Skin fibroblasts secreted the following types of GAG: hyaluronic acid (HA), chondroitin 4/6 sulfate (4/6-CS), dermatan sulfate (DS), heparan sulfate (HS) (fig.). The relative concentration of individual GAG in 7 day fibroblasts cultures reveals a prevalence of CS under the 3 experimental conditions (with serum, extract, serum + extract), but a HA prevalence in 14 day fibroblasts cultures. It was interesting to note that an accumulation of HA was detected in embryonic skin explants when serum was added[7], thus suggesting that the nutrient acts directly at the cellular level. Our experiments demonstrate that the pattern of GAG synthesis by embryonic fibroblasts is a result of a complex system of regulatory factors; on the one side by an age-dependent cell differentiation, and on the other by a modulatory effect of environmental factors.

The demonstration that environment acts on GAG production further supports the possible role of mesenchymal ground substance in regulating epithelial differentiation.

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2 To whom reprint requests should be addressed.
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Receptors for thymosin fraction V on rat thymic lymphocytes[1]

Renata Brelinska[2] and J.B. Warchoł[3]

Division of Clinical Morphology, University of Ulm, D-7900 Ulm (Federal Republic of Germany), 20 July 1981

Summary. Binding by rat thymus lymphocytes of thymosin V, labeled with colloidal gold, was studied. Under the experimental conditions employed at least 2.8% cells exhibited thymosin binding sites.

Peptides of fraction V of thymosin induce expression of T cell surface antigens (Thy, Tl, Ly)[4-6] and differentiation of presursor cells into T_1, and then T_2 immunologically competent cells[7].

Although the mechanism of action of these peptides is still controversial, data from several laboratories indicate that interaction of a target cell population with thymic peptides is mediated by cyclic AMP[8]. Until now, specific surface receptors for thymic peptides have been demonstrated on the following target cell populations; receptor on T cell lymphoblastoid lines for serum thymic factor (FTS)[9], receptors for thymus factor X (TFX, peptides from calf thymus) on rat thymocytes[10].

In this report we present evidence for surface receptors of rat thymic lymphocytes for colloidal gold-labeled peptides of thymosin fraction V.

Material and methods. Thymic lymphocytes were teased from thymuses of Wistar rats, aged 36 days. The suspension of lymphocytes was centrifuged at 2000 × g and washed in PBS. The cell suspension contained approximately 99% lymphocytes, of which 98% were viable, as evidenced by the trypan blue dye exclusion test. Washed cells were fixed with 2.5% glutaraldehyde in 0.5 M phosphate buffer, pH 7.3 for 15 min at 4 °C.

Colloidal gold was prepared by reducing chlorauric acid ($HAuCl_4ICN$, Merck) with trisodium citrate, according to

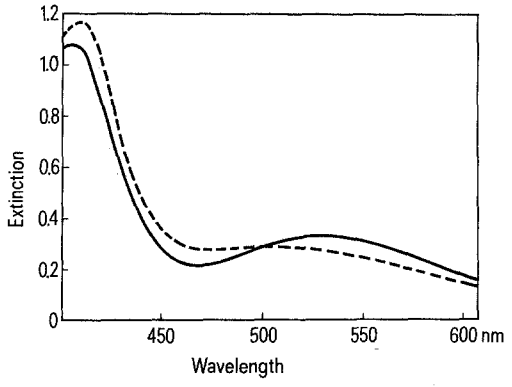

Figure 1. Demonstration of the thymosin-gold-binding sites at the plasma membrane of the thymic lymphocytes. × 33,800.

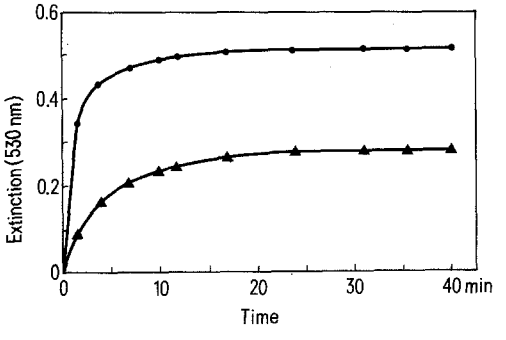

Figure 2. Thymosin-binding lymphocytes. T-Au complex covered some region of cell surface. × 10,000.

the method described by Georghegan and Ackerman[11]. The gold particles obtained were 20 nm in size. Thymosin was added directly to colloidal gold in a dose of 0.2 mg/ml, at pH 7.4. The gold-protein complex (T-Au) was centrifuged several times to wash the colloidal gold free of traces of nonadsorbed peptides and then was diluted in PBS. Thymic lymphocytes were incubated with T-Au for 60 min at room temperature. Further processing included washing, postfixation in 1% OsO_4, dehydration and embedding in resin according to Spurr, in a routine way. Ultrathin and semithin sections were contrasted in a routine way and examined in JEOL JEM 100 C electron microscope.

