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Erythrophagocytosis by brown adipocytes of rat interscapular tissue

J. Radovanovic[1], A. Korac[1], V. Davidovic[2], V. Koko[3] and V. Todorovic[3]

[1]Institute of Zoology, Faculty of Science, [2]Institute of Physiology, Faculty of Science and [3]Institute for Medical Research, Belgrade, Yugoslavia

Summary. In this investigation the following phenomena were observed: 1. Rat interscapular brown adipocytes were found to be capable of erythrophagocytosis; 2. Before leaving the capillary lumen, erythrocytes took some material from the blood plasma by endocytosis and passed the endothelial junction carrying endocytotic vacuole. Some erythrocytes were in transit: the so-called «head» was in the process of engulfment by brown adipocytes while the rest of the cell had not left the capillary lumen. Fragmentation of erythrocytes was observed during passage through the endothelial junction as well as in the cytoplasm of adipocytes. 3. In some brown adipocytes erythrocytes retained the same shape as in the capillary, but in many cases they exhibited unusual form. Intracytoplasmic erythrocytes were seen in a semithin sections stained with toluidine blue. 4. Erythrocytes either became cells which phagocytized mitochondria and lipid droplets before their transformation into lipofuscin bodies or they were degraded into ferritin-like particles observed (on unstained sections) in the mitochondrial matrix, intercristal space, on the periphery of lipid droplets and in brown adipocyte cytoplasm.

Key words: Brown adipocyte, Erythrophagocytosis, Rat

Introduction

While investigating interscapular brown adipocyte tissue we observed the presence of intracytoplasmic erythrocytes which are degraded or display phagocytic activity before death of the cell. As far as we know, this atypical erythrophagocytosis is also characteristic of a number of cell types under hemorrhagic conditions such as thyroid epithelial cells, liver cells, epidermal cells, epithelial cells of the gallbladder, mast cells (Rosin and Doljanski, 1944; Platt, 1963; Wakefield and Hicks, 1974; Spicer et al., 1975; Zeligs, 1977) as well as many carcinoma cells (Marin-Padilla, 1977; Foadi et al., 1978; Falini et al., 1980) but this phenomenon has not been described in the available literature in normal brown adipocytes.

Offprint requests to: Dr. Jelena Radovanovic, Institute of Zoology, Faculty of Science, 11000 Belgrade, Studentski trg 16, Serbia, Yugoslavia

Materials and methods

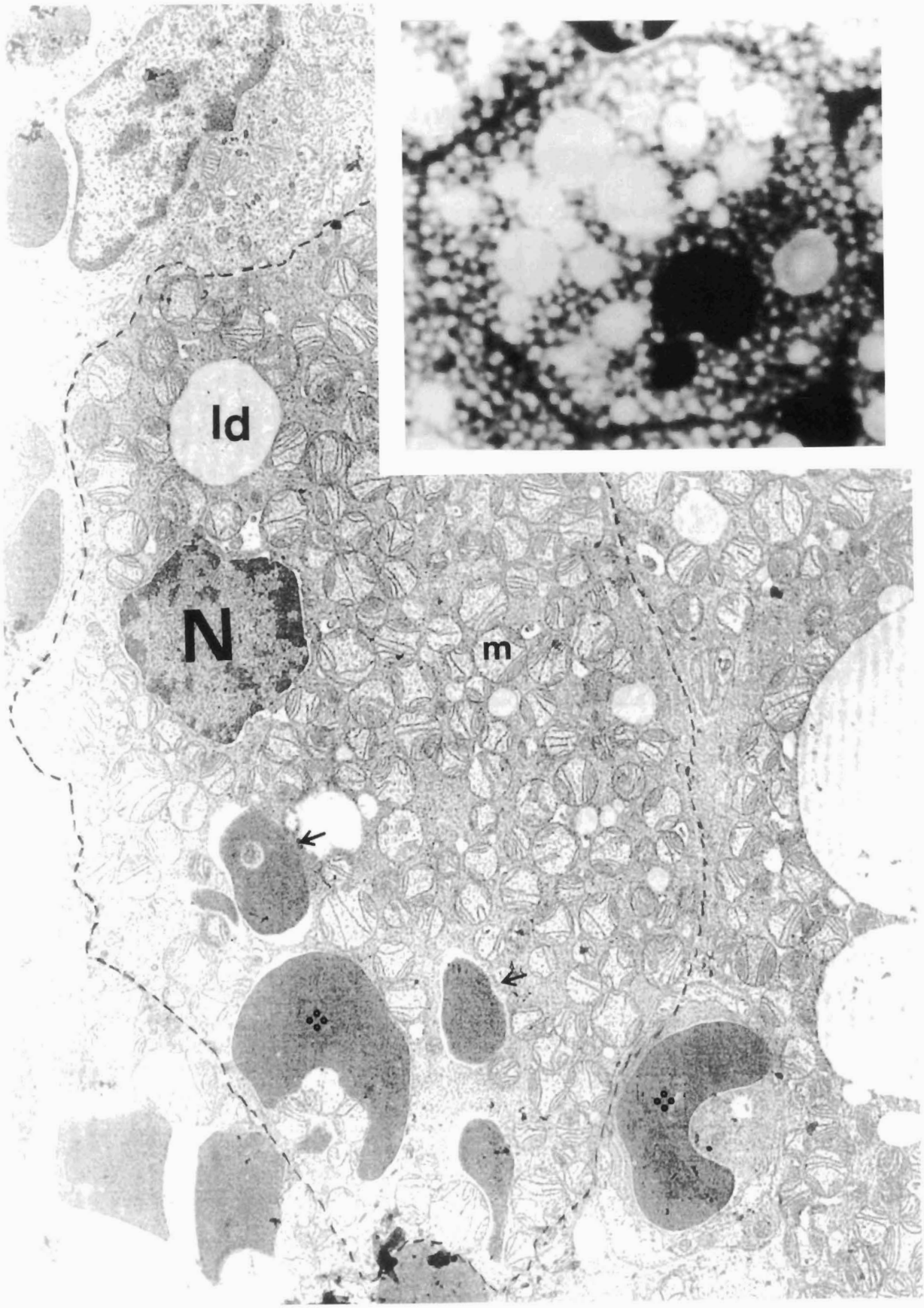
Ten adult male rats of the Wistar strain, weighing 180-200 g, were used in this study. The interscapular brown adipose tissue was dissected out and several small pieces were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7,2), and postfixed in 2% osmium tetroxide in the same buffer. After 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. Semithin sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections were mounted don copper grids, stained either with uranyl acetate and lead citrate or unstained and examined with a Philips MC 12 transmission electron microscope.

