Investigations on the Developing Chicken Bursa of Fabricius

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RECENT investigations on the bursa of Fabricius have led to the conclusion that this organ exerts some influence on the development of immunological responsiveness in birds by regulating the production of humoral antibodies (Glick *et al.*, 1956, 1964; Warner and Szenberg, 1964; Pierce *et al.*, 1966; Cooper *et al.*, 1967). Such a control operates through the production of immunologically competent cells which are able to migrate and differentiate. These processes are then followed by the onset of humoral immunologic response (Woods and Linna, 1965; Jaffe and Fechheimer, 1966; Moore and Owen, 1966). Moreover the bursa secretes a diffusible factor which acts on the trophism of lymphoid tissue (Glick, 1960; Jankovic and Leskovitz, 1965; St. Pierre and Ackermann, 1965).

Grossi *et al.*, (1967, 1968), Thorbecke *et al.* (1968) and Zaccheo *el al.* (1968) suggested another function for the bursa, that is the capacity to synthesize immunoglobulins. The immunoglobulin synthesis can be demonstrated in the late embryonic stages (the bursa produces γ M starting from the 18th day of incubation).* Then, after hatching the rate of immunoglobulin synthesis increases when both γ M and γ G are synthesized (Thorbecke *et al.*, 1968). Clawson *et al.* (1967), Cooper *et al.* (1967) and Zaccheo *et al.* (1968) have shown that the onset of immunoglobulin synthesis is related to the appearance of a differentiated lymphocytic cell type rich in polyribosomes, localized in the medulla of bursal follicles where immunoglobulins are detectable (Grossi *et al.*, 1968; Zaccheo *et al.*, 1968).

The main steps in bursal differentiation can be summarized as follows: 1) the lymphocytes appear on 14th–15th day of incubation (Ackerman, 1962; Ackerman and Knouff, 1959, 1964); 2) on 18th day of incubation, when γ M synthesis starts, a few large lymphocytes rich in polyribosomes can be detected (Thorbecke *et al.*, 1968; Zaccheo *et al.*, 1968); 3) during the first month after hatching, growth and differentiation of bursae occur at a very noticeable rate (Glick, 1956, 1960, 1964). Finally, at the end of this period, the bursa shows its characteristic structure and a remarkable decrease in the growth rate (Glick, 1956).

With the aim of investigating the phases of development and differentiation of the bursa of Fabricius we have determined the fresh and dry weight, total lipids, protein, DNA and RNA content during the first month at which time the organ undergoes its maximum growth and differentiation.

MATERIAL AND METHODS

Male and female White Leghorn chickens were used in these experiments. The bursae were examined at various developmental stages on the 1st, 5th, 10th, 15th, 20th, 25th and 30th day after hatching. For every stage the bursae from 5

* We used the immunoglobulin nomenclature proposed by WHO:19S γ globulin, γM; 7S γ globulin, γ G.

chickens were used. This series of experiments was repeated three times. The bursae were removed by careful dissection in order to free them completely from the surrounding connective tissue. Immediately after their removal, the bursae were weighed and dried at 100°C. until constant weight was reached.

Total lipids were measured by weighing after a triple extraction with 2:1 chloroform-methanol (v./v.), according to the method of Folch *et al.* (1957). This was followed by drying at 100°C. until constant weight (Manzoli and Gelli, 1968).

Proteins were measured according to the methods of Lowry *et al.* (1951).

Nucleic acids were determined according to the method of Munro and Fleck (1965).

RESULTS

Weight variations of the bursae were recorded from the 1st to the 30th day after hatching (Table 1). The mean fresh weight undergoes a considerable increase: from 48.8 mg. on the 1st day to 950.2 mg. on the 30th; the mean dry weight in the same period varies from 8.4 mg. to 177.6 mg. The growth rate is particularly rapid on 10th to 15th day; in fact during this period there is a considerable increase in dry weight (22.0 mg. to 57.8 mg. with a ratio of 2.6). An analogous trend was observed for the fresh weight (122.2 mg. to 322.6 mg. with the same ratio of 2.6).

