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Carbohydrate Cytochemistry of the Endothelium lining the Splenic Blood Vessels in the Rat

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Summary: In the endothelial cells lining the rat splenic blood vessels, neutral carbohydrates were studied by means of combined periodic acid-thiocarbohydrazide-silver protein (PA-TCH-SP) and α-amylase digestion methods. In the endothelial cells lining the central and follicular arteries of the spleen, the neutral glycoconjugate-containing surface coat of the luminal plasma membrane and related pinocytotic invaginations and vesicles in the apical cytoplasm were strikingly distinguished, as compared with those in the cells lining the splenic sinuses. In contrast, cytoplasmic glycogen particles in the sinus endothelial cells were apparently larger in amount than those in the arteriolar endothelial cells. Such cytochemical variations of neutral carbohydrates with the arteriolar and venous vessels of the rat spleen were discussed with special reference to varying cytophysiological functions of the endothelial cells with the different segments of the splenic blood vessels.

It has recently been established that blood vascular endothelial cells play a series of important physiological roles such as (1) selective permeability of blood plasmal substances, (2) anticoagulatory mechanism, (3) elaboration and release of extracellular matrices, (4) angiogenesis, (5) regulation of angiotonus and so forth (Sueishi, 1988). For the functional activities of blood vascular endothelial cells, carbohydrates are believed to be an important key substance and, in fact, carbohydrates such as heparan sulfates and related sugars are known to perform an indispensable function for the anticoagulatory mechanism ascribed to vascular endothelial cells (Shimada and Ozawa, 1985; Marcum *et al.*, 1986).

Incidentally, the mammalian spleen is provided with a highly developed microcirculation system and unusually unique, since its tissues are in part of a state of physiological bleedings (Asai and Nakayama, 1987). In view of such characteristic anatomical features of the spleen, a series of light (Snook, 1964; Chen, 1978) and electron (Pictet *et al.*, 1969; Miyoshi and Fujita, 1971; Chen and Weiss, 1972, 1973; Blue and Weiss, 1981; McCuskey and McCuskey, 1985; Seki and Abe, 1985) microscopic studies have so far been made on the mammalian splenic blood vessels, in particular on their distribution patterns and fine structures. To the best of the authors' knowledge, however, no cytochemical studies have ever been performed on carbohydrates involved in the endothelial cells lining the splenic blood vessels in mammals.

In the present study, attempts have successfully been made to investigate into neutral carbohydrates contained in the endothelial cells lining the rat splenic blood vessels by means of methods of electron microscopic cytochemistry. The present results are thought to provide precious and important clues to the precise elucidation and recognition of cytophysiological functions performed by blood vascular endothelial cells in the mammalian spleen.

Materials and Methods

In the present study, 15 male adult rats of Fischer strain (8 weeks, 100–250 g in weight) were used as

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donor animals of the spleen. These animals were perfused through their thoracic arteries first with physiological saline so as to expel blood and then with 0.05 M cacodylate buffered 1% glutaraldehyde (GA) – 2% paraformaldehyde (PFA) solution (pH 7.4), both at room temperature. From the donor animals the spleen was dissected out, immersed in 0.05 M cacodylate buffered 2.5% GA – 2% PFA solution (pH 7.4) on a sheet of dental wax and minced into tiny cubes with an approximate side of 1 mm. Subsequently these tissue blocks were immersion fixed in the 2.5% GA – 2% PFA solution at room temperature for 120 min and rinsed in 0.1 M cacodylate buffer (pH 7.4) at the same temperature for 12–24 h.

Preparation of tissues for cytology

For electron microscopic cytology, the buffer-rinsed tissue blocks were postfixed in 1% osmium tetroxide aqueous solution at room temperature for 60 min, dehydrated in an ethanol series of ascending concentrations, and embedded in Quetol 812 (modification of Luft, 1961). From the resin-embedded tissues, ultrathin sections with an approximate thickness of 800–1000 Å were prepared on an ultramicrotome (Reichert-Jung Ultracut E), mounted on copper grids and counterstained doubly with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963).

