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The diffusely-infiltrated lymphoid tissue of the bursa of fabricius of *Sturnus unicolor*. Histological organization and functional significance

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Summary. In the present study we confirm and extend previous reports about the existence of a T-dependent area in the bursa of Fabricius of some birds, analyzing ultrastructurally the cell content of the so-called diffusely-infiltrated lymphoid tissue of the bursa of the spotless starling, *Sturnus unicolor*. It consists of lymphocytes, plasma cells, macrophages (Møs) and interdigitating cells (IDCs) in a supporting reticular stroma which, apart from blood capillaries, contains postcapillary high-endothelial venules (HEVs). The presence of both IDCs and HEVs confirms the T-cell-dependent nature of this area, as previously claimed for other avian species, and emphasizes other functions, apart from its role in B-cell maturation, for the bursa of Fabricius specially in adult birds.

Key words: Diffusely-infiltrated lymphoid tissue, Bursa of Fabricius, Spotless starling, Ultrastructure

Introduction

From Jolly's pioner studies (1915, 1923) the morphology of the bursa of Fabricius, a lymphoid organ restricted to birds and involved in the generation of B-cell repertoire, has been profusely studied principally in chickens (Ackerman and Knouff, 1959; Frazier, 1974; Glick, 1982), and to a lesser extent in ducks (Glick, 1960; Ward and Midleton, 1971; Hashimoto and Sugimura, 1976), some species of Struthioniformes (von Rautenfeld and Budras, 1982) and in *Sturnus vulgaris* (Glick and Olah, 1982). The organ, a dorsal diverticulum of the cloacal protodeum, contains numerous lymphoid follicles consisting of developing B lymphocytes. In addition, partially covering the dorsal wall of the bursal central channel of both chickens and ducks a non-follicular lymphoid tissue has been identified, so-called diffusely-infiltrated lymphoid tissue, the functional significance of which remains obscure. This tissue appears in chickens after hatching and it has been claimed as a T-cell dependent area of avian bursa (Ward and Midleton, 1971; Odend'hal and Brazile, 1979a,b; Naukkarinen, 1982; Dolfi et al., 1988). It contains post-capillary high-endothelial venules (HEVs) (Odend'hal and Brazile, 1979a,b, 1980; Odend'hal and Player, 1979; Syrjanen and Naukkarinen, 1982), alpha-naphthyl acetate esterase (ANAE)-positive cells (Syrjanen and Naukkarinen, 1982), responds to T-dependent antigens (Syrjanen and Naukkarinen, 1982) and labelled T-lymphocytes home specifically on it (Odend'hal and Player, 1979). Recently, we have immunohistochemically confirmed the presence of different T-cell subsets in this region of the chicken bursa of Fabricius by using an extended battery of MoAbs specific either to T- or B-cells (Cortés et al., in preparation). Because of the lack of reagents for recognising T- and B-cell markers in other avian species, in the current study we use electron microscopy to histologically characterize the diffusely-infiltrated tissue of the bursa of Fabricius of the spotless starling, *Sturnus unicolor*. Our results demonstrate, according to the histological organization and cell components (IDC, HEV), that the bursa of *Sturnus unicolor* also contains a diffusely-infiltrated lymphoid tissue of presumable T-cell nature.

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Materials and methods

Bursae aseptically removed from both juvenile and adult starlings collected close to Madrid (Spain) were used in the present study.

By light microscopy, the samples were fixed in Bouin's solution for 24 hours, dehydrated in ethanol and embedded in paraffin. 7-µm serial sections were stained with Masson haemalum and picroindigocarmine and according to Gomori's silver impregnation method for reticulin fibres. By electron microscopy, small pieces were fixed in 2% glutaraldehyde in Milloning phosphate buffer (pH 7.4) for 4 hours at 4 °C, washed with buffer

supplemented with 10% sucrose for 12 hours, postfixed in 1% osmium tetroxide in the same buffer for 1 hour at 4 °C, dehydrated in acetone and embedded in Araldite. 1 µm semi-thick sections stained with an alkaline solution of toluidine blue were used for identifying the bursal areas which presumably contained the diffusely-infiltrated lymphoid tissue. Finally, thin sections obtained in a Reichter-UM3 ultratome were stained on copper grids with lead acetate and observed and photographed in a JEOL 100-B electron microscope.

