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ULTRASTRUCTURAL AND MORPHOMETRICAL STUDIES ON THE RETICULAR FRAMEWORK AND RETICULAR FIBERS IN THE RETICULOENDOTHELIAL SYSTEM OF RATS

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ABSTRACT

The reticular framework and reticular fibers in the thymus, cervical lymph node, spleen and bone marrow of Wistar rats were studied by transmission electron microscopy and morphometrical method. The reticular framework was usually observed in these organs as a common structure that consisted mainly of the slender cytoplasmic processes of the fibroblastic reticular cells interconnected with tihgt junction. Ultrastructurally, the reticular fibers were a mixture of collagen fibrils and amorphous materials, and were almost completely ensheathed by the cytoplasm of fibroblastic reticular cells. Such characteristic structure of the reticular fibers was found not only in the thymus, lymph node and spleen, but also in the bone marrow where it has not been clearly demonstrated previously. Morphometrical analysis revealed that the content of collagen fibrils in the reticular fiber in the lymphoid tissues (the thymus, lymph node and splenic white pulp) was much greater than that in the hematopoietic tissues (the bone marrow and splenic red pulp). Based on these evidences, it was reasonably considered that the reticular framework and reticular fiber ensheathed by the cytoplasm of the fibroblastic reticular cells were the most representative common structure in the reticuloendothelial system (RES) and played some important roles in the functions of RES.

Key Words: Fibroblastic reticular cell, Reticular framework, Reticular fiber, Reticuloendothelial system, Morphometry.

INTRODUCTION

In 1924, L. Aschoff proposed the concept of the reticuloendothelial system (RES) for a system of specific cell groups that showed prominent phagocytosis in common. Although such a cell system consisted of the reticular cells, reticuloendothelia, histiocytes and blood histiocyte (phagocytic monocyte)[1], these mesenchymal cells were considered to belong to a single cellular system sharing a common origin, structure and function. In 1952, however, Akazaki and his coworkers corrected Aschoff's original concept of RES, based on the results of their long series of studies on RES[2]. Their couclusion was that RES was defined as a functional unit composed of two groups of mesenchymal cells genetically of different origins. Furthermore, R. van Furth pointed out that RES contained various kinds of cells that did not fulfil the criteria for Aschoff's concept, and proposed a new classicification of macrophages, monocytes and their precursor cells, as the mononuclear phagocyte system separated from cell group in RES[3]. From the view point of cellular heterogeneity in RES, Furth's correction seems to be in line with Akazaki's concept.

Recently, much attention has been paid to the common architecture of RES concerning the

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reticular framework for re-evaluation of RES[4–6]. Many electron microscopic studies have been made on the reticular framework and reticular fiber in each tissue of RES [7–9]. However, there has been no overall study of these in RES previously. Moreover, no morphometrical studies have been performed on the reticualr fiber.

In the present studies, we first attempted to clarify some characteristics of the common ultrastructure of the reticular framework and the reticular fibers in RES of Wistar rats. Secondly, we tried to analyse the constitutional elements of the reticular fibers by using electron micrographs with the morphometrical method.

MATERIALS AND METHODS

Five Wistar male rats, weighing 200–250g were used for the present studies. All animals were fed a commercial diet (CE-2, Clea Japan, Inc.) under physiological condition and had water *ad libitum.*

For electron microscopic examinations, the rats were anesthetized with diethyl ether and then fixed by perfusion through the aorta with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffered saline (PBS), pH 7.4, at room temperature for 30 min. After perfusion, the spleen, cervical lymph node, thymus and bone marrow were removed from the rats. Small pieces of these tissues were fixed again with the same fixative at 4°C for 30 min and further post-fixed with 2% OsO_4 in PBS at 4°C for 1 h. Tissue blocks were dehydrated in graded alcohols and embedded in Epon 812-Araldite. Ultrathin sections were cut with a Reichert-Jung Super Nova ultramicrotome and stained with uranyl acetate followed by lead citrate. Electron micrographs were taken with a Hitachi 600 transmission electron microscope operated at 75kV.

