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DARK RETICULAR CELLS IN THE THYMUS OF MICE

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The increased knowledge of the functional importance of the mammalian thymus has caused an increased interest in the morphology of this organ in general and in its ultrastructure in particular. Thus several reports on this subject have been published in the last decade (CLARK 1963, WEISS 1963, LUNDIN & SCHELIN 1965, VAN HAELST 1967, MANDEL 1968, ABE & ITO 1970, PEREIRA & CLERMONT 1971, RAPPAY et coll. 1971). The investigations have been concentrated to the most common types of cells, the lymphocytes, the epithelial reticular cells and the mesenchymal reticular cells or macrophages. Little interest has been devoted to a less common, dark reticular cell with increased electron density (IZARD & DE HARVEN 1968). The identity of this cell is not completely known, nor is its function or its relationship to other thymic cells. This short report will elucidate some details of the morphology of this dark cell and of its distribution in normal and in irradiated thymus and in thymic lymphoma of mice.

Material and Methods. C_3H and CBA mice, aged 20 days to 4 months were used. For radiation experiment a total dose of 550 R was divided between four equal exposures and given every fifth day beginning at an age of 25 days. Lymphomas were induced by fractionated irradiation or by a combination of fractionated irradiation and injection of ^{90}Sr (JÄRPLID 1974). The irradiation

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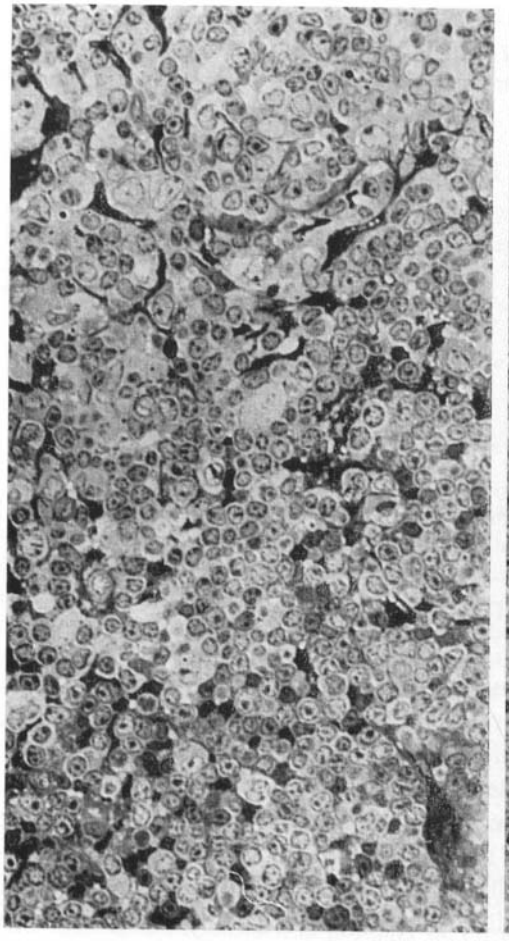

Fig. 1. Plenty of dark cells in the medulla (above) and in the cortex near the boundary between cortex and medulla. Toluidine blue stain. ×465.

