J. Anat. (1987), **150**, pp. 89–98
With 13 *figures*
Printed in Great Britain

Ultrastructural study of macrophages in the rat thymus, with special reference to the cortico-medullary zone

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(*Accepted* 7 *March* 1986)

INTRODUCTION

The cortico-medullary zone of the normal rat thymus is characterised by the presence of very large, metallophilic cells, with prominent, stocky cytoplasmic prolongations, which form a dense meshwork between the cortex and medulla (Milićević, Milićević, Piletić & Mujović, 1982). These cells, strategically positioned within the thymic parenchyma, contain numerous intracellular granules of varying size, which stain selectively with aldehyde fuchsin (Milićević, Milićević & Mujović, 1983). Enzyme histochemical studies demonstrated that these cells are macrophages. The intracellular granular content is of heterogeneous composition and is primarily lipid in nature. The enzyme characteristics of these macrophages (Milićević & Milićević, 1984) appeared to be similar to those of macrophages activated *in vitro* by soluble products of stimulated lymphocytes (Nath, Poulter & Turk, 1973; Poulter & Turk, 1975). It is possible that these cells could be involved in the process of thymocyte migration and/or maturation *in vivo* (Milićević *et al.* 1983).

Considering that there are no data in the literature on the ultrastructure of macrophages of the cortico-medullary zone, especially relating to the structure of the globular cytoplasmic inclusions of these cells, the aim of our study was to investigate the ultrastructural features of macrophages located in the cortico-medullary zone of the normal rat thymus and correlate them with those of macrophages situated in other regions of the thymic parenchyma.

MATERIALS AND METHODS

Young, adult Wistar rats of either sex, eight weeks old, with an average body weight of 210 g, were studied. Thymic tissue for examination was removed under ether anaesthesia.

The pieces of thymic tissue for light microscopy were fixed in Bouin's solution and routinely processed to Paraplast. The sections, 4–5 μm thick, were stained with aldehyde fuchsin by the method of Landing, Hall & West (1956), as a specific histochemical marker for macrophages of the cortico-medullary zone (Milićević *et al.* 1983).

For electron microscopy, the thymus tissue was quickly immersed in glutaraldehyde and cut into 1 mm^2 cubes. Thereafter, the tissue was fixed for two hours in 2% glutaraldehyde in 0·1 M cacodylate buffer (pH = 7·6) at 4 °C. Subsequently, the

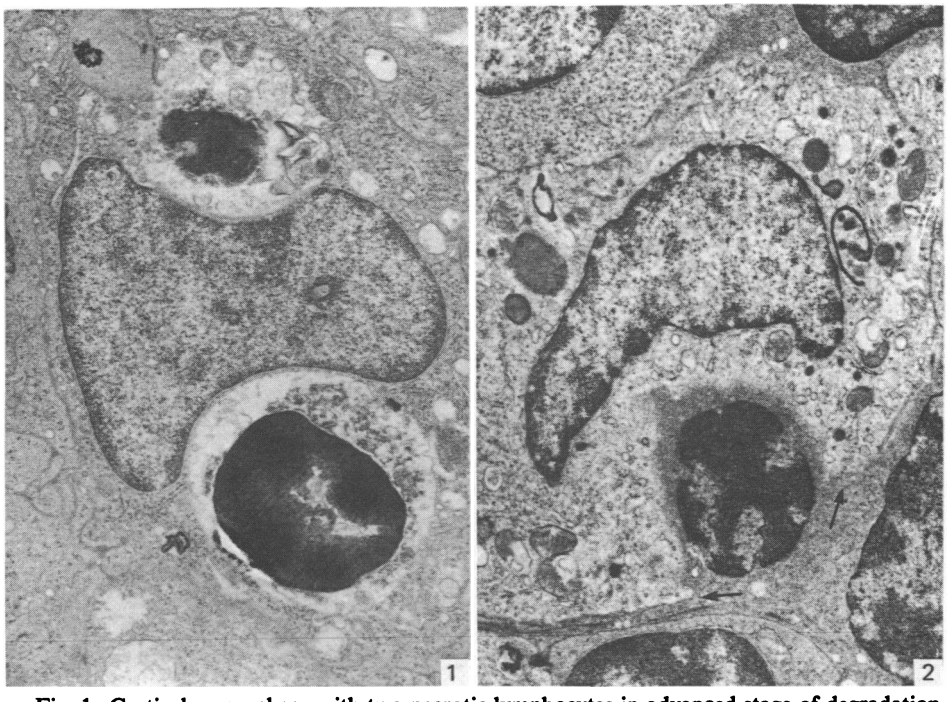

Fig. 1. Cortical macrophage with two necrotic lymphocytes in advanced stage of degradation in the cytoplasm. Uranyl acetate and lead citrate. × 8400.

