Histol Histopath (1988) 3: 419-424

Histology and Histopathology

The lectin binding pattern of normal and pathologically altered synovial tissue

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Summary. Light-microscopical lectin-binding studies were carried out in healthy and pathologically altered synovial tissue (osteoarthrosis, rheumatoid arthritis (RA)). Seven lectins were studied: Con A, DBA, PNA, RCA, SBA, UEA-I, and WGA. Con A and WGA mark all lining cells and the majority of subintimal synovial cells. RCA and SBA stain only a portion of lining cells, regardless of the basic pathology. The lectin PNA reacts only with RA and arthrotic material, and is thus suitable for the diagnosis of inflammatory changes in synovial tissue. UEA-1 is a consistent marker for capillary endothelium and large vessels.

Key words: Synovial tissue, Lining cells, Lectins, Osteoarthrosis, Rheumatoid arthritis

Introduction

Lectins are non-enzymatic proteins of non-immune origin in plants and animals, which bind carbohydrate groups with great specificity and sensitivity (Goldstein et al., 1980). The carbohydrate groups of cell surfaces are essentially involved in cell adhesion, antigen recognition, cell differentiation and malignant transformation (Damjanov, 1987).

Quantitative and qualitative changes in the structure of the synovial membrane, especially those due to inflammatory and degenerative joint diseases, can be evaluated by lectin marking (Borisch et al., 1986, Itokazu et al., 1987). The varying results reported in the literature can possibly be traced back to different preliminary treatments of the tissue. The goal of our project was now to perform a comparative study of healthy and diseased synovial tissue under controlled conditions.

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Materials and methods

Frozen and paraffin sections

Biopsies of synovial tissue were removed from the knee joints of 23 patients and divided into three groups: normal tissue (e.g. meniscus lesions) n = 5, arthrotic tissue n = 11, and rheumatoid arthritis (RA) n = 7. Immediately after removal the tissue samples were either quick-frozen in liquid nitrogen (–196°C) or fixed with 3.5% phosphate-buffered formalin solution (pH 7.4) or in Bouin's fluid and then embedded in paraffin. 5 µm thick sections were then taken of the paraffin and cryostat samples. Some cryostat sections were treated either for one minute with 100% 4°C cold acetone solution, for 20 minutes at room temperature with 3,5 formalin or Bouin solution, or fixed for one hour with 100% methanol at 4°C (Rittman and Mackenzie, 1983).

Semi-thin sections

Tissue from another group of seven patients (normal tissue n = 1, arthrotic n = 4, RA n = 2) was fixed according to Karnovsky's method (1965), after-treated with cacodylate-buffered osmium tetroxide, drained in increasingly concentrated ethanol solutions, and, after transfer to propylenoxide solution embedded in Epon (Luft, 1965). Finally the tissue samples were de-epoxified according to the method of Geleff and Bock (1984).

For comparison, additional synovial tissue was removed from the knee joints of 14 Wistar rats and 12 guinea pigs respectively, then fixed and embedded in the same manner.

Mast cell test

Bouin or formalin-fixed paraffin sections were stained with tolouidin-blue (pH 0.5; Strobel et al., 1981).

Lectin staining

The sections were treated for 30 minutes with 0,3% hydrogen peroxide in 100% methanol. All specimens were then rinsed in aqua bidest and placed in 8% phosphate-buffered swine serum for 30 minutes. After a final rinse in 1% swine serum the specimens were incubated with biotinylated lectins (Vector; Table 1) in a moist chamber for one hour at room temperature. The avidin-biotin-peroxidase test was then carried out according to the technique of Wrotnowski and coworkers (1985).

Controls

Specimens incubated with the hapten sugars of corresponding lectins (Table 1) in 0,2 molar solution served as specific controls. Subsequently the respective hapten sugars of the lectins were added to the incubation. Non-specific negative controls were incubations without added lectins or avidin.

