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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Histochemical localization of acetylcholinesterase in the glycogen body (sinus rhomboidalis) of common brown dove, *Streptopelia senegalensis* and house sparrow, *Passer domesticus*

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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.

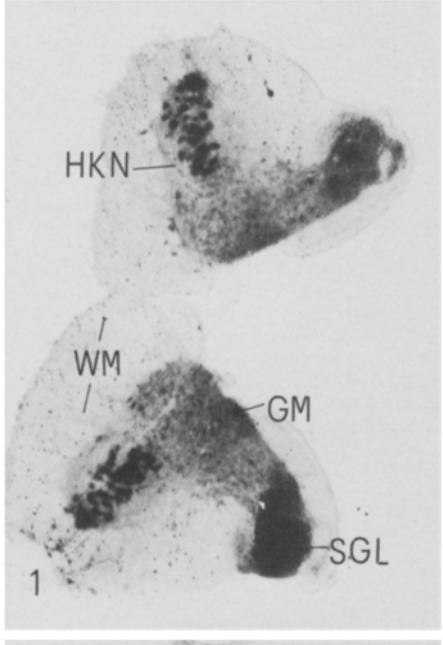
The glycogen body, variously designated as the rhomboid sinus, intumescentia lumbalis or sinus rhomboidalis forms a lumbosacral swollen part of the spinal cord in birds. It has been found to be very rich in glycogen[1]. Enzyme histochemistry of the glycogen body with particular reference to AChE distribution 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.

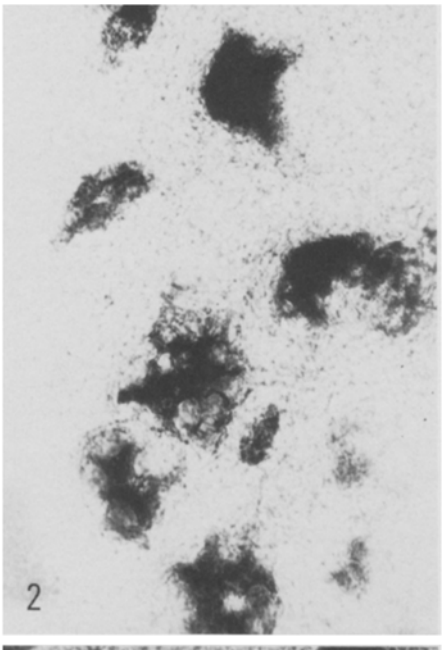
Material and methods. Adult healthy birds were collected from nearby forests. They were decapitated and the lumbosacral region of the vertebral column was cut open so as to separate the glycogen body from the rest of the spinal cord.

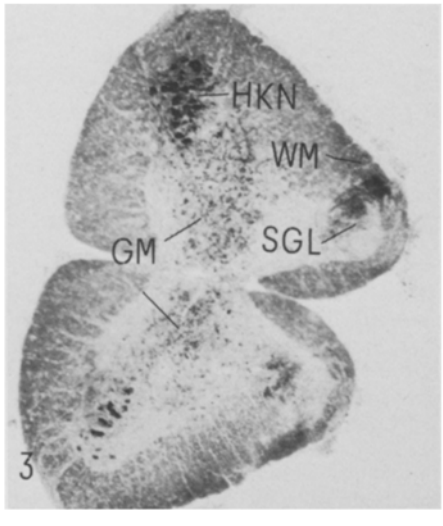
They were fixed in 10% neutral chilled formalin for 6 h. Fresh frozen sections were cut at 10-12 nm and were processed for the demonstration of AChE using the thiocholine method as devised by Gerebtzoff[8].