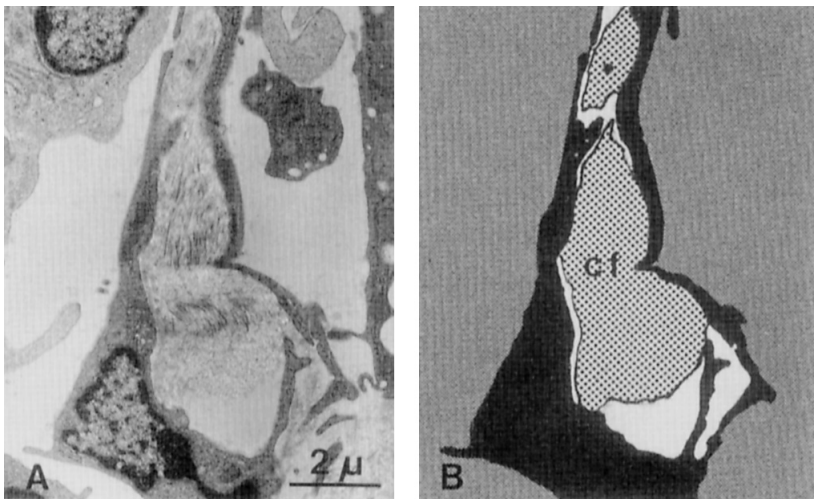

Fig. 1. Cut surface of the reticular fiber.
(A) An electron micrograph of the reticular fiber ensheathed by cytoplasm of the fibroblastic reticular cell in the lymph node. (B) Tracing figure of the reticular fiber which consists of white zone and dotted zone occupied by collagen fibrils (cf).

For morphometrical analysis of the reticular fibers, which consisted of collagen fibrils and amorphous materials, the areas occupied by collagen fibrils were traced on electron micrographs of the reticular fibers in the reticular framework of the spleen, lymph node, thymus and bone marrow (Fig. 1). Then, both areas of collagen fibrils and whole reticular fibers were measured by a computer using the personal image analysing system, LA-525(PIAS Co. Osaka). Electron micrographs (more than 70 sheets) taken at 10,000× magnification were examined for the morphometrical study of each tissue.

RESULTS

Ultrastructure of the reticular framework and reticular fibers

The reticular framework was usually observed in the stroma of the spleen, lymph node, thymus and bone marrow as a sponge-like structure composed mainly of the fibroblastic reticular cells in association with vascular or lymphatic endothelial cells, macrophages, interdigitating cells, and follicular dendritic cells. Essentially the same ultrastructures of the retiular framework in these organs were demonstrated, although they contained different kinds of homing cells. The lymphopoietic cells were located in the reticular framework of the thymus, lymph node and splenic white pulp, while the macrophages and/or hematopoietic cells nestled in that of the splenic red pulp and bone marrow. The slender adjacent cytoplasmic processes of the fibroblastic reticular cells interconnected with the tight junction (*zonula occludens*) to make a cellular meshwork (Fig. 2A). As shown in Fig. 2B, the fibroblastic reticular cells were spildle-like or dendritic in shape and often had large, round nucleoles in an oval or polygonal nucleus. The rough surfaced endoplasmic reticulum, Golgi complex, and microfilaments developed well, but no phagosomal lysosomes were observed in their cytoplasm (Fig. 2C).

Ultrastructurally, the reticular fibers were a mixture of collagen fibrils and amorphous materials, and were almost completely ensheathed by the cytoplasm of either a single or a pair of fibroblastic reticular cells (Figs. 1 & 3). Such characteristic structure of the reticular fiber was found not only in the spleen, lymph node and thymus, but also in the bone marrow. This finding was certified by examining seriated ultra-thin setions of the bone marrow. It was remarkable evidence that the contents of collagen fibrils were different in the reticular fiber of various tissue (Figs. 1 & 3). The reticular fibers in the thymus (Fig. 3A), lymph node (Fig. 1), and splenic white pulp (Fig. 3B) contained many more collagen fibrils than those in the splenic red pulp (Fig. 3C) and bone marrow (Fig. 3D). There was a tendency for collagen fibrils to distribute in the central zone of the reticular fibers and to come together compactly, as the content of collagen fibrils increased. Collagen fibrils in the reticular fiber were about 45 nm in diameter and presented a linear periodicity of 60—70 nm along their axis (Fig. 2D). The reticular fibers frequently showed structural continuity with the basement membrane of small blood vessels.

