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Diapedesis of thrombocytes from capillary into the intercellular space of interscapular brown adipose tissue and their increase by Ca-Sandoz

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Summary. Diapedetic capacity of the rat thrombocytes to leave capillaries of the interscapular brown adipose tissue (IBAT) and infiltrate the interstitium has been observed by conventional electron-microscopy.

Thrombocytes that reach IBAT interstitium are morphologically completely different from lumenary ones. The interstitial thrombocyte has a prominet head region (1.51x2.12 μm) and very long phylopodium (3.43 μm). Experimental conditions which induced drastic changes in morphology of interstitial thrombocytes were: sucrose overfeeding (10% over 2 days); a 24 hour starving after sucrose overfeeding and Ca-Sandoz drinking (480 mg/L Ca^{2+} during 2 days). The thrombocytes in the IBAT interstitium can be classified as activated according to: a) pseudopode extension; b) swollen open canalicullar system (OCS); c) endocytosis via coated pits and vesicles; and d) structural changes in α granules excreted to the interstitium through OCS.

In the IBAT interstitium of 24-hour starved rats after sucrose overfeeding, a thrombocytic layer was observed. It was suggested that thrombocyte adrenalin, stored in dense bodies, was selectevely included in the IBAT supply without mediation of the central nervous system.

Key words: Diapedesis, Thrombocyte, Brown adipose tissue, Interstitium-Thermogenesis

Introduction

The thrombocytes, along with their participation in haemostasis, are responsible for the transport of creative substances which are essential for the maintenance of the vessel wall structure. They are absorbed by the endothelial cells delivering them macromolecules contained in the thrombocytes. About 15 per cent of the blood-circulating thrombocytes are used for this purpose. With the lack of interaction with thrombocytes, vascular endothelium is subjected to dystrophy and the erythrocytes leak throught it (Skipetrov, 1990).

The role of thrombocytes in inflammatory disorders (respiratory distress syndrome, mesangial glomerulonephritis, chronic inflammatory bowel disease, disseminated intravascular inflammation and allergic vasculitis) and in immune-related diseases, is reviewed by Mannaioni et al. (1997).

Activated thrombocytes express P-selectin from α-granules, combining them with sulfatides present in collagen of lesioned endothelium, thus producing thrombocytes diapedesis (Mannaioni et al., 1997).

Examining the interrelationship between the capillaries and cells of the brown adipose tissue during the induced and suppresed thermogenesis (Ca-Sandoz drinking rats, sucrose overfed rats and in a 24-hour starved rats after sucrose overfeeding), we observed the hitherto unreported phenomenon, i.e. that thrombocytes leave capillaries by diapedesis changing their morphology, which is a clear sign of their cytological activation.

For this reason and to contribute to the study of the thrombocyte role in the develoment of various diseases, which is at present the main aim of many investigations, we focused our attention on the study of this unusual thrombocyte in the IBAT interstitium which has no pathological implications, but exerts physiological influence on the IBAT metabolic processes involved in thermoregulation.

After diapedesis the thrombocytes secrete α granules into IBAT interstitium but also endocytose some substances from their new environment which is in accordance with the expression of thrombocyte activity described by the term «two way street» (White and Clawson, 1980; White and Escolar, 1991).

The present report deals with the relationship between interstitial thrombocytes and metabolic status of IBAT as well as with the recovery process of infiltrating thrombocytes into IBAT interstitium.

Materials and methods

Two-month-old male rats of Wistar strain, weighing 180-200 g, were used in this study. The animals were fed

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with standard pelleted food ad libitum. They were divided into three groups, each consisting of six animals: Ca-Sandoz drinking (480 mg/L Ca^{2+} during 2 days); sucrose overfed (10% over 2 days); and a 24 hour-starved after sucrose overfeding. The control group was fed with standard food and given tap water ad libitum. After decapitation, the interscapular brown adipose tissue was dissected out and several small pieces fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2), and postfixed in 2% osmium tetroxide in the same buffer. After dehydration, through serial alcohol solutions of increasing strength, the specimens were embedded in Araldite. The blocks were trimmed and cut with glass knives on an LKB III ultramicrotome. Ultrathin sections were mounted on copper grids, unstained or stained with uranyl acetate and lead citrate and examined on a Philips MC 12 transmission electron microscope.