Results

The results obtained in comparative studies of brown adipocytes and capillaries under the electron microscope are presented as follows:

a) The presence of erythrocytes that either kept the shape they had in the capillaries (Fig. 1) or changed it to some unusual form (Fig. 2a) or had pseudopodia (Fig. 2b) was observed in some brown adipocytes. An intact phagosomal membrane was rarely observed. The erythrocyte membrane was surrounded by lipid-like material or with loose granular material as a result of degradative pattern seen around the periphery of intracytoplasmic erythrocytes (Fig. 2a,b).

b) Before leaving the capillary lumen, erythrocytes took hold of some material by endocytosis (Fig. 3a,b). This material was located in endocytic vacuoles in both cases, when the erythrocytes passed through capillaries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(Fig. 3c) and when the erythrocytes were situated in the cytoplasm (Fig. 3d) of brown adipocytes. While passing through endothelial junctions, the part that entered intercellular space, the so-called «head», was surrounded by an almost empty space (Fig. 3c); on the contrary, the part that did not leave the capillary lumen was in contact with some elements of blood plasma (Fig. 3c). Some erythrocytes were not fragmented (Fig. 4a) but some others were broken down as soon as they entered the cytoplasm of brown adipocytes (Fig. 4b) or later (Fig. 1).

c) In brown adipocytes, erythrocytes displayed phagocytic activity engulfing either lipid droplets (Fig. 5a) or swollen mitochondria (Fig. 5b) and were transformed into lipofuscin bodies (Fig. 5c) in which the remains of mitochondrial cristae and lipid droplets could be observed.