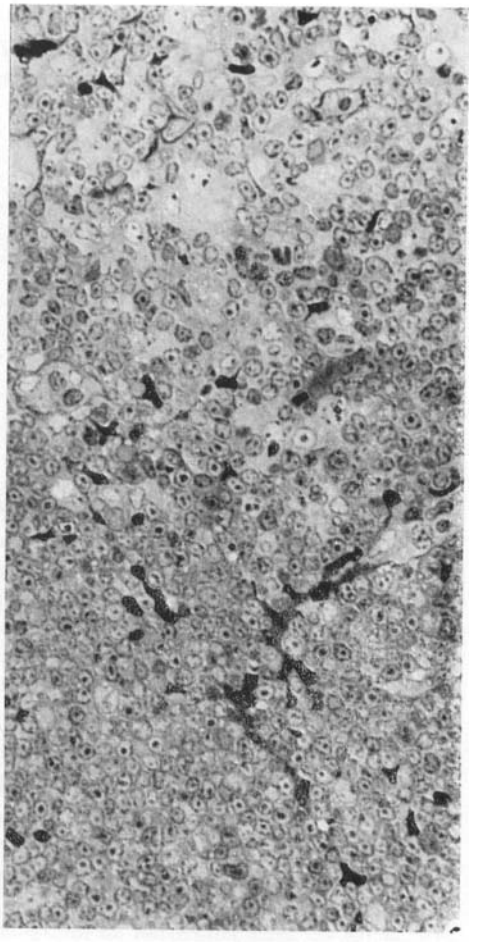

Fig. 2. Uneven distribution of dark cells in the cortex (below) and medulla. A capillary, right. Toluidine blue stain. ×465.

conditions were described previously (JÄRPLID 1968). Specimens from the thymus were fixed in Stieve's fluid and stained with Ehrlich's haematoxylin and eosin for light microscopy. For electron microscopy specimens were fixed in glutaraldehyde or formaldehyde-glutaraldehyde solution (KARNOVSKY 1965), postfixed in OSO_4 (BENNETT & LUFT 1959) and embedded in Epon (LUFT 1961). For light microscopy 1 μ sections were stained with toluidine blue (BJÖRKMAN 1962). Ultrathin sections were stained with uranylacetate in methanol (STEMPAK & WARD 1964)

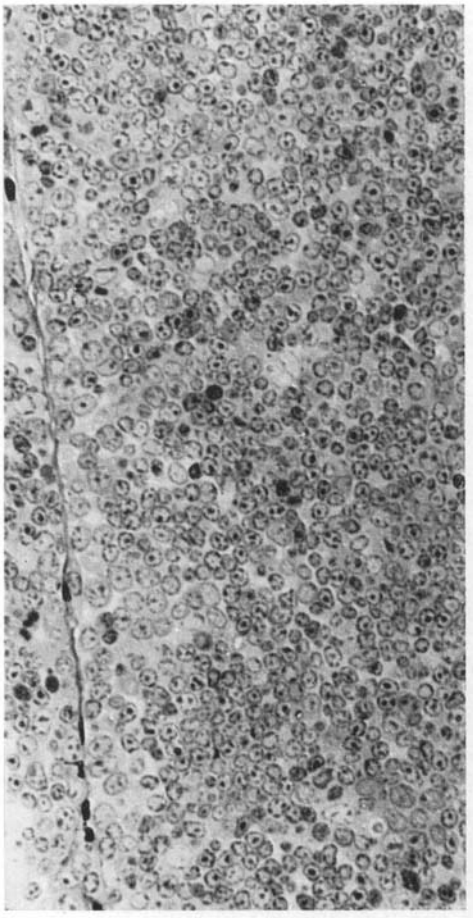

Fig. 3. Thymic cortex. Dark cells only along a connective tissue structure, left. Toluidine blue stain. ×470.

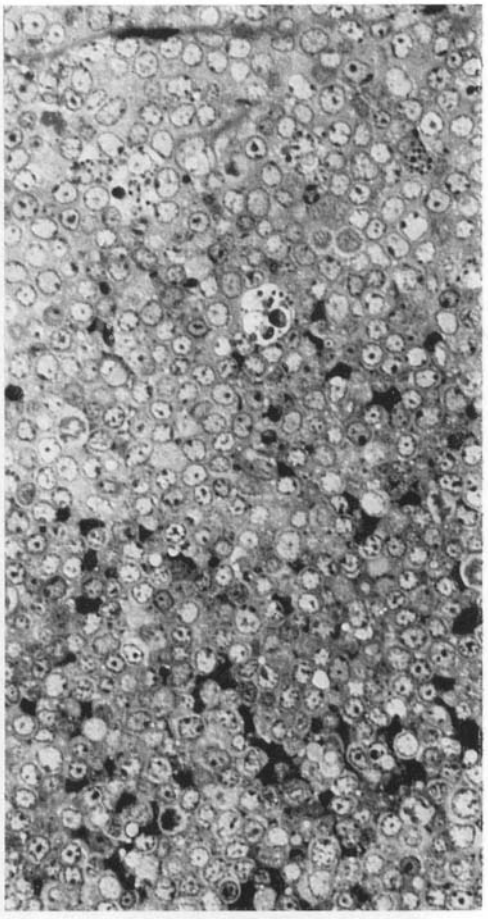

Fig. 4. Thymic lymphoma. Uneven distribution of dark cells. Toluidine blue stain. ×470.

