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Thymus and Bursal Ribosomes in the Developing Chicken

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ABSTRACT The ultracentrifugal analysis of thymus and bursal ribosomes in the developing chicken shows single ribosomes, dimers and polysomes in both organs. This pattern does not change until 60 days after hatching.

On the 100th day the thymus ribosomes remain unchanged whereas the bursal pattern shows only trace amounts of monomers and dimers. Possible relationships between the ribosomal patterns and the functions of the two organs have been discussed.

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THE thymus and the bursa Fabricii of birds are considered as immunologically competent central organs (Cooper *et al.*, 1966). Their function occurs through the production of cellular clones which participate directly in immunological responses (Papermaster and Good, 1962) and through the production of diffusible factors acting on lymphoid tissues (St. Pierre and Ackerman, 1965). Other functions have recently been ascertained, i.e. the bursa Fabricii synthesizes immunoglobulins without antibody specificity (Grossi *et al.* 1968; Thorbecke *et al.* 1968) and the thymus contains immunologically competent cellular clones which synthesize antibodies within the organ itself (unpublished observations).

The two organs examined have both lymphoid structure; however, with the electron microscope thymic lymphocytes predominantly show free ribosomes while in bursal lymphocytes polysomes are mostly detectable with a circular arrangement (rosettes) (Johnson *et al.*, 1966; Clawson *et al.*, 1967).

The structural and functional analogies of the two organs and the occurrence in both, particularly in the bursa Fabricii, of active protein syntheses promoted the present investigation of the characters of thymic and bursal ribosomes.

Because of the different developmental capacities of the two organs, the investigation has been performed starting from the 1st to the 100th day after hatching. The thymus is, in birds, a permanent organ while the bursa Fabricii undergoes

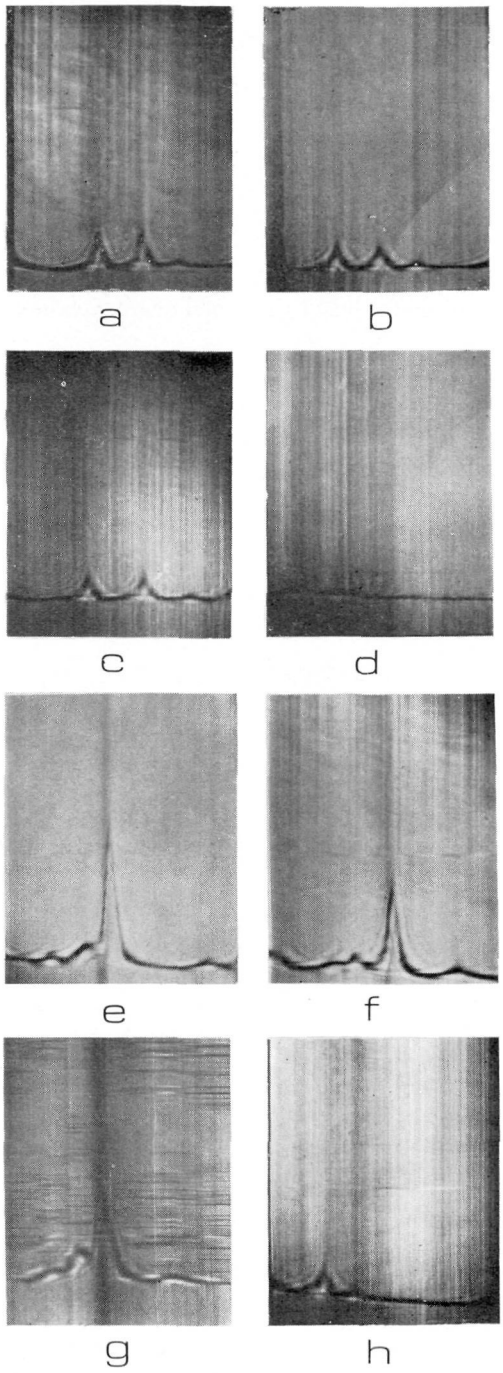

FIG. 1. (a–b) Sedimentation profile of thymus (a) and bursal (b) ribosomes from 1 day old chick in medium C. Photographs were taken after 9 minutes (thymus) and after 10 minutes (bursa) at 65,000 g. Sedimentation coefficients are left to right 67S, 102S, 123S (thymus) and 66S, 99S, 123S (bursa).

(c–d) Sedimentation profile of thymus (c) and bursal (d) ribosomes from 100 day chick in medium C. Photographs were taken 14 minutes (thymus) and after 10 minutes (bursa) at 65,000 g. Sedimentation coefficients are left to right 69S, 100S, 119S (thymus); the bursal pattern shows a flat line with a trace of two peaks (possibly 70S and 100S).

(e–f) Sedimentation profile of thymus (e) and bursal (f) ribosomes from 1 day old chick after 18 hours dialysis against buffer ($MgCl_2$ absent). Photographs were taken after 21 minutes (thymus) and 27 minutes (bursa) at 65,000 g. Sedimentation coefficients are left to right 27S, 40S, 55S, 86S (thymus) and 35S, 49S, 63S, 95S (bursa).