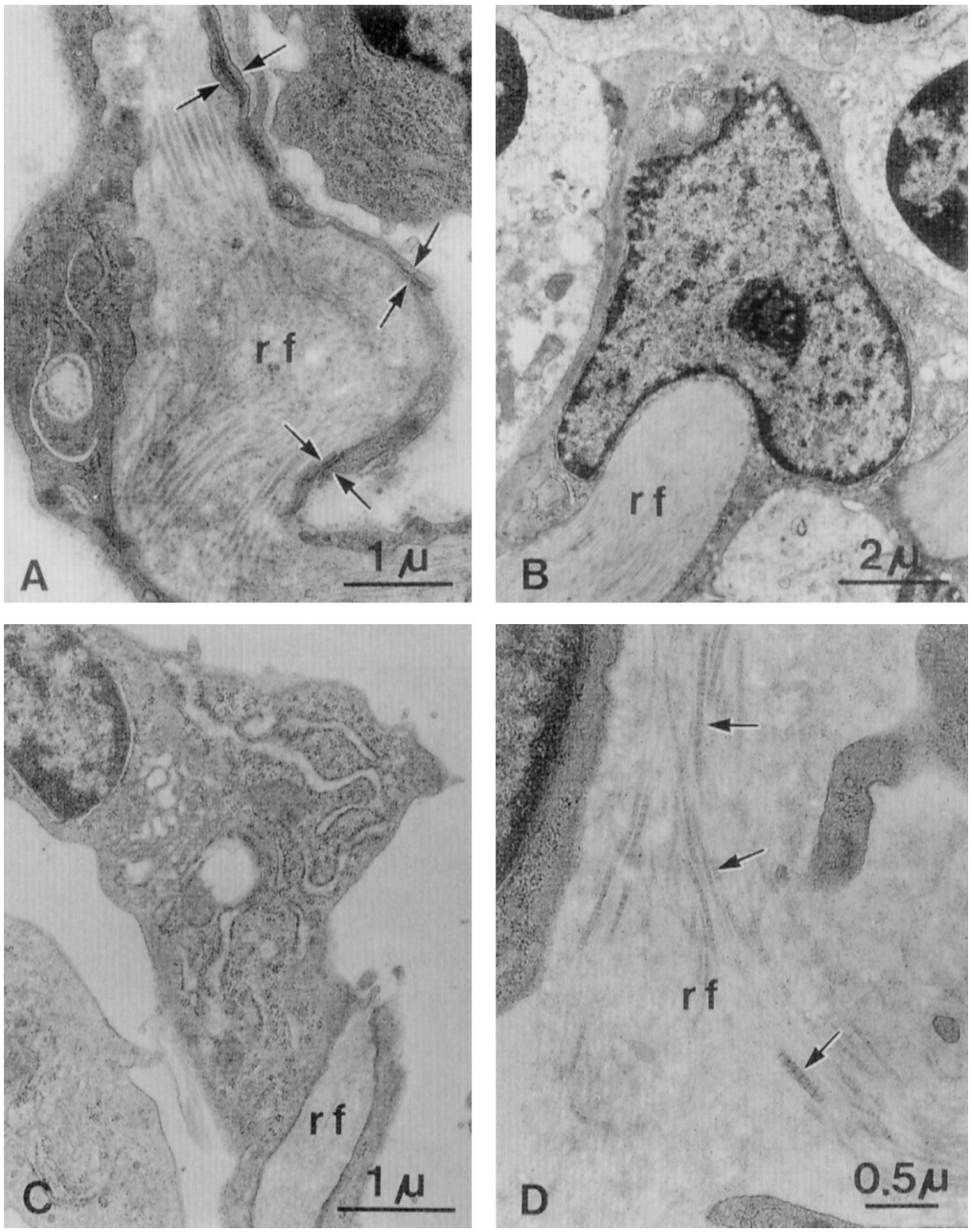

Fig. 2. Ultrasrtucture of the fibroblastic reticular cells with reticular fiber (rf). (A) Shows tight junctions (arrows) between adjacent fibroblastic reticular cells. Lymph node. (B) A prominent large round nucleole. Lymph node. (C) Well-developed r-ER and Golgi complex. Splenic red pulp. (D) Collagen fibrils (arrows) in the reticular fiber demonstrate a periodic banding pattern. Lymph node.

