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THE MORPHOLOGY OF SYNOVIAL LINING OF VARIOUS STRUCTURES IN SEVERAL SPECIES AS OBSERVED WITH SCANNING ELECTRON MICROSCOPY

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Abstract

Data concerning surface morphology of synovial lining of tendons, tendon sheaths, cruciate ligaments, infra-patellar fat pads and peripatellar synovial ridges in various species (rat, rabbit, dwarf goat, sheep, pig, dog, human) are reported on. Supportive studies with transmission electron microscopy (TEM) and light microscopy were performed.

Three principal morphological appearances of the synovium are evident. On structures with a dense fibrous architecture like tendons, tendon sheaths and cruciate ligaments the intimal cells and processes are mostly slender and may tend to orientation in the length-axis of the structure. On the peri- and infrapatellar adipose tissues two principal 'extremes' are seen: one in which the contours of the fat cells are clearly visible with fungoid shaped structures in between them, and one in which the fat cell contours are not recognizable and the intima consists of cauliflower-like cells. Transitional forms exist.

Several features observed on tendons and tendon sheaths which have not been reported on before are presented in this paper.

A consistent classification of synovium is presented.

Keywords: Synovium, SEM, normal, tendon, tendon sheath, cruciate ligament, fat pad, rat, rabbit, dwarf goat, sheep, pig, dog, human.

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Introduction

In studying the intra-articular biological transformation of processed dermal sheep collagen which to this effect was implanted as an anterior cruciate ligamentoplasty in dwarf goats, attention was paid to the morphology of the surface of the synovial membrane lining the implant. To gain understanding of that synovial lining a proper insight into the morphology of the natural lining of the authentic cruciate ligament (ACL) is mandatory.

Aim of the investigation.

The aim of this investigation was twofold: to assess whether the rapid determination of the nature of the lining of intra-articular structures such as ligamentoplasties is feasible with scanning electron microscopy (SEM), and to obtain insight in the morphology of the surfaces of normal synovial lining of various anatomical structures and to compare those with each other and with those in other species.

In support of the SEM observations light microscopy (LM) and transmission electron microscopy (TEM) were performed.

The literature.

Review of the literature on this subject presents a void; reports on observations concerning the three-dimensional morphology of the surface of normal synovium are scarce (Fujita et al. 1968; Gryfe et al. 1969; Redler and Zimny 1970; Woodward et al. 1971; Wysocki and Brinkhous 1972; Cameron and MacNab 1973; Hayashi 1976; Shively and Van Sickle 1977; Date 1979; Schmidt and Mackay 1982; Stofft and Effendy 1985; Wei et al. 1989), while the quality and quantity of the reported data often are unsatisfactory.

Studies both on the ultrastructure of synovial cells with transmission electron microscopy (TEM) as well as studies on their function are well represented in the literature.

TABLE 1. Several authors, sources of synovium, A-type/B-type ratio's, opinions concerning SLC relationships.

Species	Authors		Source	Status[1]	Predominant type SLC			Relationship[2]	
					A>B	B>A	A≈B	A=B	A≠B
Guinea pig	Okada et al.	1981b	Knee						
	Wyllie et al.	1964	"Joint"	N	+				
Dog	Greisen et al.	1981	?	N		+			
	Wysocki et al.	1972[3]	Knee (detailed)	N					
Cat	Lever et al.	1958	Knee, shoulder, jaw						
	Wassilev	1970	Knee		+				
Rabbit	Date	1979[3]	Knee						
	Davies et al.	1966	Knee					+	
	Ghadially et al.	1966	Knee					+	
	Ghadially	1983	"Joints"	N		+		+	
	Krey et al.	1973	Knee			+			
	Lever et al.	1958	Knee, shoulder						
	McDonald et al.	1988[3]	Knee	N Pressurized					
	Okada et al.	1981b	Knee						
	Wassilev	1970	Knee			+			
	Watanabe et al.	1974	Knee				+		+
	Wei et al.	1989[3]	Knee, anterior part	N	+				
	Woodward et al.	1971[3]	Knee, hip						
Human	Barland et al.	1962	"Joint"	N	+				
	Barnett et al.	1961	"Joint"	N					
	Cameron et al.	1973[3]	Hip joint	N, RA					
	Castor	1960	Knee	N					
	Coulter	1962	Knee	N					
	Dryll et al.	1980	Knee	N inflammation	+				
	Fujita et al.	1968[3]	Knee	N, RA					
	Ghadially et al.	1966	?		+				
	Gryfe et al.	1969[3]	Knee	RA					
	Lever et al.	1958	Knee	N					
(?)	Letizia et al.	1980	Knie, elbow		+				
	Okada et al.	1981b	Knee						
	Redler et al.	1970[3]	"Articular"	N, pathological					
	Roy et al.	1966	Knee	N?	+				
	Schmidt et al.	1982[3]	Tendon sheath "Synovium"	N N					
	Soren et al.	1981	"Joints"	RA				+	
	Woodward et al.	1971[3]	Knee						
	Date	1979[3]	Leg	N					

TABLE 1. Several authors, sources of synovium, A-type/B-type ratio's, opinions concerning SLC relationships. **(continued from previous page).**

Species	Authors		Source	Status[1]	Predominant type SLC			Relationship[2]	
					A>B	B>A	A≈B	A=B	A≠B
Mouse	Edwards et al.	1982							+
	Linck et al.	1978	"Joints"	N		+			
	Linck et al.	1981	Knee, MTP		+				+
	Okada et al.	1981a	Knee			+			+
	Okada et al.	1981b	Knee						
Horse	Johansson et al.	1976	"Joints"	N		+			
	Shively et al.	1977[3]	Carpus	N					
Rat	Graabaek	1984	Knee						+
	Okada et al.	1981b	Knee						
	Roy et al.	1967	Knee		+				
	Stofft et al.	1985[3]	Knee					+	
	Wassilev et al.	1976	Knee	N Loaded	+	+		+	
	Woodward et al.	1971[3]	Knee, hip						
Sheep	Cutlip et al.	1973	Knee, elbow					+	
Pig	Fell et al.	1976	MCP III and IV			+		+	
	Woodward et al.	1971[3]	Knee						
Overview	Ghadially	1980	"Joints"					+	
	Revell	1989		N					+

1) N = Normal; RA = Rheumatoid arthritis.
2) A=B same origin, different functional expressions.
A≠B different origins.
3) (including) SEM.

Most papers concern synovium of (normal and rheumatoid) human, mice, rat and rabbit knees or other joints, in the majority of cases without exact topographic specification of the sites from which the specimens were obtained (Lever and Ford 1958; Barland et al. 1962; Coulter 1962; Davies and Palfrey 1966; Ghadially and Roy 1966; Roy and Ghadially 1967; Wassilev 1970; Cutlip and Cheville 1973; Krey and Cohen 1973; Watanabe et al. 1974; Fell et al. 1976; Wassilev et al. 1976; Dryll et al. 1980; Ghadially 1980; Okada et al. 1981a,b; Ghadially 1983; Graabaek 1984; Henderson and Pettipher 1985; Wei et al. 1989). See also Table 1. No significant publications on the normal synovial lining of tendons, tendon sheaths and cruciate ligaments are known to the authors.

No reports are available on comparative research in which specimens are observed as well with light microscopy (LM), as with SEM and TEM.

In the literature concerning TEM and SEM observations hardly any comparisons between synovial linings of fibrous and adipose subsynovial structures are made (Wysocki and Brinkhous 1972). In consequence of observations based on cytochemical and TEM research Barland et al. (1962) developed a schematic three-dimensional representation of "the" normal human synovium, in which hardly any at-

tention was paid to surface morphology.
'Early' publications concerning LM of synovium of joints (a.o. Key 1932; Lever and Ford 1958; Castor 1960; Barnett et al. 1961; Barland et al. 1962; Davies and Palfrey 1966) are still regarded to be prominent (Ghadially and Roy 1966; Roy and Ghadially 1967; Krey and Cohen 1973; Fell et al. 1976; Ghadially 1983; Krstic 1984; Henderson and Pettipher 1985; Cormack 1987); hardly any observations on 'non-joint' synovium are presented apart from implications that, for example, the "fibrous type of synovial membrane" is similar throughout the body (Key 1932, p.1074), i.e. in tendons, tendon sheaths, intra-articular ligaments. Cormack (1987, p.265) states only that the surfaces of ensheathed parts of tendons and tendon sheaths lack continuous cellular lining and that they are composed of mostly collagen.

On terminology.

As early as 1763 Bonn[1] described and named a continuous synovial membrane. In the first half of the nineteenth century it was believed that joints were lined by epithelium, later replaced by the view that their lining consisted of mesothelium similar to that of the other body cavities. In 1866 Hueter[1] concluded that the supposed continuous layer of endothelial cells composing the synovial membrane, actually was an incomplete and irregular cellular connective tissue surface, a view which was to be supported by many. Others, amongst whom Steinberg[1] (1874), stuck to the view that synovial joints were lined by endothelium.

Key (1932) already remarked that it is not possible to identify a definite synovial membrane as it cannot be delimited from its substratum. Nowadays we know that a basement membrane as for example in epithelium indeed is absent. Nevertheless, it is considered convenient to use the term "membrane" and to regard it as consisting of two layers: a "lining layer (intima)", and a "subintimal layer" upon which the first rests (Barnett 1961). Generally the synovial membrane is referred to as the lining of a synovial cavity, its characteristics outlined in a descriptive manner (a.o. Key 1932; Lever and Ford 1958; Barnett 1961; Ghadially 1983; Cormack 1987).

Classifications. The customarily applied categorization of the synovial membrane into three different main types, i.e. "fibrous","areolar" and "adipose", was introduced by Key in 1932, based on the structure of the "underlying tissue" which was not clearly defined, but possibly meant to relate to the underlying anatomical structure. Castor (1960) used a classification with similar adjectives which reflected on the "subintima", defined as the area immediately beneath the intimal cells, not indicating whether considered to be part of the synovial membrane.

