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Glutaraldehyde and Osmium tetroxide fixation of thymus in effective demonstration of Hassal's corpuscles in thymus of goats

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Abstract
The scanning electron micrograph of the goat thymus was studied by glutaraldehyde and osmium tetroxide fixation. The thymus showed epithelial reticular cells, thymocytes, macrophages, Hassal's corpuscles, interdigitating cells, Dendritic cells and Thymic nursing cells. The epithelial cells form a meshwork in the thymus parenchyma. Cortical epithelial reticular cells were stellate in shape, while the medullary epithelial reticular cells were of two types, stellate and large vacuolated elements. A continuous single layer of epithelial cells separates the parenchyma from connective tissue formations of the capsule, septa and vessels. Surrounding the blood vessels, this epithelial sheath was continuous in the cortex, while it was partly interrupted in the medulla, suggesting that the blood thymus barrier might function more completely in the cortex. Osmium tetroxide being a heavy metallic compound gives higher electron beam resistance under the gold sputtered surface of the tissue.

Keywords: glutaraldehyde, osmium tetroxide, thymus, hassal's corpuscles, goat

Introduction
The thymus represented the central organ of the immune system, constituting a microenvironment for T-lymphocyte differentiation. It was now accepted that, in the embryo, precursor T-cells migrate into the thymus from the yolk sac and liver, and postnatally, from the bone marrow. They become mature T-cells after passing through the cortex and medulla of the thymus [7].
Glutaraldehyde and osmium tetroxide fixation of tissue for Scanning electron microscopy (SEM) has proven useful for revealing cell relationships showing microenvironment of T-cell differentiation and to the fine structure of inter digitating cells.

Materials and Methods
Thymus from 6 numbers of 2 year old goats slaughtered in and around thanjavur was collected. One millimeter cube tissue pieces were cut and fixed in neutral buffered formalin. The tissues were routinely processed and thick sections of 10 microns were made in Leica manual microtome. Obtained sections were dewaxed and 3 millimeter square area of study was cut with glass cutter. Then sections were hydrated and treated with 2.0% solution of glutaraldehyde followed by post fixation in osmium tetroxide and evaporation-coated with gold-palladium. The tissues were observed in a VEGE3 TESCAN in SASTRA University, Thanjavur with an accelerating voltage of 3 kV.

Result and Discussion
In low-magnification SEM views, the thymus was surrounded by a capsule (Fig 2) of connective tissue, which extended septa, dividing the thymic parenchyma into incomplete lobules [13]. Each lobule consisted of the cortex and medulla.
In the cortex, epithelial cells, conventionally called "epithelial reticular cells", were stellate in shape (Fig 2) with a central thickening and several long processes extending in various directions [12]. The cytoplasmic processes were string-like and connected with those of adjacent cells to form a reticulum. The coarse meshwork containing numerous spaces will hereafter be called the epithelial reticulum. The epithelial cells were smooth in surface. Several vacuoles, 0.5-1.5 µm in diameter, were present in the cytoplasm. They usually contain fine-granularmaterial [11]. At the periphery of the cortex, the processes of the epithelial cells constitutea continuous single layer, which rests on a basal lamina and separates the thymic

parenchyma from the connective tissue. The epithelial cells also anchor the capillaries [10].

In the meshes of the epithelial reticulum, numerous free cells were found, most corresponding to thymic lymphocytes, which were round and vary in size from 4 to 8 µm in diameter [9]. Small lymphocytes predominate, while large lymphocytes were sparsely scattered throughout the cortex (Fig 1), the latter being more numerous in the outer cortex [8]. Both types of lymphocyte were smooth in surface; they possess a few villous micro processes and occasionally exhibit knob-like cytoplasmic protrusions [6].

Another type of free cell was scattered in the cortex, especially around small vessels. These cells measure 10-15µm in diameter and correspond to cortical macrophages [5]. They were covered by numerous villous or bubble-like microprojections and ruffles, which sometimes contact both epithelial cells and lymphocytes [4]. Sometimes, the macrophages enclose one or two lymphocytes with their pseudopods [3]. Some fractured macrophages also reveal large vacuolar or cavernous inclusions, which probably correspond to phagosomes derived from the internalized lymphocytes.

Epithelial cells in the medulla, as in the cortex, form a reticulum, although the meshwork was more complicated than in the cortex. These cells present several morphologic forms, which can be classified into the following two main types. The first was stellate in shape, extending thin, thread-like processes which form the reticulum [2]. These cells display a smooth surface. The second type was a large cell usually possessing some vacuoles of varying size in the cytoplasm. These cells sometimes show a complicated profile of cellular interdigitation with the adjacent cells of the same type [1].