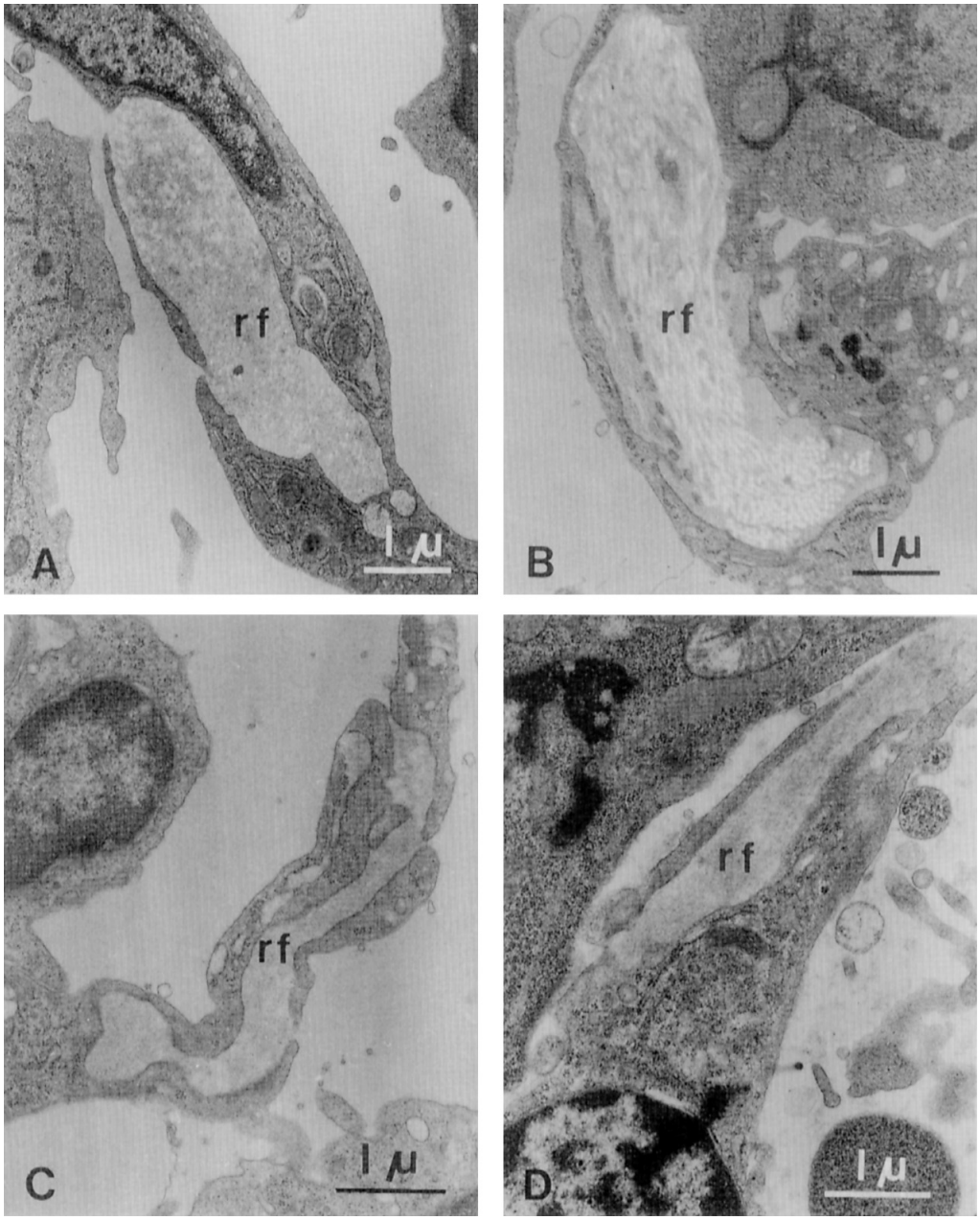

Fig. 3. Ultrastructure of the reticular fibers (rf) in a variety of lymphoreticular tissues. (A) Thymus. (B) Splenic white pulp. (C) Splenic red pulp. (D) Bone marrow. A large number of collagen fibrils are observed in Fig. A and B, but few in Fig. C and D.