In regard to these classifications in the literature the adjectives "areolar", "fibrous" and "adipose" frequently are used indiscriminately. Often it is hard to tell whether reference is made either to the anatomical structure being lined by the synovium, or to a deeper part of the synovium itself. An example of ambiguous terminology causing bafflement presents itself when comparing the use of the adjective "subsynovial" in a report of Date (1979) with that in a review by Henderson and Pettipher (1985). In the latter the adjective is considered to be identical with "subintimal", defined as belonging to the deeper part of the synovium, whereas in the first paper it is used to differentiate from "synovial", indicating its non-synovial character.

In the classifications of both Key (1932) and Castor (1960) the intrinsic values of the types' adjectives are not unequivocal either. In a linguistic sense these suggest that the synovium either has a certain architecture or is constituted of the kind of tissue denominated by them.

In the case of the "adipose type" both possibilities are incorrect as the adipose tissue actually represents the substratum, i.e. the subsynovium (see definition below) which is lined by the synovium and is not a part of it. Of the other two adjectives "areolar" refers to the architectural composition whereas "fibrous" refers to the material composition.

In order to avoid the use of terminology of ambiguous meaning or incorrect nature the above mentioned classifications of the synovium will not be adhered to by the authors of this paper.

Definitions. Nomenclature used in this paper:

Synovium: the lining of a synovial cavity (formerly also named the synovial membrane), considered to consist of an intimal layer and a subintimal layer (see below).

Subsynovium: tissue lined by the synovium (e.g. adipose tissue, collagen bundles of tendon and tendon sheath, fibrous capsular wall).

[1] As referred by Key (1932).

Synovial intima: the part of the synovium which is in direct contact with the compartment (e.g. joint space, tendon sheath cavity, bursal cavity) it frames, consisting mainly of synovial lining cells and their processes (SLC's).

Synovial subintima: that part of the synovium which is situated in between the synovial intima and the subsynovium, consisting mainly of fibrous tissue. In most cases a plane of transition is not clearly distinguishable and one layer merges gradually into another, if the subintima at that particular site exists at all.

Sizes and shapes. Sizes of structures will be presented: it is emphasized that the figures do not pretend to be absolutes, they merely give an indication of a range sizes of structures may lie in.

Sizes of structures on photographs can be deducted from the print width (P.W.) indicated in the legends.

In the literature no internationally accepted classification on thread-like structures exists. Unequivocal use of terminology should universally be pursued. In this paper the classification based on diameter (ø) as described by Ghadially (1983) is used. See Table 2.

There is no consensus on descriptive terminology related to cell processes (e.g. filopodia, lamellipodia) and shapes. In this paper cell processes simply are called "processes" without any further specification. To describe shapes of structures the following terms were used in this paper:

Fungoid: a relatively thin rim-like structure which rises from a surface in an angle and which may have a wavy, wrinkly appearance. The length of the rim may vary from very long to short; in case of the latter it often looks semicircular.

Polygonal: structures of which the length/width ratio is closer to 1 than is the case in elongated ones and to which the descriptions round, oval, globoid, etcetera, do not fit.

Materials and Methods.

From the species dog (dog 1 and dog 2, both mongrels), rat (Wistar), rabbit (New Zealander), pig (Yorkshire), sheep (Texel), dwarf goat and man, several structures, i.e. tendon and tendon sheath, anterior cruciate ligament (ACL; not from man), posterior cruciate ligament (PCL; human[2]), infrapatellar fat pad or peripatellar capsular ridge were obtained (see Figs.1-16 for representative examples of the anatomical structures). During the process of fixation and preparation traumatizing of the synovial surfaces was painstakingly avoided.

TABLE 2. Categorization of thread-like structures by diameter (after Ghadially 1983).

Unit	Non-collagen	Collagen
Protofilaments[1], tropocollagen:	0.15 - 0.3nm	1.5nm
Filaments:	2 - 20nm	3 - 5nm
Fibrils[2]:	20nm - 2µm	30 - 180nm
Fibres[3]:	2 - 100µm	1 - 100µm
Bundles :	≥ 100µm	≥ 100µm

1) Being a part of filaments or microtubuli.
2) Aggregates of filaments.
3) Aggregates of fibrils.

The specimens were obtained from one individual per species (except for the dog). The animals were scrutinized for disorders which might have afflicted the structures which provided the specimens; none were found.

In order to demonstrate that the inspected surfaces indeed represented the synovial linings of the specimens at hand, preparations for TEM and LM observations were made in the fashion described below.

Fixation and excision.

Under general anaesthesia the femoral artery and vein, or the abdominal aorta and inferior vena cava were cannulated, a vasodilator and heparin were introduced through the arterial cannula. Under perfusion with saline 0.9% regional exsanguination took place, followed by perfusion with fixative (containing 1% glutaraldehyde and 1% formaldehyde in 0.12M phosphate buffer with 0.02mM $CaCl_2$, Palay and Chan-Palay, 1974) for half an hour. The excised structures were placed in receptacles containing the same fixative.

Because in some cases it was impossible to perform perfusion several specimens were excised after local application of fixative for 15-30 minutes.

The human specimens were excised approximately 12 hours post-mortem; macroscopically the synovial surfaces appeared as in vivo.

2.The ACL (woman, aged 70) was completely absent; no evidence that it had ever been present was available and the knee joint looked completely normal.

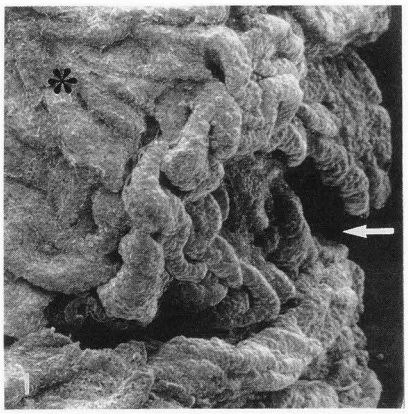
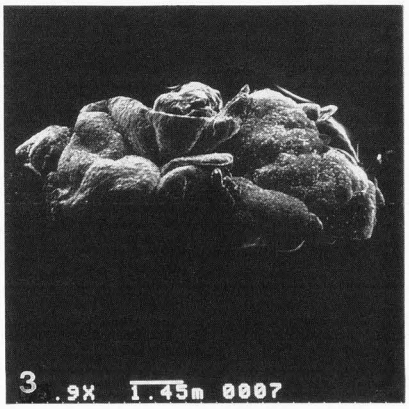


Fig.1. Infrapatellar fat pad, dog. Asterisk: transverse plane of section. Arrow: studied side. Print Width (P.W.)= 5.1mm.

Fig.2. Infrapatellar fat pad, human. Lobule. Rests on cut surface (reflected light macrophotograph). P.W.= 15.7mm.



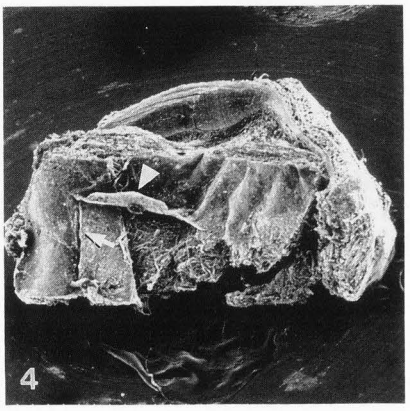
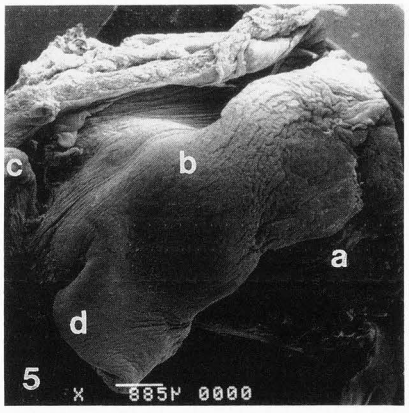


Fig.3. Peripatellar ridge, rabbit. View at lobulated surface. P.W.= 11mm.

Fig.4. Peripatellar ridge, rat. Arrowhead: torn and lifted synovium. Arrow: see fig.58. P.W.= 6mm.



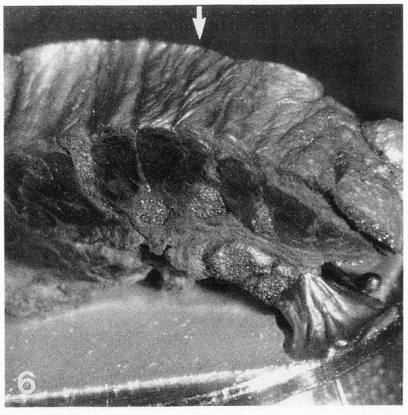
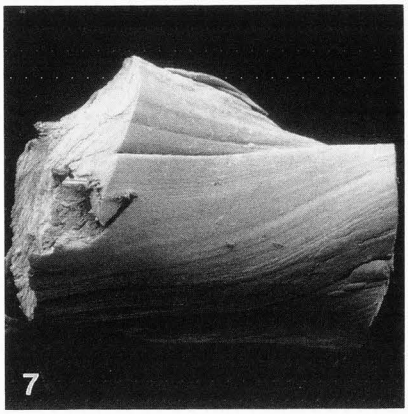


Fig.5. Peripatellar ridge, dwarf goat.
a-d: see figs.50,54,56,57.
P.W.= 7mm.

Fig.6. Posterior cruciate ligament (PCL), human. Transversal slice. Arrow: synovial surface (reflected light macrophotograph).
P.W.= 9.7mm.