Results

The bursa of Fabricius of the spotless starling, *Sturnus unicolor*, is a sac-like, cylindrical organ which constitutes a dorsal diverticulum in the wall of cloacal proctodeum. The centre of this sac consisted of an enlarged channel ramified in secondary channels and in continuity with the intestinal lumen (Figs. 1, 2). Although most of the bursal wall was occupied by a follicular lymphoid tissue, close to the contact of the central channel with the proctodeum and lining its lumen various masses of lymphoid tissue were identified diffusely infiltrated in the loose connective tissue (Figs. 1, 2). The light microscopic study confirmed the diffuse rather than follicular nature of that lymphoid tissue, its irrigation by blood vessels coming from the deeper regions of mucosa and, as shown by silver impregnation, an irregular pattern of reticular fibres which constituted the supporting network in the tissue (Figs. 2, 3).

By electron microscopy, the lymphoid masses consisted of closely-packed, various-sized lymphocytes and lymphoblasts, some of them in division, arranged in a supporting reticular network formed both by electron-lucent and, sometimes, dark reticular cells and their cell processes (Figs. 4, 5). Numerous mature and developing plasma cells occurred in the tissue, frequently forming subepithelial cell clusters (Fig. 5).

Apart from lymphoid cells the free cell population found in the bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor* consisted predominantly of IDCs and macrophages (Møs). IDCs had irregular electron-lucent elements with an irregular nucleus which exhibited a few clumps of condensed chromatin in the periphery and a patent nucleolus (Figs. 6, 7). Most cytoplasmic organelles occurred grouped close to the nucleus, whereas the peripheral cytoplasm appeared empty. On the surface, IDCs established labyrinth cell contacts with the neighbouring cells, including other IDCs, lymphocytes or plasma cells (Fig.