Results

Tissue structures that stained positively for lectins showed a dark brown color. The paraffin sections showed a significantly higher lectin concentration than the cryostat sections. Because of better tissue preservation in the case of paraffin embedding, precise histological evaluation of the section was possible. Formalin-fixed tissue showed a weaker degree of lectin-marking than the Bouin-fixed samples. De-epoxified tissue required higher concentrations of lectins than the paraffin sections. In the control sections that were pre-incubated with the corresponding hapten sugars, the reaction was completely inhibited. The nonspecific control reaction was also negative.

In humans Con A marked all lining cells, as well as the majority of the subsynovially located cells of the healthy and pathologically altered synovialis (Fig. 1). In two patients with osteoarthrosis the small blood vessels were also stained. The fibrous connective tissue structures showed a weakly positive reaction. Results regarding differences in the density of marking or in the distribution of lectin-binding sites were not available for the three groups of human subjects nor for the animal material.

The synovial cells and the interstitium showed no

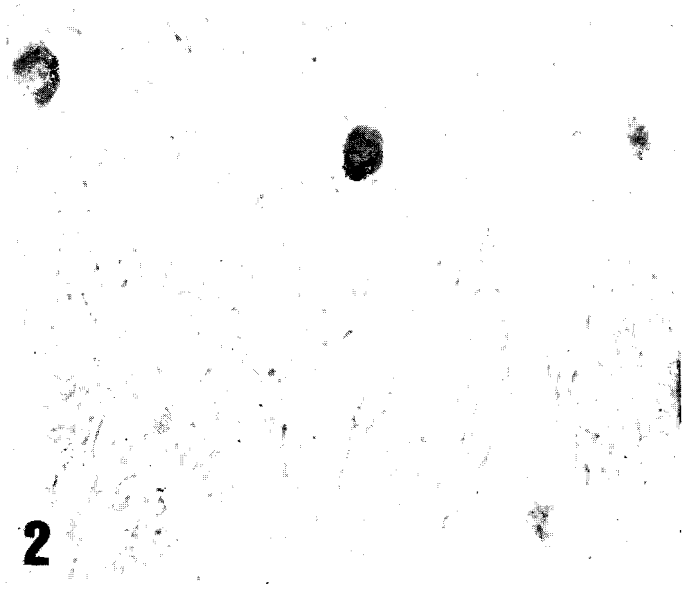

Fig. 2. Binding of DBA to mast cells lying in the subsynovium (Human, osteoarthrosis). Paraffin section. × 1.000 (Oil inmersion)

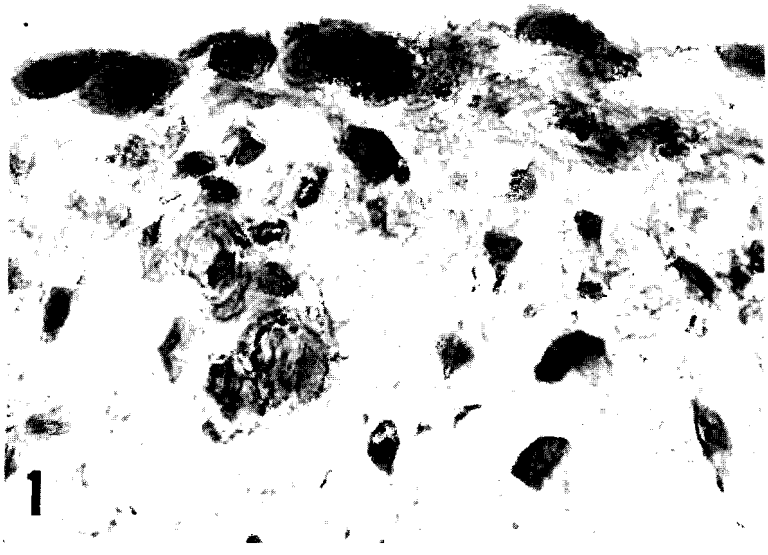

Fig. 1. Staining of lining cells and subsynovial cells with Con A (Human, normal tissue). Paraffin section. × 1.000 (Oil inmersion)

