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Carbohydrate cytochemistry on hypodermic lymphatic endothelium of the green lizard, *Lacerta hispanica*

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Summary

A cytochemical study on the endothelium of the hypodermic lymphatic capillaries of the green lizard, *Lacerta hispanica*, has been carried out. The dialysed iron method produced a homogeneous precipitate on the surface of the endothelial cells and on the inside of the endocytic vesicles. The periodic acid–thiocarbohydrazide–silver proteinate, low pH phosphotungstic acid and high iron diamine techniques gave negative results. The carbohydrates in the capillaries thus seem to be glycosaminoglycans with carboxyl groups. The possible role of these glycosaminoglycans in the formation of the endocytic vesicles is discussed.

Introduction

It is now generally accepted that the transfer of macromolecules for lymph formation in the lymphatic capillaries is carried out via endocytic vesicles which are present in the endothelium (Dobbins & Rollins, 1970; O'Morchoe *et al.*, 1980; Yang *et al.*, 1981); both in the interior of the cytoplasm and open on the two endothelial surfaces. In both cases tracing molecules accumulate in its interior (Casley-Smith, 1965; Yang *et al.*, 1981).

Plasma membrane components play a decisive role in the formation of endocytic vesicles, particularly the carbohydrates associated with their external surfaces (Brandt & Pappas, 1960; Goldstein *et al.*, 1979; Waksman *et al.*, 1980). In this paper, we report the cytochemical characterization of the complex carbohydrates associated with the plasma membrane of the endothelial cells of the hypodermic lymphatic capillaries of the green lizard, *Lacerta hispanica*, and relate these components with the transfer of macromolecules during lymph formation. The hypodermic lymphatic capillaries are concerned with the transport of lipids from skin reserve adipose cells.

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Materials and methods

Small fragments of dorsal skin were removed from the green lizard, *Lacerta hispanica* (Reptilia, Sauria), and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, and embedded in Epon. Thin sections of the fixed tissue were mounted on gold grids and stained with the periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-Ag) method of Thièry (1967). Oxidized control sections were treated either with silver proteinate without previous thiocarbohydrazide (TCH) treatment or with TCH alone. Other sections were floated on 1% phosphotungstic acid in 1 M HCl for 40 to 60 min according to Flechon (1970), and washed with 1.25 M HCl. The epidermis of the newt *Pleurodeles waltlii* (Amphibia, Urodela) was used as a positive control (Bueno *et al.*, 1981).

Small fragments of skin fixed in buffered 2.5% glutaraldehyde were stained with dialysed iron (Wetzel *et al.*, 1966) or high iron diamine (Spicer *et al.*, 1978), dehydrated and embedded in Epon.

Results

The hypodermis of *Lacerta hispanica* is formed by a loose connective tissue with characteristic blood and lymphatic capillaries and abundant adipose cells.

The PA-TCH-Ag proteinate method for the identification of *vic*-glycol groups in hexoses, and the low pH phosphotungstic acid method for determining the existence of hydroxyl groups, gave negative results for the lymphatic capillaries, whose morphological characteristics are similar to those described for mammals (Casley-Smith & Florey, 1962).

Dialysed iron produced a precipitate on the surface of the lymphatic endothelium (Figs. 1, 2), but the HID method gave negative results. The colloidal iron precipitate was homogenous over the whole endothelial surface and in the interior of the endocytic vesicles (Fig. 1), both in those which were free in the cytoplasm and in those which were open towards one of its surfaces. The colloidal iron precipitate was also observed on the plasma membrane in the overlapping contact zones and in the vesicles present in this region (Fig. 2).