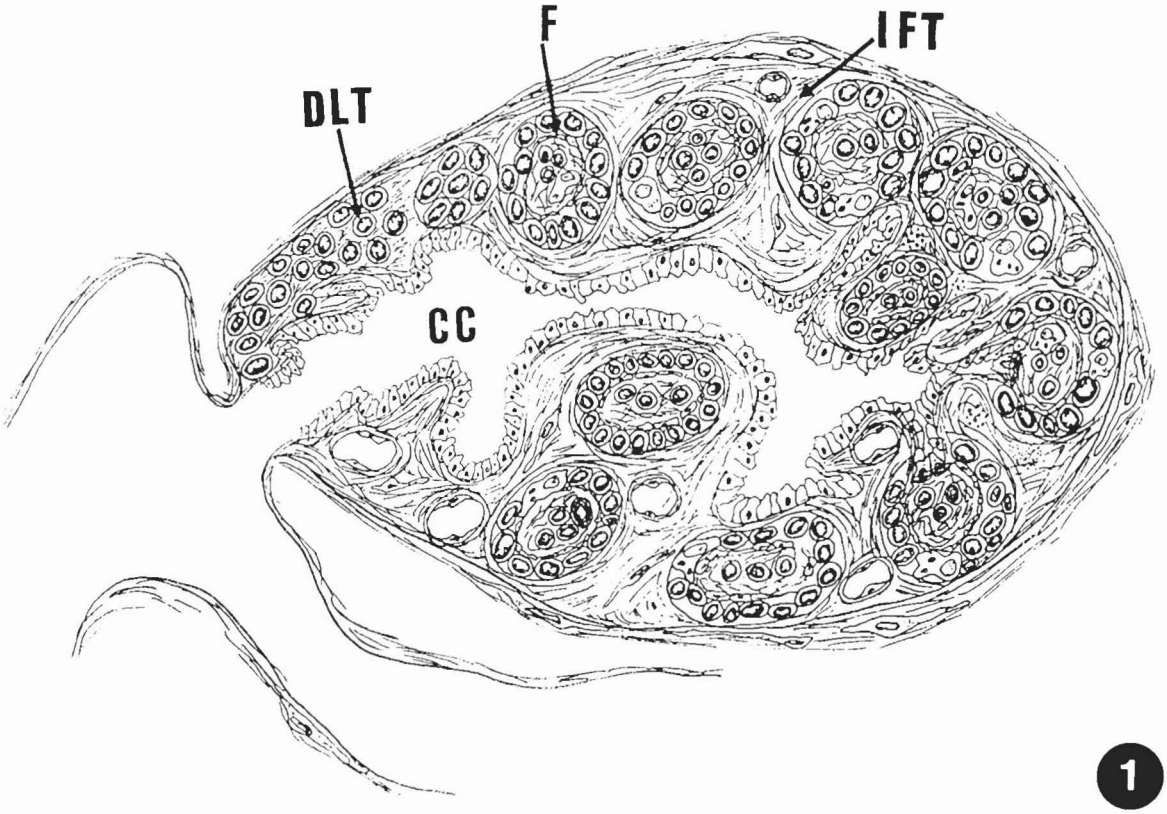

Fig. 1. Drawing of the different components of the bursa of Fabricius of *Sturnus unicolor*. F: lymphoid tissue; IFT: interfollicular tissue; DLT: diffusely infiltrated lymphoid tissue; CC: central channel.

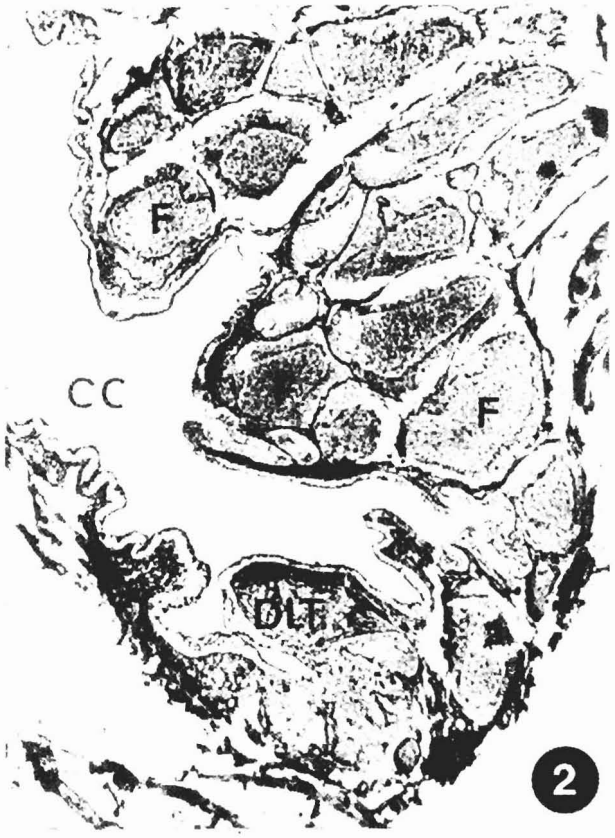

Fig. 2. Longitudinal section of the bursa of Fabricius of *Sturnus unicolor* stained according to Gomori's silver impregnation method. F: lymphoid follicles; CC: central channel; DLT: diffusely-infiltrated tissue. x 90

Fig. 3. Diffusely infiltrated lymphoid tissue (DLT) in the bursa of Fabricius of *Sturnus unicolor*. Gomori's silver impregnation method. Note the irregular pattern exhibited by the reticular network of this bursal region. x 600

