

Ultrastructural changes in the synovial membrane in experimentally-induced osteoarthritis of rabbit knee joint

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Summary. Rabbit knee joint osteoarthritis was induced by intraarticular injections of a 10% sterile NaCl solution. Within 30 days the synovial membrane had undergone hyperplasia resulting in activated synovial fibroblasts. Transitional forms of synoviocytes as well as activated synovial macrophages were a very common finding. At 60 days a thickening of the synovial intima was perceptible. Most of the synoviocytes were of the fibroblast type. Transitional cell forms abounded. An increase in collagen fibres and capillaries of the fenestrated type occurred in the intercellular spaces. In the deep layer collagen bundles had formed between which activated fibroblasts and macrophages were noticed. The described changes point to an active participation of the synovial membrane in the destruction of articular cartilage in osteoarthritis.

Key words: Electron microscopy, Synovial membrane, Experimental osteoarthritis, Knee joint, Rabbit

Introduction

Osteoarthritis (OA) is considered to be a degenerative joint disease with primary damage to the articular cartilage (Fuzesi, 1984; Munte and Bjele, 1984). The balance between synthesis and disintegration of the cartilaginous matrix is disturbed; this disturbance becomes manifest in a reduction of proteoglycans and a demasking and destruction of collagen fibres (Sandy et al., 1987). The reduced cellular activity of chondrocytes plays an important role in these changes (Stiller, 1982; Fassenberg, 1987).

The problem whether the synovial membrane (SM)

participates in these processes has not yet been solved. The concept that the changes in SM are secondary prevails, but the data of biochemical studies point to an activation of synovial cells in osteoarthritis (Dingle et al., 1979; Steinberg, 1983; Swierstra, 1983; Pelletier et al., 1985). The morphological equivalent of these changes has so far not been specified. Destructive changes in SM have been demonstrated in advanced forms of coxarthrosis (Roy, 1967; Hulth et al., 1973), but the issue of the dynamic patterns of such changes has not been fully elucidated as yet (Burmester et al., 1983; Revel et al., 1988). A fair chance of such a morphological analysis is afforded by experimental OA models, like the ones used in evaluating articular cartilage changes (Hulth et al., 1970; Marshall and Olson, 1971; Kalbhen, 1984; Moskowitz, 1984). The present study outlines the changes in the ultrastructure of SM, accompanying articular cartilage destruction in osteoarthritis induced by an original experimental model (Vidinov et al., 1989).

Materials and methods

Ten rabbits, mean weight 2.5 kg, aged 8 - 12 months, received a daily injection of 2 ml of a 10% sterile NaCl solution in water into the right knee joint for 5 days. The left knee served as control. All animals were kept on a free unrestricted motor regime. At days 30 and 60 after the first injection parapatellar arthrotomy of both knees was performed (5 animals per group) under deep ether anaesthesia. SM pieces from the infrapatellar region, measuring 1 x 5 mm, were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 h at 4° C. Postfixation was done in 1% OsO₄ in 0.1 M cacodylate buffer, pH 7.4 for 2 h at 4° C. Further processing of the material was carried out according to protocol for transmission electron microscopy. The material was embedded in Durcupan (Fluka). Identification of the objects was done on semithin (0.1 µm) sections, stained with 1% methylene blue and 1% Azure II. The ultrathin sections were

