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Thymosin-producing Cells of the Thymus

GOLDSTEIN *et al.*[1,2] have succeeded in isolating a hormone-like substance, thymosin, from the thymus which exhibits specific biological activity *in vivo* and *in vitro*[3,4]. It elicited lymphocytosis in neonatally thymectomized rats and significantly increased their growth rate and survival.

All the thymic extracts prepared so far by Goldstein *et al.* seem to be chemically pure and very active biologically. It was, however, not clear which of the different cell types present in the thymus, namely, thymocytes, epithelial reticulum, and mesenchymal reticulum cells, are the sites of production of thymosin. To answer this problem, experiments were performed using immunohistochemical methods.

Thymosin was obtained from bovine thymus tissue using the method of Goldstein *et al.*[3,5]. Five rabbits were immunized with thymosin in complete Freund's adjuvant (Difco) given in weekly injections for 4 months. The total amount of thymosin administered during the immunization period was 300 mg per rabbit. After 4 months, the presence of thymosin antibodies was tested in the blood serum of the rabbits by Ouchterlony's technique and immunoelectrophoresis (Fig. 1). For further assay, only the sera of the three rabbits which gave a precipitation arc with 1 mg ml^{-1} thymosin were used.

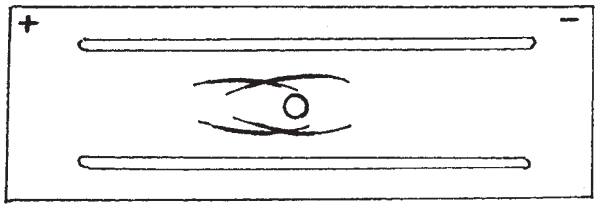

Fig. 1 Immunoelectrophoresis of thymosin. Purified thymosin (central well) shows alpha$_2$ mobility. Anti-thymosin immune serum (upper well) gives two precipitation arcs. Anti-thymosin IgG conjugated to Rhodamin B isothiocyanate (lower well) preserves its antibody nature.

For the direct immunohistochemical method, IgG was precipitated from the sera and conjugated to Rhodamin B isothiocyanate (Michrome). This stain seemed to be more suitable than the fluorescein isothiocyanate, as the latter yields a greenish-yellow fluorescence very similar to that exhibited by the autofluorescent cells of the thymus[6]. The labelled anti-thymosin IgG was applied to frozen thymus sections cut at 5 μm in a cryostat. After incubation for 30 min and washing, the slides were examined under a HBO 200 lamp using a combination of filters BG-12 and BG-3. As controls, the slides were treated with unlabelled anti-thymosin IgG (30 min) followed by rinsing and treatment with labelled anti-thymosin IgG (30 min) or with labelled IgG (30 min) obtained from a non-immunized rabbit.

In untreated sections, large cells with processes showing a greenish-yellow, coarsely granulated autofluorescence were well visible. The cells were arranged in bundles or in groups localized in a nest-like way in the region of the medulla, or on the border of the cortical and medullar layers.

In sections treated with anti-thymosin IgG, the number of fluorescent cells increased. In the cytoplasm of autofluorescent cells, in addition to coarse granules showing a greenish-yellow colour, fine granules displaying a rust red fluorescence were visible. Similar fluorescence was observed in the large non-autofluorescent cells as well. Staining of the same sections with haematoxylin-eosin and haematoxylin-PAS showed that Rhodamin B isothiocyanate was bound to the epithelial cells of the medullar layer. No fluorescence was exhibited by the thymocytes. In the control sections only the greenish-yellow autofluorescence was visible.

The results of our examination seem to be the first direct evidence that thymosin, a well identified chemically hormone-like substance, is produced by the epithelial cells of the thymus gland. As we know from earlier electron microscopic investigations[7–9] that the epithelial cells of the thymus are of glandular character, it is most probable that thymosin is not stored but produced by these cells.

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Origin and Renewal of Lymphocytes in Avian Embryo Thymuses studied in Interspecific Combinations

DIFFERENCES in the structure of the interphase nucleus between two species of birds, the japanese quail (*Coturnix coturnix japonica*) and the chick (*Gallus gallus*), may be used to distinguish cells of different origins in interspecies combinations as we have shown previously[1–3]. This method has been adapted to the study of certain problems in developmental biology. In the quail, an important part of the chromatin is condensed during interphase either in a single central mass or in several heterochromatic masses associated with the nucleolar RNA. In the chick the chromatin is dispersed in a fine network. When quail cells are transplanted into a chick embryo, or associated with chick tissues *in vitro*, the cells from each species retain their nuclear characteristics and can be identified in the chimaera. This is the case for the various cell types in the thymus: lymphocytes, reticular cells and connective elements, the origins of which can easily be distinguished after Feulgen and Rossenbeck staining. The reticular cells have large nuclei containing in the quail, one central prominent heterochromatic mass (Fig. 1*a*), and in the chick, a fine network of evenly dispersed chromatin (Fig. 1*b*). The same characteristics are found in the connective elements, except that their nuclei are smaller than those of reticular cells. In quail lymphocytes, each nucleus contains a large mass of DNA, irregular in shape and