Results

General features of diapedesis of capillary thrombocytes in the IBAT interstitium

Thrombocytes, along with erythrocytes, in most cases migrated through open capillary junctions. The leading part of the thrombocyte, which was the first to enter the interstitium, lacked both thrombocyte granules and other structural components (Fig. 1A). However, at the later stage this leading part of diapedetic thrombocyte could be observed in the IBAT interstitium as an independent vacuolar structure (Fig. 1B). At the beginning of diapedesis, thrombocytic granules were shifted to the thrombocyte center (Fig. 1A) whereas at its end they were within the interstitium (Fig. 1B).

In sucrose overfed rats interstitial thrombocyte morphology was changed: head and long thin pseudopodium (phylopodium) or pseudopodies were present

The interstitial thrombocyte (Fig. 2A) in sucrose overfed rats was distinguished by: big head region (1.51x2.12 μm); long, thin phyllopodium i.e. tail region (3.43 μm); endocytic coated pits and vesicles as well swollen OCS with many α granules (Fig. 2B).

Interstitial thrombocytes possessed two different large granules: one with internal vesicles and another angular with filial twig-like material situated in the vacuole

In 24 hour-starved rats after sucrose overfeeding, the activated thrombocyte with endosomal vesicles, pseudopodium, large granule, angular granule and OCS were observed (Fig. 3A). As for Ca-Sandoz drinking rats high endocytic activity of the thrombocytes was reflected in a great amount of coated vesicles. However, the interstitial thrombocyte had a large α granule, close to the OCS, and 3 pseudopodiae (Fig. 3B).

Interstitial thrombocytic layer observed in 24 hour-starved rats after sucrose overfeeding

At the beginning of OSC forming, its membrane, visible in the interstitial thrombocytic layer, had a geometric shape. This suggested that angular granule with internal structure originated from the OCS (Fig. 4).

Discussion

Diapedetic activity of thrombocytes is usually accompanied by erythrocytic migration and is enhanced by Ca-Sandoz administration

Our findings, that diapedetic activity of thrombocytes is accompanied by erythrocytic diapedesis, are in agreement with those of other authors who found that cooperative biochemical interaction between erythrocytes and thrombocytes enhances thrombocytic activation (Santos et al., 1991). Since adenosine diphosphate (ADP) from dense bodies is virtually not present either in the SDP (storage pool deficiency) or in HPS (Hermansky-Pudlak syndrome) platelets, the non-granular ADP source, such as platelet cytosole and/or red cells has been proposed (Lages and Weiss, 1997). Our morphological results, indicating the close relationship between thrombocyte and erythrocyte, support this assumption. Recent evidence (Mannaioni et al., 1997) suggests a key role for platelets in mediating the diapedesis of neutrophils and platelets in inflammation. Our results indicate a cooperative diapedesis in sucrose-overfed and in 24 hour-starved rats after sucrose overfeeding.

Diapedetic activity of thrombocytes was enhanced especially in the Ca-Sandoz group. In platelets, as in many other cells, one of the earliest responses to stimulation by a variety of antagonists is an increased cytosolic Ca^{2+} concentration. This increase in $[Ca^{2+}]_I$ might be due to Ca^{2+} release from the intracellular stores or from an influx of extracellular Ca^{2+}, or from the combined effect of these processes (Sage et al., 1993). Thrombocytes contain calcium-dependent, sulphydryl, neutral proteases calpains which preferentially cleave cytoskeletal proteins, particularly actin-binding protein and talin (Fox et al., 1985). It has been reported that calpains are involved in cytoskeletal reorganization upon platelet activation. We have observed during diapedetic activity the centralization of organelles, including thrombocytic secretion activity, the latter also being observed by White (1984), and cytoskeletal reorganization.