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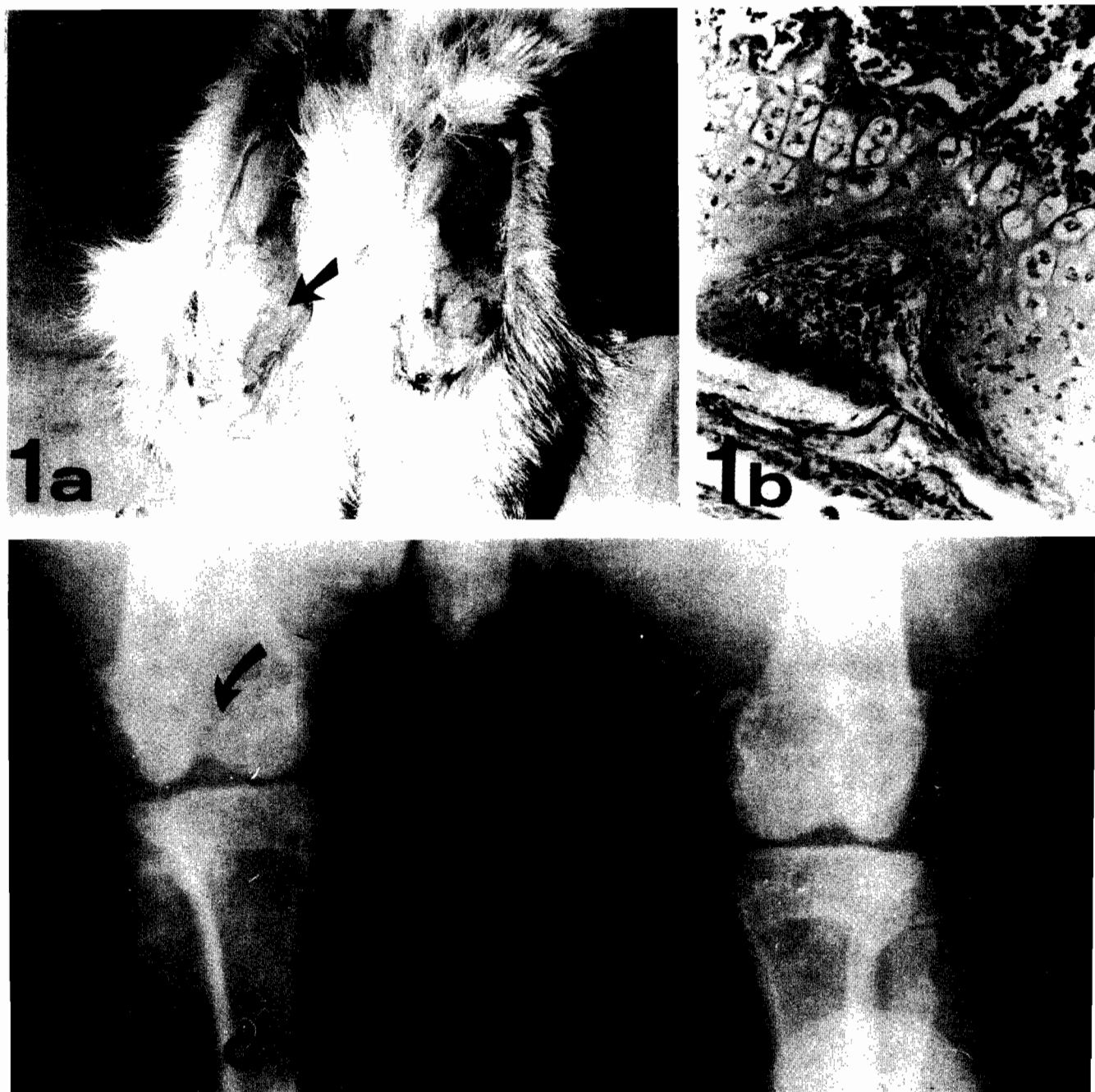


Fig. 1. Rabbit knee joint. **a)** Right knee joint deformation 30 days after induced osteoarthritis (arrow). **b)** A cleft (arrow) on the articular cartilage surface, filled with fibrous tissue 30 days after the experiment. $\times 150$. **c)** Bone changes (arrow) in the right knee joint 60 days after the beginning of the experiment. The left knee joint is used as control.

counterstained with 2.5% uranyl acetate for 20 min and 2.6% lead citrate for 20 min. A Zeiss 10 AB served for electron microscopic inspection.

Results

Since changes in the articular cartilage produced by the experimental OA model were established after 25 days (Vidinov et al., 1989) when abatement of the

reactive synovitis takes place (Revel et al., 1988), our first investigations were carried out within 30 days of the experiment. The knee joint was deformed. The articular cartilage lacked its normal lucency, becoming turbid with irregular thickenings appearing along its margins (Fig. 1).

In the control knee joints the synovial membrane was made up of a thin marginal layer (synovial intima), composed of elongated, overlapping,