The main control reaction involved preincubation of the cells with a solution of thymosin (0.2 mg/ml) prior to incubation with T-Au. For other control reactions the following media for incubation of cells were used: bovine albumin-Au complex prepared in the same way as T-Au and a solution of uncoated colloidal gold.

Results. The site of interaction of thymosin peptides, labeled with colloidal gold, and the cell surface were localized by electron microscopy. The reaction product was detected as electron dense particles on the surface of rat thymic lymphocytes (fig. 1). Occasionally encountered non-lymphoid cells (red cells, macrophages, eosinophils) remained unlabeled. Only 2.8% cells were positive as counted in electron micrographs. The intensity and distribution pattern on the surface of the cells varied markedly. In some thymic lymphocytes gold particles covered only some region of their surface, in others the label in small patches covered a large fraction of cell membrane outline. Therefore, the real percentage of labeled cells may be higher and could be estimated exactly only on analysis of serial sections or by examining smears of labeled thymocytes. Lymphocytes with a narrow rim of cytoplasm, containing scanty organelles and medium sized nuclei, rich in condensed chromatin, were most intensely labeled (fig. 2). Lymphocytes with large nuclei and prevalent euchromatin were found to bind T-Au complexes only on part of their cell surface. Small numbers of T-Au grains were noted also on cell membranes of some dividing cells. The control reactions were negative throughout.

Discussion. The experiments showed that the lymphocyte surface labeling, observed in the EM, involved a specific reaction of binding thymosin peptide(s) by cell surface receptors. The reaction could be blocked by preincubating the cells with thymosin before incubation with T-Au complex. Lack of T-Au binding by erythrocytes as well as by other non-lymphoid cells in the thymus seems to exclude T-Au binding by putative free glutaraldehyde groups following glutaraldehyde fixation. Also, no labeling could be obtained when T-Au was substituted by albumin-Au or uncoated colloidal gold while similar labeling results were noted when formaldehyde-fixed or unfixed, cold treated thymocytes were labeled with T-Au. The labeled cells seem to represent a certain subpopulation of lymphocytes with free receptor sites for xenogeneic thymic hormones. If bovine and rat thymic hormones are trapped by identical receptors on rat thymocytes, the unlabeled majority of rat thymocytes may represent cells the receptors of which have been saturated in vivo by the endogenous hormone.

The morphological observations of this paper indicate that thymosin-binding lymphocytes are heterogenous both in ultrastructure and the cell cycle stage. Further characterization of such thymocytes is in progress.

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3 Present address: Department of Histology and Embriology, Institute of Biostructure, Medical Academy, 60-781 Poznan (Poland).
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Histochemical localization of acetylcholinesterase in the glycogen body (sinus rhomboidalis) of common brown dove, *Streptopelia senegalensis* and house sparrow, *Passer domesticus*

U. Kumar and S.P. Singh

Department of Zoology, University of Jodhpur, Jodhpur-342001 (India), 20 February 1981

Summary. Acetylcholinesterase (AChE) activity was studied in the glycogen bodies of the spinal cords of 2 birds namely *Streptopelia senegalensis* and *Passer domesticus*. A possible functional significance of AChE in the light of relative enzymatic localization especially in Hoffmann-Kolliker nuclei (motor cell groups), substantia gelatinosa and other regions of gray matter of 2 avian glycogen bodies has been discussed.

The glycogen body, variously designated as the rhomboid sinus, intumescentia lumbalis or sinus rhomboidalis forms a lumbosacral swollen part of the spinal cord in birds. It has been found to be very rich in glycogen[1]. Enzyme histochemistry of the glycogen body with particular reference to AChE distribution has rereived the attention of few investigators[2,3] although a lot of literature is available on mammalian nervous tissue[4–7]. In the present article, histochemical localization of AChE has been made in the glycogen body of the spinal cord of a granivorous bird, the common brown dove, *Streptopelia senegalensis* (Columbidae) and the omnivorous house sparrow, *Passer domesticus* (Ploceidae) with a view to correlating the relative AChE activities in various lumbosacral neurophysiological processes.

Material and methods. Adult healthy birds were collected from nearby forests. They were decapitated and the lumbosacral region of the vertebral column was cut open so as to separate the glycogen body from the rest of the spinal cord.