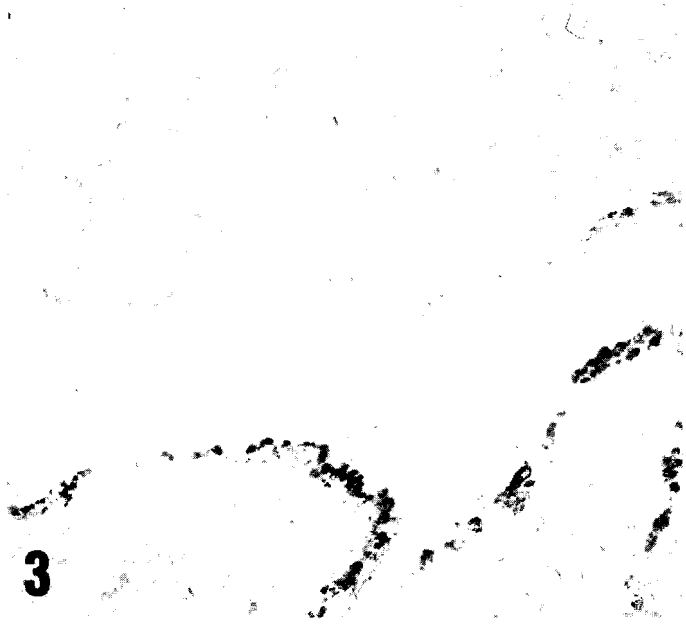

Fig. 3. PNA-labelling of lining cells separated from the joint lumen by unstained cells (Human, RA). Paraffin section. × 200

Table 1. Lectins used for staining synovial tissue

Lectins	Abb.	Concentr.+	Sugar specification*	Binding inhibitor
Concanavalia ensiformis	Con A	4 µg/ml	α-D-Glc; α-D-Man	mannose
Dolichos biflorus	DBA	10 µg/ml	α-D-GalNAc	N-acetyl-galactosamine
Arachis hypogea	PNA	7 µg/ml	Gal-ß-(1-3)-GalNAc	N-acetyl-galactosamine
Ricinus communis	RCA_{120}	4 µg/ml	ß-Gal	Lactose
Glycine max	SBA	10 µg/ml	αD-GalNAc; α-D-Gal	N-acetyl-galactosamine
Ulex europaeus I	UEA-I	5 µg/ml	α-L-fucose	α-1-fucose
Triticum communis	WGA	7 µg/ml	ß-(1-4)-D-GlcNAc; NeuNAc	N-acetyl-glucosamine

+ Paraffin sections
* Gal = galactose; Glc = glucose; Man = mannose; GalNAc = N-acetyl-galactosamine; GlcNAc = N-acetyl-glucosamine; NeuNAc = N-Acetylneuroaminic acid (sialic acid)

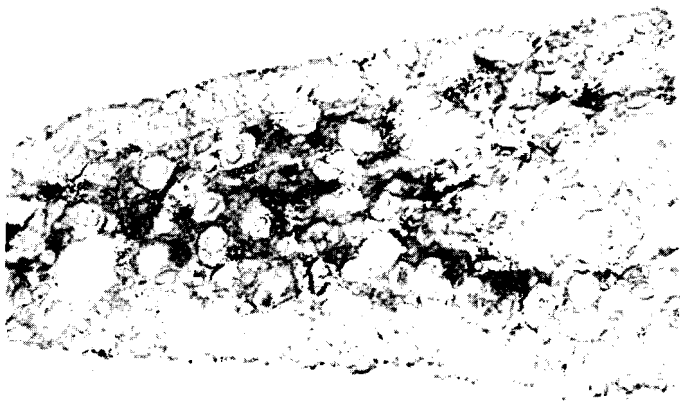

Fig. 4. Binding of PNA to extracellular matrix of connective tissue. The cells are not marked (Human, arthrosis). Paraffin section. × 400