Discussion

The various ultrastructural techniques that have been applied in this study to the hypodermic lymphatic capillaries of *Lacerta hispanica* for the analysis of glycoconjugates enable carbohydrate-rich substances present in these features to be characterized in them (Spicer *et al.*, 1979). The surface of the lymphatic endothelium shows an affinity

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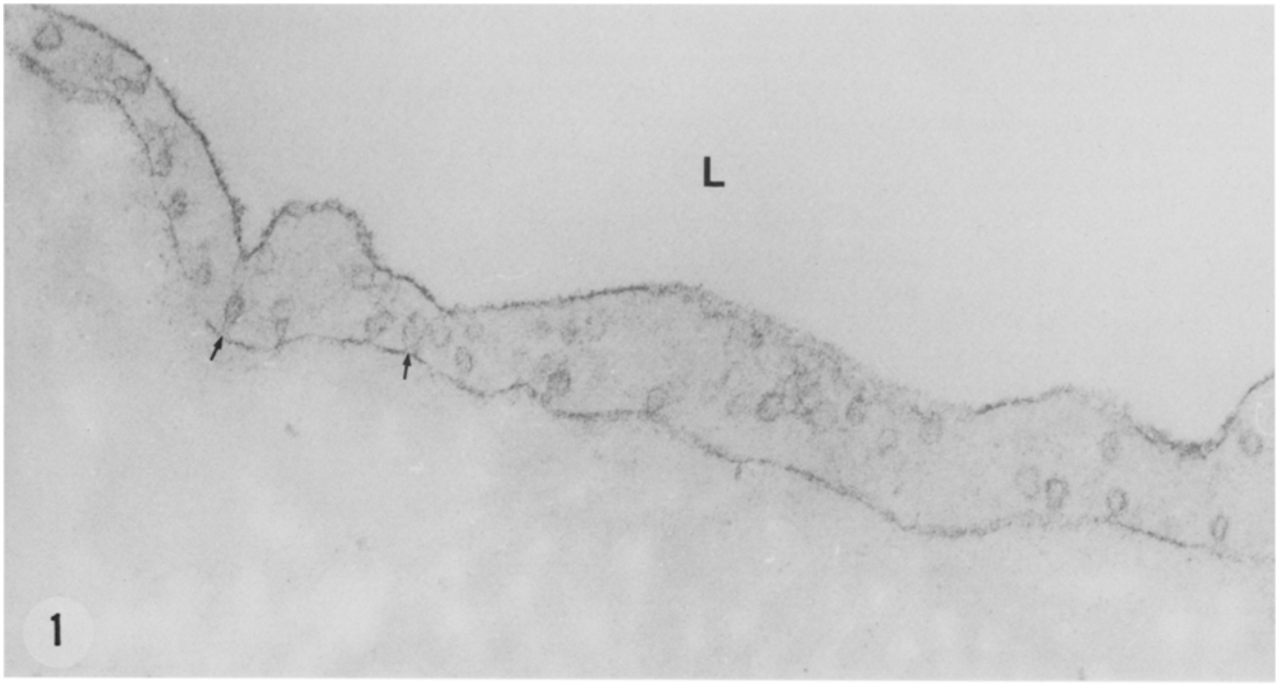
Fig. 1. Hypodermic lymphatic endothelium of *Lacerta hispanica* stained with dialysed iron procedure. Note the precipitate on cell surface and into the endocytic vesicles (arrows). L, lumen. × 40 000