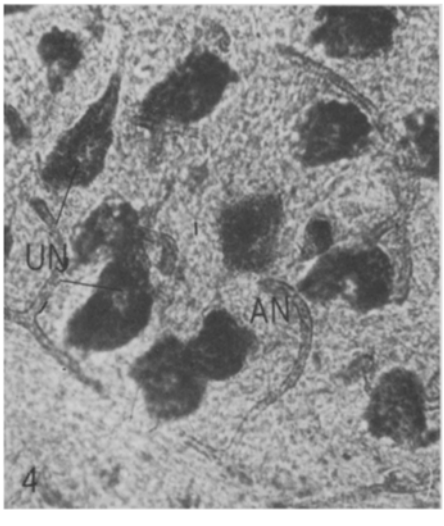
Results and discussion. As is evident, AChE activity is mainly confined to the gray matter of the glycogen bodies of the 2 birds. White matter seems to be devoid of AChE staining. In the glycogen body of *S. senegalensis,* motor cell groups forming Hoffmann-Kolliker nuclei of the gray column are particularly positive for AChE activity, showing mostly multipolar neurons; there is less AChE activity in the substantia gelatinosa and the middle column of the gray matter (figs 1 and 2). However, AChE staining in the glycogen body of *P. domesticus* appears to be relatively less marked as compared to *S. senegalensis.* Here also distribution of the reaction product appears to be similar throughout the gray matter of the glycogen body of *P. domesticus.* In it, blood vessels and substantia gelatinosa of the gray matter show very weak AChE activity or none (fig. 3) whereas Hoffmann-Kolliker nuclei are AChE positive (fig. 4) showing various kinds of neurons but the intensity of AChE staining appears to be at a lower level in comparison to those of the small brown dove. Among the neurons exhibiting AChE activities are unipolar ones and a few appear to be apolar (axons not visible).

AChE activity has usually been associated with the integration and transmission of nerve impulses, neurogenesis and related synaptic physiology[9,10]. The glycogen body is supplied with the nerve fibers of the lumbosacral region meant for the limbs and wings[11,12]. The occurrence of relatively greater AChE activity in the Hoffmann-Kolliker nuclei of the ventral horn of *S. senegalensis* as compared to *P. domesticus* might be attributed to the fact that these sites are more actively involved in the neurotransmission of synaptic impulses required for coordinating the movements of wings and tail feathers during flight, which appear to be somewhat stronger in the small brown dove than in the house sparrow. Moreover, diffused AChE staining in substantia gelatinosa and marginal cells of the dorsal horn of these glycogen bodies might be associated with their respective neuronal physiological processes as ascending fibers enter the gray column via the dorsal horn. Thus it may be concluded that strong AChE staining, especially in the ventral motor neurons of the gray matter of the glycogen body,









Photomicrographs of cross sections of the glycogen body showing AChE activity.

Figure 1. Cross sections of glycogen body of *S. senegalensis,* ×90. Note the AChE staining in gray matter (GM) especially substantia gelatinosa (SGL) and Hoffmann-Kolliker nuclei (HKN). White matter (WM) is negative.

Figure 2. A part of HKN magnified × 1000 showing AChE activity in nuclear group.

Figure 3. Cross sections of glycogen body region of spinal cord of *P. domesticus* showing AChE activity mainly in Hoffmann-Kolliker nuclear group, ×90. Note weak AChE activity in substantia gelatinosa (SGL).

Figure 4. Hoffmann-Kolliker group (HKN) of same magnified ×1000. Note the diffused AChE activity in blood vessels and various kinds of neurons namely unipolar (UN) and probably apolar (AN) as well.

might indicate a possible role in flight in these birds, apparently by its cholinergic activity which is necessary for intergrating synaptic impulses; these are apparently better developed in the small brown dove, *S. senegalensis* than in the house sparrow, *P. domesticus* as denoted by reduced AChE staining in the glycogen body of the latter.