The release of thrombocytic histamine, situated in dense bodies (Wurzinger, 1990) and the expression of P-selectin almost invariably takes place during thrombocyte activation. The causal relationship during thrombocyte activation is either platelet-derived growth factor or histamine release and the expression of P-selectins on thrombocyte surfaces. According to Mannaioni et al. (1997) in addition to thrombin, as aggregational

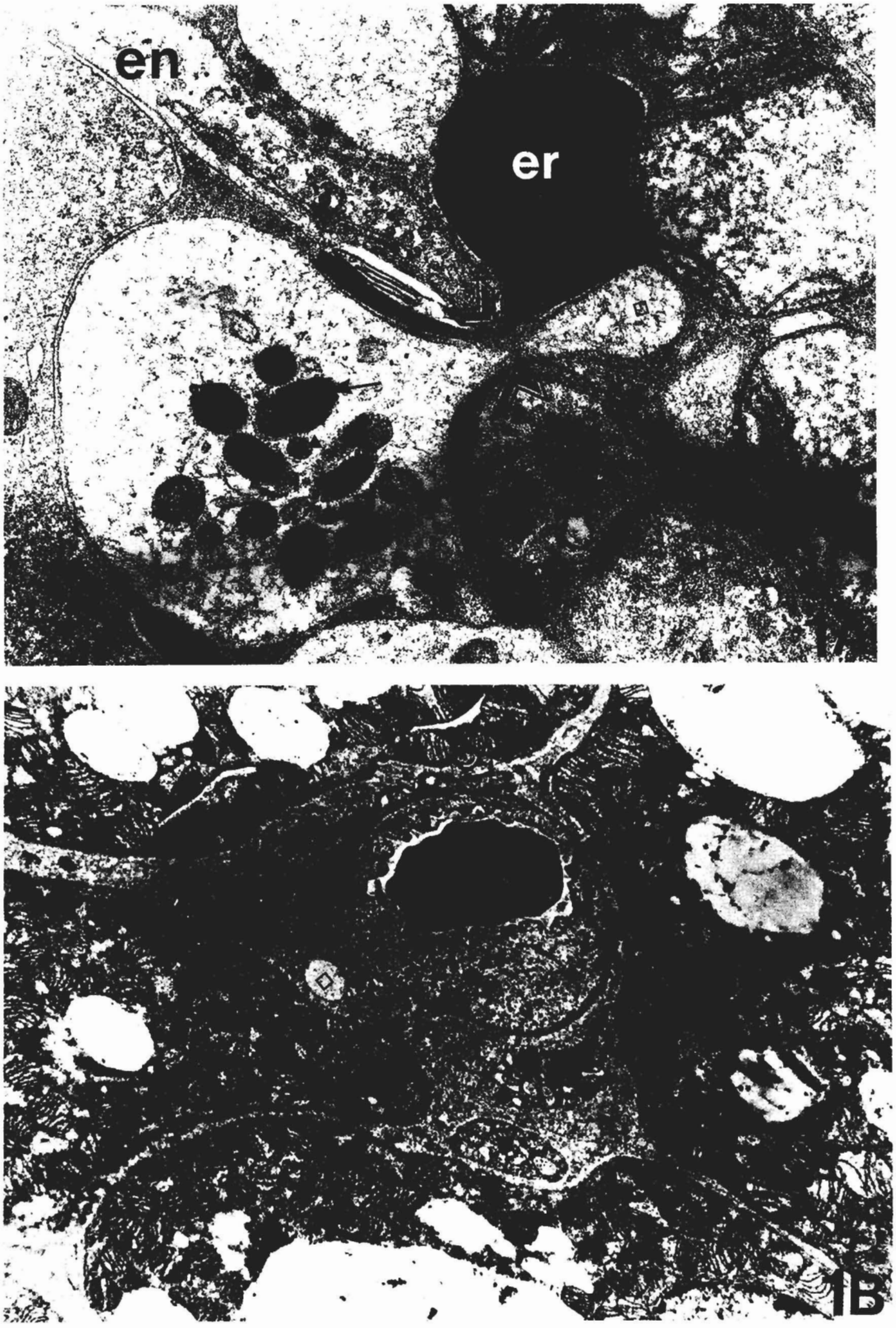

Fig. 1. Diapedetic activity of capillary thrombocytes to migrate into the IBAT interstitium. **A**. Non granulated part of thrombocyte (square) accompanied by diapedesis of erythrocyte (er) in Ca-Sandoz drinking rats. Arrow: α-granule; triangle: dense body; big X: open endothelial junction; en: endothelium. **B.** The IBAT interstitium occupied by shedding thrombocytic bleb or vesicle (square) from thrombocyte and two thrombocytes (arrow) under experimental conditions of a 24 hour starvation after sucrose overfeeding.
A, x 26,000;
B, x 76,000