Morphometrical analysis of the reticular fibers

The reticular fibers ensheathed by the cytoplasm of the fibroblastic reticular cells were approximately 1−3 μm in width and showed no significant difference in their width among various tissues of RES.

Fig. 4 is the histogram of ranged ratios which indicate percentages of area occupied by collagen fibrils in the reticular fibers in the thymus, lymph node and spleen. In the thymus and lymph node the ratios of area occupied by collagen fibrils showed a similar distribution and revealed one peak within the range of 40% to 80%. On the contrary, the ratios in the spleen

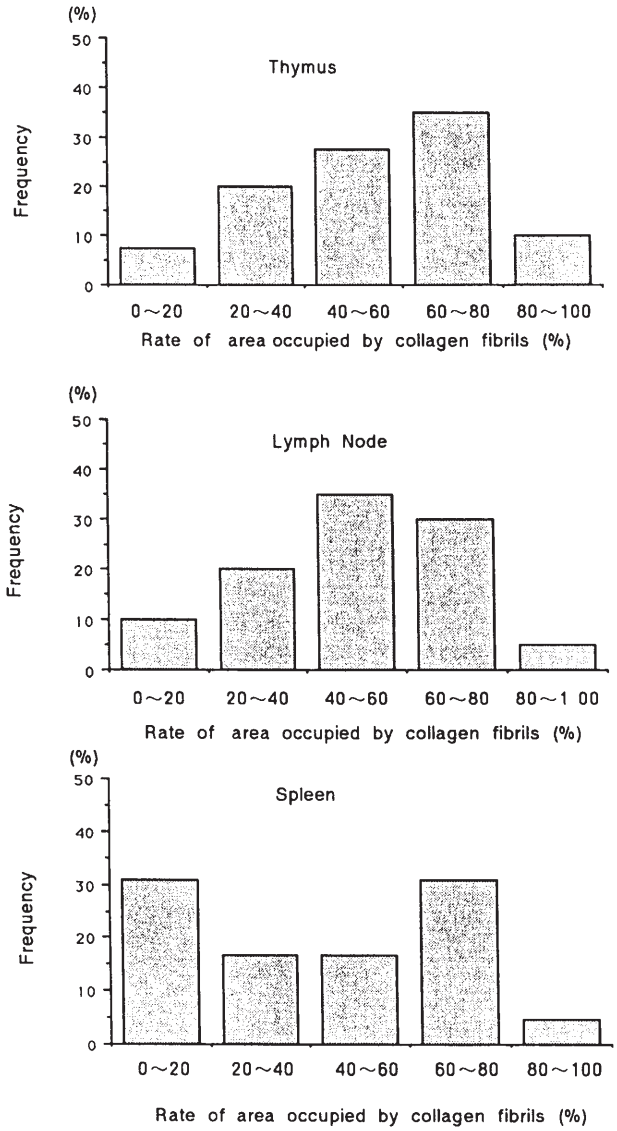

Fig. 4. The histogram of distributional ratios of collagen fibrils on the cut surface area of the reticular fibers in the thymus, lymph node and spleen.

showed a complicated distribution, revealing a remarkable difference from those in the thymus and lymph nodes. Therefore, the reticular fibers in the splenic white and red pulps were separately measured. As shown in Fig. 5, the white pulp had one peak of distribution in the range of 60% to 80% same as the thymus, and the red pulp also had one peak in the range of 0% to 20%.