Preparation of tissues for cytochemistry

For electron microscopic cytochemistry, the buffer-rinsed tissue blocks were dehydrated in an ethanol series of ascending concentrations, and embedded in LR-White, according to a technique derived by Newman *et al.* (1983). On the ultramicrotome mentioned above, ultrathin sections at the thickness mentioned were prepared and mounted on gold grids. The grid-mounted sections were reacted with periodic acid-thiocarbohydrazide-silver protein (PA-TCH-SP) (Thiéry, 1967; Katsuyama, 1978; Yamada, 1987). These sections were not counter stained with uranyl and lead salts following the cytochemical staining so as to close up reactive ultrastructures. Prior to the PA-TCH-SP staining, some ultrathin sections were subjected to digestion with α-amylase (Seikagaku Kogyo Co., Japan) (0.001 g/ml of 0.002 M calcium acetate) at 40°C for 18 h in order to remove and identify glycogen particles. As controls for such an enzyme digestion procedure, other sections were incubated in the calcium acetate medium without the enzyme under the conditions of the same temperature and duration, whereas other sections were kept intact without any incubation procedures.

Results

For the cytology and carbohydrate cytochemistry of their endothelium, electron microscopic observations were made on three segments of the rat splenic blood vessels; the central and follicular arteries and splenic sinuses.

Cytology and carbohydrate cytochemistry of the endothelium in the arteries:

In the central arteries of the rat spleen, the endothelium consisted of a single layer of flat cells (Fig. 1). These endothelial cells were provided with a slender nucleus surrounded by a flat cytoplasm, which contained usual cell organelles and inclusions such as mitochondria, Golgi complex, endoplasmic reticulum elements, free ribosomes, lysosomes and pinocytotic vesicles (Fig. 1). The endothelial cells lining the central arteries rested upon a relatively thin basal lamina and their luminal plasma membrane was thrown into relatively tiny sparse microvilli (Fig. 1).

If the endothelial cells lining the central arteries were subjected cytochemically to the PA-TCH-SP staining, a series of their ultrastructures were found to exhibit positive reactions of varying intensities. These structures included the surface coat of the plasma membrane, pinocytotic invaginations and resultant vesicles, certain elements of Golgi complex and lysosomes together with the subjacent basal lamina (Fig. 2). It was of particular note that the surface coat of the luminal plasma membrane was relatively thick and exhibited strong positive PA-TCH-SP reaction, which was, however, discontinued intermittently along the plasma membrane (Figs. 2,2-inset). In keeping with such a cytochemical nature of luminal plasma membrane, pinocytotic invaginations and resultant vesicles in the apical cytoplasm showed vivid positive reactions (Figs. 2,2-inset). In the cytoplasm of the arterial endothelial cells, however, glycogen particles were not pronounced in amount (Fig. 2).

In the follicular arteries of the rat spleen, the cytology of the endothelial cells was more or less comparable in ultrastructural features to that of the cells lining the central arteries of the organ (Fig. 3). The endothelial cells were uninucleate and provided with a thin cytoplasm, which contained usual cell organelles and inclusions similar in quality and quantity to those involved in the cells of the central arteries (Fig. 3).

When the endothelial cells lining the follicular arteries were stained with PA-TCH-SP, positive reactions were obtained in such ultrastructures as those which are comparable to PA-TCH-SP reactive structures in the cells of the central arteries; the surface coat of the plasma membrane, pinocytotic

invaginations and resultant vesicles, certain elements of Golgi complex, lysosomes and subjacent basal lamina (Fig. 4). As in the case with the endothelial cells lining the central arteries, the surface coat of the luminal plasma membrane was rather pronounced in thickness and showed pronounced positive PA-TCH-SP reaction, which was likewise discontinued intermittently along the plasma membrane (Figs. 4,4-inset). However, such a discontinuity of the positive reaction was less marked, as compared with that in the cells lining the central arteries (Figs. 4,4-inset). As in the apical cytoplasm of the endothelial cells lining the central arteries, pinocytotic invaginations and resultant vesicles exhibited distinct positive reactions (Fig. 4). In the cytoplasm of the endothelial cells lining the follicular arteries, further, glycogen particles were not pronounced in amount (Fig. 4).