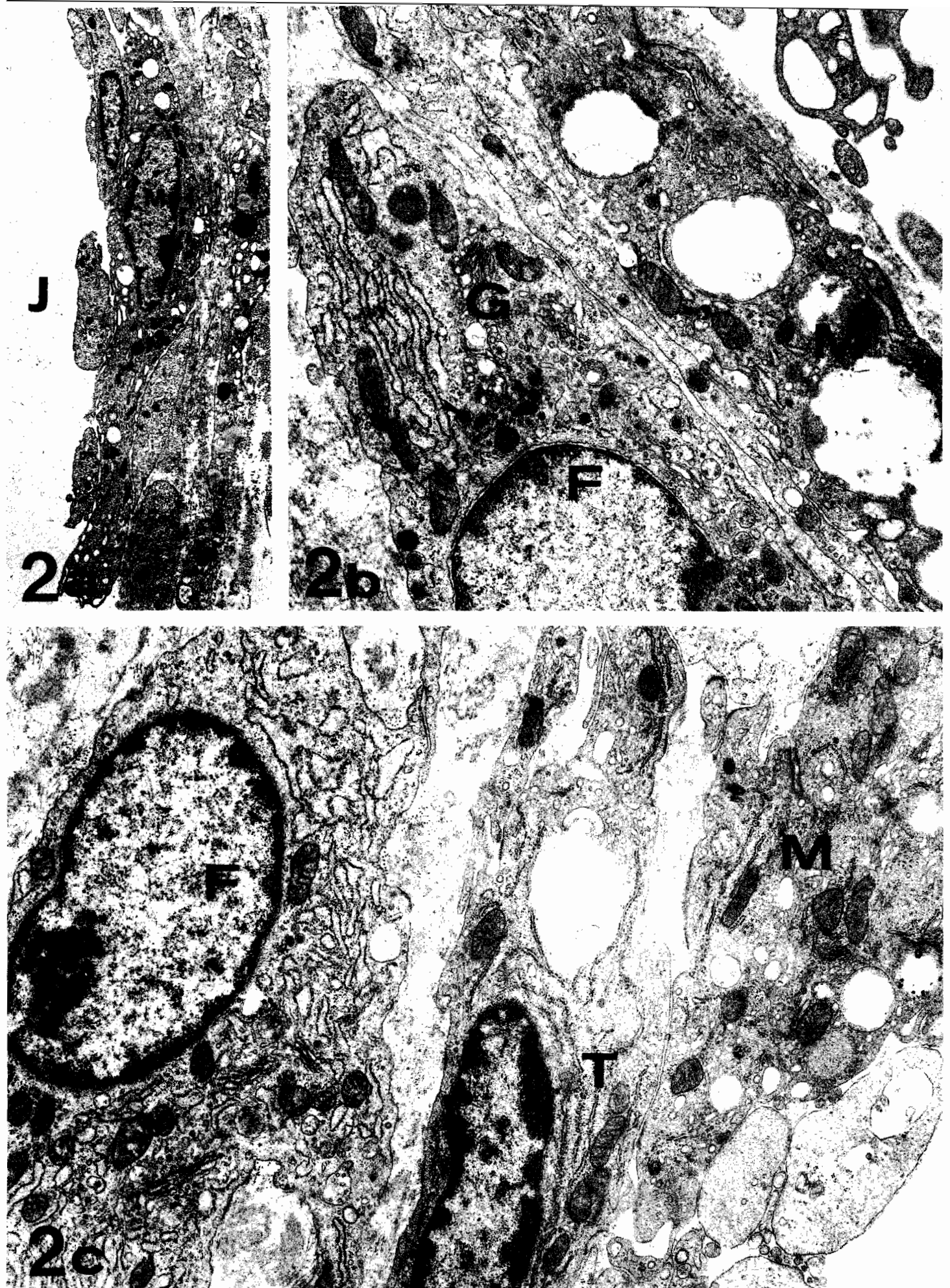


Fig. 2. Synovial membrane from untreated rabbit knee joint (control). **a)** Synovial intima. Synovial macrophages (M) predominate. A basal lamina is absent. J = articular cavity. $\times 5,100$. **b)** Synovial macrophage (M) with underlying synovial fibroblast (F) with adequately developed rough endoplasmic reticulum and wide Golgi zone (G) with osmiophilic granules (arrow head). $\times 12,500$. **c)** Synovial fibroblast (F), cell of transient type (T) and synovial macrophage (M). $\times 15,200$