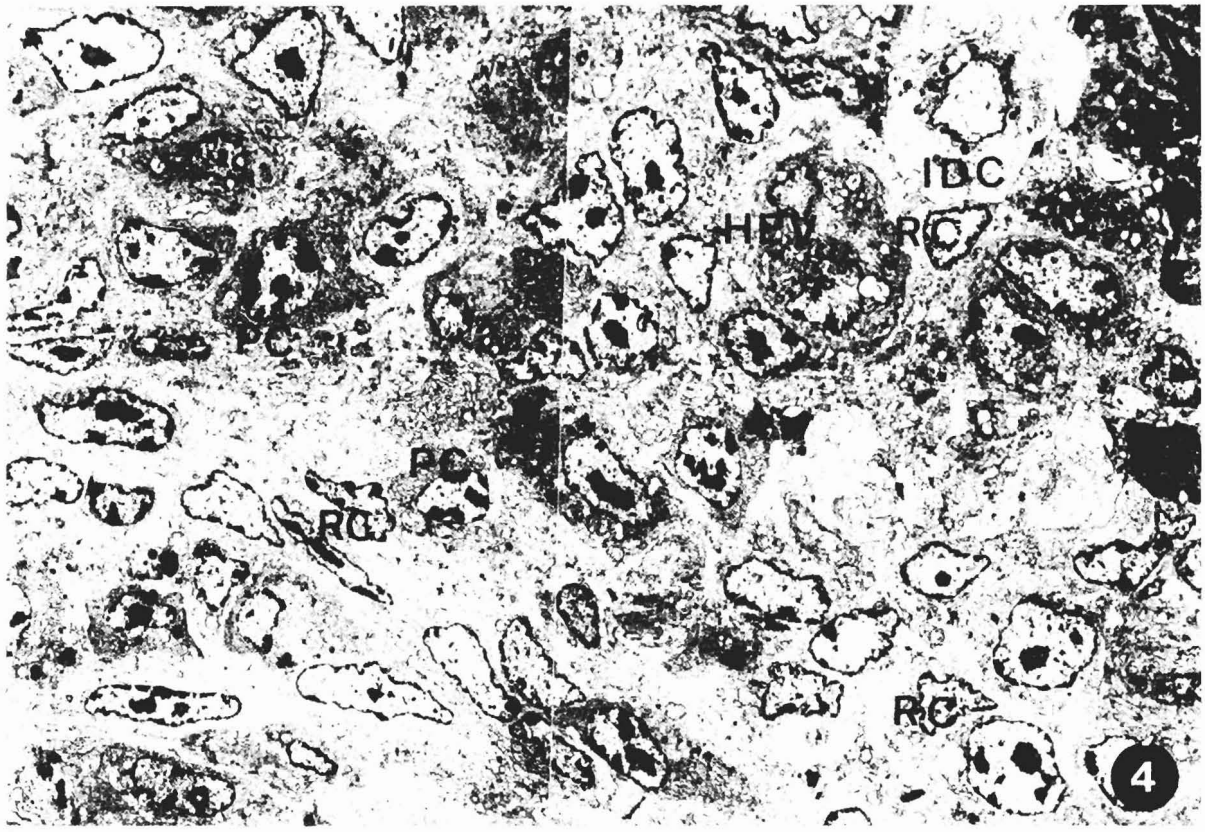

Fig. 4. Cell components ultrastructurally identified in the bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor*. RC: reticular cells; L: lymphocytes; PC: plasma cells; IDC: interdigitating cell; HEV: post-capillary high-endothelial venules. x 3,000