(g–h) Sedimentation profile of thymus (g) and bursal (h) ribosomes from 100 day old chick after 18 hours dialysis against buffer ($MgCl_2$ absent). Photographs were taken after 20 minutes (thymus) and after 6 minutes (bursa) at 65,000 g. Sedimentation coefficients are left to right 35S, 42S, 53S, 82S (thymus) and 60S, 89S (bursa).

All sedimentation coefficients are reported as $S^{\circ}_{w,20}$ values.

complete atrophy (Glick, 1954; Stefoni *et al.*, 1971).

MATERIAL AND METHODS

White Leghorn male chickens 1, 30, 60 and 100 day old were employed. Thymuses and bursae were taken in a cold room at 4°C. and homogenized in medium A (0.010 M-KCl, 0.005 M-$MgCl_2$, 0.001 M-tris-HCl at pH 7.6) containing bentonite 2% (Elson, 1959; Tal and Elson, 1963; Petermann and Pavlovec, 1967; Stefoni and Facchini, 1969). The homogenate was centrifuged for 10 minutes at 8,700 g at 4°C. (Phywe, mod. U 50 L ultracentrifuge) to remove nuclei, mitochondria and cell debris. The supernatant was treated with 2% DOC (Natrium deoxycholat) (4:1 v/v), centrifuged at 20,000 g for 5 minutes, layered on a double volume of medium B (0.25 M-sucrose, 0.010 M-KCl, 0.005 M-$MgCl_2$, 0.001 M-tris-

HCl at pH 7.6) and again centrifuged for three hours at 75,000 g. Pellets were washed in medium C (0.010 M-KCl, 0.005 M-$MgCl_2$, 0.001 M-tris-HCl at pH 7.6) and centrifuged again for 180 minutes at 75,000 g. Ribosomes were resuspended in medium C at 4°C. overnight. After the first analytical ultracentrifugation, the ribosomal fraction was dialyzed for 18 hours against the buffer 0.001 M-tris-HCl at pH 7.6 containing 0.010 M-KCl (Alberghina and Suskind, 1967) and examined by a second analytical ultracentrifugation.

RESULTS AND DISCUSSION

The sedimentation analyses of ribosomes (0.005 M-$MgCl_2$) show in the bursa and in the thymus three fractions whose sedimentation coefficient corresponds to that of single ribosomes, dimers and polysomes (Fig. 1, a–b). The sedimentation pattern is practically unchanged in the two organs 30 and 60 days after hatching. On the 100th day the thymus still shows the same three fractions, while the bursa has only minor traces of monomers and dimers (Fig. 1, c–d).

Sedimentation analyses after dialysis show, both in the thymus and the bursa on the 1st day, four components corresponding to three subunits and monomers (Fig. 1, e–f). This pattern of subunits does not change on the 30th and 60th day. On the 100th day it is possible to detect in the thymus the dissociation into three subunits and monomers, while bursal ribosomes show the presence of 60 S subunits and monomers (Fig. 1, g–h).

The ultracentrifugal analysis does not show significant differences in all the developmental stages examined, until 60 days after hatching. This applies to both ribosomal and subunit patterns. The lack of apparent changes in the physical state of the ribosomes together with the absence of variations in their protein composition (unpublished observations) confirm the functional analogies concerning the protein syntheses of thymus and bursa.

Some differences can be detected between the thymus and the bursa on the 100th day since ribosome and subunit pattern remain unchanged in the former while in the latter ribosomal aggregates and subunits disappear.

The subunits, as demonstrated by Nomura and Lowry (1967), Schlessinger *et al.* (1967), and Guthrie and Nomura (1968) participate in the formation of the initiation complex of protein synthesis. Thus, these changes could possibly be related to a decrease in protein synthesis (perhaps immunoglobulin) which occurs during the atrophy of the bursa.

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The Influence of Growth Rate on the Development of Marek's Disease in Chickens[1]

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ABSTRACT The response of chickens to the JM strain of Marek's disease virus was studied following directional selection for body growth rate. Comparisons were made for four pairs of selection lines, two pairs resulting from long term selection for growth rate and two pairs following a single generation of selection. In all cases, it was observed that the faster growing lines were more susceptible to the development of Marek's disease than their corresponding lines exhibiting a slower growth rate. These observations suggest that selection for rapid growth rate would result in increased susceptibility, while reverse selection would result in decreased susceptibility. This correlated response can, however, be influenced by the residual genotype present.

The results of IHA tests showed that the injected group had a significantly higher JM-antigen titer and incidence of Marek's disease compared with the naturally exposed groups, indicating the parallel relationship between the virus level and the incidence of tumor formation. The accompanying higher titer of antibody did not provide protection against neoplasia. It appeared to be produced in response to the antigen level, since antigen and antibody titers were found to be positively correlated.

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MAREK's disease is a neoplastic disease of the fowl caused by a transmissible DNA virus belonging to group B of the herpes viruses. It is characterized by lymphoid proliferation in the nervous system and other organs and by the formation of tumors in the viscera. Although the disease was described by Marek as early as 1907, fruitful research did not begin until after the Marek's disease transmission studies by Sevoian *et al.* (1962) and Biggs and Payne (1963). Recent studies of Marek's disease have centered around its pathogenesis, etiology, epizootiology, genetic control and immunolog-

[1] Contribution of the Massachusetts Experiment Station, Amherst, Massachusetts 01002.