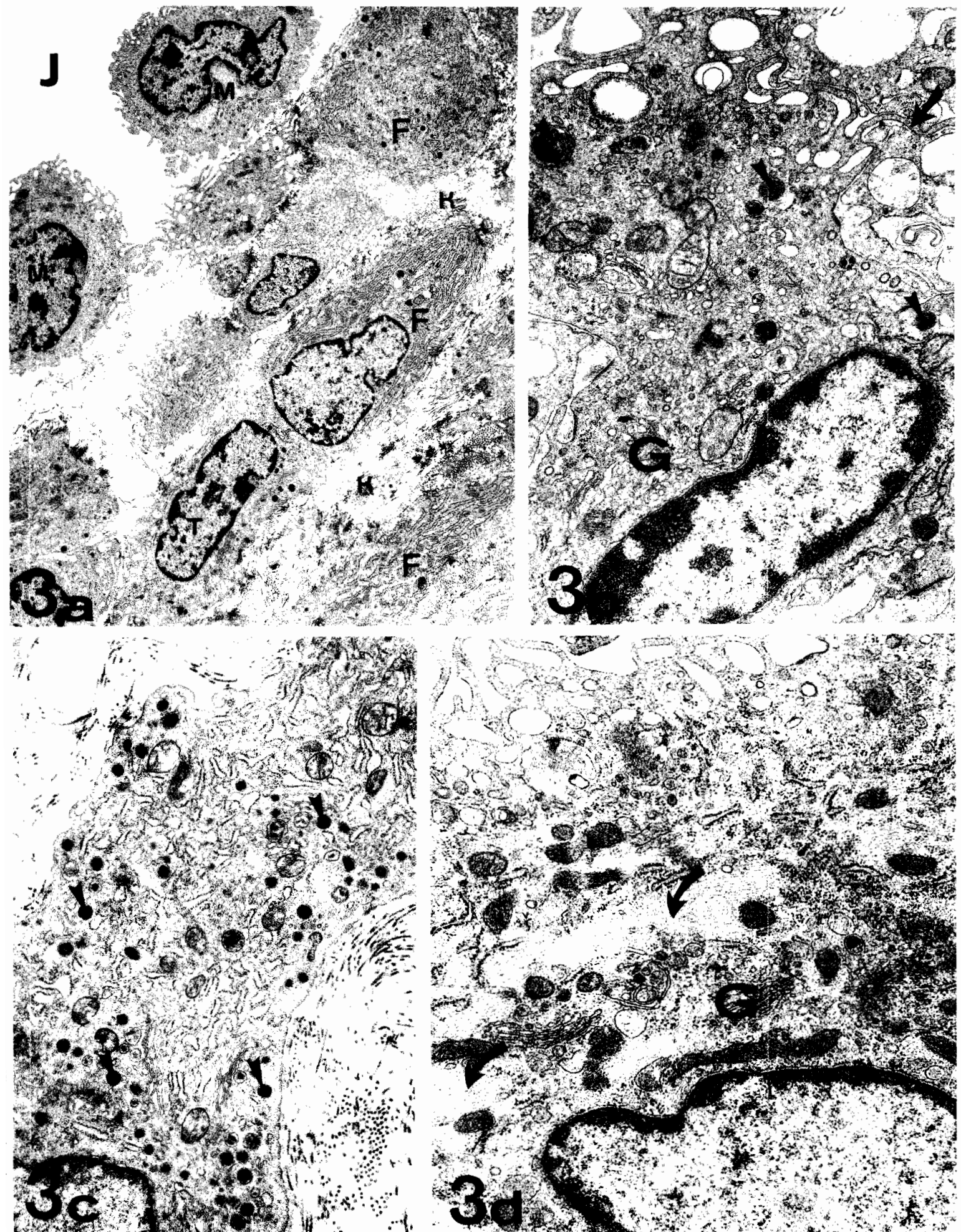


Fig. 3. Synovial membrane 30 days after induced osteoarthritis. **a)** Synovial intima. Synovial macrophages (M), cells of transient type (T) and synovial fibroblast (F). In the intercellular spaces collagen fibres (K). J = joint cavity. $\times 10,000$. **b)** Activated synovial macrophage. Filopodia establish contact with neighbouring cells (arrow). Clear-cut Golgi apparatus (G), lysosomes (arrow head). $\times 19,500$. **c)** Synovial fibroblast with numerous osmiophilic granules (arrow head). $\times 22,000$. **d)** Cell of transient type with filaments (arrow) and well-developed Golgi apparatus (G). $\times 25,000$