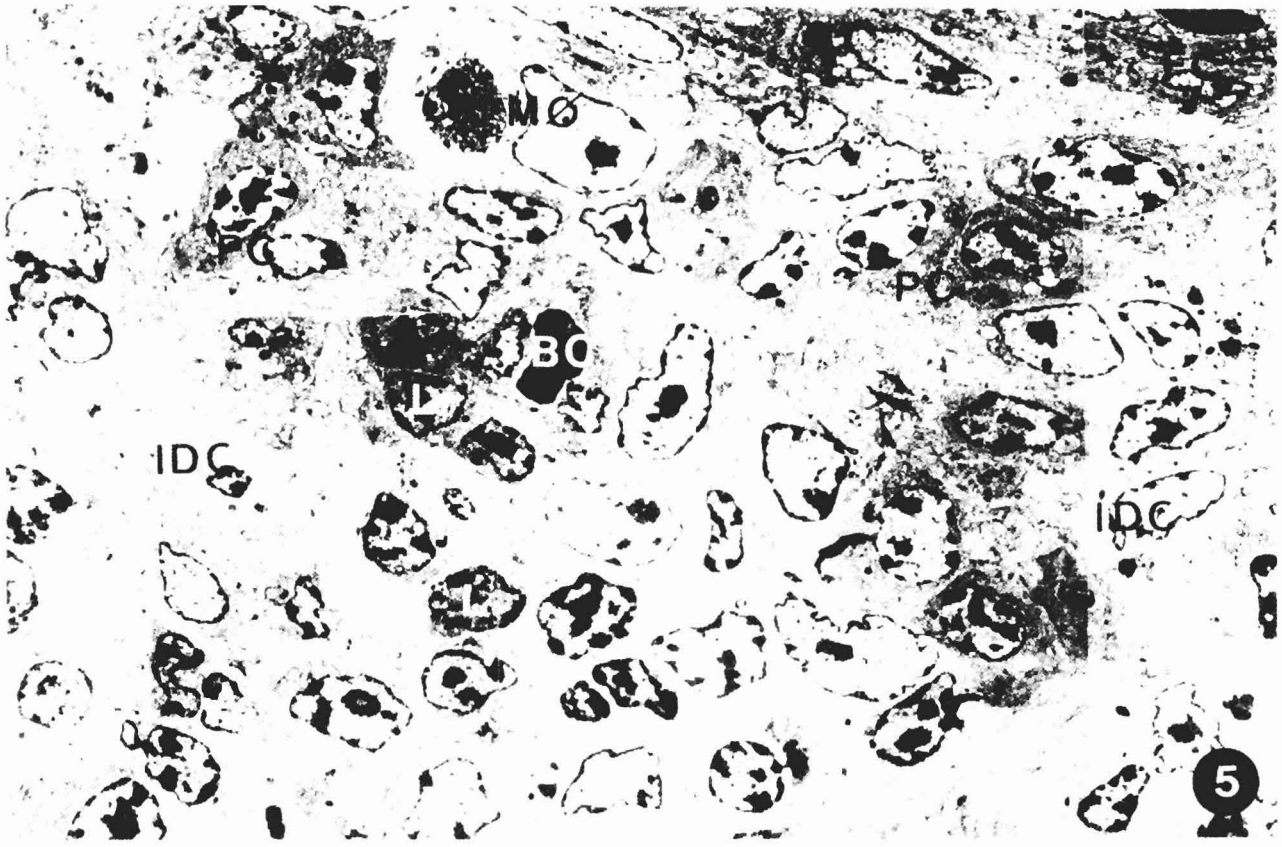

Fig. 5. Survey of bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor*. L: lymphocytes; Mø: macrophages; FC: fibrocytes; PC: plasma cells; IDC: interdigitating cells; BC: Blood capillaries. x 3,800

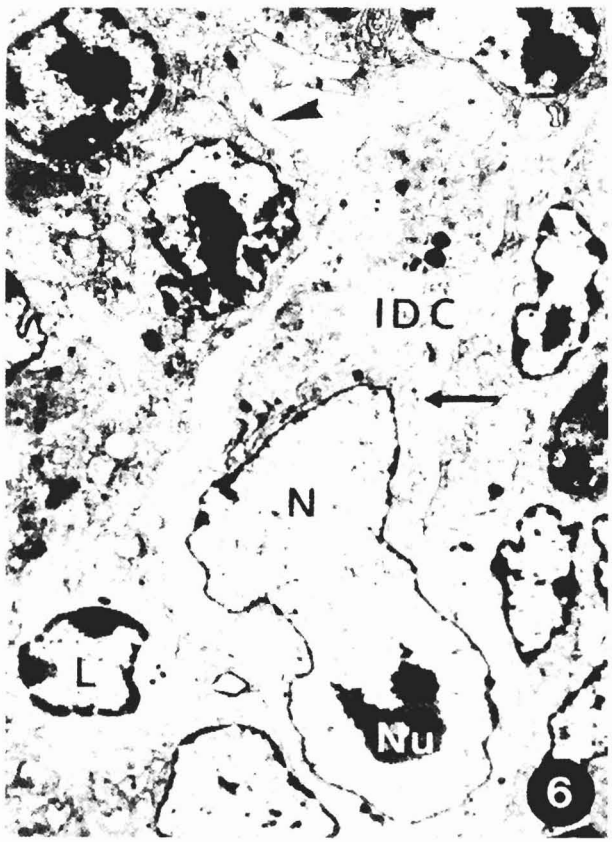

Fig. 6. Interdigitating cells (IDC) in the bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor*. Note the arrangement of membranous organelles close to the nucleus (N), and the electron-lucent peripheral cytoplasm (arrow). Arrowhead: surface interdigitations; L: lymphocytes; Nu: nucleolus. x 6,000