Fig. 2. Overlapping contact zone of lymphatic endothelium in *Lacerta hispanica* hypodermis. The colloidal iron precipitates on the surface of two cells. L, lumen. × 45 000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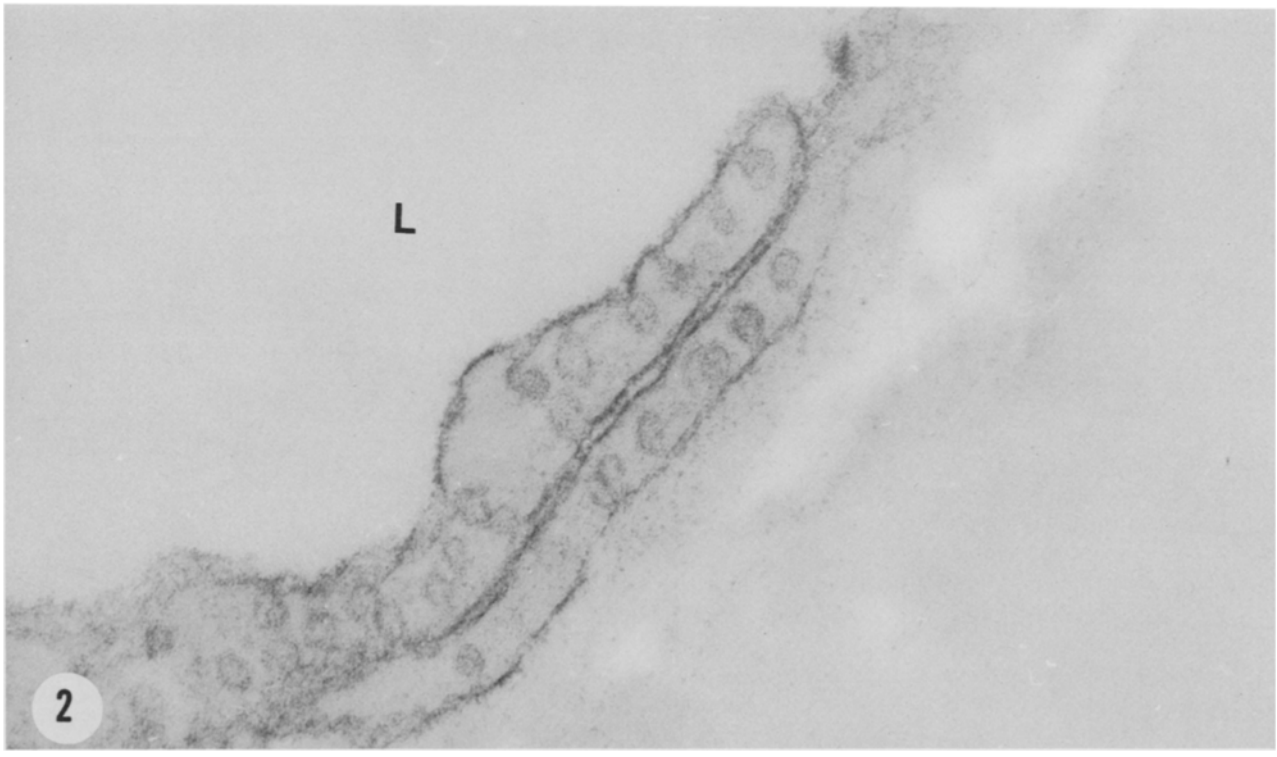
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for dialysed iron but does not react with PA–TCH–SP, phosphotungstic acid at a low pH, or HID. For this reason, the carbohydrates of this cell surface can be considered to be glycosaminoglycans with carboxyl groups. The latter show an affinity for dialysed iron but not for HID (Spicer *et al.*, 1978).

These glycosaminoglycans form a continuous layer associated with the plasma membrane of the lymphatic endothelial cell. The fact that it is negatively charged facilitates the adhesion of macromolecules which are fixed by weak electrostatic links to the surface of the endothelium in the first phase of the endocytosis (Gosselin, 1967; Waksman *et al.*, 1980). Since coated vesicles have not been observed, it seems reasonable to suppose that the adhesion zones are evenly distributed over the whole cell surface, as occurs in the amoeba (Brandt & Pappas, 1960), and not as in receptor-mediated endocytosis that occurs in other cell types (Goldstein et al., 1979).

Because it is accepted that the movement of macromolecules across the lymphatic endothelium is realized from the abluminal to the luminal surface, we consider that the glycosaminoglycans which appear on the luminal surface originate from those transferred by endocytic vesicles from the abluminal surface and which are incorporated in the luminal cell border by exocytosis.

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