This cell type often accumulates in small groups. Transitions between the two types were also present. Hassall's corpuscles, which were formed by a concentric arrangement of epithelial cells (Fig 3), were also encountered in the present study. This precision was achieved by fixing the specimen in Osmium tetroxide a heavy metallic compound that rendered higher electron beam resistance under the gold sputtered surface of the tissue.

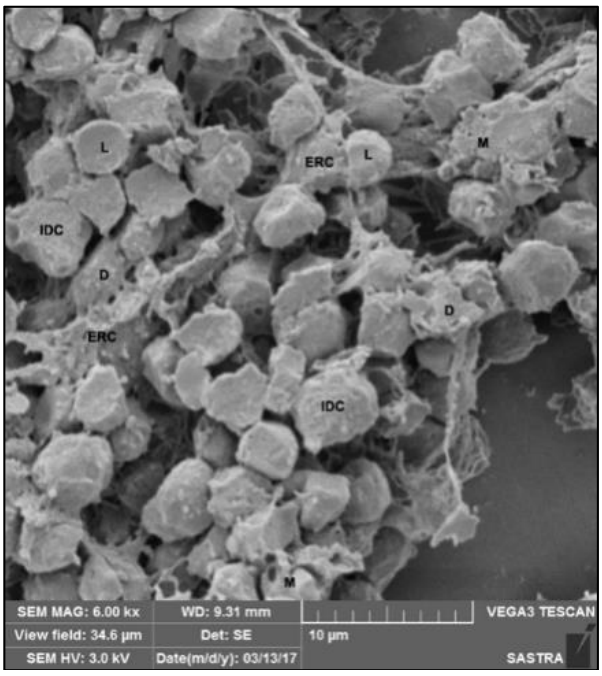

Fig 1: L – Lymphocyte, M – Macrophage, D – Dentritic Cell, ERC – Epithelial Reticular Cell and IDC – Inter Digitating Cell

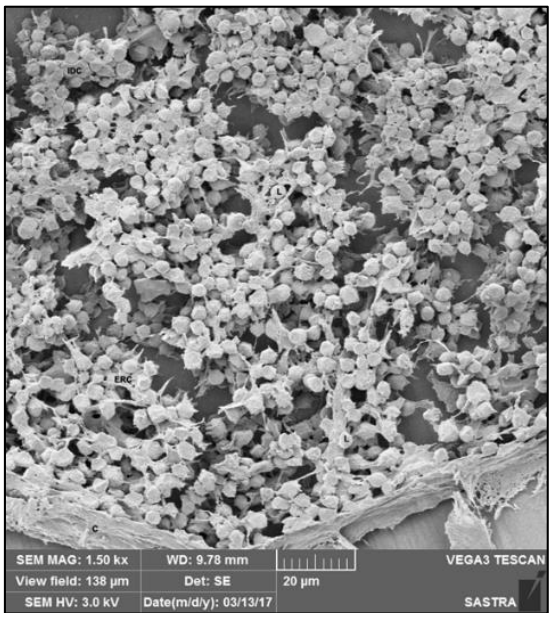

Fig 2: IDC – Inter Digitating Cell, L – Lymphocyte, ERC – Epithelial Reticular Cell and C – Capsule

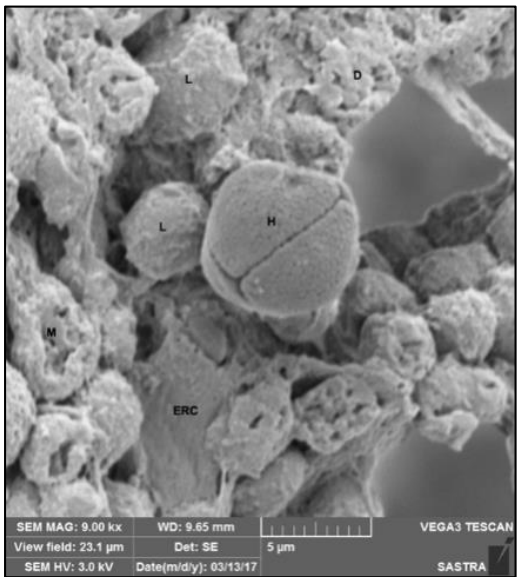

Fig 3: L – Lymphocyte, D – Dendritic Cell, H – Hassel's Corpuscle, M – Macrophage and ERC – Epithelial Reticular Cells.

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