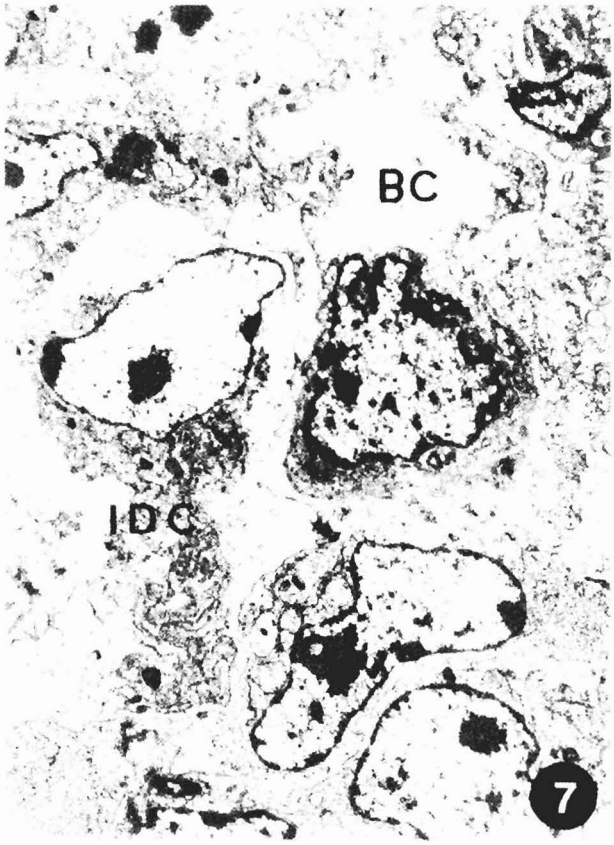

Fig. 7. Interdigitating cell (IDC) near a blood capillary (BC) of the bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor*. x 5,500

6). Furthermore, they frequently occurred close to blood vessels (Fig. 7). Møs, although numerous, occurred in smaller numbers than in other areas of the bursa (Fig. 5). They appeared under the epithelium, in the region in which plasma cells predominated, and exhibited abundant engulfed materials in their cytoplasm (Fig. 5).

As mentioned above, the diffusely-infiltrated lymphoid tissue was irrigated by an extensive network of blood vessels. In the deeper regions, near to the limits of lymphoid tissue, close to the muscularis, the blood vessels showed flat endothelial cells, similar to those observed in the mucosal capillaries. In those cases, they appeared to be surrounded by a continuous basement membrane and a sheath of reticular fibres and cells (Fig. 5). On the contrary, the blood vessels occurring inside the lymphoid masses corresponded to HEVs, the lumen of which frequently appeared obliterated (Fig. 8). They contained cuboidal endothelial cells with irregular, indented nuclei showing patent nucleoli, a cytoplasm rich in membranous organelles and lateral interdigitations (Fig. 8).