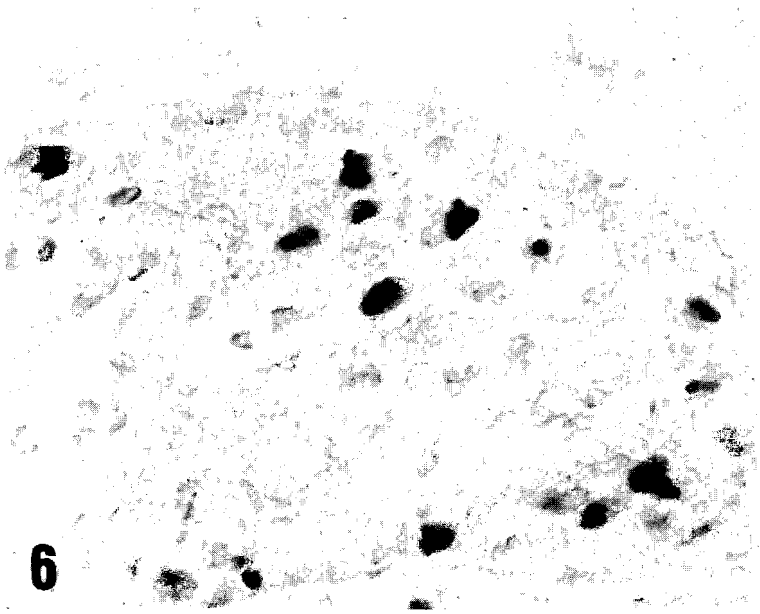

Fig. 6. SBA-lectin binding of lining cells not directly bordering on the joint lumen (Human, osteoarthrosis). Paraffin section. × 200

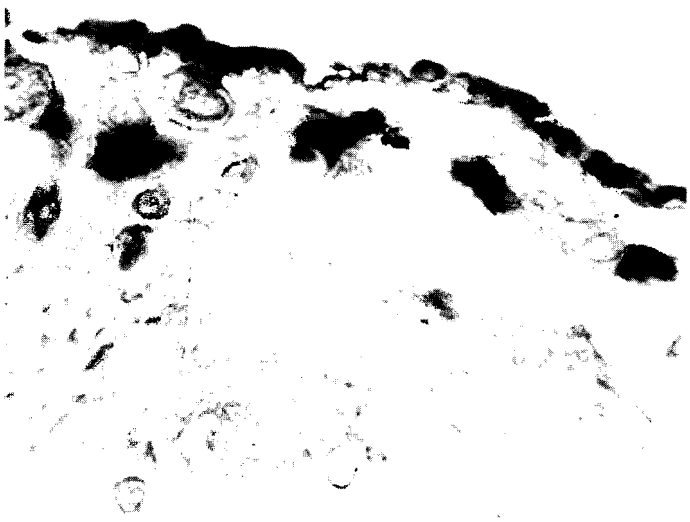

Fig. 5. RCA-staining of lining cells in the deeper intimal layers and of extracellular matrix (Human, normal tissue). Paraffin section. × 400