and lead citrate (VENABLE & COGGESHALL 1965) and examined in a Siemens Elmiskop IA at 60 kV.

Results

In light microscopy the most evident characteristics of the dark cells were the affinity to toluidine blue stain. The cells were irregularly stellate and sometimes had long slender processes. Both the nucleus and the cytoplasm were very dark and the nuclear membrane was often indistinct (Figs 1—4).

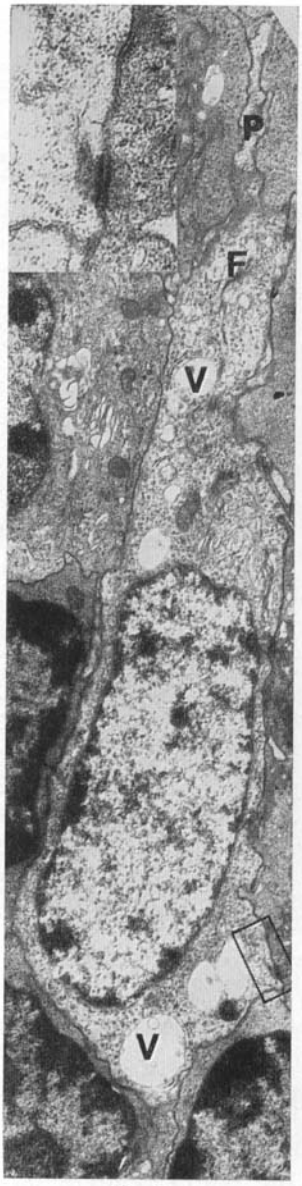

Fig. 5

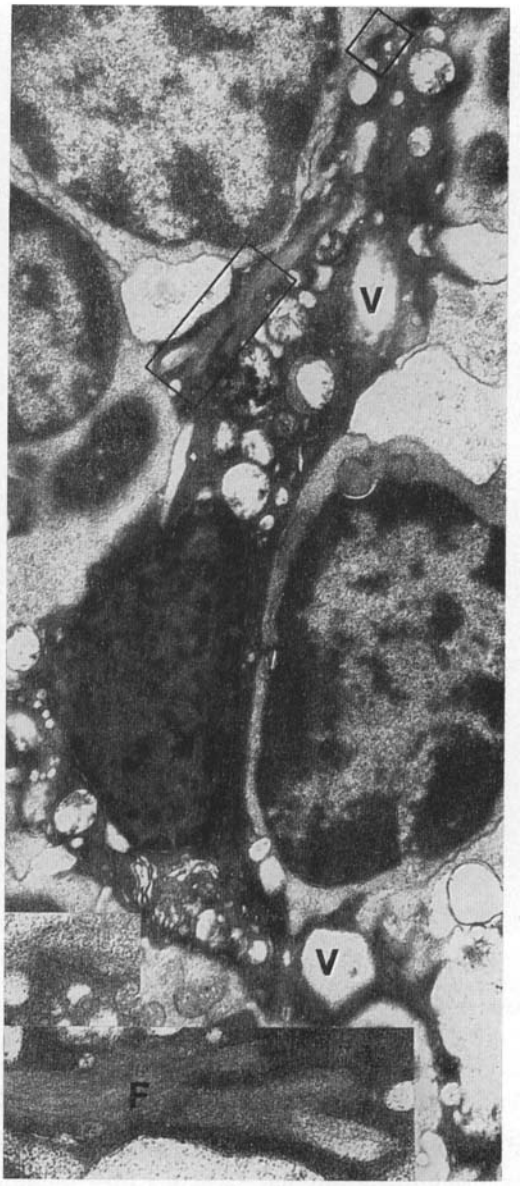

Fig. 6

Fig. 5. Light epithelial reticular cell with cytoplasmic processes (P), intracytoplasmic fibrils (F), a desmosome (inserted, × 23 250), and vacuoles (V). × 6 720.