Fig. 2. Macrophage of the outer cortex. The cytoplasmic prolongations (arrows) enclose the necrotic lymphocyte, which is still not completely engulfed. Uranyl acetate and lead citrate. × 8400.

tissue was washed three time in 0·1 M cacodylate buffer and postfixed in 1 % OsO_4 for two hours. After the repeated washing the tissue was dehydrated in graded alcohols and embedded in Epon 812. The blocks were cut on an LKB Ultramicrotome III.

Semithin sections, 1 μm thick, were routinely stained with methylene blue and impregnated with silver methenamine (Rambourg, 1967) for light microscopy.

Ultrathin sections were serially cut and adjacent sections were routinely contrasted with uranyl acetate and lead citrate or treated with silver methenamine (Veldman, 1970). This enabled the precise identification of subcellular structures with affinity for silver methenamine. For demonstration of polysaccharides, the ultrathin sections were treated by the thiocarbohydrazide-silver proteinate method (Thiéry, 1969), and sections of normal hepatic tissue were used as a positive control. The material was examined with a Philips EM 201 electron microscope.

RESULTS

Cortex

Typical cortical macrophages are distributed throughout the cortex. By light microscopy, these cells are readily recognised owing to the presence of intracytoplasmic nuclear remnants of phagocytised lymphocytes, which stain deep blue with methylene blue. Cortical macrophages are relatively large cells; their nuclei are large and have clumps of heterochromatin on the inner surface of the nuclear membrane.

Most of the cytoplasm is occupied by easily recognisable necrotic lymphocytes in various stages of degradation (Figs. 1, 2) which compress the nucleus. These remnants do not contrast with silver-methenamine and thiocarbohydrazide-silver proteinate methods. The cytoplasm and organelles are scanty. Only occasionally several typical primary lysosomes are seen.

Cortico-medullary zone

Light microscopy shows that, in contrast to the cortex and the medulla, the cortico-medullary zone is populated with a specific type of macrophage, which contains numerous cytoplasmic granules of varying size, selectively stained with aldehyde fuchsin. These cells form a garland positioned between the cortex and medulla (Fig. 3). Sometimes aldehyde fuchsin-positive granules are very large and compress the nucleus, which acquires an irregular shape, but remains markedly euchromatic, with a very prominent nucleolus (Fig. 4). In semithin sections these cells contain abundant inclusions (which correspond to aldehyde fuchsin-positive granules) stained brilliantly a pale blue-green colour with methylene blue. Sometimes, the large inclusions almost completely fill the cytoplasm of macrophages located in the cortico-medullary zone, and these acquire the appearance of 'soap bubble cells'. The inclusions compress the nucleus, which acquires a very irregular shape. It is still very euchromatic, with one or two prominent, large nucleoli (Fig. 5; compare with Fig. 4). Very rarely, in addition to the described inclusions, a single phagocytosed necrotic lymphocyte (stained dark blue), identical to those observed in cortical macrophages, is seen within the cytoplasm of macrophages of the cortico-medullary zone. Smaller, granular inclusions selectively impregnate with silver-methenamine (Fig. 6).

With the electron microscope, several typical features always enable the precise identification of these macrophages: (*a*) their cytoplasm contains the vacuolar inclusions of varying size, (*b*) the nucleus is markedly euchromatic, irregularly shaped, with a very prominent nucleolus, (*c*) phagocytosed lymphocyte remnants are very rarely present within their cytoplasm and (*d*) these cells have neither tonofilaments nor desmosomes.