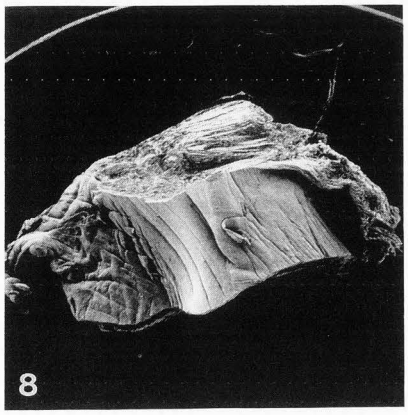
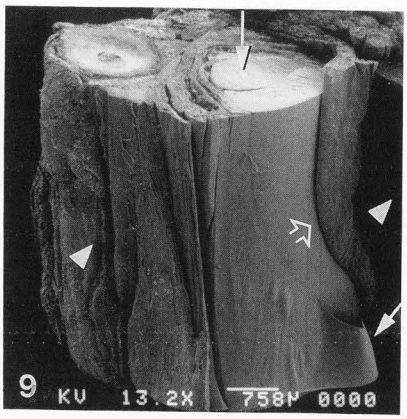


Fig.7. Anterior cruciate ligament (ACL), dog.
P.W.= 6.5mm.

Fig.8. ACL, sheep. Transversal slice. P.W.= 7.9mm.



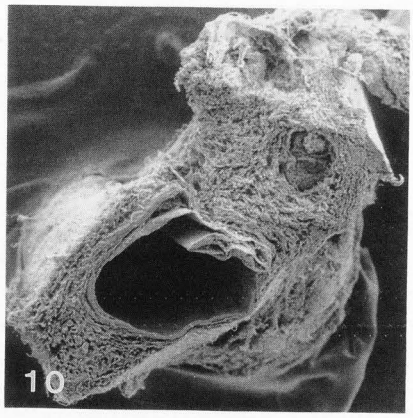
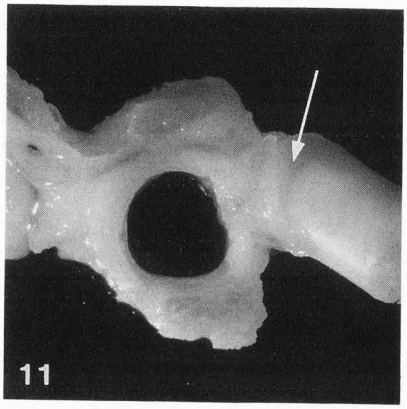


Fig.9. Tendon sheath, dog. Upper arrow: tendon in situ. Lower arrow: thin fold. Cut tendon sheath (arrowheads). Open arrowhead: adipose fold.
P.W.= 6mm.

Fig.10. Tendon sheath, rat.
P.W.= 1677µm



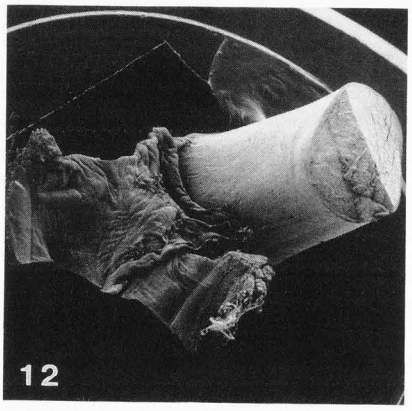
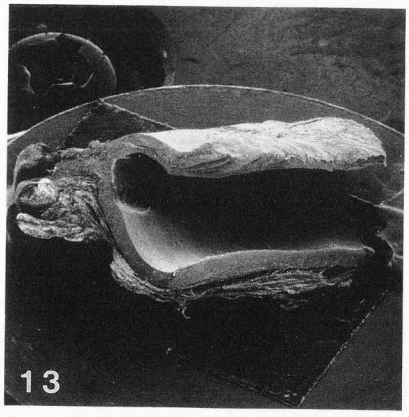


Fig.11. Tendon sheath with luxated tendon; dwarf goat. Attached to one another by synovial doubling (arrow) (reflected light macro-photograph).
P.W.= 8mm.

Fig.12. Tendon with tendon sheath; dwarf goat. Specimen shown in fig.11., after CPD, in SEM. Half of the sheath's wall was excised.
P.W.= 10mm.



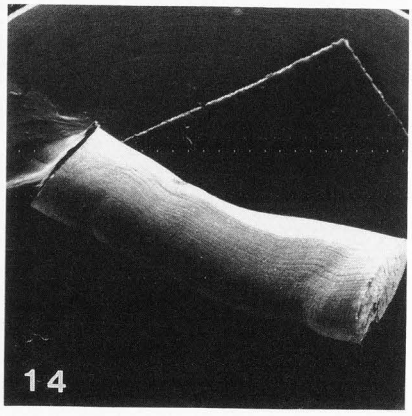
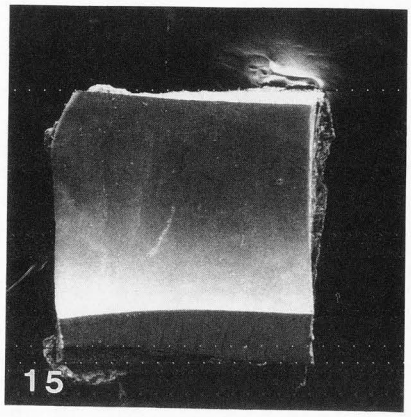


Fig.13. Tendon sheath, pig.
P.W.= 11.4mm.

Fig.14. Tendon, pig. From sheath shown in fig.13.
P.W.= 9.9mm.



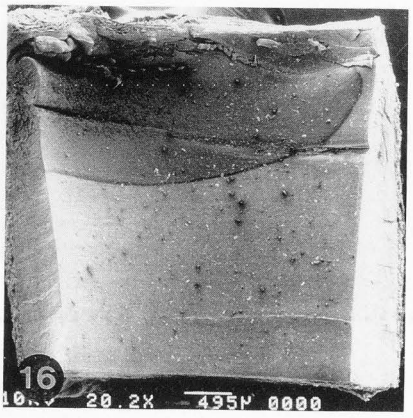


Fig.15. Tendon sheath, sheep. Part of wall.
P.W.= 6.4mm.

Fig.16. Tendon sheath, sheep. Part of wall showing gullies as described in text. See figs.35 & 36.
P.W.= 4mm.

After a fixation period of about seven days the specimens were carefully reduced in size under a dissecting microscope, and photographically documented.

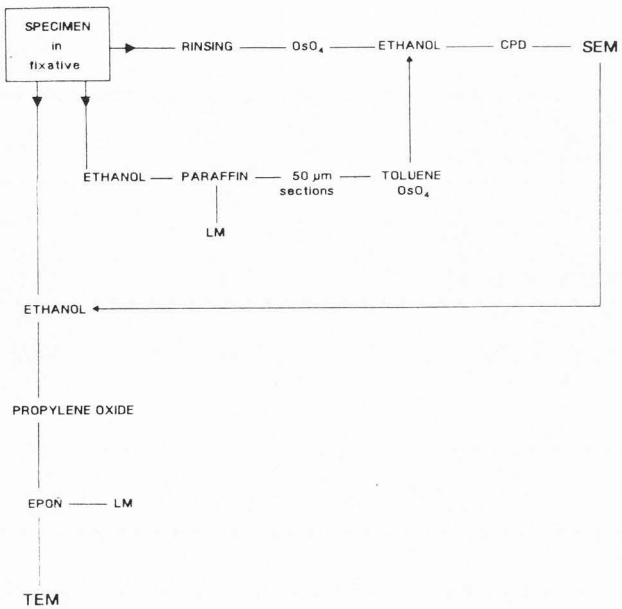
Preparation for SEM. Specimens for SEM were taken out of the fixative and rinsed in a container with phosphate buffer containing $CaCl_2$ and 8% dextrose, by shaking them gently. The rinsing buffer was changed three times in a period of 15 minutes, after which post-fixation with 2% OsO_4 in phosphate buffer was done.

The specimens were dehydrated in a series of ethanol starting at 70%, and subsequently critical point dried from ethanol 100%.

The dried specimens were mounted on SEM stubs either by double coated sticking tape, carbon glue or malleable conductive plastic and rendered conductive by sputtering them with gold-palladium at 1 kV and 40 mA (Polaron SEM coating unit E5000) during four periods of 30 seconds separated by 30 second intervals to prevent heating of the specimens.

Preparation for serial LM, SEM and TEM. See the diagram for a representation of the ways by which TEM and LM sections were obtained.

The specimens, obtained from dog 2, were prepared as follows: after fixation as described above they were embedded in paraffin and serially cut in slices of 5 and 50µm. The 50µm slices were deparaffinised by placing them at room temperature during 30 minutes in toluene in which 1% crystalline OsO_4 was dissolved to effectuate post-osmication (modified from Van den Bergh Weerman and Dingemans 1984). Subsequently the specimens were put in 3 changes of ethanol 100% in a period of 15 minutes, after which they were dried, mounted and sputtered.



After SEM inspection the 50µm specimens from dog 2 were prepared for TEM: via ethanol 100% and propylene oxide they were embedded in LX 112-epon. After inspection of 0.5-1µm sections to check the cutting angle ultrathin sections were made and stained with 0.01% tannic acid at 60°C for 3 minutes - this method developed in and routinely used by the department of Pathology of the Academic Medical Center of the University of Amsterdam - after which staining with uranyl magnesium-acetate for 20 minutes and lead citrate for 10 minutes, both at 60°C, concluded the procedure.

LM sections were stained with hematoxylin-eosin (5µm paraffin sections), toluidine-blue or methylene-blue with basic fuchsin (0.5-1µm epon sections).

Preparation for TEM only. Several specimens from dog 2 (tendon sheath and infrapatellar fat pad) were prepared for TEM directly from fixative in the routine manner of the laboratory: via osmication and impregnation with uranyl magnesium-acetate the specimens were dehydrated in ethanol as described, followed by the above mentioned routine via propylene oxide.

A specimen from a remainder of the infrapatellar fat pad from dog 1 was also prepared for TEM from fixative. SEM specimens were inspected in an ISI-DS 130 Scanning Electron Microscope at 9 or 10 kV accelerating voltages at magnifications ranging from 12 to 4000. Ultra-thin sections were observed in a Philips EM-201.