Fig. 6. Dark epithelial reticular cell characterized by cytoplasmic processes and increased density of both nucleus and cytoplasm. Vacuoles (V), fibrillar structures (inserted (F), × 23 040) and plenty of ribosomes in the cytoplasm. Desmosome (inserted, × 23 040). × 9 600.

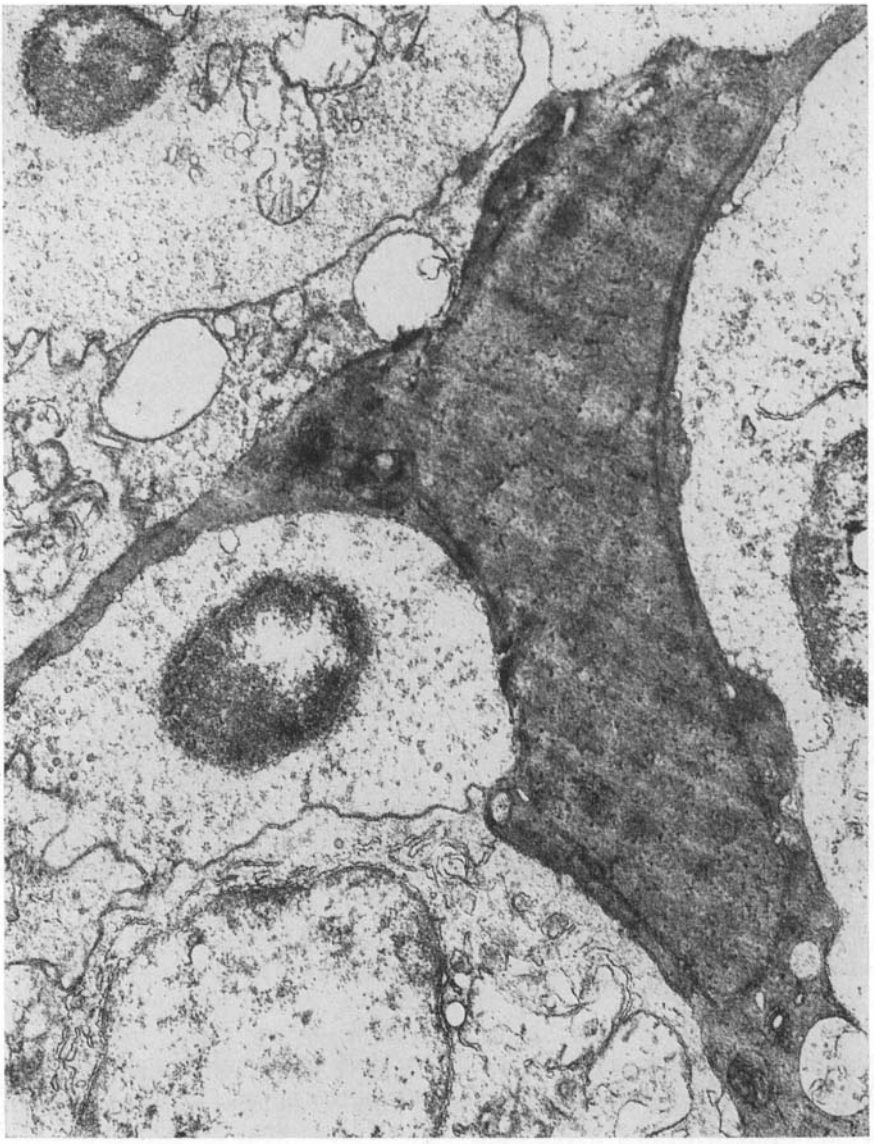

Fig. 7. Dark reticular cell with slender cytoplasmic processes. The nucleus is homogenous and the density is equalized between nucleus and cytoplasm. Possible cellular degeneration. ×16 100.