stimulus, histamine is also involved in diapedesis and infiltration of thrombocytes as inflammatory stimulus. Histamine induces thrombocyte expression of P-selectin combined with collagen sulfatides of lesioned endothelium. In our case, physiological activation during thermogenesis, usually accompanied with erythrocytes, also has an activating role on thrombocytes whereby lesioned endothelium is not visible since physiological activation is in play.

IBAT interstitial thrombocytes show recovery morphology as compared with the luminal one

When we discovered in the that thrombocytes

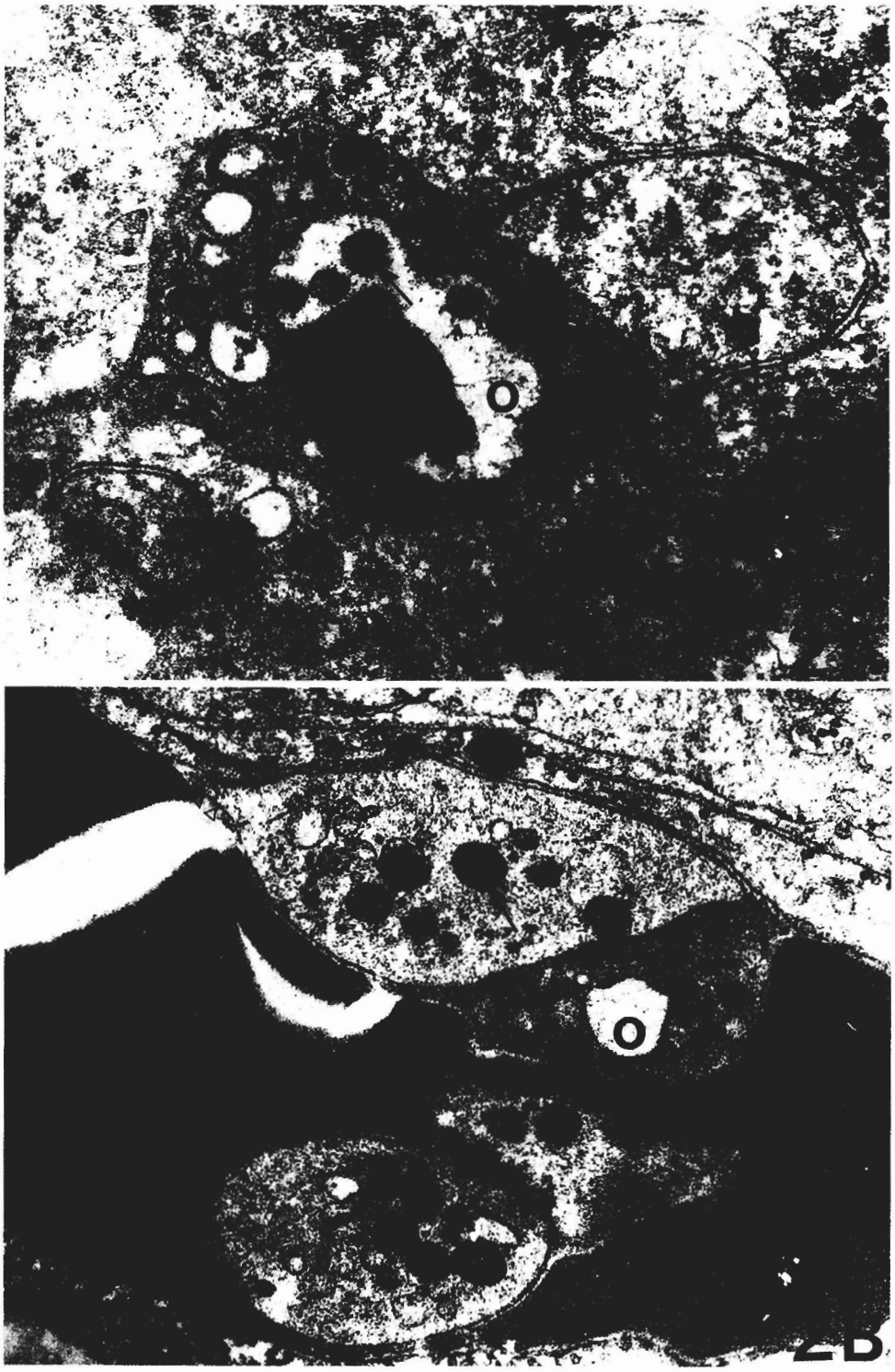

Fig. 2. A. The IBAT interstitial thrombocyte morphology is changed in comparison with the luminal one. Cytogenesis of interstitial thrombocyte with a extremely big tail region (three triangles) and head region where OSC (o) with α granules (arrow) dominate in sucrose overfed group. Coated pit and vesicles (arrowheads). **B.** Group of luminal thrombocytes in sucrose overfed group. Thee triangles: pseudopodium; o: OCS; arrow: α granule; open arrow: dense body. A, x 3,300; B, x 22,000