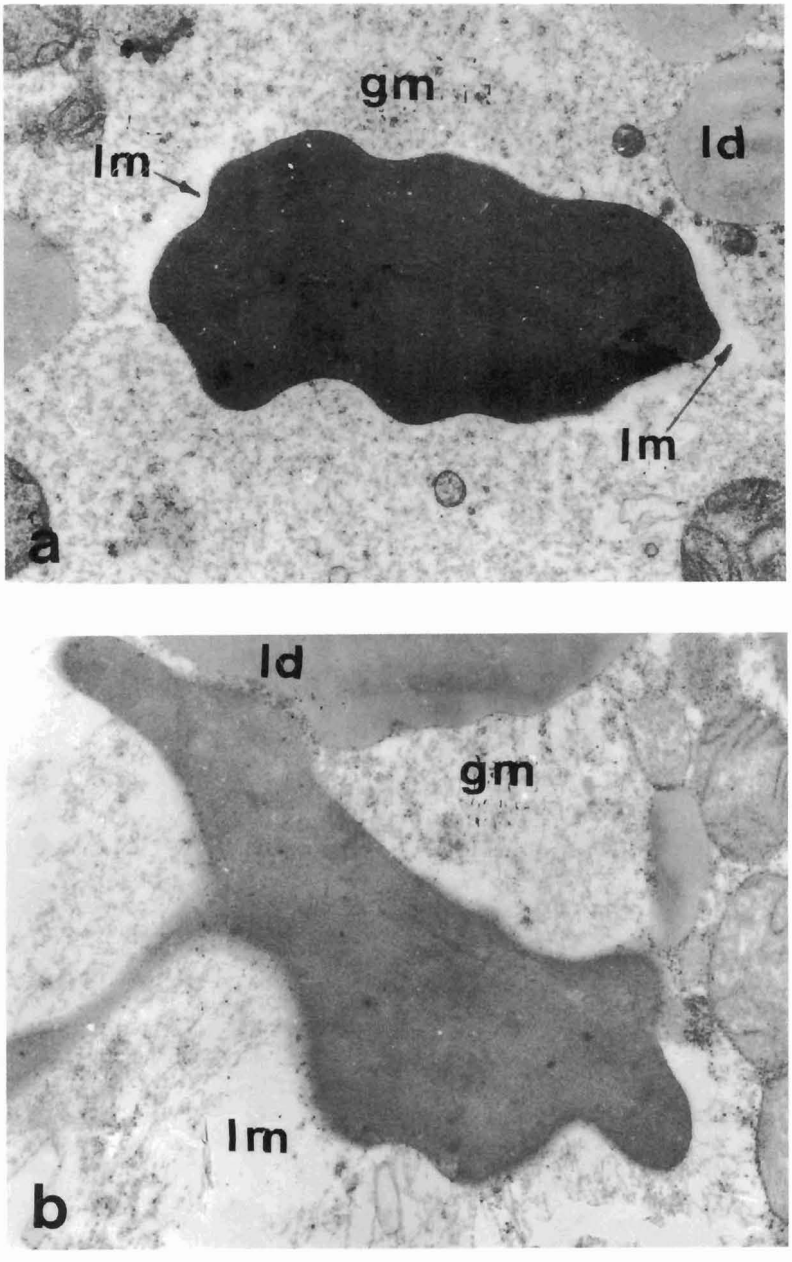


Fig. 2a,b. Different unusual forms of intracytoplasmic erythrocytes. gm: granular material; lm: lipid material; ld: lipid droplets. Uranyl acetate and lead citrate. a, x 15,000; b, x 16,000

Fig. 1. The gross subcellular appearance of a brown adipocyte (marked area). Note the presence of similar erythrocyte kidney forms either in the cytoplasm of the brown adipocyte or in the capillary lumen next to the adipocyte (circles). Fragmented parts of erythrocytes are also present in the cytoplasm of the same adipocyte (arrow). The insert (semithin sections; toluidine-blue, x 1,200) shows the presence of an erythrocyte and a fragmented particle in the cytoplasm of the brown adipocyte. N: nucleus; ld: lipid droplets; m: mitochondria. Uranyl acetate and lead citrate. x 7,860