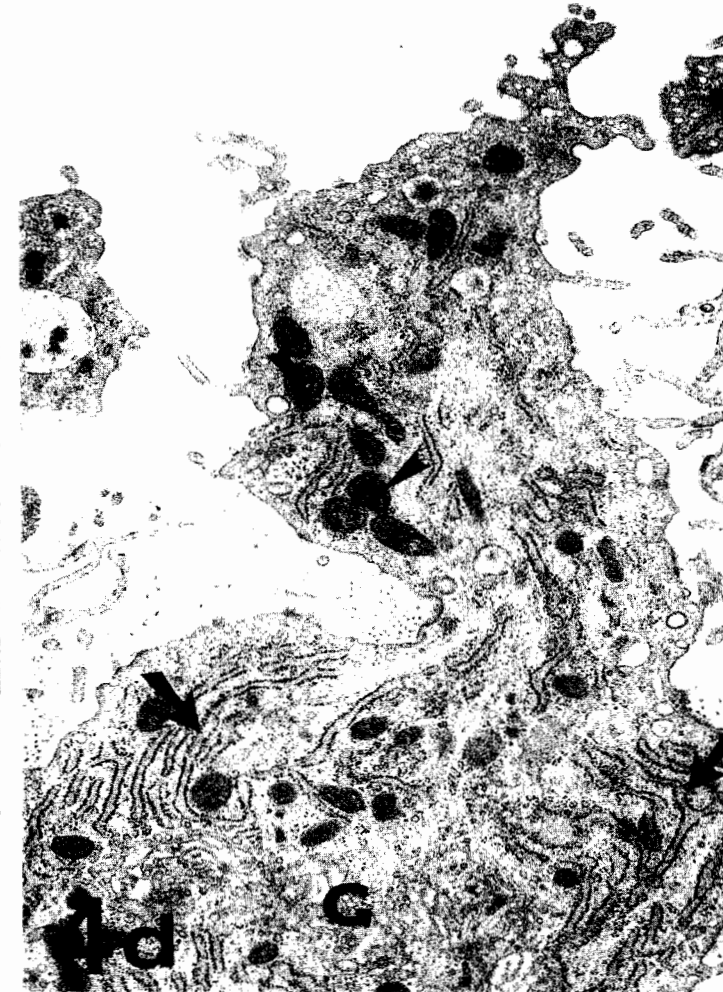
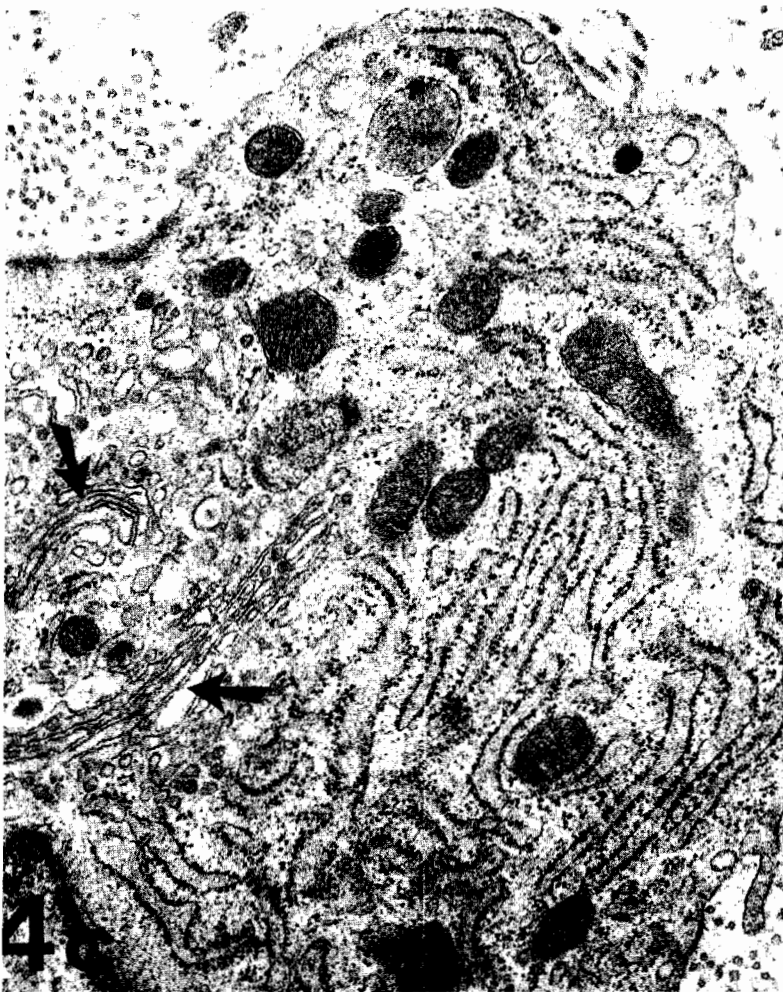
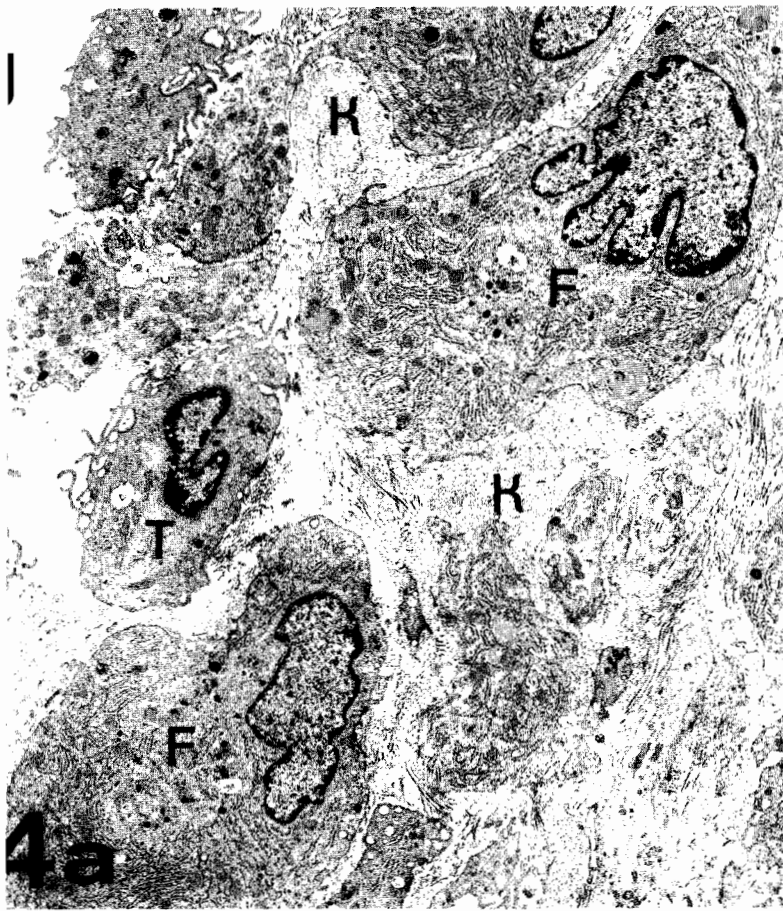