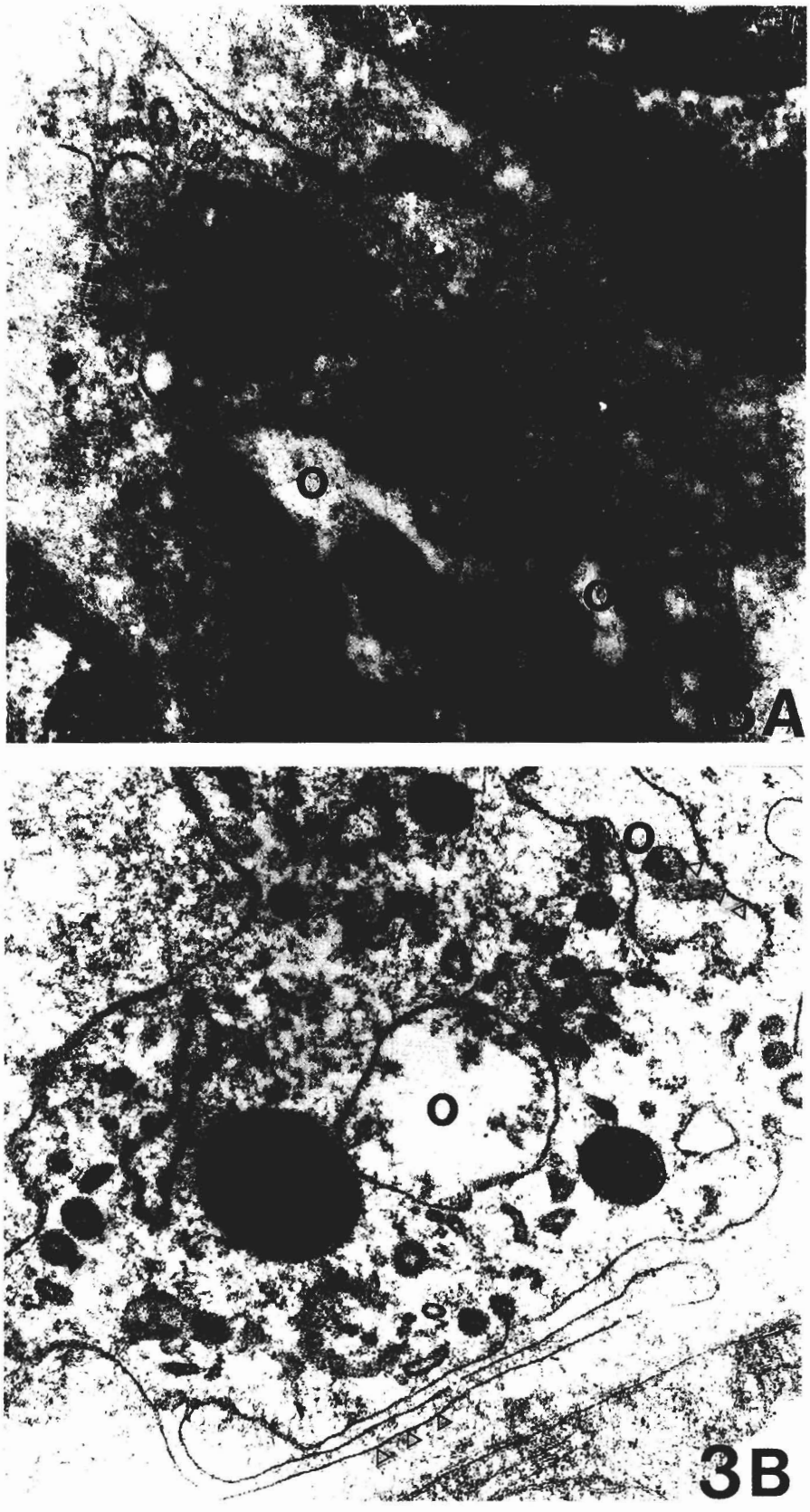

Fig. 3. Interstitial thrombocytes have two large granules: an angular one and one with internal vesicles. **A.** Activated thrombocyte shows presence of large α granule (lg) with vesicular structure (triangle), as well as angular granule (ag) containing hexagonal membrane space with internal structure, in sucrose-overfed group. o: OCS; three triangle: pseudopodium. **B.** Pinocytotic-coated vesicles (arrowheads) and large α granule (lg) in close contact with OCS (o) in Ca-Sandoz drinking group. Three triangles: pseudopodium. A, x 44,000; B, x 17,000

infiltrated the IBAT intracellular spaces by diapedesis in the control rat IBAT capillary, our first conclusion was that thrombocytes entered IBAT to be phagocytosed by brown adipocytes, given that we have previously revealed that brown adipocytes have phagocytic capacity (Radovanovic et al., 1996). However, under all our experimental conditions the recovery of interstitial thrombocytes was observed.

It is well known that the brown adipose tissue is thermogenically activated by sucrose overfeeding and suppresed by starvation, whereby neurohumoral control of the IBAT thermogenic function is mediated by adrenaline (Landsberg and Young, 1978, 1984).

According to our results, it seems that diapedetic thrombocytes supply the IBAT interstitium with their own adrenaline, along with noradrenaline, dopamine, histamine, ADP and ATP (Wurzinger, 1990). This «thrombocytic» adrenaline was endocytosed from blood plasma where it is present with other substances secreted by various cells: white blood, endothelial cells, hepatocytes, and alike (Handagama et al., 1987, 1990; Harrison et al., 1989). This implies that thrombocytic adrenaline was selectevely included in the IBAT adrenaline supply without CNS mediation. Besides, thrombocytic α granules, observed in OCS during sucrose induced thermogenesis, were the major store for adhesive