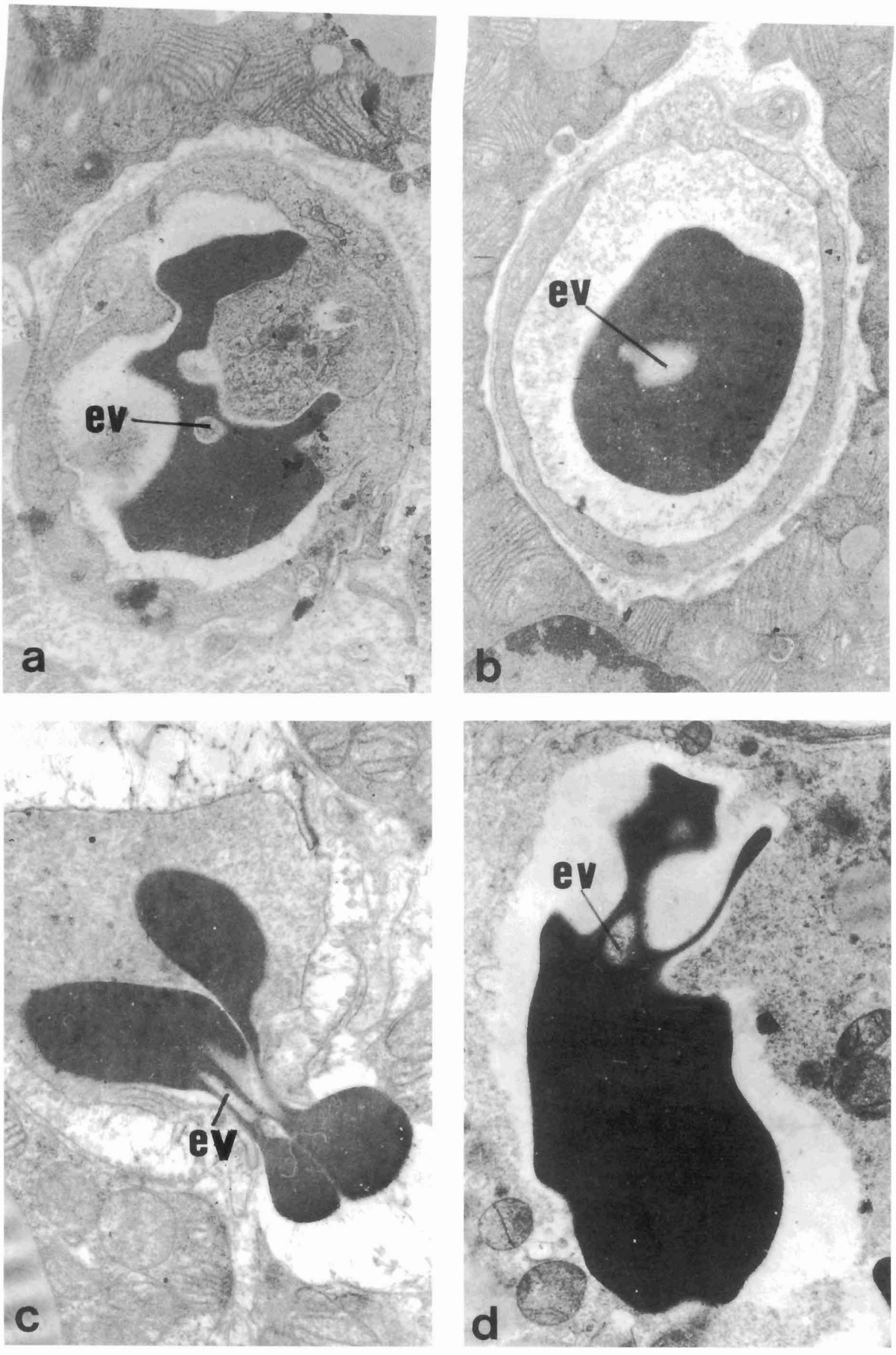
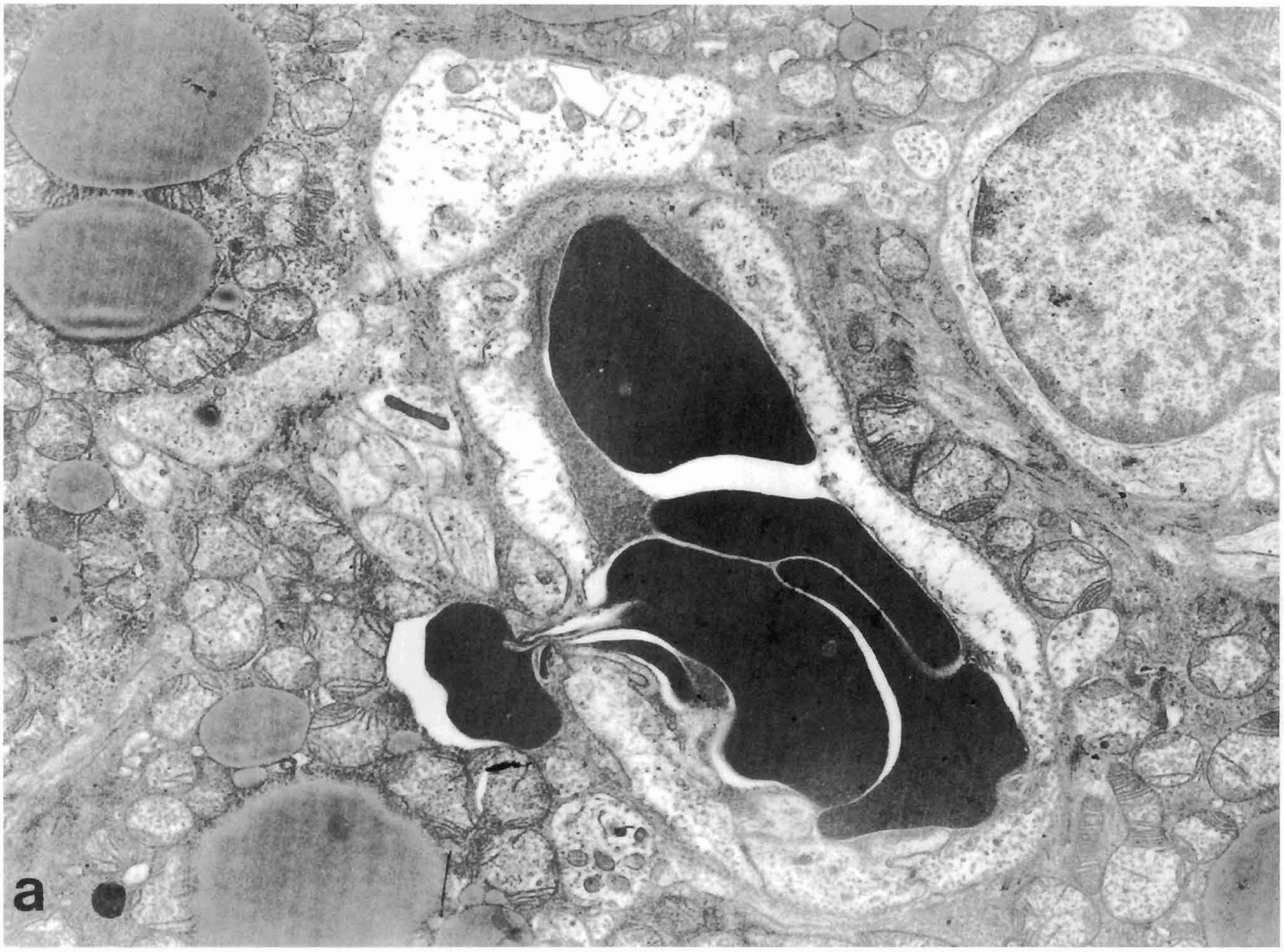