The vacuolar cytoplasmic inclusions, of different size and shape, which correspond to the aldehyde fuchsin-positive granules observed at light microscopic level, are always present within the macrophages of the cortico-medullary zone (Fig. 7). The vacuoles are surrounded by trilaminar membrane and contain homogeneous flocculent material of very low electron density. In some cells the vacuolar inclusions occupy the greater part of the cytoplasm (Fig. 8). Occasionally, vacuoles of giant size fill up the cytoplasm almost completely, compressing the nucleus, which acquires a bizarre shape. However, because of the prominent nuclear euchromasia and the abundance of polyribosomes, these cells retain a very active appearance (Fig. 9; compare with Figs. 4–5). The dense bodies, which are sometimes closely positioned on the inner side of the vacuolar membrane (Fig. 8), selectively contrast with silver-methenamine and thiocarbohydrazide-silver proteinate methods. Occasionally, in addition to the vacuolar cytoplasmic inclusions, some of the macrophages of the cortico-medullary zone contain inclusions predominantly composed of numerous dense bodies (Fig. 10). Between the dense bodies, compressed membranes are observed, which sometimes form dilated cisternae filled with flocculent, electron-lucent material. The dense bodies and membranes selectively contrast with silver-methenamine (Fig. 11; compare with Fig. 10). Polysaccharides are selectively demon-

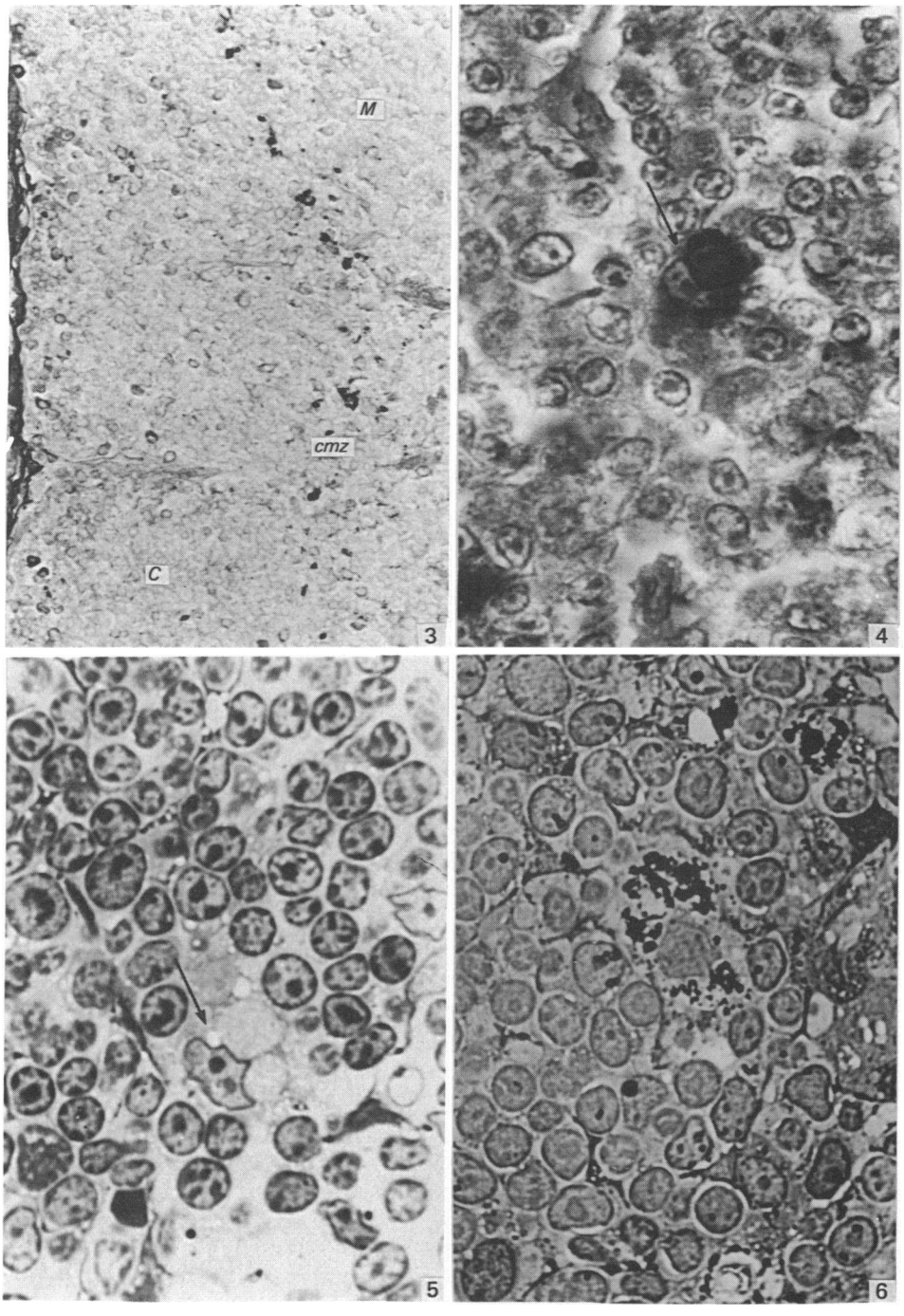

Fig. 3. Aldehyde fuchsin-positive macrophages in the cortico-medullary zone (*cmz*). No counterstain used. *C*, cortex; *M*, medulla. × 280.