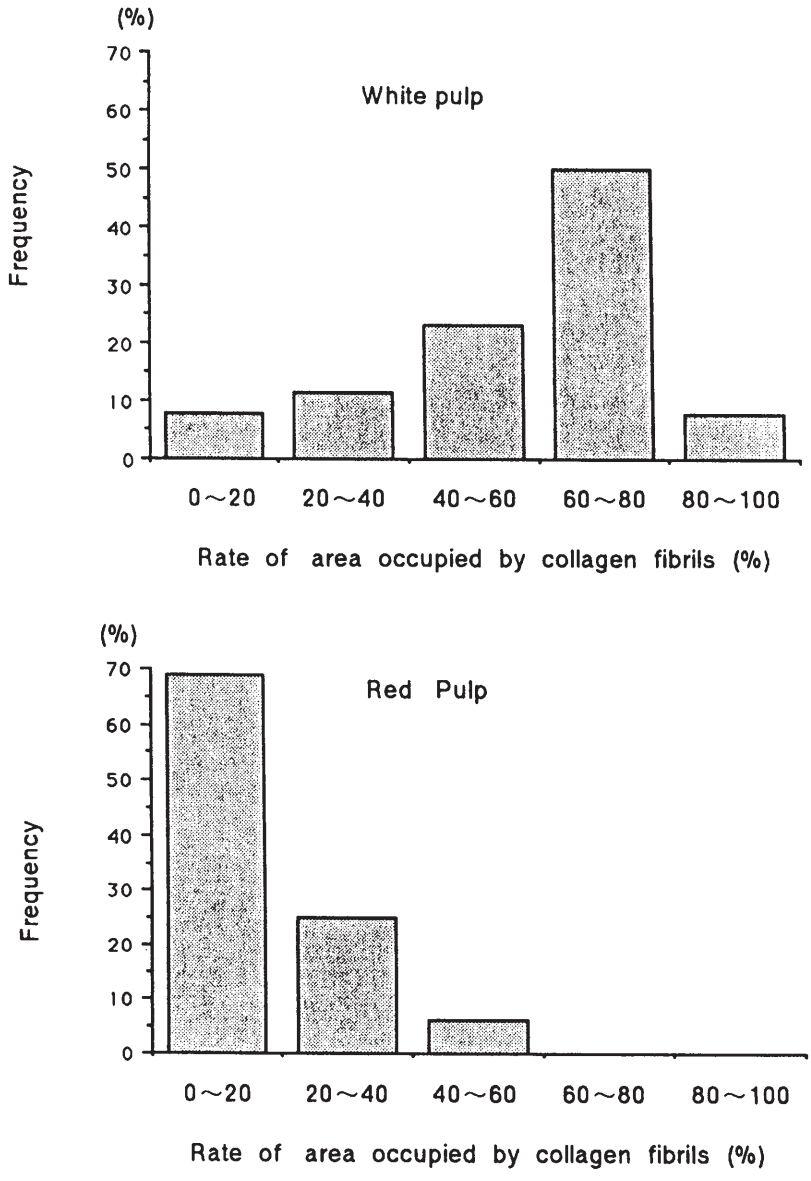

Fig. 5. The histogram of distributional ratios of collagen fibrils on the cut surface area of the reticular fibers in the white and red pulps.

The mean values of distributional ratios occupied by collagen fibrils in the reticular fibers of each tissue were shwon in Fig. 6. In the bone marrow, there were a few collagen fibrils in the reticular fibers. However, they were so finely scattered in the reticular fiber that the area occupied by collagen fibrils could not be traced and measured on electron micrographs of the bone marrow. It was clear-cut evidence that the mean values of the distributional ratios of area occupied by collagen fibrils in the reticular fibers in the lymphoid tissues (the thymus, lymph node and splenic white pulp) were remarkably higher than those in the non-lymphoid tissues of RES (the splenic red pulp and bone marrow), with a significant difference recognized between them ($p<0.001$).