Fig. 4. Synovial membrane 60 days after induced osteoarthritis. **a)** Synovial intima with abundance of synovial fibroblasts (F) and transient type cells (T). Numerous collagen fibres (K). J = joint cavity. $\times 10,000$. **b)** Synovial macrophage with nucleus rich in chromatin and cytoplasmic filaments (arrow). $\times 15,000$. **c)** Synovial fibroblast with a wide Golgi zone (arrow). $\times 31,000$. **d)** Cell of transient type with irregular surface. Well-developed Golgi apparatus (G) and rough endoplasmic reticulum (arrow). Numerous mitochondria (arrow head). $\times 16,000$

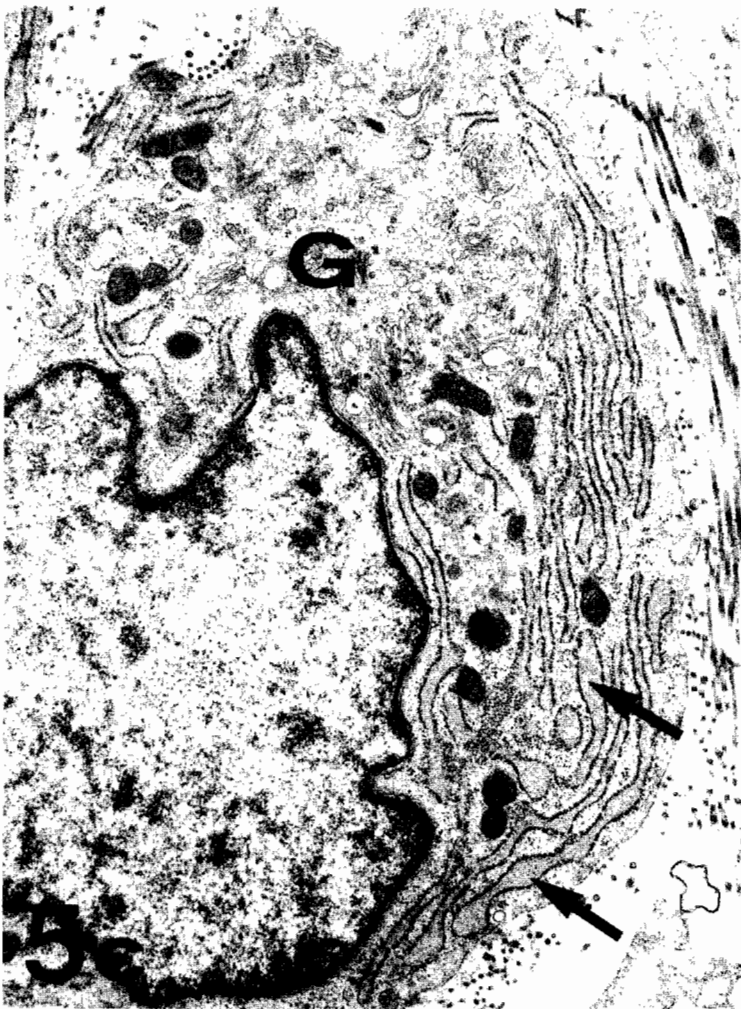
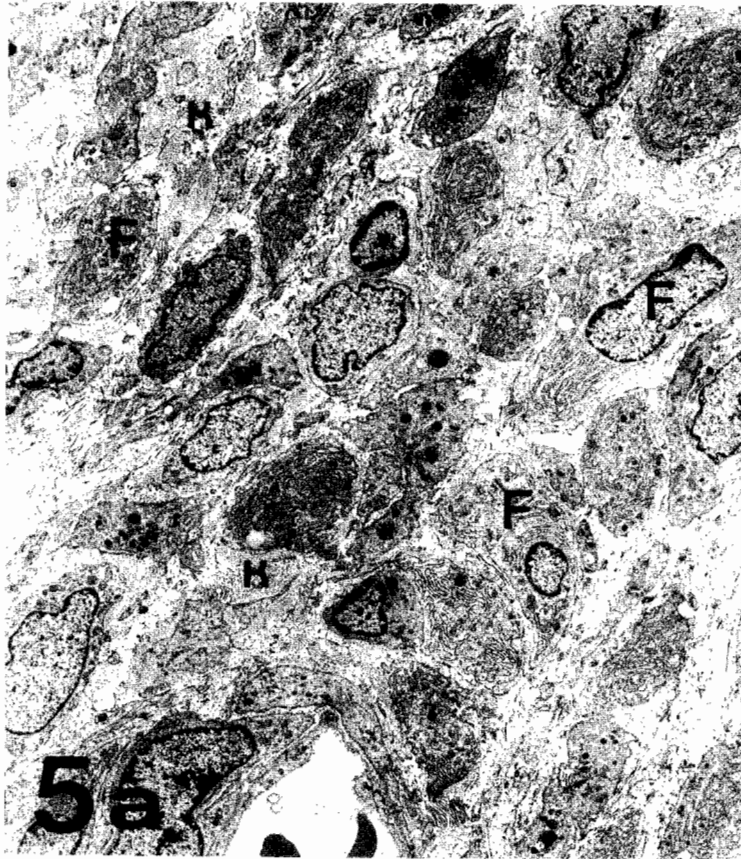