Fig. 4. Macrophage of the cortico-medullary zone (arrow). The characteristic nucleus has a prominent nucleolus and aldehyde fuchsin-positive cytoplasmic granules of varying size. Counterstained with haematoxylin. × 1650.

Fig. 5. Macrophage of the cortico-medullary zone, with a characteristic nucleus and cytoplasmic inclusions (arrow). Semithin plastic section, methylene blue. × 1650.

Fig. 6. The smaller granules selectively impregnate with silver methenamine. × 1650.

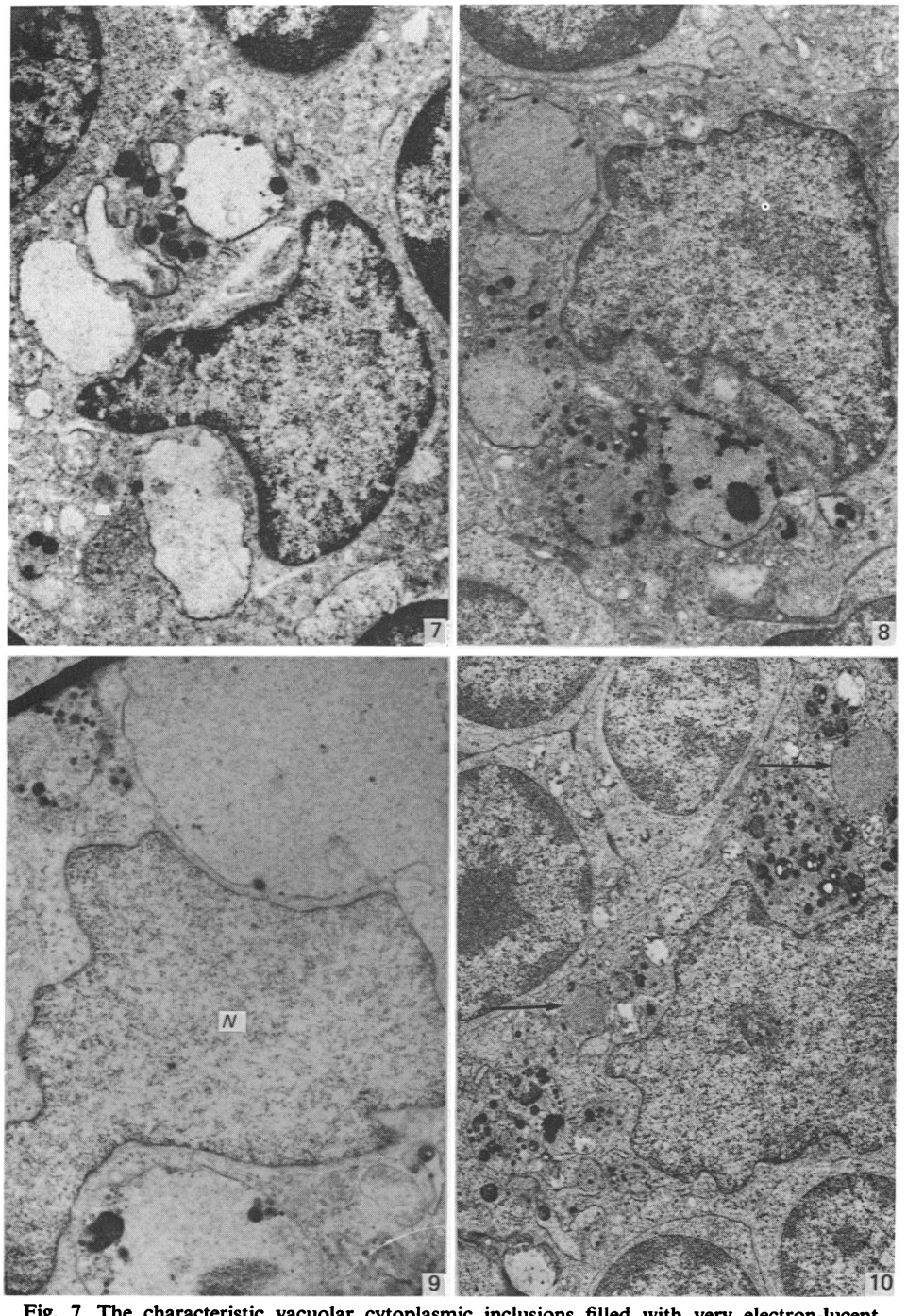

Fig. 7. The characteristic vacuolar cytoplasmic inclusions filled with very electron-lucent content. The nucleus is irregularly shaped. Uranyl acetate and lead citrate. × 8400.

Fig. 8. Numerous vacuolar inclusions in the cytoplasm, occasionally with dense bodies on the inner aspect of their membranes. Polyribosomes are abundant. Uranyl acetate and lead citrate. × 8400.

Fig. 9. Giant vacuoles compress the nucleus (*N*), which is markedly euchromatic. Uranyl acetate and lead citrate. × 12000.

Fig. 10. In addition to the vacuolar cytoplasmic inclusions (arrows), others predominantly composed of dense bodies are also seen. Uranyl acetate and lead citrate. × 7840.

