretation of results, is a tenuous one. The present experiments indicate that subtle changes of the thymus, as seen by a retardation of morphogenesis while easily overlooked by gross inspection or weighing of whole thymus organs, can be detected by use of quantitative size analysis.

It is necessary to draw attention to the considerable differences between our studies and those of Peterson and Good. Their description of thymus development corresponds in general terms to that presented here but it differs in timing, modal cell volume and detail of pattern. The differences in cell volume for a given stage could represent differences between embryos, but since both studies used similar incubation temperatures and similar breeds of chickens, this is an unlikely source for the observed differences. A more likely explanation may be found in differences in calibration of the Coulter Counter. Methods of calibration are still relatively inaccurate, depending on use of "sized" pollen grains and latex particles. Since pollen grains are usually much larger than the actual cells to be analyzed and since latex particles are usually much smaller, determination of volume in the significant size range is apt to be inadequate unless a large number of reference points are used. Comparison to standard cells such as red blood cells is generally more reliable but usually not made. In the present study, four reference points were used in obtaining initial calibration, and the calculated volume of mouse red blood cells obtained from plotter data agrees with published microscopic measurements. Unfortunately, Peterson and Good give no details concerning their mode of calibration.

Peterson and Good describe thymus differentiation as it occurs in a 3-day
period. In keeping with our own histologic preparations, differentiation of the thymus is a process requiring about 9 days, and while the major shifts do occur in a short period of time, lesser shifts can be found throughout the period of morphogenesis. Careful examination of thymic lobes, moreover, indicates that not all lobes of the thymus are at the same stage of differentiation at a given time.

Unfortunately, the data obtained by Peterson and Good for development of the bursa is not comparable, since they do not describe bursal cells in the embryo or newly hatched chick. When cells from these stages are examined with the plotter, it can be seen that the bursal cell population undergoes maturation in a manner analogous to that seen in the thymus. The starting cell is a larger one than that observed in the thymus, but the pattern of maturation, although it occurs in slightly older embryos, is quite similar and the ratio of cell volume of the immature to mature type is almost identical.

The conclusion drawn by Peterson and Good that the bursa has immature cells, whereas the thymus has mature ones, is thus not confirmed by our experimental evidence. It is true that the mature thymic lymphocyte is smaller than the mature bursal lymphocyte (c.f. Figure 3), but this finding is not unexpected since in the mouse the mature thymic lymphocyte is correspondingly smaller than the mature lymph node lymphocyte (c.f. Figure 4). As has been emphasized by Ruth, there may be at least two types of lymphocytes responsible for two distinct functions in the animal. If, in fact, this is true, the size differences observed between bursal and thymic lymphocytes in the chicken, as well as between lymph node and thymic lymphocytes in the mouse, may serve for their identification.
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SUMMARY

1. Critical size analysis of the lymphoid cell populations of the developing chick bursa of Fabricius and thymus indicates that characteristic shifts in cell size distribution, modal cell volume and mean cell volume occur during morphogenesis of these organs.

2. Comparison of bursa and thymus shows that while there are clear differences between the lymphoid cells of these two structures, the pattern of morphogenetic change is similar.

3. 19-nortestosterone injected in low doses can partially inhibit bursal development without affecting thymus morphogenesis. Depending on the age of the embryo at time of injection, however, doses sufficient to produce complete bursal inhibition may also retard thymic morphogenesis.

4. The size differences found between bursal and thymic lymphocytes in the chicken parallel the size differences found between lymph node and thymic lymphocytes in the mouse.

SUMMARIO IN INTERLINGUA

1. Le analyse del magnitude critic del populationes de cellulas lymphoide in le bursa de Fabricius e le thymo de gallinettas in stato de disveloppamento indica que caracteristic modificationes in le distribution del magnitude cellular, le modal volumine cellular, e le volumine cellular medie occurre durante le morphogenese del organos mentionate.
2. Un comparation del bursa con le thymo monstra que, ben que ii existe nette differentias inter le cellulas lymphoide de iste duo structuras, le configuration del modificationes morphogenetic es simile.

3. Le injection de basse doses de 19-nortestosterona pote inhibir partialmente le disveloppamento bursal sin afficer le morphogenese del thymo. Tamen, in dependentia del etate del embryon al tempore del injection, doses sufficiente a produced un complete inhibition bursal es etiam capace a retardar le morphogenese del thymo.

4. Le differentias in magnitude constatate inter lymphocytos bursal e lymphocytos thymic in le gallina es parallel al differentias in le magnitude trovate inter le lymphocytos de nodos lymphatic e del thymo in le mus.

ACKNOWLEDGMENTS

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REFERENCES


Quantitative Characterization of Chick Thymus and Bursa Development

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