Results

SEM

When the intimal surface is labelled "continuous" this indicates that the synovial intima consists of intertwining synovial lining cells and their processes (SLC's). Table 3. provides more detailed information on morphological features of SLC's.

Special features observed in several specimens will be described in detail.

Tendon. In pig (Fig.17), rabbit (Fig.18), dwarf goat (Fig.19) and dog (Fig.20) the synovial lining of the ensheathed part of the

tendon is continuous; in all specimens but the dwarf goat's the SLC's show an orientation which is approximately parallel to the longitudinal axis of the tendon. The SLC's are elongated and flat; their discernable length is mostly of the same order of magnitude (15-20µm). In the rabbit this is somewhat smaller.

The synovium of the rats' tendon is similar to that of its tendon sheath: see there.

In all species the cell-surfaces are irregular because of wrinkles, protrusions and the like. The subintima - when visible - generally consists of thin fibrils.

To the surface of the dwarf goat's tendon specimen a 1.1 mm long, slender, stalked polyp-like structure is attached (Fig.21). Its surface is wrinkled and seems to be covered by SLC's.

Tendon sheath. The SLC's form a continuous layer in all species and are, excluding sheep (Fig.22) and dwarf goat, orientated in the length axis of the sheath.

In the dwarf goat, pig, rat and man (Fig.23) the synovial intima shows elongated and slender structures; in the other species their form is polygonal. The size of SLC's in rat and rabbit (Fig.24; Fig.25) is smaller than in the other species; in the dwarf goat and pig larger SLC's are found also. In the dog, sheep and pig large and small fungoid shaped cells were observed on the free surfaces of the synovial folds which do not lie parallel to the surface of the sheath (see below).

The cell surfaces are similar to those seen on the tendons.

In the walls of the tendon sheaths of rabbit (Fig.26) and sheep some clusters of fat cells are evident.

In the tendon sheath of the rat a longitudinal fold is present which kept two tendon slips separated from each other (Figs.10,27). The surface of the fold is lined by the same kind of predominantly lengthwise orientated cells and cell-processes as the rest of the sheath's surface (Fig.28).

In the dog's tendon sheath several folds are present intra-lumenally (Fig.29): one consists of a synovial duplication (Fig.30), others have a stuffing of one or more layers of fat cells.

The lining of the tendon sheath consists of a continuous layer of SLC's, which are orientated in the length-axis of the sheath and have slender processes (Fig.31).

TABLE 3. Representation of SEM acquired data of synovial lining as observed in this study.

Subsynovium and Species	Orientation SLC's 1)	Form SLC's 2)	Sizes SLC's (µm)
TENDON:			
Pig	l	e	15-20 x 2-5
Rabbit	l	e p	10-17 x1-1.6 20 x 8
Dog	l	e-p	20 x 6-10
Rat	l	e-p	20 x 10
Human	-	e	15-20 x 8-10
Dwarf goat	-	e	20-25 x 1.5-3
TENDON SHEATH:			
Sheep	-	p f	12-20 x 8-12 40 x 8
Pig	l	e-p f	20-25 x 5-6 10 x ?
Rabbit	?	p	16 x 8
Dog	l	e p f	15-20 x 4 15-20x10-15 >=30 length
Rat	l	e	2-16.x0.6-2.6
Human	?	e	?
Dwarf goat	-	e	25 x3; 25x8-10
PERIPATELLAR RIDGE:			
Sheep	-	f	120 x 10 x 3
Rabbit	-	f c	400 x 10 to 12 x 3 9-12 Ø
Rat	-	p	17 x 15
Dwarf goat	-	e p f c	20 x 2-4 20 x 10-20 10-15 x9 x2.5 10 Ø
INFRAPATELLAR FAT PAD:			
Pig	-	f	<250 x 16-25
Dog 1.	-	f	25-60 x 17 x3
2	-	c	10 Ø
Human	-	f f	75-100 x 13 15 Ø
LCA/LCP:			
Sheep	-	p	8-10 x 4-7
Pig	?	? p	? 13 x ?
Rabbit	-	p e	30 x 17 13 x 2
Dog	?	p	?
Rat	l	e	30 x 7
Human (LCP)	-	e p	15-20 x 4 24 x 24
Dwarf goat	l	e	15 x 8

1) l = parallel to the length axis of subsynovium.
2) c = cauliflower-like; e = elongated; - = no preferred orientation; f = fungoid; p = polygonal.

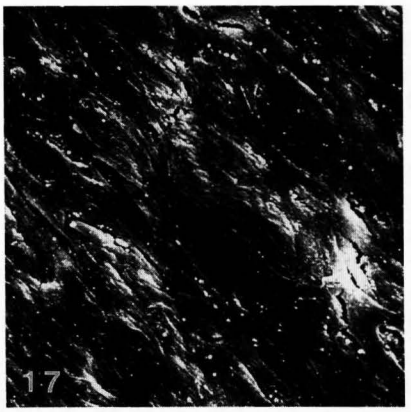


Fig.17. Tendon surface, pig (ensheathed part of m.flexor dig.tendon, see also fig.14). Elongated branching SLC's, orientated in length axis of tendon.
P.W.= 50µm

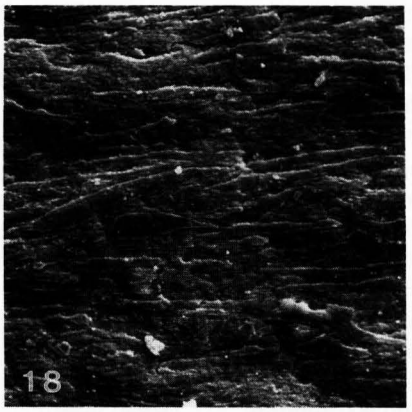


Fig.18. Tendon surface, rabbit (ensheathed part of m.flex.dig. tendon). Elongated SLC's orientated in length axis of tendon.
P.W.= 26.5µm

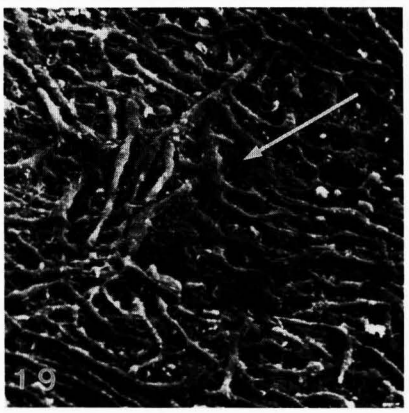


Fig.19. Tendon surface, dwarf goat. See also fig.12. Elongated SLC's and wider ones (arrow).
P.W.= 53µm

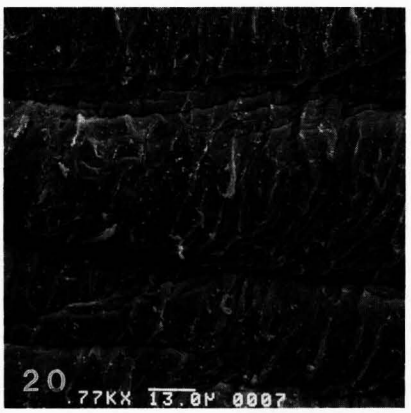


Fig.20. Tendon surface, dog (ensheathed part of m.tibialis ant.tendon). Furrowed subsynovium; length axis from top to bottom. Continuous surface. Arrow: SLC process.
P.W.= 58µm

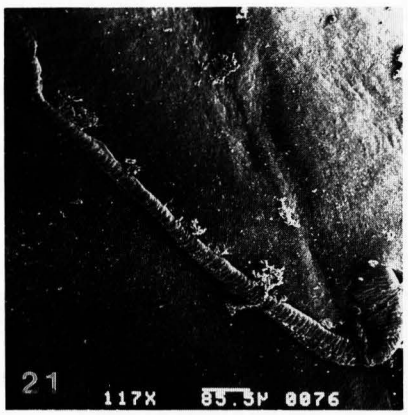


Fig.21. Tendon surface, dwarf goat. Length axis in direction of furrow. Polyp-like structure.
P.W.= 748µm

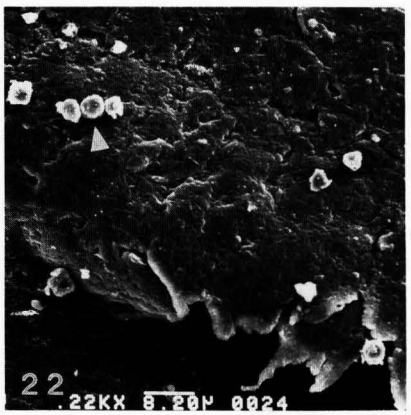


Fig.22. Tendon sheath surface, sheep. SLC's in continuous layer, partially torn from subsynovium. Arrowhead: debris.
P.W.= 67µm

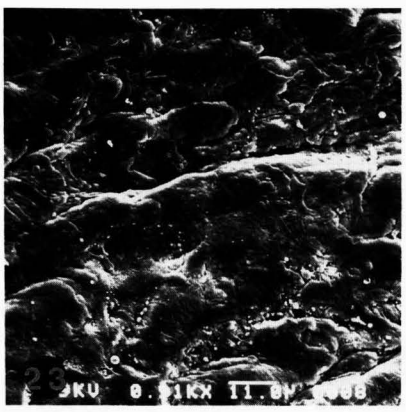
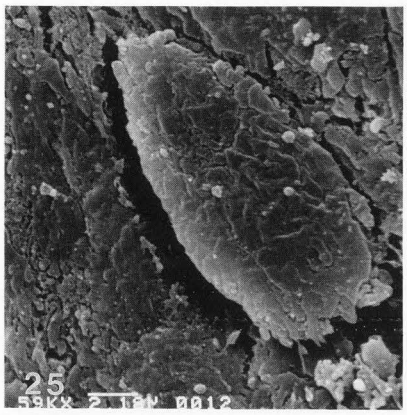


Fig.23. Tendon sheath surface, human. Continuous layer of SLC's. See also fig.49 for subintimal surface.
P.W.= 64µm