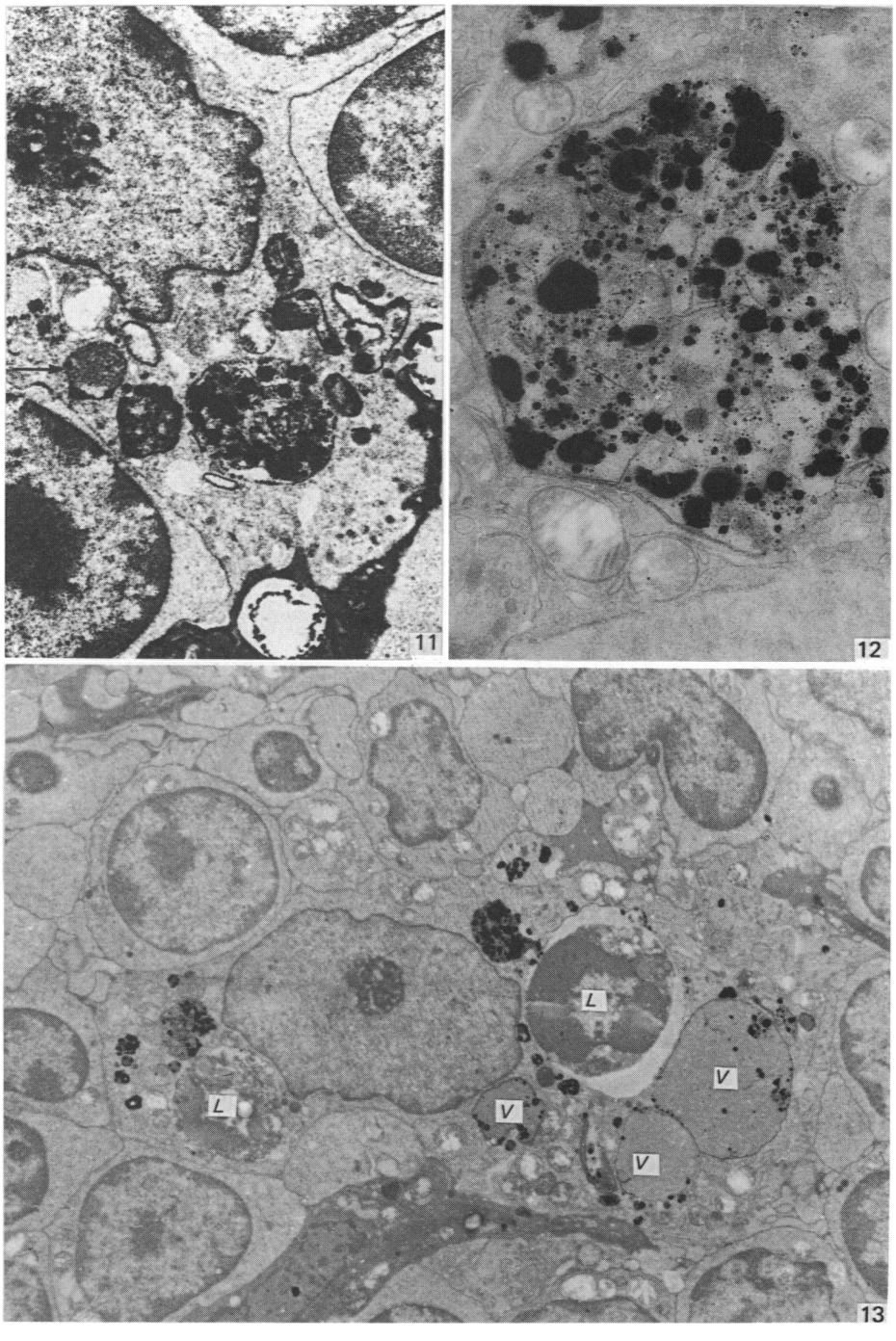

Fig. 11. Electron-dense bodies and membranes of the cytoplasmic inclusions contrasted with silver methenamine. Vacuolar inclusion is indicated by an arrow. The section is adjacent to that in Figure 9. × 12000.

Fig. 12. Polysaccharides in the dense bodies of the cytoplasmic inclusion. Membranes are only faintly positive. Thiocarbohydrazide-silver proteinate, uncontrasted. × 16425.

Fig. 13. Two phagocytosed necrotic lymphocytes (*L*) are morphologically and histochemically different from vacuolar inclusions (*V*). Thiocarbohydrazide-silver proteinate, uncontrasted. × 7840.

strated in the dense bodies, whereas the membranes are negative (Fig. 12). Only very rarely do the macrophages of the cortico-medullary zone contain phagocytosed lymphocyte remnants, which are morphologically, as well as histochemically, readily distinguished from the cytoplasmic inclusions and do not contrast with silver-methenamine and thiocarbohydrazide-silver proteinate methods (Fig. 13).

Numerous polyribosomes are scattered in the cytoplasm of macrophages of the cortico-medullary zone. The granular endoplasmic reticulum is often associated with the cytoplasmic inclusions. Mitochondria are scanty.

The type of thymic macrophage described here is not topographically related to the blood vessels of the cortico-medullary zone.

Typical interdigitating cells are positioned closer to the outer medulla. Their cytoplasm is abundant, electron-lucent and contains Birbeck granules. The cytoplasmic prolongations are prominent and their membranes interdigitate with the cell membranes of the neighbouring lymphocytes. The nucleus has the typical bizarre shape.

Medulla

Typical interdigitating cells represent the predominant type of mononuclear phagocytes in the thymic medulla. Sporadic macrophages containing phagocytosed material are also encountered.

DISCUSSION