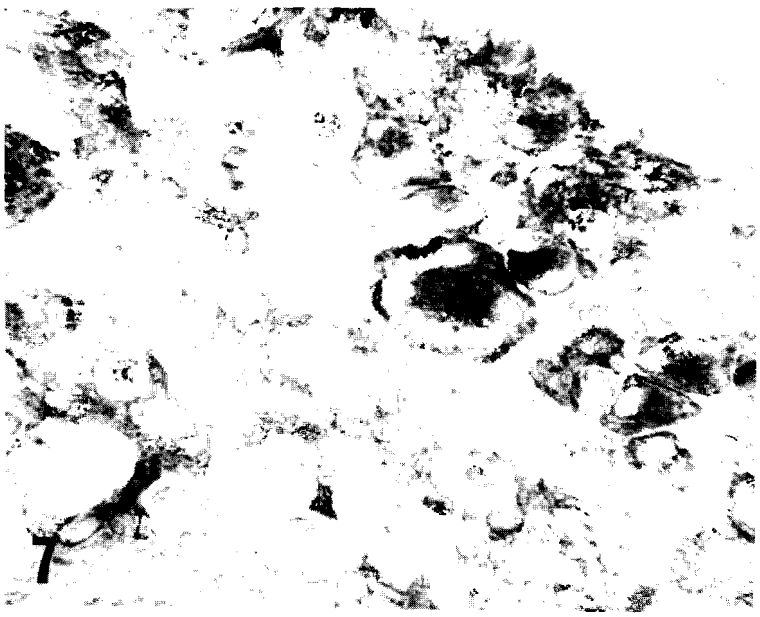

Fig. 7. WGA-labelling of lining cells and extracellular matrix. Note the lectin binding within the cytoplasm of giant cells (Human, RA). Paraffin section. × 1.000 (Oil immersion)

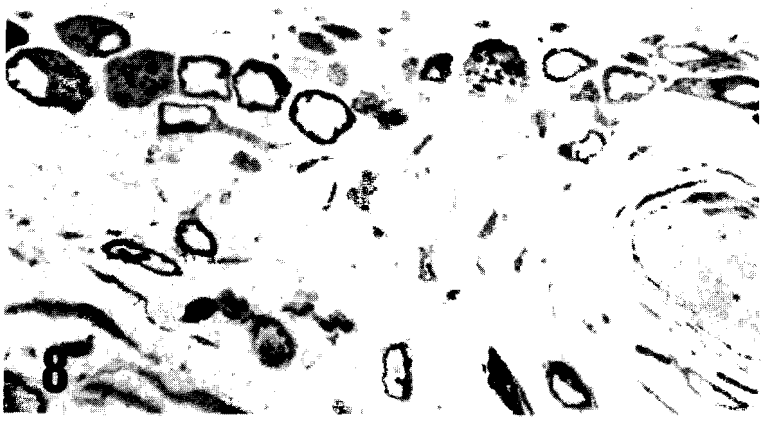

Fig. 8. Staining of the cytoplasm of synoviocytes and endothelial cells with WGA. The nucleoplasm is constantly negative (Human, osteoarthrosis). Semi-thin section (Epon). × 400

lectin-stain with DBA. On the other hand, in three patients mast cells were stained (Fig. 2). In the rest of the patients, and in the rats and guinea pigs, the binding reaction in lining cells and in the subsynovial regions was consistently negative.

In all patients with RA and in six patients with osteoarthrosis, PNA significantly stained a portion of the lining cells in circumscribed regions. The marked cells bordered uniformly on the joint lumen or were separated from it by one or two rows of unstained cells (Fig. 3). At the same time additional areas with extracellular substance were marked with this lectin. In these regions the cells themselves showed no test reaction (Fig. 4). In a portion of the arthrotic materials that showed no cellular PNA-test (n = 2), a narrow extracellular edge could be distinguished directly bordering on the joint cleft. In animal materials the PNA-test was negative.

In human samples RCA marked 40-60% of the lining cells regardless of basic type of pathology. Cells in the deeper intimal layers mostly showed a positive reaction (Fig. 5). Isolated subsynovial cells were also tested, having shown significant differences in staining capacity among individual cells. A significantly positive reaction was also given by a homogeneous, extracellularly located ribbon-shaped region of synovial tissue, bordering on the joint lumen. In rats and guinea pigs the portion of marked border cells was smaller than in the human species, while the number of stained subsynovial cells was greater. The luminal upper region of the synovial tissue also showed no extracellular sugar test.