Fig.24. Tendon sheath surface, rabbit. Continuous synovial intima.
P.W.= 57µm



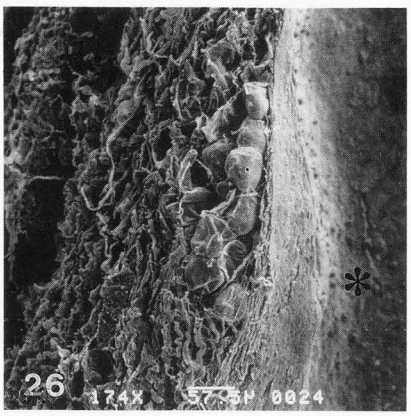
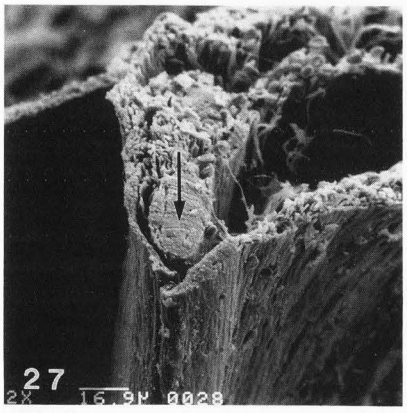


Fig.25. Tendon sheath surface, rabbit. Ovaloid SLC. Globular protrusions, flat wrinkles and finger-like processes are clearly recognizable.
P.W.= 18µm

Fig.26. Tendon sheath, rabbit. Fat cell cluster beneath synovium. Asterisk: sheath lumen.
P.W.= 312µm



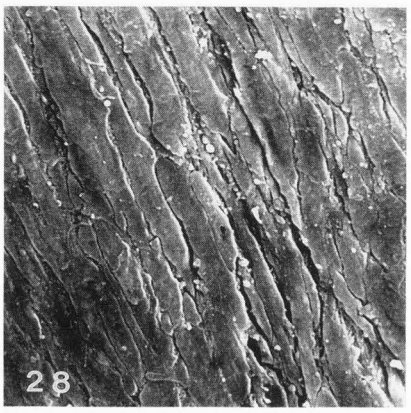
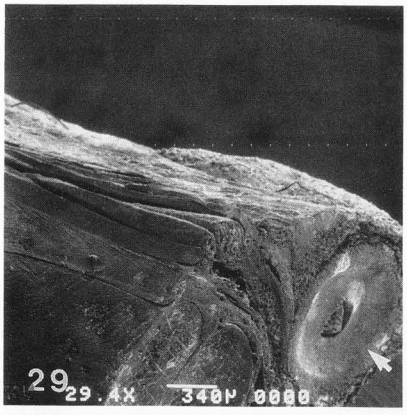


Fig.27. Tendon sheath, rat. Inter-tendinous plica. Arrow: longitudinal collagenous 'rib'.
P.W.= 139µm

Fig.28. Tendon sheath surface, rat (see fig.10). SLC's and processes orientated in length axis of sheath.
P.W.= 19.6µm



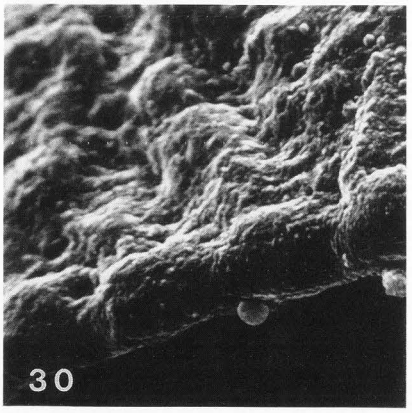
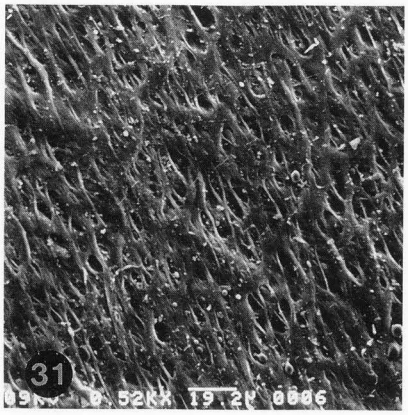


Fig.29. Tendon sheath dog, overview of folds. Specimen from fig.9: rotated 90 degrees clock-wise. Arrow: artery.
P.W.= 2.8mm.

Fig.30. Tendon sheath, dog. Natural free rim of thin synovial fold, see fig.9.
P.W.= 19.5µm



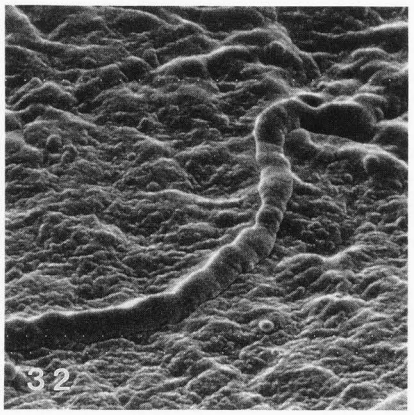


Fig.31. Tendon sheath surface, dog. Continuous layer of SLC's. Length-axis sheath from top to bottom.
P.W.= 54µm

Fig.32. Tendon sheath, dog. Tendon-facing surface of thin fold (see fig.9); capillary-like structure disappearing in intimal surface.
P.W.= 64µm

One part of a thin fold was lifted from the tendon and at its lower surface several long (125-275µm) and slender (5µm Ø.) tubular structures with several circular constrictions are observed, the ends of which blend into the surface (Fig.32).

Three adipose folds are in evidence, one of them quite voluminous (Fig.33) and stuffed with several layers of fat cells (Ø. 45-60µm), appearing as large bumps in the surface. At the convex side of this fold rows of globular structures are seen (Fig.33).

In the wall of the pig's sheath a hollow (Figs.34,35) is in evidence with an adipose fold covered with SLC's extending to one side. In the cut surface (Fig.35) proximal to the hollow a single layer of fat cells (55 x 20µm) lends a bumpy appearance to the surface.

Over the contra-lateral rim of the hollow lies a polyp-like structure (200 x 60 x 4µm), lined by SLC's which are continuous with the sheath's surface in the hollow (Figs.35,36).

At another location on the synovial surface a cable-like structure of 2mm length and 6.5µm diameter is present, composed of three entwined fibres (Fig.37). The cable is spliced and the three fibres run in different directions, each becoming enfolded from the loose end onwards in cell-like structures which are continuous with the synovial intima.

Several more thread-like structures are observed to be in contact with the cable; all are wrapped in cell-like structures.

The tendon sheath synovial lining of another pig (compare Fig.38) is different in appearance (Fig.39).

Perpendicular to the length axis of the tendon sheath of the sheep runs a ridge which originates in two gullies with a depth of 135-250µm. In one of the gullies (Fig.40) a synovial doubling originates (Fig.41); near this a flipper-like structure (250 x 80µm), covered by SLC's, protrudes into the synovial cavity (Fig.42).

On the free surface of a part of the doubling fungoid shaped structures are present (Fig.43), consisting of smooth-surfaced rims enclosing rough granulated centers.

The synovial doubling between the tendon and its sheath in the dwarf goat (Figs.11,12) covers approximately 40% of the tendon's circumference with the synovium of which it merges seamlessly (Fig.44). In comparison to its native state as seen under the dissecting microscope (Fig.11) after preparation for SEM (Fig.12) the doubling shows a larger amount of transverse creases. The SLC's are similar to those of the tendon (Fig.45); closer to the sheath's surface they become somewhat wider.

The intimal surface near the doubling shows SLC's similar - albeit wider and flatter - to those already described (Fig.46).

From the convexity of the doubling dangles a polyp-like structure of 250µm length and 20µm diameter (Fig.47) covered by SLC's, which near its free end seem to possess an increasing amount of thin processes surrounding the top like a network (Fig.48).

The visible part of the subintima consists mostly of a network of fibrils. The subintima of the human tendon sheath is a nice example (Fig.49).

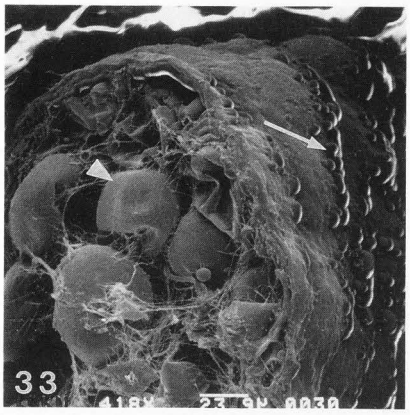
Peripatellar ridges. In all studied species the cells of the peripatellar synovial intima form a continuous layer. The SLC's do not show a preferred orientation.

In the dwarf goat (Fig.50), sheep (Figs.51,52) and rabbit (Fig.53) large and small fungoid cells of similar form which lie in between and partially on top of fat cells are observed. They also appear on surfaces where fat cells are not in evidence (Figs.54,55). Their rims are smooth and surround a coarse granular center. In the dwarf goat such cells are somewhat smaller.

In the rabbit small fungoid cells with a more roundish form are in evidence as well. In the dwarf goat cauliflower-like (Fig.56) and elongated cells (Fig.57) are also observed.

In the rat cells with a different form are in evidence: they are polygonal and flat and possess many small processes on their surfaces (Figs.58,59); fat cells are present but only at some depth below the intima. A part of the surface of the rat's specimen was peeled off (Fig.9) and a fibrillar network overlaying globular structures (50 x 45µm) became visible. At several locations tubular structures (Ø. 7-8µm) show through the network (Fig.60). In the rabbit some long, flat and slender, free running structures are observed, lined at two sides by SLC's bordering a granular center (Fig.53).

Sometimes in the sheep a globular dome was seen, surrounded by fungoid structures, protruding from the surface (Fig.61). The cell-surfaces often seem to contain fibril-like structures (Ø. 0.7µm) which may cross over to another fat cell or a fungoid cell through the joint space (Fig.62).