Fig. 3. Different phases of formation of endocytotic erythrocyte vacuole either in the capillary **(a,b)**, in the «neck» during transit **(c)** or in the brown adipocyte **(d)**. ev: endocytotic vacuole. Uranyl acetate and lead citrate. a, x 16,000; b, x 13,000; c, x 16,000; d, x 12,000



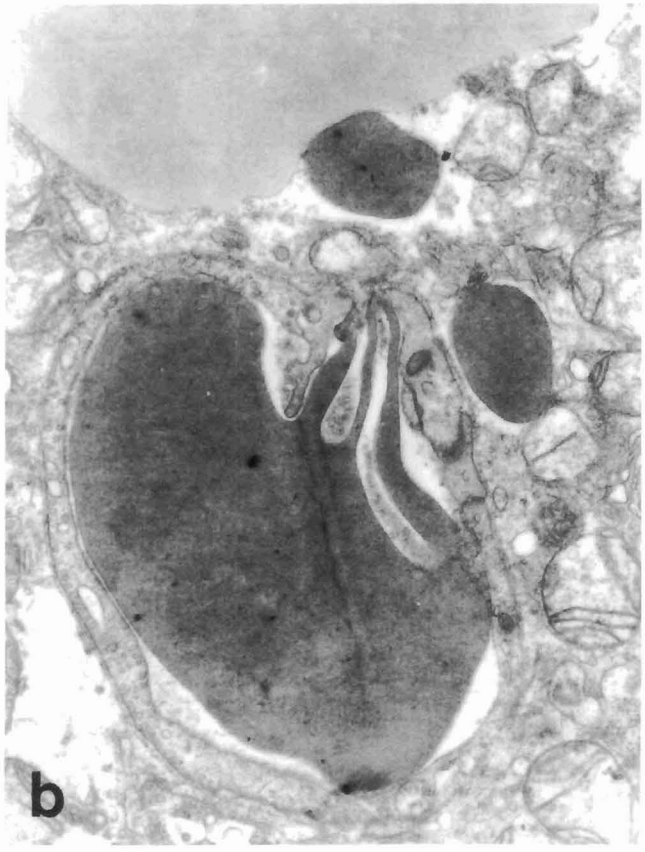


Fig. 4. Transit of erythrocyte from capillary to brown adipocyte. Some erythrocytes are not fragmented **(a)**. but some others are **(b)**. Uranyl acetate and lead citrate. a, x 8,400; b, x 16,000

d) Some erythrocytes were degraded by the granular pathway: they disintegrated into ferritin-like particles (Fig. 6) which could later be noticed in the mitochondrial matrix (Fig. 7a), intracristal space (Fig. 7a), and especially on the periphery of lipid droplets (Fig. 7b). The recent process was observed in nonstained section.

Discussion

Under hemorrhagic conditions many cell types such as thyroid epithelial cells (Zeligs, 1977), liver cells (Rosin and Doljanski, 1944), epidermal cells (Platt, 1963), epithelial cells of the gallbladder (Wakefield and Hicks, 1974), mast cells (Spicer et al., 1975), undifferentiated lung carcinoma cells (Falini et al., 1980), acute lymphoblastic leukeaemia cells (Foadi et al., 1978) and epithelial cells of a breast carcinoma (Marin-Padilla, 1977) have been found capable of erythrophagocytosis.

In the present study hemorrhagic areas were not seen in rat interscapular brown adipose tissue, and erythrocytes passed through the capillary endothelial junction in a similar way as that seen in the spleen (Satodate et al., 1986; Athens, 1993). In our laboratory, sugar-induced thermogenesis of brown adipocytes was studied recently.

A remarkable number of intercytoplasmic erythrocytes are seen in stimulated brown adipocytes (unpublished results). This adaptive change in the number of engulfed erythrocytes strongly suggests that the presence of erythrocytes in brown adipocytes is characteristic of the cell biology of brown adipocytes.

Some erythrocytes are fragmented during passage through openings in the endothelial junction and that may mean that the structural integrity of the membrane is disturbed and elastic recovery after deformation