Cytology and carbohydrate cytochemistry of the endothelium in the splenic sinuses:

In the rat, the splenic sinuses were lined by a single layer of endothelial cells (Fig. 5). The endothelial cells lining the sinus were provided with a slender nucleus embedded in a relatively thin cytoplasm, where usual cell organelles and inclusions were detected, such as mitochondria, Golgi complex, free ribosomes, endoplasmic reticulum elements, lysosomes, pinocytotic invaginations and resultant vesicles and glycogen particles (Fig. 5). A rather thin and discontinuous basal lamina was interposed between the endothelial and subjacent reticular cells (Fig. 5). The luminal plasma membrane of the sinus endothelial cells was more or less flat in contour, without being thrown into any apparent microvilli (Fig. 5).

The PA-TCH-SP staining procedure resulted in distinct positive reactions of a variety of ultrastructures in the endothelial cells lining the splenic sinuses, as in the cells lining both the central and follicular arteries; certain elements of Golgi complex, lysosomes, glycogen particles and subjacent basal lamina (Figs. 6,6-inset). It deserved, however, special attention that the surface coat of the plasma membrane, in particular, of the luminal plasma membrane was unusually thin and thus pinocytotic invaginations and resultant vesicles in the apical cytoplasm exhibited exceedingly feeble or negative PA-TCH-SP reaction (Figs. 6,6-inset), unlike the pronounced thickness and distinct positive reaction of the corresponding ultrastructures noted in the endothelial cells lining the central and follicular arteries (Figs. 2,2-inset, 4,4-inset). In the endothelial cells lining the splenic sinuses, another finding of importance was an appreciable abundance of glycogen particles in their cytoplasm (Figs. 6, 7), and these inclusions varied in diameter and reaction intensity with individual particles (Figs. 6, 7). Digestion with α-amylase resulted in the abolition of nearly all the glycogen particles within the cytoplasm of the endothelial cells (Fig. 8).

Finally, it was of particular interest that neighboring reticular cells were closely similar in not only the PA-TCH-SP reactivity of the luminal plasma membrane and pinocytotic images but in the amount of glycogen particles to the endothelial cells lining the splenic sinuses (Figs. 6,6-inset, 7).

Discussion

In the central and follicular arteries and venous sinuses of the rat spleen examined in the present study, the endothelial cells together with their subjacent basal lamina have been shown to be largely comparable in ultrastructural cytology to those reported previously (Pictet *et al.*, 1969; Miyoshi and Fujita, 1971; Chen and Weiss, 1973).

In the present study, the surface coat of the luminal plasma membrane of the endothelial cells lining the central and follicular arteries of the rat spleen was relatively thick and exhibited strong positive PA-TCH-SP reaction with an intermittent discontinuity along the plasma membrane, whereas the corresponding ultrastructure of the cells lining the splenic sinuses was unusually thin and showed exceedingly feeble or negative reaction. In view of their stability against digestion with α-amylase, the PA-TCH-SP reactive substance involved in the surface coat of the luminal plasma membrane of these endothelial cells is regarded as a glycoprotein (Lillie and Fullmer, 1976; Pearse, 1985; Yamada, 1987). It deserves special discussion how is the mechanism underlying the difference in thickness of the reactive luminal surface coat and in intensity of its positive PA-TCH-SP reaction in the endothelial cells between the two types of the splenic arteries and the sinuses. Such a mechanism remains to be fully elucidated. However, at least three possible factors (physical, chemical and developmental) could appropriately be considered for plausibly comprehending the mechanism. First, the surface coat of the luminal plasma membrane of the arterial endothelial cells could be exposed to a higher speed of blood streams, as compared with that to which the corresponding ultrastructure of the sinus endothelial cells is exposed. Secondly, the surface coat of the luminal plasma membrane of the former cells is apparently in direct contact with arterial blood with respective higher and lower tensions of oxygen and carbon dioxide, as compared with venous blood with which the corresponding ultrastructure of the latter

cells is in contact. Thirdly, the difference in the surface coat of the luminal plasma membrane of the endothelial cells between the two types of arteries and venous sinuses could reasonably be related to a developmental factor, in view of the fact that the splenic sinuses are embryologically originated from spaces between reticular cells, unlike the splenic arteries (Robinson, 1930; Blue and Weiss, 1981). These three factors are believed to give rise to a neutral glycoprotein-rich thick luminal surface coat of the arterial endothelial cells, as compared with that of the sinus endothelial cells, in view of the known cytophysiological functions of surface coat glycoproteins, such as protection, ion exchange, specific filtration and so forth (Shinohara, 1977; Schute *et al.*, 1984).