The dry/fresh weight ratios do not change (Table 1). These data indicate constancy of the water content of the bursa during the thirty day period considered.

We have evaluated in the solid residues total lipids, proteins, DNA and RNA.

Total lipids increased from 1.7 mg. to 19.9 mg.; a particularly noticeable increase occurs on 15th to 20th day. However, the total lipids/dry weight ratio decreases from 21.1 to 11.2, showing that the growth of the bursa cannot be attributed to lipid storage (Fig. 1).

The protein content increased in a linear fashion, moving from 520.0 mg./g. on the 1st day to 780 mg./g. dry weight on the 30th day. Thus, the weight increase can be related to protein storage.

The nucleic acids determinations are given as mg./g. dry weight (Table 2).

The RNA values do not show any important variation during the whole period (38.9 to 42.5 mg./g. dry weight). The DNA values decrease from the 1st to the 10th day (63.7 to 48.6 mg./g. dry weight); however from the 10th to the 30th day a progressive increase of DNA values (48.6 to 71.3 mg./g. dry weight) is observed.

In each developmental stage, the DNA/RNA ratio is always greater than 1 (Table 2). This finding is characteristic for cell populations consisting primarily of lymphocytes.

The RNA/proteins ratio decreases from the 1st to the 30th day (0.74 to 0.50)

TABLE 1.—*Weight and total lipids values during first month after hatching*

Days	Fresh weight*	Dry weight*	Total lipids*	D.W./F.W.	T.L./D.W.
1	48.8±4	8.4±0.5	1.7±0.4	17.2	21.1
5	90.6±10	16.0±3	2.5±0.4	17.6	16.0
10	122.2±12	22.0±4	3.6±0.3	18.0	16.3
15	322.6±32	57.8±7	4.3±0.6	17.9	7.5
20	535.2±38	90.8±10	12.9±2	16.9	14.2
25	653.3±15	133.6±13	14.3±3	20.4	10.7
30	950.2±30	177.6±7	19.9±3	18.6	11.2

* Given as mg.

TABLE 2.—*Protein, RNA and DNA content of the bursa valued during the first month after hatching*

Days	Proteins*	RNA*	DNA*	DNA/RNA	RNA/Prot.	DNA/Prot.
1	520±25	38.9±3	63.7±3	1.6	0.74	1.22
5	560±20	41.2±5	51.7±1	1.2	0.73	0.92
10	630±20	42.6±3	48.6±6	1.1	0.67	0.77
15	670±10	40.1±4	58.2±6	1.4	0.59	0.86
20	700±30	40.6±6	60.5±6	1.4	0.58	0.86
25	730±15	46.8±5	63.3±3	1.3	0.64	0.86
30	780±20	42.5±2	71.3±6	1.6	0.54	0.91

* Given as mg./g. dry weight.

as does the DNA/proteins ratio; in the latter case a considerable decrease occurs especially from the 1st to 10th day (1.22 to 0.77).

DISCUSSION

The fresh weight determinations performed on the bursa of Fabricius during the first month after hatching (Fig. 1) demonstrate a very rapid growth of this organ. The growth is particularly noticeable in the White Leghorn strain which we used (Glick, 1956). The dry weight values and the fresh/dry weight ratio indicate that the weight increase depends on an actual dry mass increment.

The total lipids determinations are not consistent with the hypothesis of a weight increase due to lipid storage (Fig. 1), while the progressive and conspicuous rise of the protein content observed in the bursa of Fabricius can be related to a protein involvement in the organ growth processes. Furthermore, during this first month of development γ G synthesis is already detectable in the bursa (Grossi *et al.*, 1968).

The percentage diminution of DNA in the first 10 days after hatching (Fig. 2) is an expression of the great increase in protein production during this period. There is also indirect evidence for the percent decrease of DNA as expressed by the diminution of mitotic index (Prochazka *et al.*, 1967).