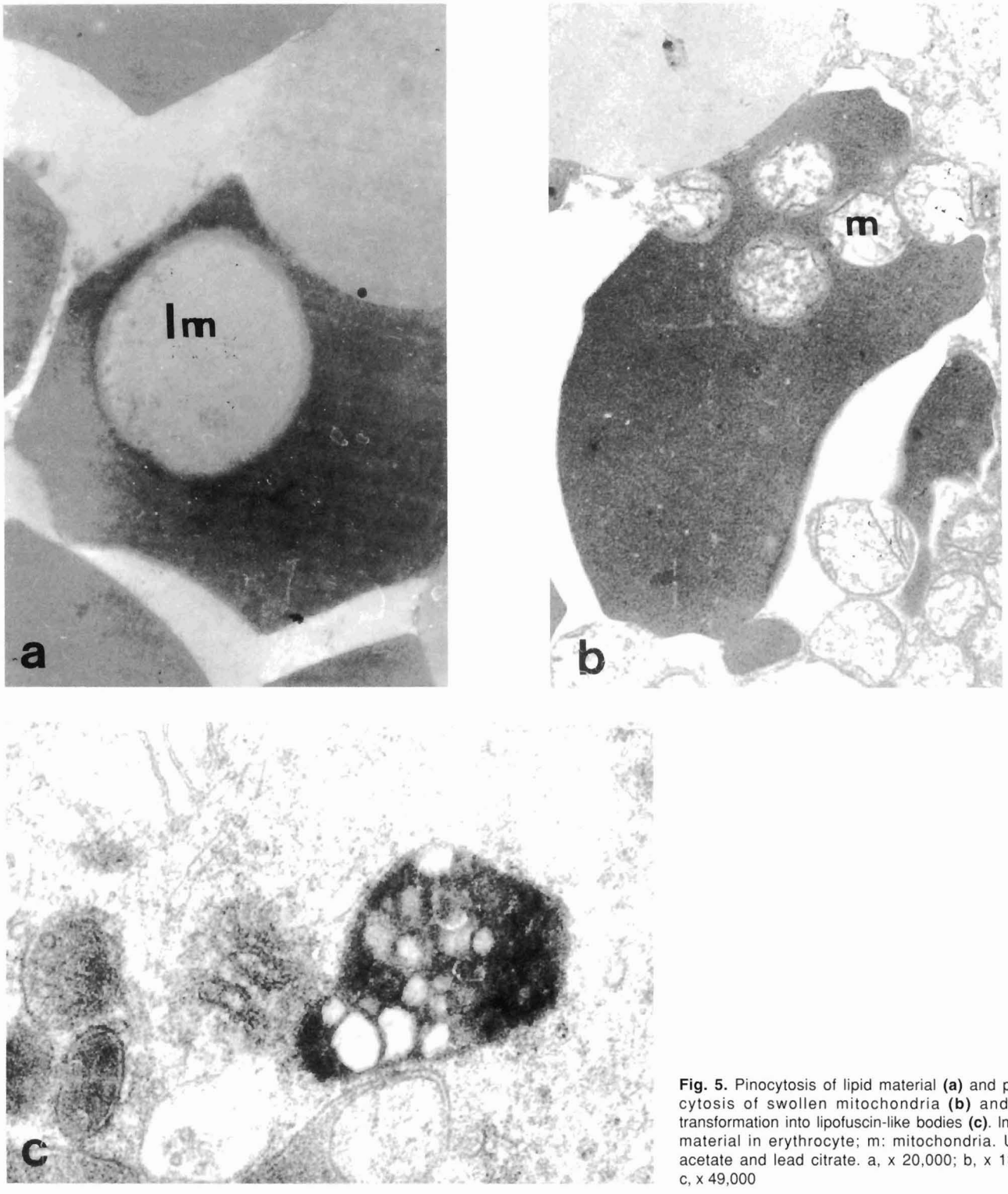


Fig. 5. Pinocytosis of lipid material **(a)** and phagocytosis of swollen mitochondria **(b)** and their transformation into lipofuscin-like bodies **(c)**. lm: lipid material in erythrocyte; m: mitochondria. Uranyl acetate and lead citrate. a, x 20,000; b, x 13,000; c, x 49,000

during passage does not occur. On the contrary, other erythrocytes pass without damage. This means that individual erythrocytes enter into brown adipocytes with different physiological status and have a different activity before cell death. In our case some of them recover showing one kind of biological activity (phagocytosis), while others are degraded to ferritin-like particles. Phagocytosis and degradation of injured, senescent cells or erythrocytes depleted of ATP stores constitutes an important normal hemoregulatory process and provides for the recycling of iron to new erythrocytes (Athens, 1993). Erythrocytes depleted of ATP stores are unable to perform their function of transporting oxygen and carbon dioxide.

In stimulated brown adipocytes during thermogenesis when anaerobic glycolysis takes place and ATP is extremely increased (Himms-Hagen, 1991), the erythrocytes which contain many of the components of the calcium-dependent contractile mechanism of muscle (Palek, 1995) are capable of revitalisation using glycolytic ATP both for cell movement and phagocytic activity. In normal erythrocytes, ATP does not pass across the cell membrane. However, in deoxygenated sickled cells uncoupling of the lipid bilayer from the underlying skeleton is found on the top of pseudopodia (Franck et al., 1985) and this provides some openings for the entry of ATP. During the ageing process endogenous activation of some enzymes and proteolysis is associated with an increase in the amount of cytosolic calcium or maybe calcium which is situated in so called «silent vacuoles» (Allan and Raval, 1987). We suppose that some vacuoles formed before the erythrocyte enters a brown adipocyte contain a certain amount of calcium which activates the erythrocyte movement and phagocytosis.