Independently of basic pathology, 20-50% of human lining cells were marked with SBA (Fig. 6). It is noteworthy that in the majority of the sections the positive-staining cells were arranged in a hem-formation in the deeper layers of the lining cell band, parallel to the surface cell groups that bordered on the joint cleft lumen. In other patients the marked cells could be found in linear formation directly on the joint lumen.

In all human samples, UEA-I consistently stained the endothelium of capillaries and larger vessels, while other structures did not stain with this lectin. In animal samples no positive reaction with UEA-I was observable.

WGA likewise stained the endothelium of capillaries, veins, and arteries. This test reaction could likewise be observed in animal specimens. In all paraffin and cryostat sections WGA also stained lining cells, as well as the overwhelming majority of the cells of the subsynovium (Fig. 7). The lectin also bound within the cytoplasm, while the nucleoplasm was consistently binding-free. This effect was especially significant in the semi-thin sections (Fig. 8), where all the cells were stained. At the same time the extracellular connective tissue also showed lectin binding, preferentially in the neighborhood of the joint cleft. While in normal tissue the intensity of stain was not marked, synovial tissue with inflammatory alterations demonstrated a significant reaction.

Discussion

It can be demonstrated that different fixation and processing techniques can yield differing alterations of lectin bindign patterns (Jeffrey et al., 1987). Different interpretations can be given for the choice of suitable method. While Rittman and Mackenzie (1983) proceed from the assumption that the reactive groups are masked by fixing and embedding, Allison (1987) and Sturgess and co-workers (1987) claim that during tissue handling of cryostat sections, the carbohydrate components of glycolipids, glycoproteins, and smaller oligosaccharides are released. A parallel study involving variously preteated tissue thus seemed meaningful to us. With the lectins we used we achieved uniform results in paraffin and cryostat and semi-thin sections respectively, excluding those obtained with the WGA test. We hypothesize that in the semi-thin sections, fundamentally more intensely fixed (with glutaraldehyde) a greater retention of stainable cell structures would result. Nonspecific lectin binding with the aldehyde groups of the fixative could be ruled out by after-treatment with swine serum, glycine or NH_4Cl respectively (Gilboa-Garber and Mizrahi, 1980).

Comparisons of the results for human and animal (rat or guinea pig) tissue yielded correspondence in the binding pattern demonstrated for some lectins (e.g. Con A, WGA). We interpret the significant differeneces that were found with the other lectins (such as UEA-I, PNA) to mean that the lectin binding pattern in animals could only be applied with reservations to human synovial tissue (Holthöfer, 1983).

It has been postulated that the border cells of synovial tissue derive from monocytes (A cells) and fibroblasts (B cells) (van Furth, 1981). Numerous sources have reported a positive sugar test with Con A and WGA in fibroblasts (Collard and Temmink 1976) and in monocytes/macrophages (Welsh and Schumacher

1983, Kreipe et al. 1986) in specimens of both human and animal origin. In normal tissue we were consistently able to test all lining cells with these lectins, and our results could thus be used to support the above hypothesis. Discrimination between different lining cell forms was not possible, however. Our findings contradict those of Itokazu and coworkers (1987), who found Con A binding only in subsynovial lymphocytes, and a consistently negative test for WGA in normal tissue.

Our research shows that a discrimination among lining cells is possible using RCA and SBA. Whether or not both lectins mark the same cell population is not answerable from the results of our study. the different proportion of marked cells in individual patients would argue against such an interpretation, however. Kreipe and coworkers (1986) claim that monocytes and macrophages stain for SBA. The fact that in many patients the cells directly bordering on the joint cleft are not marked would not support an SBA staining capacity in A cells. In electron-microscopic studies A cells could be consistently tested in immediate neighborhood of the joint lumen (Ghadially, 1983; Stofft and Effendy, 1985). RCA binding on human macrophages has so far not been substantiated in the literature. B cells could possibly be tested with this lectin. Proteoglycan production in the cytoplasm of this cell type has been verified (Roy and Ghadially, 1967).