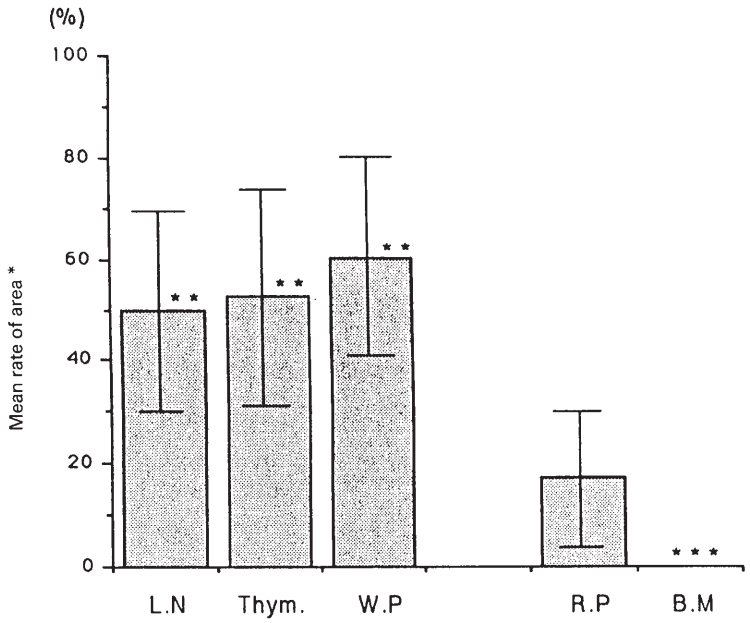

Fig. 6. Mean values of ratios of area occupied by collagen fibrils on the cut surface of the reticular fibers in the lymphoreticular tissues. L.N: Lymph node. Thym: Thymus. W.P: White pulp. R.P: Red pulp. B.M: Bone marrow.

* Area occupied by collagen fibrils in reticular fibers

** $P<0.001$

*** Unmeasurable

DISCUSSION

Since the reticular fibers were first identified as a component of connective tissue by Kupffer in 1876[10], many studies on their structure and distribution in various tissues have been carried out light microscopically with the silver impregnation method[11]. Electron microscopic studies of the reticular fibers have shown that they consist of collagen fibrils and low electron dense materials[7]. In addition, electron microscopy has revealed that the reticular fibers are ensheathed by cytoplasm of the fibroblastic reticular cells in the thymus, lymph node and spleen [8,9,12]. However, such a characteristic ultrastructure of the reticular fibers was not clearly demonstrated in the bone marrow in previous papers[13]. In the present studies we were able to observe the well-defined structure of the reticular fibers in seriated ultra-thin sections of the bone marrow. It was

evident that the reticular framework was usually observed in those organs including the bone marrow and its ultrastructure was essentially the same among them in RES. These results suggest that the reticular framework and the reticular fiber are the repreasentative common structures of RES tissues.

It has been reported that collagen fibrils are one of the factors inducing blood coagulation[14–16]. The reticular framework of RES tissues is immersed with blood plasma even under physiological condition. Blood coagulation occurs easily in the reticular tissues, if collagen fibrils of the reticular fiber were exposed to blood plasma. Therefore, the ultrastructure of the reticular fiber ensheathed by the cytoplasm of the fibroblastic reticular cell seems to be profitable to prevent blood coagulation in RES tissues. A morphological analysis of the reticular tissue has not been completed, and additional studies on the reticular framework are needed concerning the relationship with other kinds of cells such as vascular endothelial cells, macrophages, and interdigitating cells.

Hematopoiesis appears in the splenic red pulp of rat embryo and is taken over by the bone marrow after birth. It is well known that extramedullary hematopiesis occurs often in the spleen under certain pathological conditions. Therefore, the splenic red pulp has latent hematopoietic activity and seems to be an equivalent hematopoietic tissue to the bone marrow.

Previously, no morphometrical studies had been done on the reticular fibers in the tissued of RES, although several investigations on the immunohistochemical distribution pattern of type V or III collagen in the splenic reticular fibers have been reported[17,18]. The present studies revealed that the content ratios of collagen fibrils in the reticular fibers in the thymus, lymph node and splenic white pulp were significantly higher than those in the splenic red pulp and bone marrow. This evidence indicates a qualitative difference in the reticular fibers between the lymphoid tissues and the hematopoietic tissues. The difference in constitutional elements of the reticular fiber in various tissues may reflect partially, to certain functions of the fibroblastic reticular cells that manufacture and maintain the reticular fibers. We do not know why there is a constitutional difference in the reticular fiber between lymphoid and hematopoietic tissues. This problem should be resolved in further studies.

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