In the previous ultrastructural studies of the thymus in the rat (Brelińska, Kaczmarek, Warchol & Jaroszew, 1985; Duijvestijn & Hoefsmit, 1981; Duijvestijn, Köhler & Hoefsmit, 1982; van Haelst, 1967; Hwang, Ho, Luk & Simon, 1974; Oláh, Röhlich & Törö, 1975), mouse (Bartel, 1979; Clark, 1963; Hoshino, 1963), hamster (Ito & Hoshino, 1966), guinea-pig (Klug & Mager, 1979; Mandel, 1968) and man (Bearman, Levine & Bensch, 1978; von Gaudecker, 1978; von Gaudecker & Müller-Hermelink, 1980; Goldstein, Abbot & Mackay, 1968; Haar, 1974; Pinkel, 1968; van de Wijngeart *et al.* 1984) attention was mainly focused on epithelial cells, lymphocytes and recently, also, on interdigitating cells. On the other hand, detailed ultrastructural studies of thymic macrophages are lacking in the literature (only occasional, parenthetical notes are found). In the earlier electron microscopic studies of the normal rat thymus it was reported that thymic macrophages are loaded with lymphocyte remnants in various stages of degradation (Duijvestijn & Hoefsmit, 1981; van Haelst, 1967; Hwang *et al.* 1974; Oláh, Röhlich & Törö, 1975). However, our electron microscopic findings, which are in agreement with the results of our previous enzyme-histochemical investigations of the rat thymus (Milićević & Milićević, 1984, 1985), demonstrate that there is a distinct type of macrophage for each of the three thymic regions, namely the cortex, cortico-medullary zone and medulla. These results show that the macrophages containing lymphocyte debris are confined to the thymic cortex, whereas the macrophages of the cortico-medullary zone contain cytoplasmic vacuolar inclusions, which are unlikely to have originated from the digested residues of phagocytosed lymphocytes so that the macrophages of the cortico-medullary zone differ from the cortical macrophages. The third type of thymic mononuclear phagocytes are the interdigitating cells. Our findings concerning the distribution and ultrastructure of these cells accord with the results of previous studies in the rat (Duijvestijn & Hoefsmit, 1981; Duijvestijn Köhler & Hoefsmit, 1982; Duijvestijn *et al.* 1982; Oláh *et al.* 1975). *In vitro* studies have similarly

shown that long term cultures of murine thymic macrophages are composed of three different populations of cells (Gallily & Savion, 1983).