Fig. 5. Deep layer of synovial membrane in OA. **a)** Abundant fibroblasts (F) and collagen fibres (K). V = blood vessel. $\times 6,000$. **b).** Macrophage with numerous lysosomes (arrow). $\times 12,000$. **c)** Fibroblast with a wide Golgi zone (G), cisternae of the rough endoplasmic reticulum (arrow). $\times 20,000$. **d)** Fenestrated capillary with markedly expressed cell organelles (O). Endothelial pores with diaphragm (arrow), thin interrupted basal lamina (arrowhead). $\times 7,800$.

macrophage-like and fibroblast-like cells (A- and B-cells according to Barland et al. [1962]). A basal lamina was missing, and the intercellular spaces opened into the articular cavity (Fig. 2a). Due to the morphological and functional features of these cells, they were termed synovial macrophages and synovial fibroblasts (Vasilev, 1981).

The synovial macrophages (SMa) showed numerous cytoplasmic processes on their surface and contained a great number of vesicles, vacuoles and lysosomes. Their nucleus was rich in chromatin, oriented mainly towards the nuclear membrane (Fig. 2b). The synovial fibroblasts (SFi) contained an adequately developed rough endoplasmic reticulum and Golgi apparatus wherein single, dense osmiophilic granules occurred. The nucleus was comparatively poor in chromatin (Fig. 2b). Transitional cell forms (AB-cells according to Ghadially, 1983), showing ultrastructural signs characteristic of the basic types of synoviocytes, were also observed (Fig. 2c).

At 30 days after the beginning of the experiment the cells of the synovial intima had increased in height and number and were arranged in several rows. In the intercellular spaces fibrin and thin collagen fibres lacking orientation were noted (Fig. 3a). The synovial macrophages exhibited a very well-developed Golgi apparatus, an abundance of vesicles, and primary and secondary lysosomes (Fig. 3b). The synovial fibroblasts contained a well-developed rough endoplasmic reticulum and a clear-cut Golgi zone. In their cytoplasm numerous osmiophilic granules, measuring about 200 nm, were observed. They were delimited by a membrane, and had a narrow halo (Fig. 3c). Transitional cell forms were often seen. The latter were characterized by a rich vesicular system, very well-developed Golgi apparatus, increased number of membranes of the rough endoplasmic reticulum, numerous mitochondria and considerable aggregations of intracytoplasmic filaments (Fig. 3d).

On the 60th day, SM contained a much greater number of cellular elements, as compared with the controls. Within the intercellular spaces the number of collagen fibres had also increased. The synovial intima was composed mainly of fibroblast-like cells and transient forms (Fig. 4a). Synovial macrophages were situated predominantly on the SM surface. The contour of the nucleus was undulated, rich in chromatin with clear-cut nucleoli, and often contained nuclear inclusions. Within the perinuclear space aggregations of filaments were found (Fig. 4b). The major part of SM cells was represented by synovial fibroblasts. They had a very well-developed rough endoplasmic reticulum, a wide Golgi zone, a large amount of mitochondria varying in size, and a multitude of vesicles exhibiting different phases of pinocytotic transport. Osmiophilic granules of diverse types were located within the Golgi zone. The nucleus contained evenly dispersed chromatin (Fig. 4c). In this group of animals, also, transitional cell forms were a common finding. Their cytoplasm containing

bundles of intermediary filaments and microfilaments, numerous mitochondria, clearly outlined rough endoplasmic reticulum and a broad Golgi zone (Fig. 4d). They showed morphological signs pointing to migration capability.

In the deep layer of SM a noticeable increase in collagen fibres of the extracellular matrix was observed. Compared with the controls, the number of cells was also augmented, especially that of the fibroblast type. They exhibited morphological signs indicating an enhanced synthetic activity. These were: numerous cisternae of the rough endoplasmic reticulum; properly developed Golgi apparatus forming several dictyosomes; increased number of mitochondria (Figs. 5a,b). Macrophages with irregularly-shaped, chromatin-rich nuclei, and numerous primary and secondary lysosomes were located close to the blood vessels (Fig. 5c). In osteoarthritic joints the synovial membrane contained a large number of blood vessels. Parallel to capillaries with continuous endothelial layer, fenestrated capillaries with an active-looking endothelium were also encountered - markedly expressed cell organelles and vesicular structures, heavily waved luminal surface and discontinuous basal lamina (Fig. 5d).