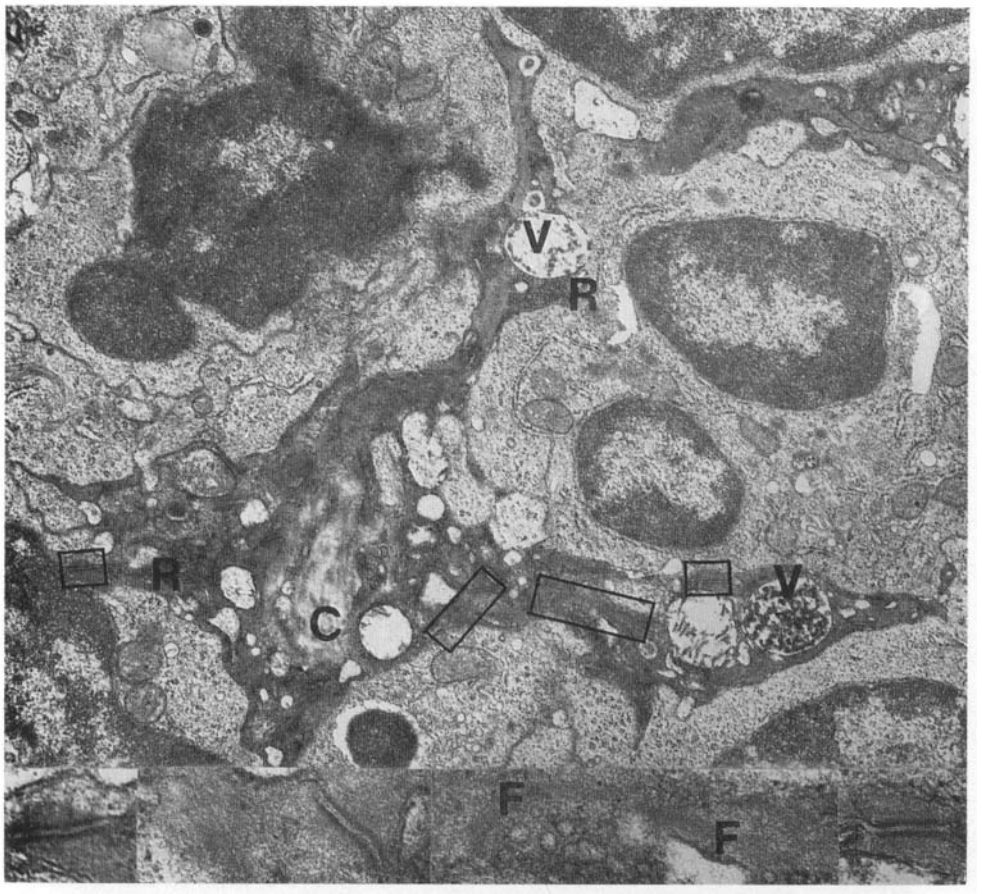

Fig. 8. Cytoplasmic processes of dark epithelial reticular cell in contact with connective tissue structures (C) and containing vacuoles (V), accumulations of ribosomes (R), fibrillar structures (inserted, F, ×36 720) and several desmosomes (inserted, ×36 720). ×12 240.

Also in electron microscopy the cells were dark as a result of increased electron density compared to surrounding cells, both in the nucleus and in the cytoplasm (Figs 5—7). The nucleus was polygonal, ovoid or somewhat elongated. In some cases the nucleus was homogeneous with a density similar to that of the cytoplasm and with an indistinct nuclear membrane (Fig. 7). The density of the cytoplasm could be referred to the uniformly granular cytoplasmic matrix (Fig. 9). The number of organelles varied. Plenty of ribosomes and some mitochondria were generally seen. Many cells had vacuoles, sometimes containing an amorphous material of moderate density (Figs 6, 8). The slender processes extending between