In the endothelial cells of the rat splenic follicular arteries, the discontinuity in the positive PA-TCH-SP reaction of the luminal surface coat was less marked, as compared with that in the cells of the central arteries. It remains to be known why such a difference occurs between the endothelial cells of the two types of the splenic arteries in the rat.

In the present study, another unique finding deserving special comments was the appreciable abundance of glycogen particles in the cytoplasm of the endothelial cells lining the splenic sinuses of the rat. It is cytologically accepted that accumulations of glycogen in the cytoplasm can well be correlated with particular functional activities of cells such as contractility of cells (eg. muscular cells) (Fawcett, 1994), energy and/or nutrition stores of cells (eg. hepatic cells) (Fawcett, 1994), hypofunction of cells (eg. certain types of endocrine cells) (Hara and Yamada, 1965; Roth and Raisz, 1966; Coleman, 1969) and so forth. The cytophysiological significance of the abundance of cytoplasmic glycogen in the sinus endothelial cells can not precisely be determined in the present study. It seems, however, likely that such an abundance of cytoplasmic glycogen could appropriately be correlated with the possible contractility of the endothelial cells, in particular, since the walls of the splenic sinuses are devoid of any smooth muscle cells and are to be provided with other architectures capable of contractions (Chen and Weiss, 1972, 1973; De Bruyn and Cho, 1974). Such a concept is in keeping with the fact that the splenic arterial endothelial cells do not contain any pronounced amounts of glycogen particles but are, instead, surrounded by a layer of smooth muscle cells.

As the present results reveal, neighboring reticular cells were closely similar in both the PA-TCH-SP reactivity of the luminal plasma membrane and pinocytotic images and the amount of cytoplasmic glycogen particles to the endothelial cells lining the splenic sinuses. Such findings are taken to indicate that the both cell types are more or less similar in cytophysiological functions to each other and that the lumen of the splenic sinuses appears to be equivalent in functions to the spaces of the red splenic cords enclosed by reticular cells. Thus, such a concept could be favorable to the so-called "open circulation theory" largely accepted generally in the physiology of blood circulation in the mammalian spleen (McCuskey and McCuskey, 1977, 1985; Cilento *et al.*, 1980).

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Explanation of Figures

Plate I

Fig. 1. Part of the intima and media of a central artery in the spleen of a rat. Endothelial cell (EC), Basal lamina (BL), Smooth muscle cell (SM). Uranyl Acetate-Lead Citrate stained. ×20,000.

Fig. 2. Part of the intima and media of a central artery in the spleen of a rat. Endothelial cell (EC), Basal lamina (BL), Smooth muscle cell (SM). PA-TCH-SP stained. ×22,500. Inset: A higher magnification of the surface coat (SC) of the luminal plasma membrane in an endothelial cell. ×60,000.

Fig. 3. Part of the intima and media of a follicular artery in the spleen of a rat. Endothelial cell (EC), Basal lamina (BL). Uranyl Acetate-Lead citrate stained. ×20,000.

Fig. 4. Part of the intima and media of a follicular artery in the spleen of a rat. Endothelial cell (EC), Basal lamina (BL), Smooth muscle cell (SM). PA-TCH-SP stained. ×25,000. Inset: A higher magnification of the surface coat (SC) of the luminal plasma membrane in an endothelial cell. ×60,000.