The enzyme capacity (increased activity of hydrolytic and respiratory enzymes) of macrophages in the cortico-medullary zone (Milićević & Milićević, 1984) is similar to that of macrophages activated by soluble products of stimulated lymphocytes *in vitro* (Nath, Poulter & Turk, 1973; Poulter & Turk, 1975). This type of thymic macrophage is also morphologically similar to the macrophages activated *in vitro*, which after five days in culture contain many granules and abundant vacuoles, in some cases acquiring a 'soap bubble' appearance (Nath, Poulter & Turk, 1973).

The polysaccharides within the cytoplasmic inclusions of macrophages in the cortico-medullary zone, detected by thiocarbohydrazide-silver proteinate method, may represent the components of the enzyme molecules (Martin, 1981), which are abundant in these cells (Milićević & Milićević, 1984, 1985).

It is of interest that macrophages of the cortico-medullary zone ultrastructurally, as well as histochemically (Milićević and Milićević, 1984), show some similarity to the macrophages of the marginal zone in the white pulp of the rat spleen (Streefkerk & Veerman, 1971). The affinity of electron-dense bodies, which are present in the macrophages of the cortico-medullary zone, for silver-methenamine further emphasizes this resemblance (Streefkerk & Veerman, 1971).

In our previous studies (Milićević, 1984; Milićević *et al.* 1983) we suggested that macrophages of the cortico-medullary zone could be identical to the macrophages which produce the thymocyte differentiating factor and control one step of thymocyte differentiation *in vitro* (Beller & Unanue, 1978). The ultrastructure of these cells, however, is not suggestive of intensive protein secretion. Still, the paucity of engulfed lymphocyte debris and presence of dilated, vacuolar cytoplasmic inclusions, which resemble accumulations of endogenous material, further confirm that these cells could have some function or functions other than phagocytosis.

Recently, we suggested that the positive histochemical reactions for ceroid of the macrophages in the cortico-medullary zone could reflect the presence of some other type of partially oxidized, unsaturated fats within their cytoplasmic granules (Milićević, Milićević & Mujović, 1986). Indeed, the electron microscopic results presented here demonstrate that the cytoplasmic vacuoles, corresponding to the aldehyde fuchsin-positive granules in light microscopy, contain a flocculent, electron-lucent material that is morphologically different from ceroid. Considering that the most prominent morphological characteristic of activated macrophages producing elevated amounts of prostaglandin E_2 and thromboxane B_2 *in vitro* is the appearance of large vacuolar cytoplasmic inclusions (Brune, Glatt, Kälin & Peskar, 1978), it seems possible that the ultrastructural features of macrophages of the cortico-medullary zone indicate intensive metabolism of arachidonic acid. *In vitro* studies confirm that the thymic phagocytic cells, in additon to interleukin 1, produce prostaglandin E_2, thus controlling the proliferation of thymocytes (Papiernik & Homo-Delarche, 1983).

SUMMARY

Electron microscopic study of the normal rat thymus has demonstrated that macrophages with different ultrastructural features are positioned in the thymic cortex, in the cortico-medullary zone and in the medulla.

Phagocytic cells, containing necrotic lymphocytes in various stages of degradation, are distributed throughout the thymic cortex. The cortico-medullary zone, in con-

trast, is populated with macrophages displaying specific ultrastructural features. These cells contain numerous vacuolar inclusions of different size, filled with homogeneous, flocculent material of very low electron density. The dense bodies, occasionally positioned to the inner side of the vacuolar membrane, selectively contrast with silver methenamine and contain polysaccharides, as demonstrated by the thiocarbohydrazide-silver proteinate method. Very rarely, these cells contain phagocytosed lymphocyte remnants.

The predominant type of mononuclear phagocytic cells in the thymic medulla are the interdigitating cells.

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