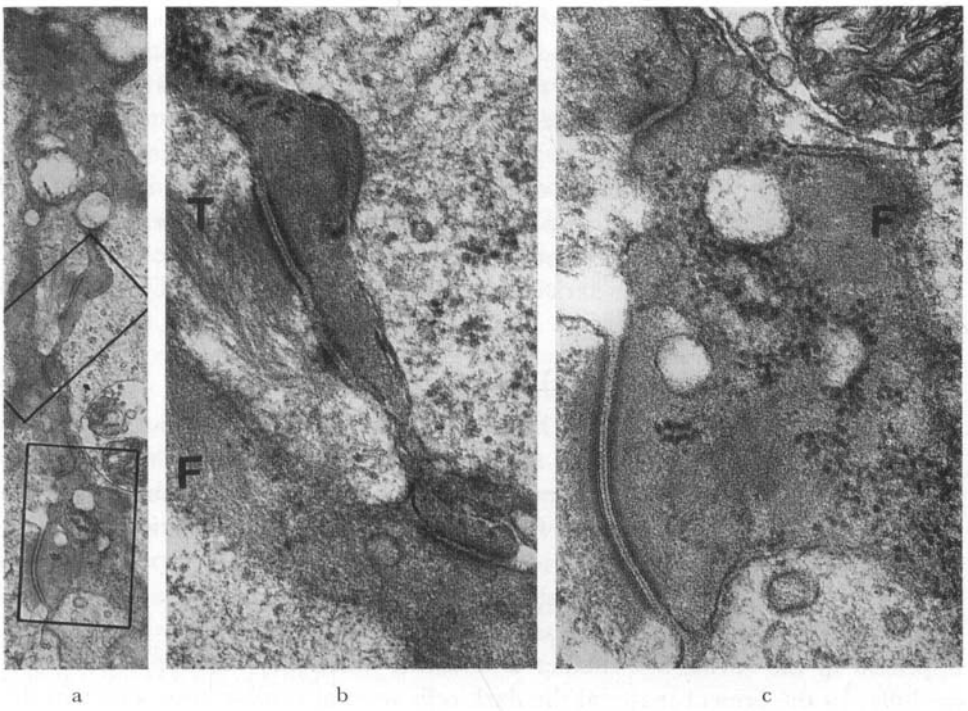

Fig. 9. Cytoplasmic processes of dark epithelial reticular cell with desmosomes, fibrillar structures (F) and accumulations of ribosomes in the condensed and finely granular hyaloplasm. Tonofilaments (T) in an adjacent epithelial cell. b) (×59 200) and c) (×59 200) are details of a) (×14 800).

surrounding cells had a finely granular cytoplasmic matrix with plenty of ribosomes, often unevenly distributed, and sometimes fibrillar structures (Figs 8, 9). Desmosomes were sometimes observed in connection with adjacent epitheliar cells or within the dense matrix (Figs 6, 8, 9).

The frequency and the distribution of the dark cells varied greatly both between different animals and between different sections from the same thymus (Figs 1, 2). In some sections no dark cells were observed. In other sections they generally appeared as single cells, in the cortex often related to light epitheliar cells, to capillaries or to connective tissue structures (Figs 2, 3, 8). Generally they were more common near the boundary between the cortex and the medulla than near the capsule. Sometimes the cells appeared in accumulations or in lines along interlobular septa (PEREIRA & CLERMONT 1971) in the cortex (Fig. 3). In the medulla the dark cells generally appeared as single cells often in contact with epithelial cells.

After irradiation the frequency of the dark cells did not seem to increase during the different phases of depletion and regeneration of the thymus (JÄRPLID 1968); whether the frequency decreased was difficult to establish because of the normally irregular and low frequency of these cells.

In the thymic lymphomas the dark cells had the same appearances as they did in normal thymus (Fig. 4). Three out of six cases of localized thymic lymphoma had a few dark cells and one had plenty of them. Among 10 cases of generalized thymic lymphoma one had numerous dark cells and another case only a few. Eight of these tumours had no dark cells.