Contrary to what was expected on the basis of protein increase RNA values do

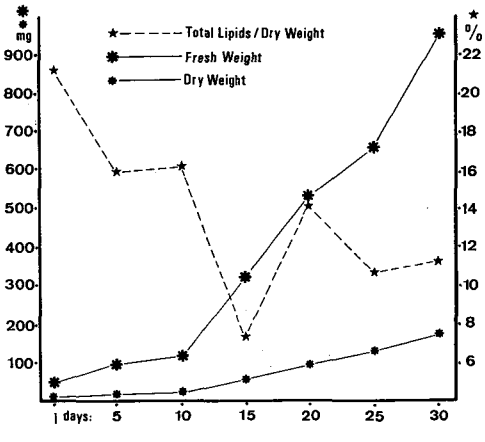

FIG. 1. Weight values during development of the bursa and their relationships with lipids content.

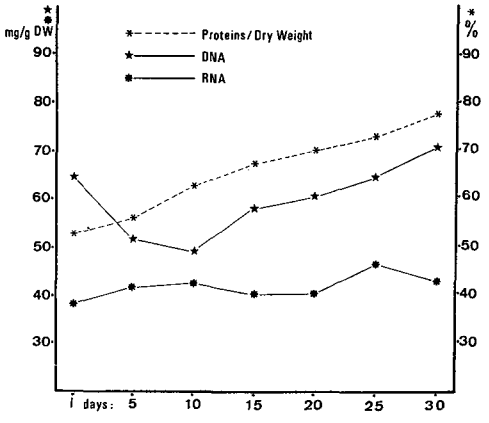

FIG. 2. Developmental pattern of RNA, DNA and proteins in the bursa of the chick.

not show significant variations. These data are not in agreement with the histochemical observations of basophilia changes in medullar follicle cells.

Perhaps this apparent disagreement is due to a rearrangement of lymphocyte RNA, resulting in the formation of polyribosomes from persistent free ribosomes. Recent electron microscope observations support this hypothesis by demonstrating that the bursal lymphocytes contain single ribosomes in early developmental stages (Zaccheo *et al.*, unpublished data).

However the ultracentrifugal analysis does not show significant differences of ribosomal pattern within the bursa until the 60th day after hatching (Stefoni and Facchini, 1969; Stefoni *et al.*, 1971). The lack of apparent changes in RNA values and in the physical state of ribosomes, together with the absence of variations in their protein composition, suggests that the activity of the bursa Fabricii has a constant feature.

SUMMARY

A study was carried out on the bursa of Fabricius during first month after hatching in White Leghorn chickens, which in this period have a very rapid growth of the bursa.

Fresh and dry weights, total lipid, protein, RNA and DNA content were valued.

The bursal dry mass appeared conspicuously raised mainly to protein increase.

During the first days after hatching a diminution of DNA content was noticed, parallel to a decrease in the mitotic activity of bursal lymphocytes. An increase of RNA was not observed.

The possible relationship between the high protein content and the onset of lymphocytic immunoglobulin synthesis is considered.

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Semen Production in Turkeys

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SEMEN production of turkey males is extremely important since most modern turkey hens are artificially inseminated. It has been well established that semen production of turkey males is influenced by several environmental factors.

Significant seasonal changes in semen production of naturally lighted Bronze turkeys were observed by Carson *et al.* (1955a, c). The average semen yield increased from maturity in October and November to a maximum in March and May with a decline in volume in June and July. In artificially lighted toms, seasonal changes in semen production and quality were noted by Law and Kosin (1958), Payne *et al.* (1960) and Harper and Ascott (1969). These seasonal changes may be the result of variation in temperature, lighting, age of birds and other environmental factors.

There has been limited research on lighting and semen production of turkey males. Marsden *et al.* (1962) noted that fertility and hatchability of eggs laid by