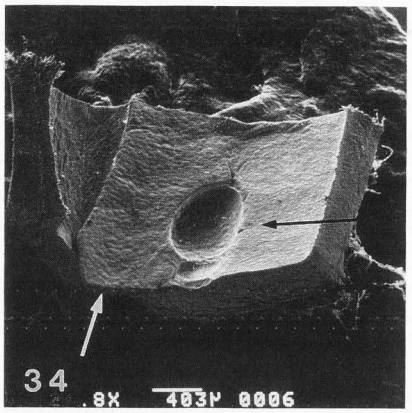
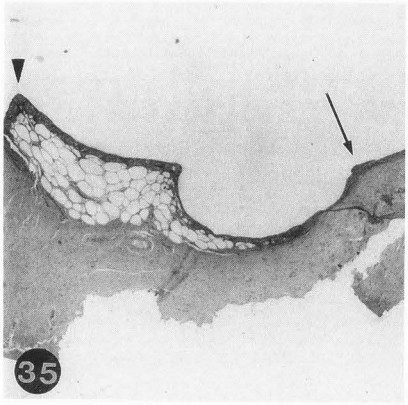
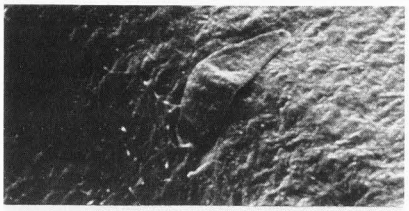


Fig.33. Tendon sheath surface, dog. Large adipose fold. Arrowhead: fat cells. Arrow: rows of fungoid structures.
P.W.= 197µm

Fig.34. Tendon sheath, pig (section from specimen of fig.13). Hollow, adipose fold (white arrow) and polyp-like structure (black arrow). See figs.35,36.
P.W.= 3.3mm.





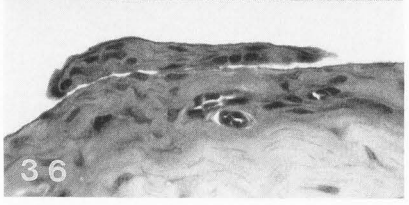
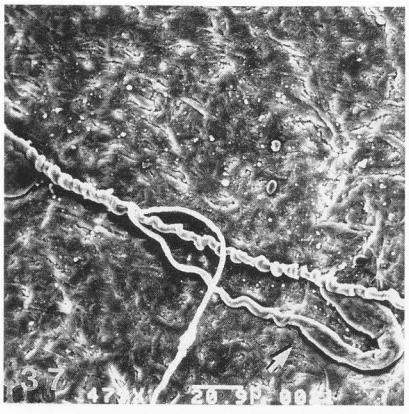


Fig.35. Tendon sheath, pig. Transversal section. Hollow and adipose fold (see fig.34). Arrowhead: free rim of fold. Arrow: polyp-like structure. Stain: hematoxylin eosin.
P.W.= 1.3mm.

Fig.36. Tendon sheath, pig. Top: SEM; bottom LM. Polyp-like structure stalked in hollow (fig.34) and lying over its rim. Stain: hematoxylin eosin.
P.W.(top)= 460µm



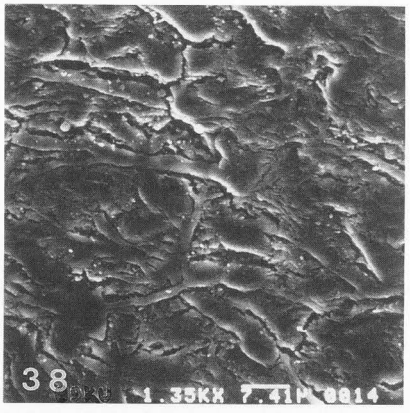
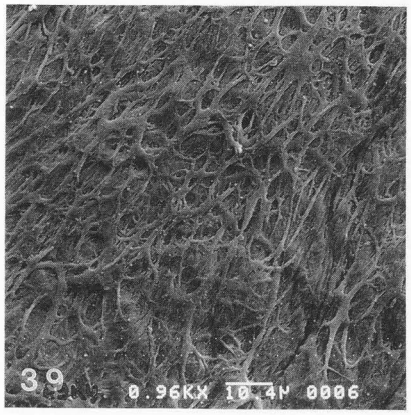


Fig.37. Tendon sheath, pig. Length axis sheath left to right. Twined cable-like structure on top of synovial intima. Arrow: enfolded fibre-end.
P.W.= 172µm

Fig.38. Tendon sheath surface, pig (see also fig.13). Continuous layer of SLC's.
P.W.= 59µm



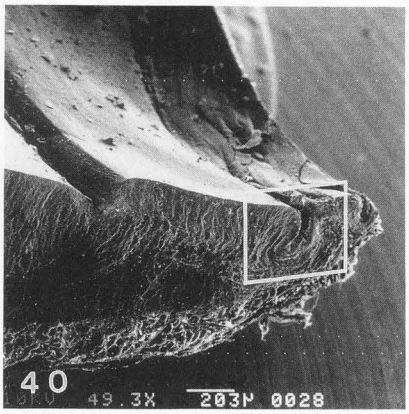
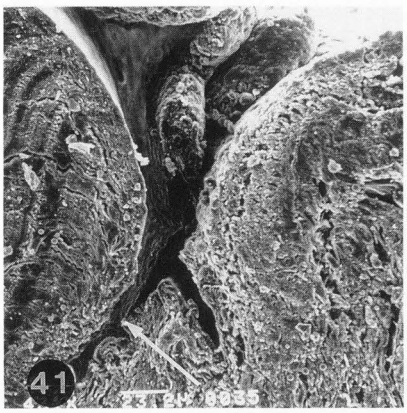


Fig.39. Tendon sheath surface, pig (a different pig than the one from fig.38). Length axis sheath from top-right to bottom-left.
P.W.= 90µm

Fig.40. Tendon sheath, sheep. Gullies. Rectangle refers to fig.41.
P.W.= 1.6mm.



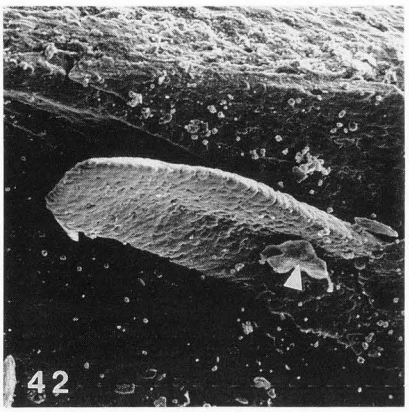
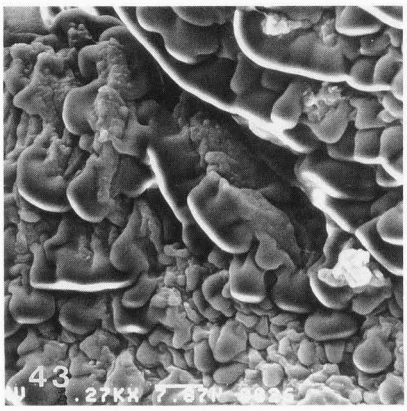


Fig.41. Tendon sheath surface, sheep (see fig.40). Arrow: site where doubling originates.
P.W.= 191µm

Fig.42. Tendon sheath, sheep. Flipper-like intimal protrusion. Arrowhead: debris.
P.W.= 325µm



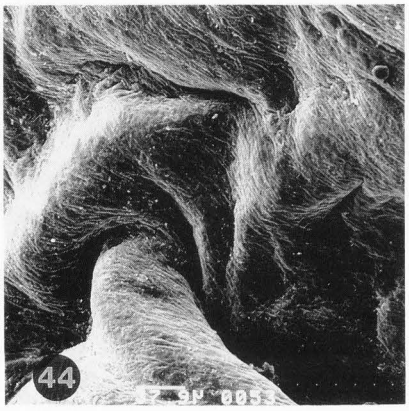
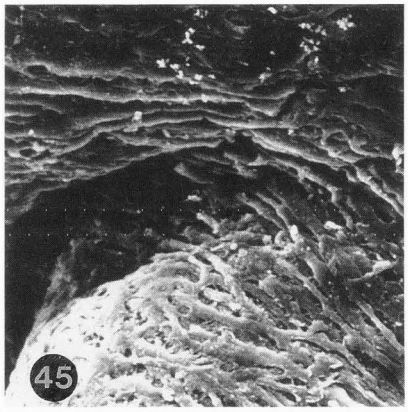


Fig.43. Tendon sheath, sheep. Free surface of synovial doubling or fold. Fungoid rims bordering coarse granular surfaces.
P.W.= 65µm

Fig.44. Tendon sheath surface, dwarf goat. Site of merger of synovial doubling and tendinous intima, bottom and top of figure respectively.
P.W.= 312µm



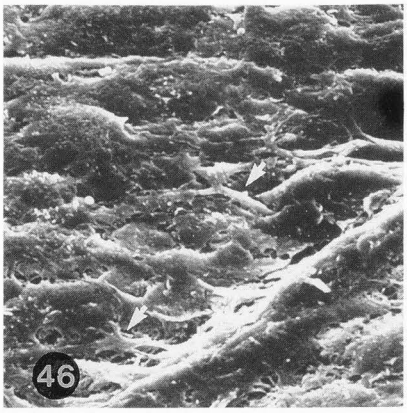
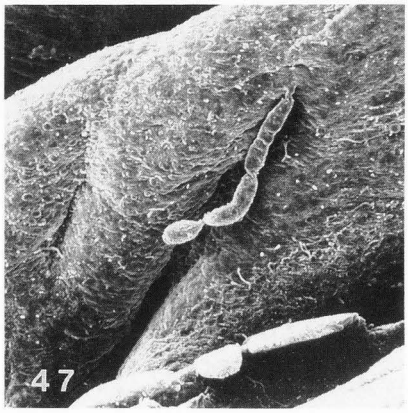