Discussion

Cells similar to these dark cells have previously been described as 'dense reticular cells' in thymus and lymph nodes of mice (IZARD & DE HARVEN 1968) and as 'dark reticular cells' in lymphadenitis and lymphomas of man (MOLLO et coll. 1969). HIROKAWA (1969) found two types of epithelial reticular cells in the thymus of human foetus and newborns, one 'hypertrophic' type which was seen only in the medulla and one 'slender' type. This slender type had long, cytoplasmic projections and a nucleus which was large and pale when the cell appeared in the cortex but smaller and darker when the cell appeared in the medulla. In the present material the dark cells were of similar appearance in the cortex and in the medulla. RAPPAY et coll. (1971) found it difficult to analyse the fine structure of slender cells in the thymus of perinatal rats because of the great density of these cells.

The dark cells have several characteristic features suggestive of light epithelial reticular cells, viz. long, slender cytoplasmic processes, desmosomes, vacuoles and fibrillar structures in the cytoplasm, and a similar distribution in cortex and medulla. A close relationship between these two cells was also suggested by the observation of many intermediate forms of variable size and density in the same sections (IZARD & DE HARVEN). These authors did not notice any occurrence of desmosomes, however, and the densities found by MOLLO et coll. between different contacting dark processes could not with certainty be interpreted as desmosomes. In the present material, however, the desmosomes both within the dense cytoplasmic matrix itself and between dark cells and light epithelial cells are evident and indicate that these dark cells are true epithelial reticular cells. Similar dark cells in the thymus of chicken have also been interpreted as epitheliar cells (FRAZIER 1973).

The epithelial reticular cells constitute the supporting framework of the cortex and outer medulla and separate these regions from the connective tissue of the capsule, interlobular septa, blood vessels and inner medulla (PEREIRA & CLER-

MONT 1971). The frequent observation of dark cells related to capillaries and connective tissue structures (Figs 2, 8), which have also been reported previously (IZARD & DE HARVEN, MOLLO et coll.), and the observation of many intermediate forms between dark and light epithelial reticular cells (IZARD & DE HARVEN) mentioned above, indicate an intimate relationship between these cells.

The homogenisation of the nuclear structures and the equalized density between nucleus and cytoplasm in some dark cells may indicate degeneration. No similar or other appearances indicating degeneration were observed in light epitheliar cells. This may suggest that dark cells are degenerating epithelial reticular cells (MOLLO et coll.).

Dark cells did not increase during regeneration of the radiation injured thymus, nor did they seem to be more common in thymic lymphomas than in normal thymus. In contrast to this IZARD & DE HARVEN found an increased number of dark cells in thymic lymphomas of AKR mice.

In conclusion the hypothesis may be suggested that dark and light epithelial reticular cells may be different modes of expression of the same cell type.

Acknowledgement

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SUMMARY

The morphology and distribution of dark reticular cells in the thymus of normal mice, of irradiated mice and of mice with thymic lymphoma are described. It is concluded that dark cells are epithelial reticular cells and the hypothesis is suggested that dark and light epithelial reticular cells may be different modes of expression of the same cell type.

ZUSAMMENFASSUNG

Die Morphologie und Verteilung der dunklen retikulären Zellen im Thymus von normalen und bestrahlten Mäusen und von Mäusen mit einem Lymphom des Thymus werden beschrieben. Der Verfasser kam zum Schlusssatz, dass die dunklen Zellen epitheliale retikuläre Zellen sind und legte die Hypothese vor, dass die dunklen und hellen epithelialen retikulären Zellen verschiedene Formen des selben Zelltypus darstellen.

RÉSUMÉ

Description de la morphologie et de la distribution des cellules réticulaires sombres dans le thymus de souris normales, de souris irradiées et de souris atteintes de lymphome thymique. L'auteur conclut que les cellules sombres sont des cellules réticulaires épithéliales et il émet l'hypothèse que les cellules réticulaires épithéliales sombres et claires peuvent être des modes différents de l'expression du même type de cellules.

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