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Fine structures of the basophil infiltration in regional lymph nodes of the guinea-pig after the intradermal injection of T cell mitogens

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Summary. Basophil-rich infiltrates in regional lymph nodes of guinea-pigs were demonstrated by electron microscopy after the intradermal injection of T cell mitogens (PHA and Con A). Basophils infiltrated the stroma of the lymph node via the postcapillary venules (PCV) and migrated to the paracortex. Prior to infiltration of the lymph nodes a cutaneous basophil hypersensitivity reaction was seen in the mitogen-injected skin. B cell mitogen (LPS) injection did not induce this response.

Cutaneous basophil hypersensitivity (CBH) characterized by basophil-rich infiltrates in the skin can be induced in men and guinea-pigs by immunization with a protein antigen in incomplete Freund's adjuvant followed 2–3 weeks later by skin testing with the antigen[1,2]. Recent experiments have revealed that CBH is elicited by T cell mitogen sensitization[3,4], suggesting that sensitized T cells participate in the induction of CBH reaction. Previous investigators concentrated on CBH of the skin, and only a few have examined the regional lymph nodes[1,2]. This paper describes infiltration of regional lymph nodes of guinea-pigs by basophils after T cell mitogen injection in the skin, studied using electron microscopy.

Materials and methods. Female guinea-pigs of the Hartley strain, weighing 350–550 g, were injected intradermally with 0.05 ml of T cell and B cell mitogens in physiological saline in the right foot pad. Phytohemagglutinin P (PHA) and concanavalin A (Con A; Sigma) were used as T cell mitogens and lipopolysaccharide B (LPS; Sigma) was employed as a B cell mitogen. PHA and Con A concentrations used were 100 µg/ml in sterile saline, respectively[3,5]. The left foot pad injected with an equal volume of saline served as control.

Electron microscopy. Groups of animals were sacrificed 2–120 h after the injection. The skin of the injected sites and the popliteal lymph nodes (regional lymph nodes) were immediately removed and trimmed in Karnovsky's fixative[2]. They were then fixed in the fluid for 4 h, post-fixed in 2% OsO_4 in 0.1 M cacodylate buffer for 2 h, dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a JEM 100 S electron microscope at an accelerating voltage of 80 kV.

Basophil numbers per postcapillary venule (PCV). Semithin sections (200–250 nm) were also made from specimens obtained at 48, 72 and 96 h after PHA-injection. They were mounted on glass slides and stained with 1% toluidine blue O in 1% borax solution. They were examined with a Nikon optical microscope at magnification × 800. Basophil numbers within the PCV lumina and the perivascular areas around 100 PCVs of cortico-medullary border[6] selected at random were counted in 20–30 typical sections obtained from each of 5 lymph nodes from 4 animals.

Proportion of paracortex to entire lymph node. Another group of animals was decapitated at 0, 48, 72 and 96 h after the injection. Four animals were used at each period. The regional lymph nodes were fixed in Baker's fixative at 4 °C and processed for cryostat sectioning. The sections (6 µm) were mounted on gelatin-coated glass slides, stained with 0.01% toluidine blue O solution and examined with the optical microscope. The volume proportion of paracortex per lymph node was examined according to Myking's description[7]. Prior to estimation, 5 typical sections of the lymph node were photographed and copied at magnification × 16. The area of the entire lymph node on the photographic paper was cut and weighed with a Sauter analytical auto-bal-

Table 1. Mean numbers of basophils in paracortex of regional lymph nodes at various intervals following PHA-injection

Hours after PHA-injection	No. of basophils in 100 PCV lumina (mean ± range)	No. of basophils around 100 PCVs* (mean ± range)
48	5 ± 2	4 ± 2
72	65 ± 7	132 ± 34
96	57 ± 13	257 ± 22

*Areas (200 µm × 200–400 µm).

Table 2. Volume proportion of paracortex per lymph node following PHA-injection

Hours after PHA-injection	$\frac{\text{paracortex}}{\text{lymphnode}} \times 100$
0	8.4 ± 2.0%
48	18.7 ± 10.0%
72	36.2 ± 2.0%
96	36.5 ± 2.0%