In contradiction to Shoda et al. (1985) and Itokazu et al. (1987) we could consistently demonstrate UEA-I binding in endothelial cells of normal synovial tissue. These findings are in agreement with the reports of Holthöfer (1982) and Alroy and coworkers (1987). Our research shows that in order to obtain consistent tests for this lectin, immediate fixation during the surgical procedure is necessary. A differentation between osteoarthrosis and RA, as is specified in the literature (Shoda et al., 1985), is thereby made impossible. Heretofore WGA binding has been demonstrated in the vessels of eight mammal species and humans. These findings point to terminal sialyl residues in the glycocalix of endothelial cells.

In agreement to the findings of Söderbaum (1987) on skin and uterine tissue bands, we have found that the extracellular matrix shows a positive reaction with Con A and WGA, and a negative reaction with SBA, UEA-I, and PNA. Our results differed from Söderbaum's in the case of RCA staining. While Söderbaum demonstrated RCA in skin collagen fibers, in our studies this lectin bound only in a sharply circumscribed area parallel to the joint cleft. The RCA was possibly produced and excreted by neigboring cells that likewise tested positive for it.

Normal tissue can be differentiated from RA tissue through the demonstration of cells with a positive PNA test. This is in agreement with the studies of Itokazu and coworkers (1987). The sugar test with this lectin can likewise be found in circumscribed areas of extracellular connective tissue. We interpret this constellation of findings as the expression of an inflamatory reaction with proof of histiocytes (Howard and Batsakis, 1982) and deposition of inflamatory mediators (complement factors ?, fibrin ?). In cases of RA, WGA gave a significantly more intense stain in the connective tissue matrix, which could point to an increased production of hyaluronic acid (Menghi et al., 1986) in inflamed synovial tissue (Itokazu et al., 1987).

Arthrotic tissue showed PNA positive cells in only a portion of the patients. This lectin test correlates with the histological picture of a cell-rich subsynovial tissue. Numerous studies have described the extremely variable histological picture of synovial tissue in osteoarthrosis, that can range from absent to marked inflammatory reactions (Sokoloff and Hough, 1985). While Itokazu et al. (1987) were able to find PNA positive cells in a portion of samples with osteoarthrosis, Borisch and coworkers (1986) were not successful in this demonstration. They suggested the possibility that PNA could be a specific marker for RA. In our view the inconsistent test for this lectin in osteoarthrosis suggests rather the demonstration of an inflammatory condition. As to the meaning of the Con A binding in the vessels of two arthrotic patients, nothing specific can be ventured. In the literature positive (Söderbaum, 1987) as well as negative (Alroy et al., 1987) results have been reported in normal tissue.

Our research shows that in contradiction to reports in the literature (Borisch et al., 1986; Itokazu, 1987), lectin-binding patterns permit no unequivocal differentation between the inflammatory conditions of osteoarthrosis and RA. On the other hand, there are indications that with the help of PNA, an acute inflammatory condition of the synovial tissue can be morphologically verified. With conventional histological staining methods this can only occasionally be achieved (Fassbender, 1975). Further, the cell population in the lining cell layer can be unequivocally differentiated. Such findings argue against the interpretation of Ghadially (1983) that synoviocytes correspond to a uniform cell population that shows only graded variations. Should it be proved that PNA represents a consistent macrophage marker (Howard and Batsakis, 1982), the monocyte derivation of A cells must be critically reconsidered.

Acknowledgements. We thank Ms. Disque-Kaiser. Mrs von Graevenitz and Ms. Kolbe for their technical assistance and the financial suport of the Deutsche Forschungsgemeinschaft (Sto 165/2-1).

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Accepted June 7, 1988