Fig.45. Intimal doubling merging with tendon intima, dwarf goat (detail from fig.44). Elongated SLC's.
P.W.= 59µm

Fig.46. Tendon sheath surface, dwarf goat. Site near doubling. SLC's are flat and wide. Arrowhead: cell-processes.
P.W.= 55µm



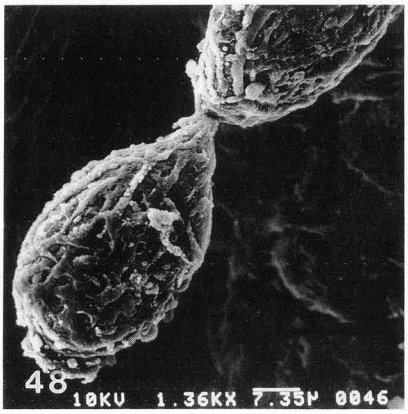
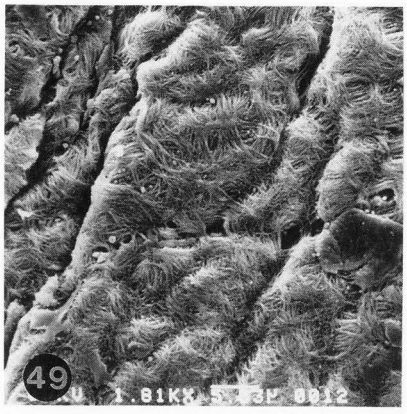


Fig.47. Tendon sheath doubling, dwarf goat. Polyp-like structure.
P.W.= 426µm

Fig.48. Tip from polyp-like structure, dwarf goat (see fig.47).
P.W.= 65µm



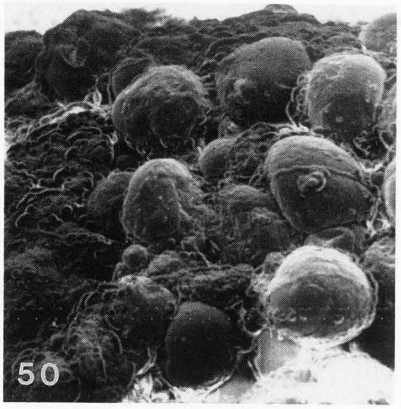
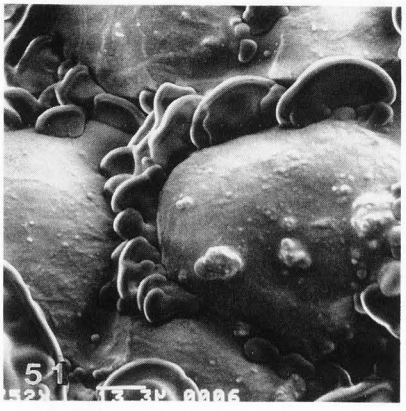


Fig.49. Tendon sheath, human. Subintimal fibrous network. See also fig.23.
P.W.= 100µm

Fig.50. Peripatellar ridge, dwarf goat. See fig.5:a. Synovium on adipose subsynovium. The surface at the left side is similar to that of fig.54.
P.W.= 300µm



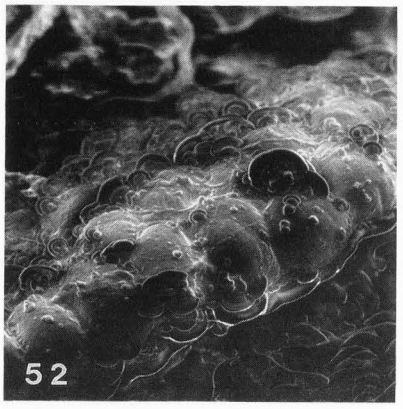
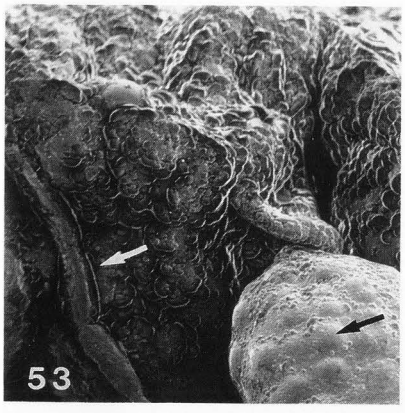


Fig.51. Peripatellar ridge, surface, sheep. Fat cells and fungoid structures. P.W.= 110µm

Fig.52. Peripatellar ridge, sheep. Fat cells in ridge, covered by fungoid structures.
P.W.= 393µm



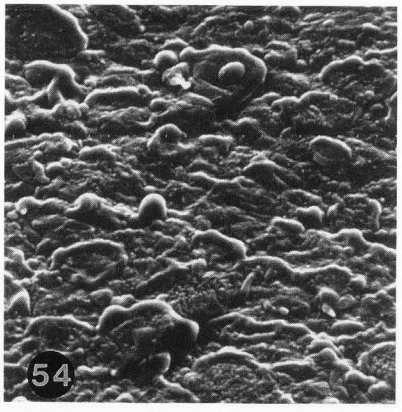
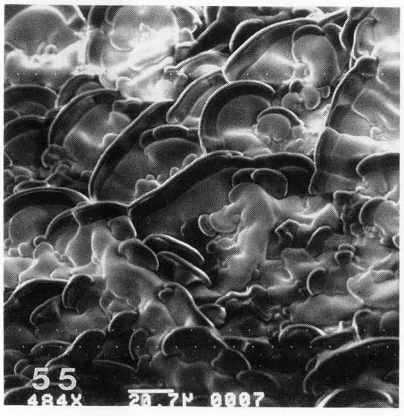


Fig.53. Peripatellar ridge, rabbit. White arrow: capillary-like structure. Black arrow: fat cell contours.
P.W.= 772µm

Fig.54. Surface of peripatellar ridge, dwarf goat. See fig.5:b. SLC's partially rising from the surface.
P.W.= 53µm



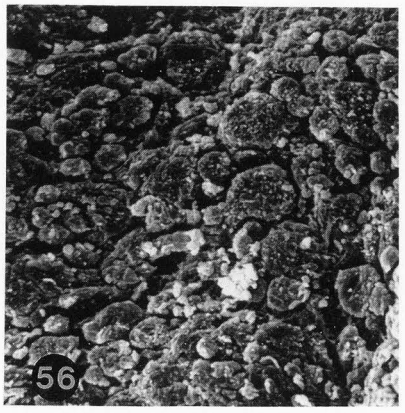
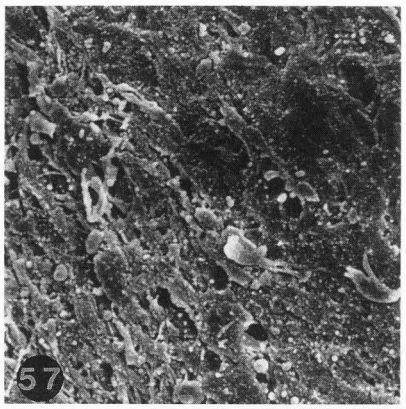


Fig.55. Peripatellar ridge, surface, sheep. Fungoid structures.
P.W.= 171µm

Fig.56. Peripatellar ridge, dwarf goat. See fig.5:c. Cauliflower-like SLC's.
P.W.= 58µm



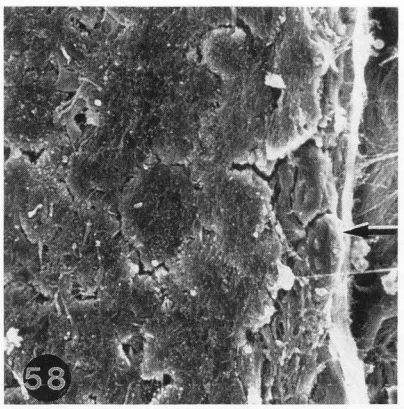
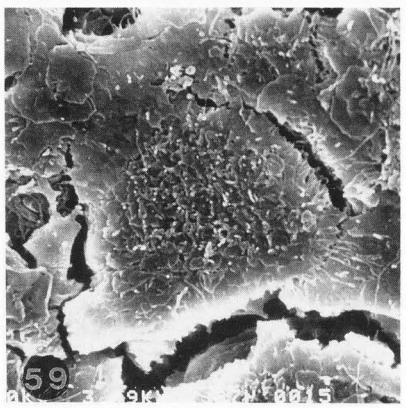


Fig.57. Surface of peripatellar ridge, dwarf goat. See fig.5:d. Elongated and (top right quadrant) polygonal SLC's.
P.W.= 55µm

Fig.58. Peripatellar synovial ridge, surface, rat. See fig.4. for exact location (arrow). Continuous layer of polygonal SLC's. Along the torn edge a deeper layer of cells is visible (arrow).
P.W.= 74µm



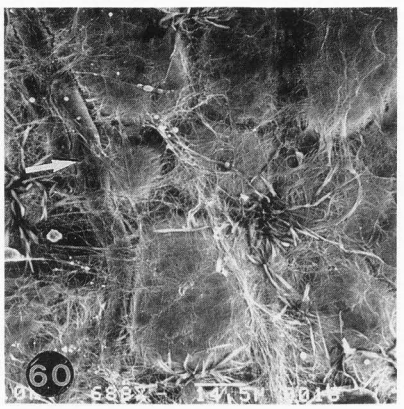
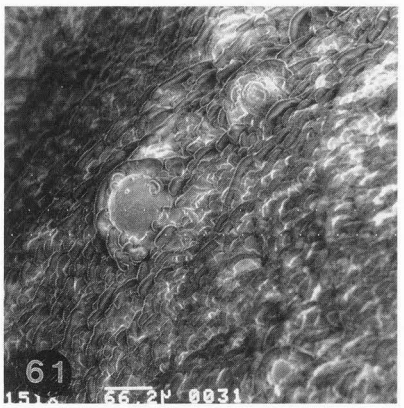


Fig.59. Peripatellar ridge, rat. Surface of individual SLC.
P.W.= 29µm

Fig.60. Peripatellar ridge, rat. Sub-synovial layer (continuous with right side of fig.58). Contours of fat cells visible. Arrow: capillary-like structure.
P.W.= 119µm