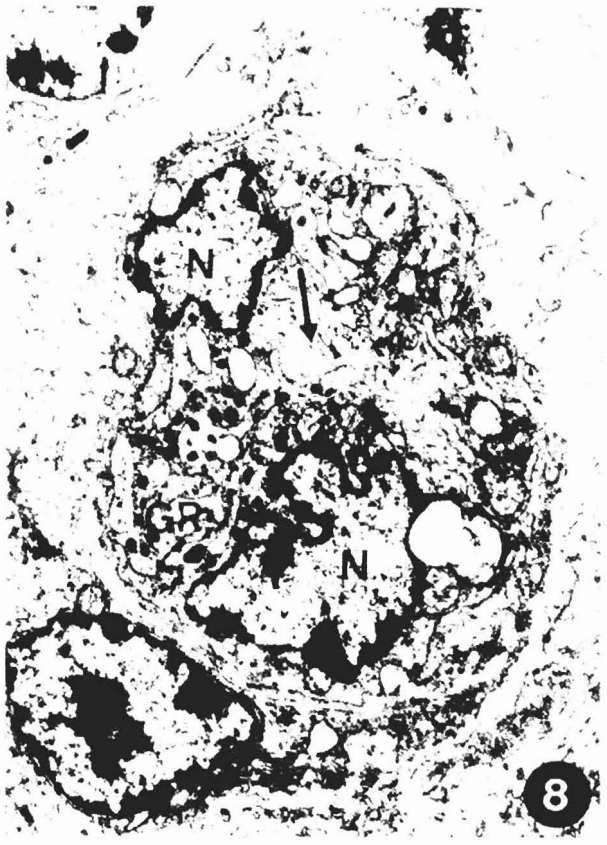

Fig. 8. High endothelial venules in the bursal diffusely-infiltrated lymphoid tissue of *Sturnus unicolor*. The endothelial cells, which exhibit irregular nuclei (N), cytoplasmic electron-dense granules (Gr) and numerous lateral foldings (arrow), completely obliterate the blood vessel lumen. x 6,800

Discussion

The presence of a non-follicular diffuse-infiltrated lymphoid tissue in the dorsal wall of bursal central channel close to the proctodeum has been previously described in chicken (Odend'hal and Brazile, 1979a,b, 1980; Naukkarinen, 1982; Dolfi et al., 1988) and duck (Ward and Midleton, 1971), but not in other avian species. In *Sturnus unicolor*, several lymphoid accumulations, histologically similar to those found in chickens and ducks, occur not only in the dorsal but also in the ventral wall of the bursal region close to the proctodeum. Likewise, the cell content of these areas in *Sturnus*, in agreement with what has been previously observed, mainly consits of various-sized lymphocytes, plasma cells, IDCs and Møs arranged in a reticular network formed by reticular cells and fibres.

With respect to the significance of this tissue, various authors have emphasized its T-dependent nature on the basis of the following indirect evidence:

1) It appears only after hatching, whereas the bursal follicular lymphoid tissue grows principally throughout embryonic life (Odend'hal and Brazile, 1980).

2) It contains positive cells for ANAE, a histochemical marker for T-cells (Syrjanen and Naukkarinen, 1982).

3) Autologous radioactive-labelled thymocytes home specifically to this bursal region after intravenous injection (Odend'hal and Player, 1979).

4) The diffusely-infiltrated lymphoid tissue, contrary to the follicular lymphoid tissue of bursa, contains HEVs (Odend'hal and Brazile, 1979a,b, 1980; Odend'hal and Player, 1979; Syrjanen and Naukkarinen, 1982), as demonstrated in agreement with *Sturnus*, described only in the T-dependent areas of peripheral lymphoid organs. Moreover, increased numbers of migrating lymphocytes through HEVs occur in the bursal region after cloacal stimulation with T-dependent antigens (Syrjanen and Naukkarinen, 1982).

Nevertheless, Dolfi et al. (1988) reported B-lymphocytes in the diffusely-infiltrated lymphoid tissue of chicken bursa, the number of which apparently increases during ageing. Recent immunohistochemical data obtained by our group confirms the presence of B-cells in that area of chicken bursa, but T-cell predominates largely (Cortés et al., in preparation).

According to the exhibited pattern of reticular fibres after silver impregnation, and principally to the demonstration of HEVs and IDCs, two morphological markers for T-dependent areas of mammalian lymphoid organs, the diffusely-infiltrated lymphoid tissue of the bursa of Fabricius of *Sturnus unicolor* may also be a T-dependent area, as previously reported in both chickens and ducks. On the other hand, the strategic location of this tissue in the bursa and its apparent histological resemblance to other gut-associated lymphoid tissues, such as Peyer's patches and caecal tonsils, involved in avian antigen-processing suggests that the diffusely-infiltrated lymphoid tissue could be involved in the

mounting of immune responses to antigens reaching the gut via the cloaca. In agreement, Dolfi et al. (1988) emphasized that both diffusely-infiltrated lymphoid tissue and lymphoid aggregates occurring in the chicken cloaca are concerned with the antibody production of local antigens preventing their access to the bursal lymphoid follicles.

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