Discussion

The results of our studies should first be discussed in view of the experimental set-up used. The chemical model described brought about changes in the articular cartilage characteristic of OA (Vidinov et al., 1989). These changes may be explained both by the direct NaCl effect on chondrocytes or by alterations in SM. When other sodium salts are employed (Na-iodo-acetate), blocking of chondrocyte glycolysis, leading to impaired neosynthesis of matrix molecules, has been demonstrated (Kalbhen, 1984).

As shown by our results there is evidence of activation of either of the two basic types of synoviocytes responsible for homeostasis within the articular cavity (Revel, 1989). The mentioned cells establish contact and interact through cytoplasmic processes. In group one animals the intercellular spaces contain only few fibrous elements regardless of the fact that they are considerably wider than those in the control knee joints. This may be attributed to the increased content of proteoglycans/glycosaminoglycans for which indirect evidence was established in our previous studies. The latter hypothesis is also supported by biochemical and immunohistochemical studies, demonstrating an increase in hyaluronic acid and fibronectin in the SM in osteoarthritis (Mayston et al., 1984; Myers and Brand, 1987; Sandy et al., 1987).

The obtained results show that there are changes in the ratio between the basic types of synovial cells. Normally SMa predominate with a ratio of 3:1 relative to the SFi (Greisen et al., 1982). In induced OA synovial fibroblasts prevail and reveal

morphological signs of enhanced synthetic activity. Moreover, these cells contain an elevated number of osmiophilic granules which, as generally accepted, are not characteristic of this particular animal species (Link and Porte, 1981). They contain glycoproteins and show negative reaction for acid phosphatase; thus, their lysosomal nature is ruled out (Okada et al., 1981). The abundance of granules of the kind seen in our experimental material corroborates the idea that they represent secretory granules which transport the synthesized products, most likely glucosaminoglycans, to the extracellular spaces and from there into the articular cavity (Scott et al., 1981; Carney et al., 1985).

Among the macrophage-like synoviocytes, the transitional cell forms are much more common in the synovial intima of arthritic joints. Their cytoplasmic organization indicates that apart from phagocytic-catabolic functions, characteristic of macrophages, these cells exhibit signs of active synthesis and secretion, typical of SFi. The latter fact complies with the data on the increased enzyme content in the articular fluid in OA (Swierstra, 1983; Pelletier and Martell-Pelletier, 1985). Part of these enzymes with lysosomal origin (acid phosphatase, β -glucuronidase, 5-nucleotidase) and others - non lysosomal (neutral proteases, collagenase, elastase) are likewise increased in OA and contribute greatly to cartilage destruction (Pelletier et al., 1985). Usually, these enzymes are found in non-active form owing to the presence of inhibitors and inadequate pH of the joint fluid. An essential role in their activation may be played by catabolic factors released from synoviocytes that exert a direct effect upon the chondrocytes of articular cartilage (Dingle et al., 1979; Ridge et al., 1980; Steinberg, 1983). Our results warrant the assumption that the transient cell types in the synovial intima may be considered to be the probable source of such stimulation of the enzymes responsible for cartilage degradation in OA.

It is of interest to note the aggregation of cytoskeletal elements in the macrophage-like cells of the synovial intima. They are discovered in the synovial cells in rheumatoid arthritis (Ghadially, 1983) and in the articular cartilage chondrocytes in OA (Weiss, 1973). Their presence in synovial cells of the macrophage group and in OA corroborates the concept that accumulation of intracytoplasmic filaments of a vimentin nature is an expression of cell metabolism impairment (Ghadially, 1983).

Thickening of the joint capsule and deformation of the knee joints in the experimental animals may be related to changes in the deep layer of SM. Here, not merely an increase in fibrous structures of the matrix is involved, but also proliferation of fibroblasts and macrophages. Local origin of these cells is not likely, since mitoses are very seldom seen and studies using labelled thymidine yield evidence of a weak DNA synthesis (Coulton et al., 1980). The round cellular infiltrations at the early stages of experimental OA,

as described by other authors (Revel et al., 1988) and our results demonstrating a large number of transitional forms point to the hematogenic origin of these cells (Dreher, 1982; Edwards and Willonghby, 1982). Positive data along these lines were obtained with immunological studies showing that synovial cells have receptors for immunoglobulins, marked with the same monoclonal antibodies marking the monocyte-macrophagal series (Theofilopoulos et al., 1980; Hogg et al., 1985). Under the experimental set-up described, the results showing a marked increase in transient type cells suggest a similar possibility for synovial fibroblasts which should be considered as a functionally specialized form of one and the same cell type.

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