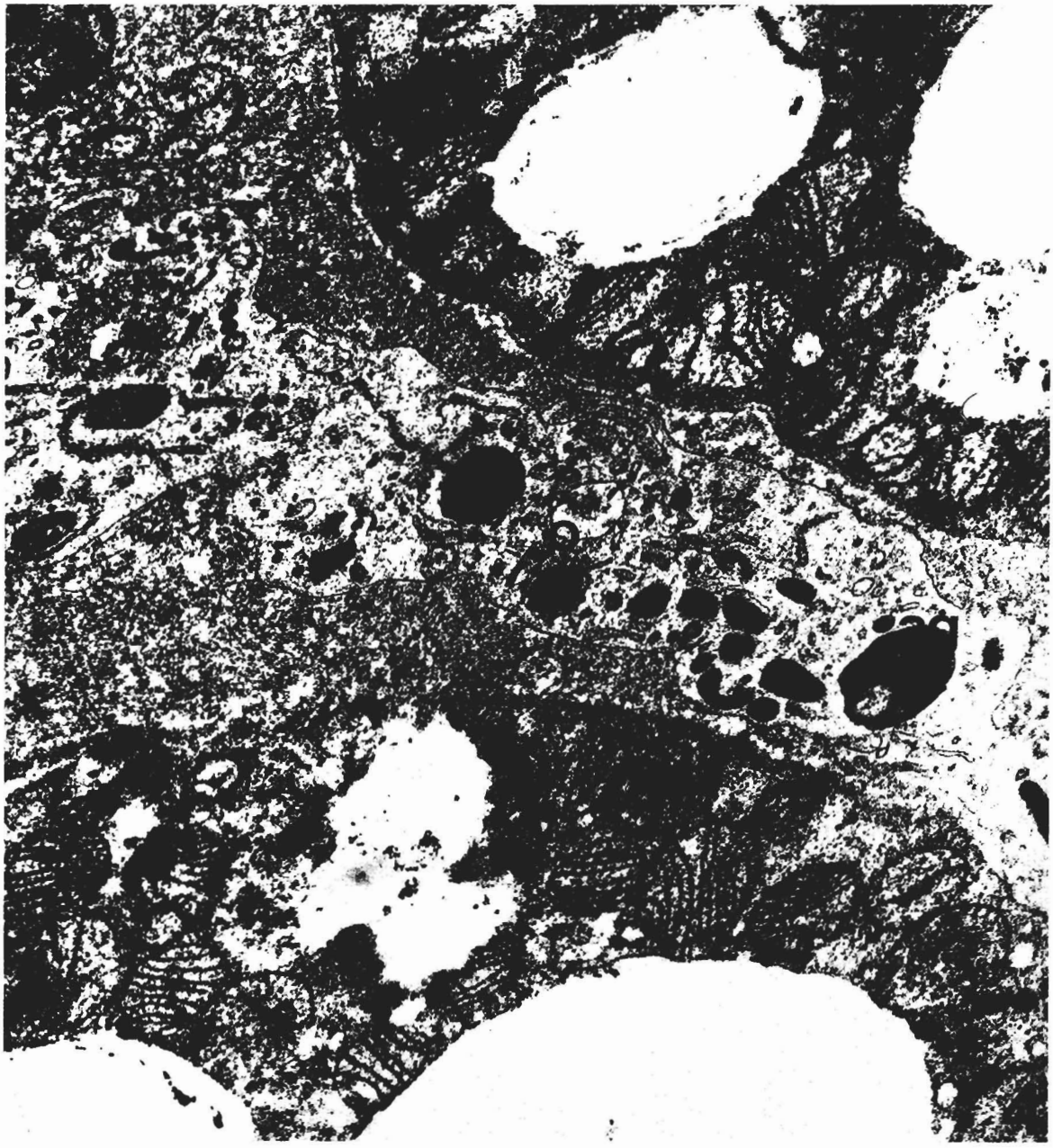

Fig. 4. Interstitial thrombocytic layer between two IBAT cells with many α granules (arrow) and one angular granule (ag) originating from OCS (o). x 18,900

proteins, various inflammatory substances, growth factors, immunoglobulins, and alike (Harrison and Cramer, 1993). Interstitial thrombocytes in sucrose-overfed rats have the ability to endocytose via coated pits and vesicles, which is a possible way of restoring α granular and dense body content (Behnke, 1989, 1992; Klinger and Klüter, 1995). All our obtained results support the conclusion that a recovery process exists in the IBAT interstitium both during and after thermogenesis.

The detailed analysis of thrombocyte survival patterns shows that the majority of thrombocytes survive until senescence while some are randomly removed from the circulation (George and Dale, 1995). We suggest that diapedesis of thrombocytes into the IBAT interstitium is a unique mode of removing them from circulation not to be destroyed but to be recovered in accordance with the IBAT metabolic requirements.

The identification of two structurally different large granules has been done according to Klinger (1996) who gave an ultrastructural explanation and functional aspect of changes during storage. He observed that three days after storage, the α granule internal structure contained von Willebrand's factor. These α granules correspond to our large angular α granule, originating from OCS, the internal structure of which resembles a foliate twig.

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