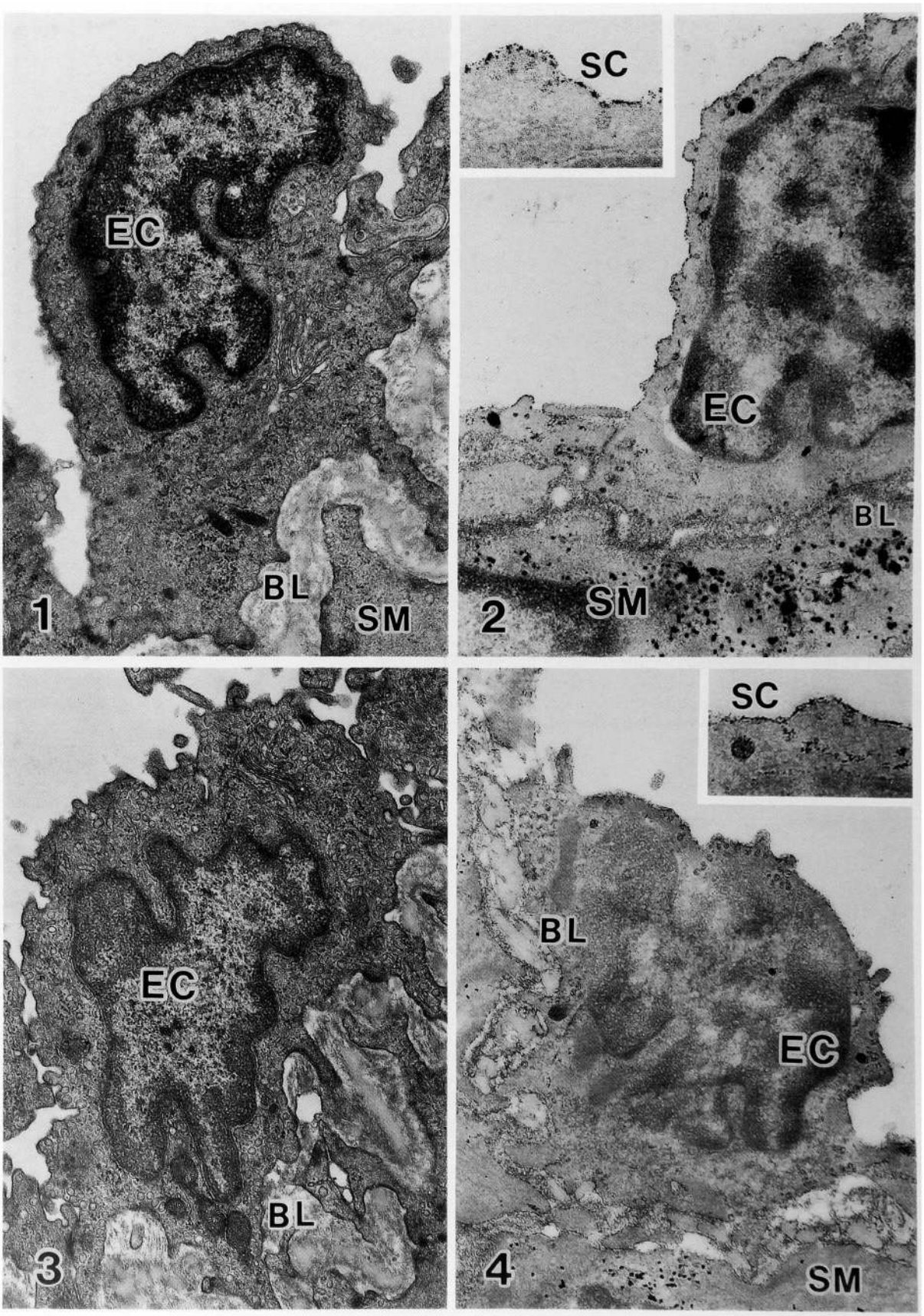
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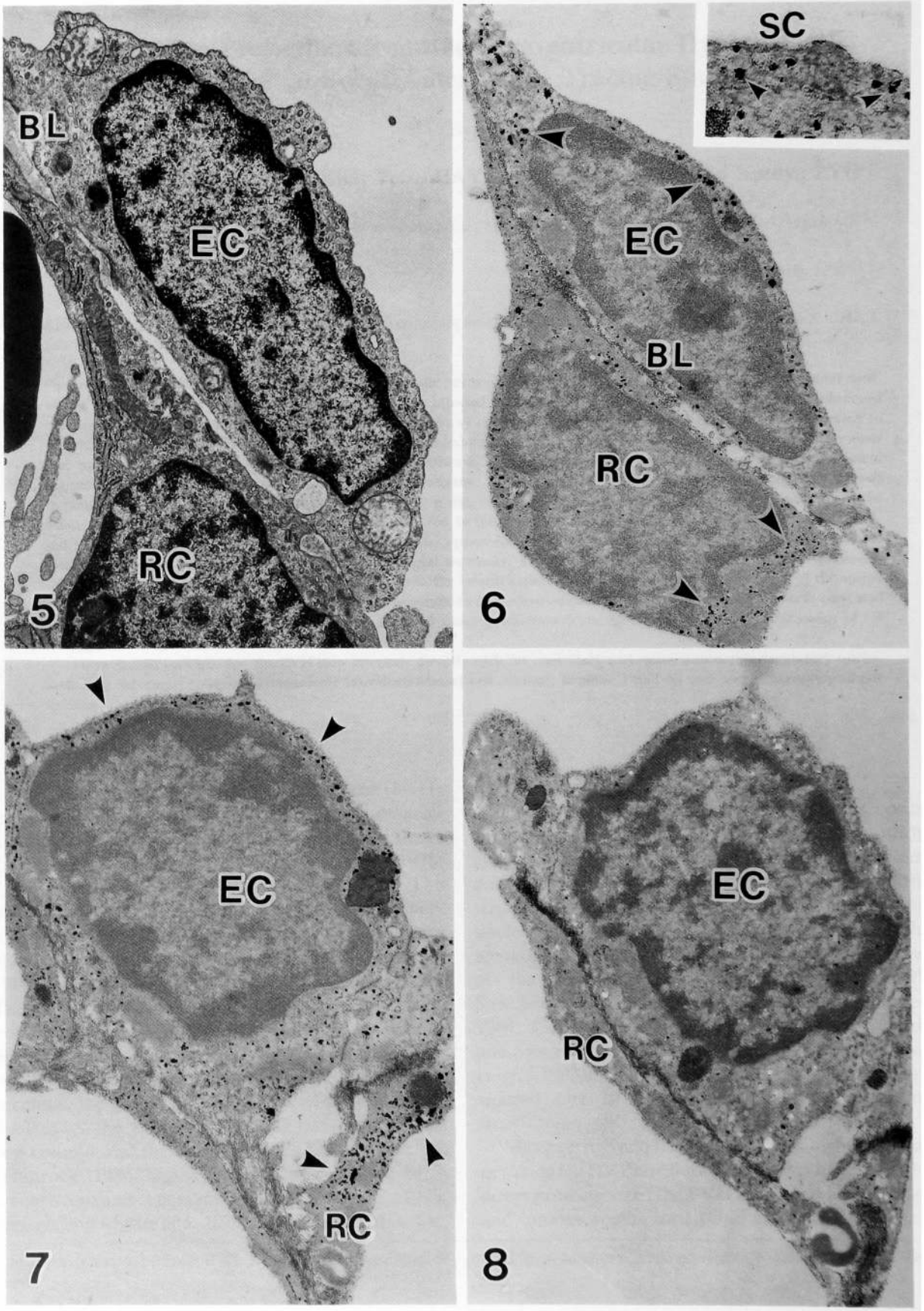
Plate II

Fig. 5. Part of the wall of a splenic sinus. Endothelial cell (EC), Basal lamina (BL), Reticular cell (RC). Uranyl Acetate-Lead Citrate stained. ×12,000.

Fig. 6. Part of the wall of a splenic sinus. Endothelial cell (EC), Basal lamina (BL), Reticular cell (RC), Glycogen particles (arrowheads). PA-TCH-SP stained. ×22.500. Inset: A higher magnification of the surface coat of the luminal plasma membrane (SC) in an endothelial cell lining the sinus. Glycogen particles (arrowheads). ×60,000.

Fig. 7. Part of the wall of a splenic sinus. Endothelial cell (EC), Reticular cell (RC), Glycogen particles (arrowheads). Stained with PA-TCH-SP following treatment with α-amylase free medium. ×15,000.

Fig. 8. Part of the wall of a splenic sinus. Glycogen particles are not detected in the cytoplasm of the endothelial (EC) and reticular (RC) cells. Stained with PA-TCH-SP following digestion with α-amylase. ×15,000.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