Several proteolytic systems have been defined in erythrocytes; for example, dipeptidases (Kaplan, 1961), neutral protease (Vettore et al., 1983) and calpain which is activated by calcium (Melloni et al., 1982). In addition, other enzymes are found, such as acid phosphatase (Dissing et al., 1991) and ribonuclease (Yasuda et al., 1990) which means that erythrocytes might play a role like lysosomes. Moreover, the proteosomes could be present in the erythrocytes, as well as in all eucariotic cells (Tanaka and Ichihara, 1990; Driscol, 1994). In this study, it is shown that erythrocytes phagocytize swollen mitochondria and lipid droplets and transform adipocytes into lipofuscin-like bodies in which iron is found (Van Eijk and De Jong, 1992). It is interesting to note that the erythrocytes, besides their diminished activity, engulf particles observed by some authors as dark vesicles (Tsang et al., 1982; Lončar and Afzelius, 1989). In addition, some other authors also documented that erythrocytes can

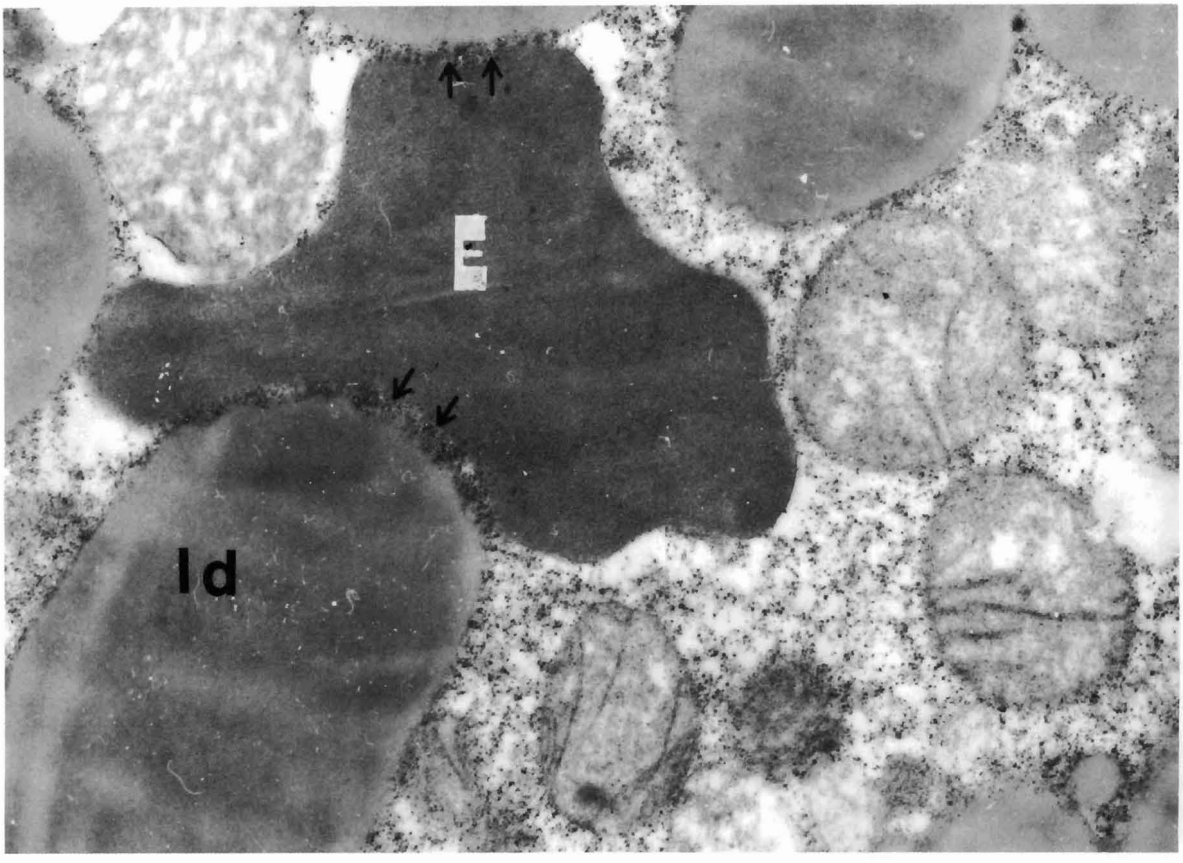


Fig. 6.
Erythrocyte degradation to ferritin-like particles in close contact with the lipid droplet. In the border line zone note the granular ferritin-like material. E: erythrocyte; ld: lipid droplet; arrows: granular ferritin-like material. Uranyl acetate and lead citrate. x 26,000

