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%

ance. Subsequently, the paracortical area in the same lymph node was marked, cut and weighed as above. The proportion of paracortex per lymph node was roughly calculated by dividing the weight of the cut-out pieces of paracortex by the total weight of those of the same lymph node.

Results. In the PHA-injected skin of guinea-pigs, mononuclear cell infiltrates were found in the papillary dermis 4–6 h after the injection[5]. Basophils were visualized only after 12 h. Their number increased with time, reaching a peak 48 h after injection, and had almost disappeared at 96 h. Basophils phagocytized by macrophages were frequently encountered.

In the regional lymph nodes of the mitogen-injected site, basophil numbers increased remarkably 72 h after injection of PHA, reaching a peak after 96 h, and then declined (table 1). A marked proliferation was observed in the paracortex (T cell dependent) region 48 h prior to infiltration by basophils (table 2). The basophils were localized in the lymphoid stroma around the postcapillary venules (PCV) (fig. 1). Some basophils remained occasionally in the portion between PCV-endothelium and the basement membrane. The infiltrating basophils in the stroma were closely attached to lymphoid cells which contain electron-dense granules and numerous mitochondria in their extensive cytoplasm (fig. 2). At a higher magnification, the dense granules were surrounded by a membrane unit. The morphological features of the lymphoid cells showed a similarity to those of human T lymphocytes bearing receptors for the Fc portion of IgG[8]. A small number of basophils were detected in the germinal center region. Since no basophils were found in the marginal sinuses or in the afferent lymphatics, it is supposed that blood-borne basophils infiltrate the lymphoid stroma only via PCV. Basophils in the CBH-reacted skin exhibited signs of degeneration up to cell death[5], while those in the regional lymph node retained an intact structure. Intradermal injection of Con A also induced a similar CBH reaction[3,5] and basophil infiltrates in the lymph node. Intradermal injections of LPS, a B cell mitogen, and of saline induced no CBH response in skin or regional lymph nodes.

Mast cells are found in a small number in the normal guinea-pig's skin and the regional lymph nodes. They were easily distinguished from basophils by the difference in granular structure[5,9]. After T cell mitogen injection, in the present study, the guinea-pig mast cells increased in number in skin and regional lymph nodes.

Discussion. Peculiar basophil infiltrates were found in guinea-pig regional lymph nodes after T cell mitogen injection. The animals were involved inevitably in a typical CBH reaction in the skin, prior to the infiltration of basophils in the lymph nodes.

Basophils are exceedingly rare in the peripheral blood of the guinea-pig, comprising about 0.5% total leukocytes[10], but analogously to mast cells they play a significant role in allergic reactions as IgE target cells. The large number of basophils in the node are presumably participating in a local allergic reaction. Participation of sensitized T cells in induction of the guinea-pig CBH reaction has been noted by many investigators[1-3,10], and might be the basis for the induction of lymph nodal basophil infiltrates described above, since infiltration took place only after proliferation in the T cell dependent paracortex and since the infiltrating basophils showed close contacts with lymphoid cells.

Ward et al.[4] have recently demonstrated that cultured lymphocytes from guinea-pigs immunized with incomplete Freund's adjuvant release a basophil chemotactic factor into the supernatant of the culture medium. Although it is premature at this stage to assume participation of a similar factor in the induction of the peculiar basophil infiltrates in lymph node and skin, we consider that T cells stimulated with T cell mitogens initially release a chemotactic factor to induce the CBH reaction. Subsequently the activated T cells might emigrate into the regional lymph nodes and mediate secondary infiltration by basophils. Further studies with immunological analysis are necessary to confirm this hypothesis.

In mice and rats, mast cells reach the lymph node via the afferent lymphatics and increase in number after skin immunization[11]. In the case of guinea-pigs, an increase of basophil numbers in the node was more predominant than an increase of mast cell numbers and these basophils reached the node probably via PCV. This variation might be due to species differences.

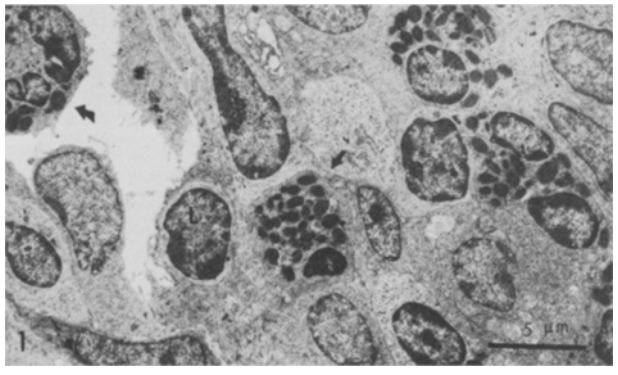

Figure 1. A postcapillary venule (left of the figure) and lymphoid stroma of a popliteal lymph node 72 h after PHA-injection. Arrows show basophils. The bar is equivalent to 5 µm. Lymphocyte (L).

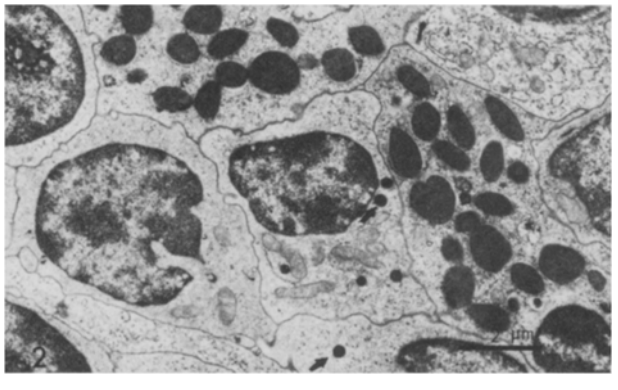

Figure 2. The paracortical region 96 h after PHA-injection. Arrows show electron dense-granules in lymphoid cells. The bar is equivalent to 2 µm.

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