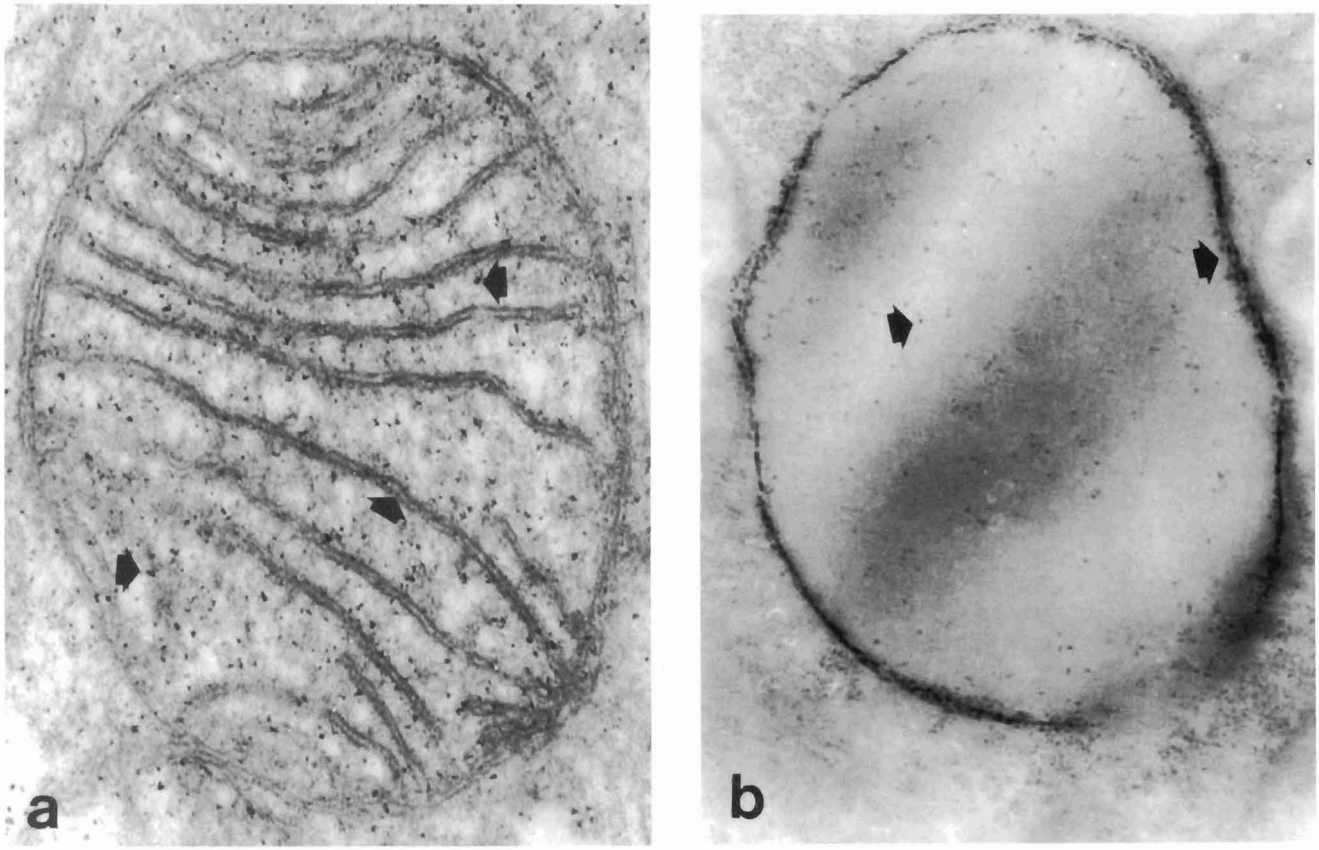


Fig. 7. Ferritin-like particles in a mitochondrion **(a)** and in a lipid droplet **(b)**. Note the dense concentration of these particles (arrow) at the periphery of the lipid droplet. Unstained sections. x 70,000

undergo receptor-mediated endocytosis under special conditions (Matovcik et al., 1985). Lipofuscin bodies have also been observed in brown adipocytes (Nnodim and Lever, 1985).

The granular pathway is characterized by fine mottling of erythrocytes first observed about the periphery and later extending to the entire cell (Zeligs, 1977). Ferritin-like particles are associated with the peripheral area of degradation, but cell lysosomes are always connected with the phenomenon of granular degradation. In one case (Zeligs, 1977) fragmented parts which adhere to the erythrocytes and which have the same density as erithrocytes are named as lysosomes. According to recent investigations sickled and ageing cells showed cellular dehydration, which is connected by an internal cell «fixative» (Palek et al., 1978; Liu et al., 1991), or cross inner membrane proteins. These internal conditions activate endogenous protease. The deleterious effect of increased cytosolic calcium is known in ageing cells or cells under shear stress (Larsen et al., 1981).

Ferritin, a protein shell with a molecular weight of about 500 kDA made up of 24 subunits, is mainly localized intracellularly, where it plays a major role in storing and detoxifying iron which is highly toxic to cells. Ferritin provides a soluble store of iron within body cells and in erythrocytes, and one molecule may contain up to 4,500 iron atoms in the form of ferric-hydroxyphosphate. A morphologist can see ferritin-molecules at a magnification of x 500,000 on stained (on unstained) micrographs (Bessis and Breton-Gorius, 1957; Tanaka and Goodman, 1972).

Iron participates in living processes by incorporation into heme and many other cytoplasmic enzymes and proteins (Van Eijk and De Jong, 1992) as well as into some subunits of four enzymes of the electron transport system in the mitochondrial inner membrane encoded in the mitochondrial genome (Ozawa et al., 1987). How ferritin-like particles enter into the mitochondrial matrix of the intracristal space is unknown. The periphery of lipid droplets where lipolysis takes place and many protein enzymes and even half leafted membrane is present is also poorly investigated and may be connected with the anaerobic metabolism of brown adipocytes (Lončar and Afzelius, 1989).

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