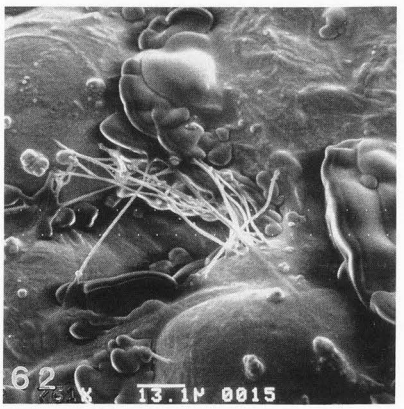
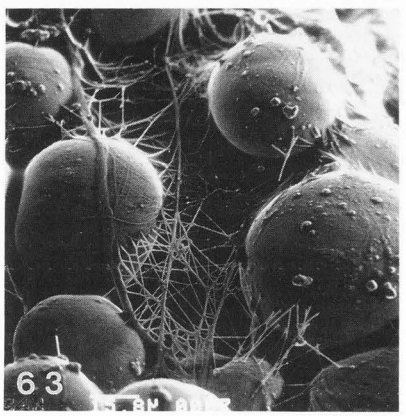


Fig.61. Peripatellar ridge, sheep. Solitary fat cell surrounded by fungoid structures.
P.W.= 545µm

Fig.62. Peripatellar ridge surface, sheep. Fibrils, continuous with fibrils in pericellular networks, crossing through joint space.
P.W.= 108µm



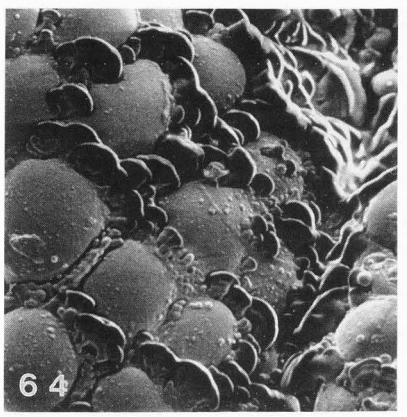
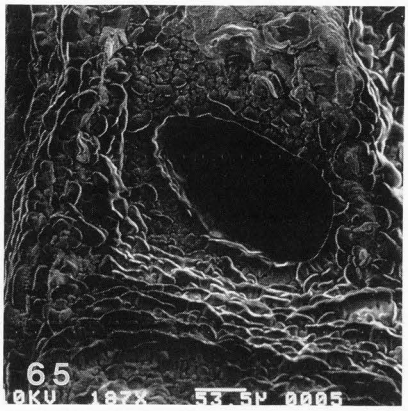


Fig.63. Infrapatellar fat pad, surface, dog 1. Fat cells enclosed in fibrous networks, continuous with intra-articular open woven network.
P.W.= 130µm

Fig.64. Infrapatellar fat pad, surface, dog 1. Fat cells and fungoid structures. P.W.= 278µm



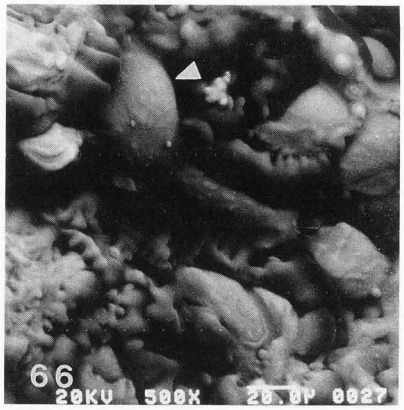
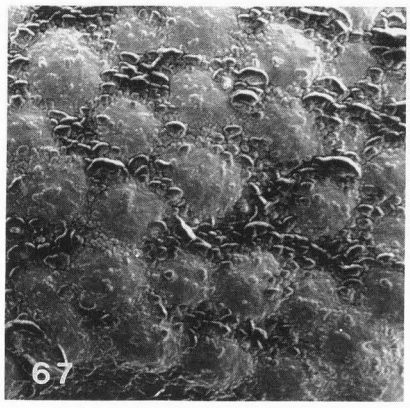


Fig.65. Infrapatellar fat pad, dog 1. Fungoid rims surrounding pore-like hole (near attachment to patellar tendon). P.W.= 416µm

Fig.66. Infrapatellar fat pad, dog 1. Bottom of deep furrow. Fungoid structures. Fat cell contour (arrowhead). P.W.= 440µm



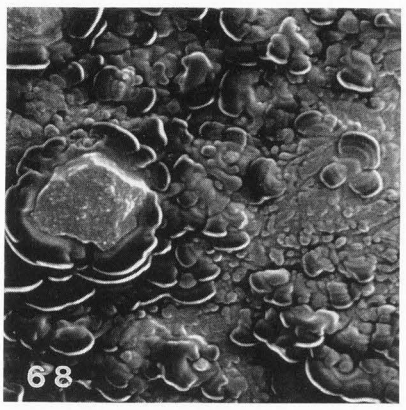
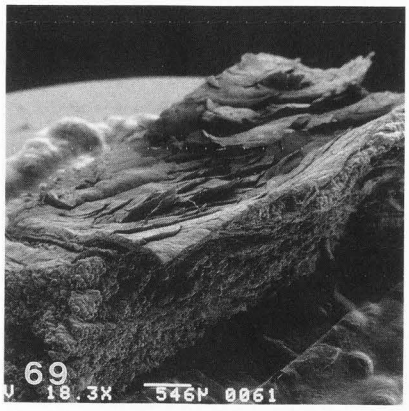


Fig.67. Infrapatellar fat pad, surface, human. Fat cell contours, fungoid structures. P.W.= 520µm

Fig.68. Infrapatellar fat pad, surface, human. Cell-like structure with fungoid rim. Small fungoid structures. P.W.= 180µm



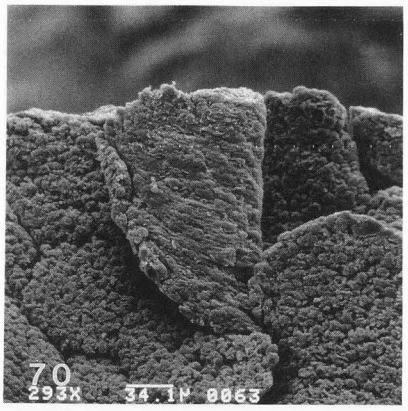
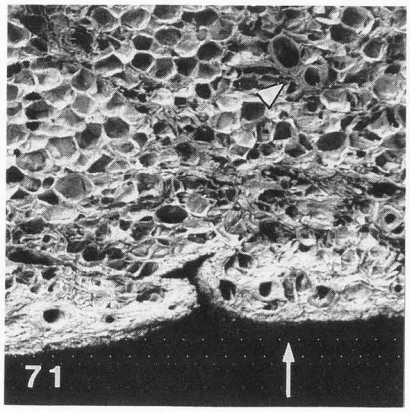


Fig.69. Infrapatellar fat pad, pig. Flaky pastry-like synovial surface. Fat cells of the subsynovium are visible at the cut edges. See also fig.70. P.W.= 4.8mm.

Fig.70. Infrapatellar fat pad, pig. View of top from specimen of fig.69, detail. Cauliflower-like cells are discernable. P.W.= 300µm



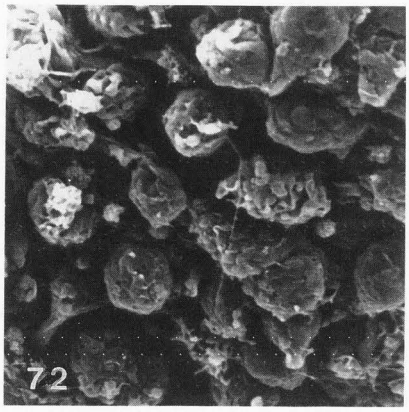
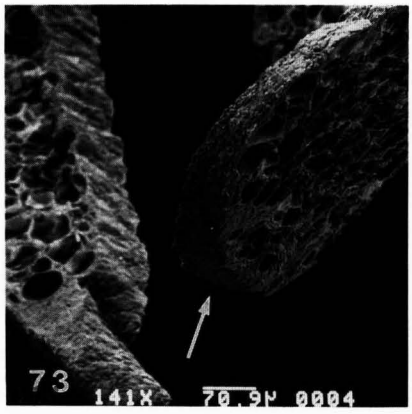


Fig.71. Infrapatellar fat pad, dog 2. 50µm section. Fat cells. Arrow: synovial lining. Arrowhead: blood-vessels. See also figs.93,94. P.W.= 910µm

Fig.72. Infrapatellar fat pad surface, dog 2. 50µm section. Cauliflower-like SLC's. P.W.= 41µm



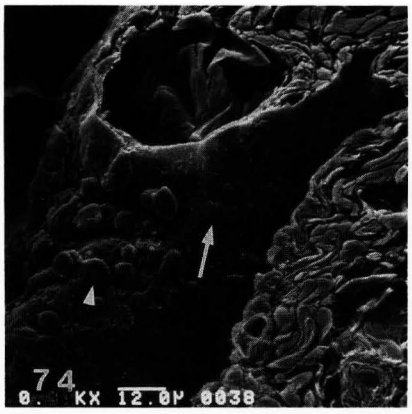
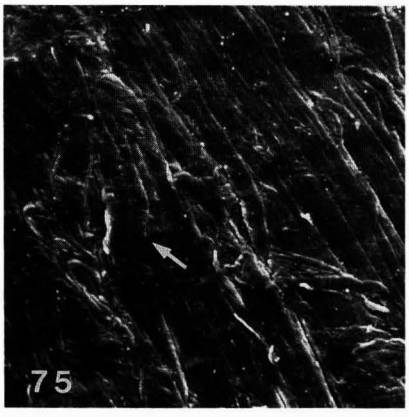


Fig.73. Infrapatellar fat pad, dog 1. 50µm section. Arrow: intimal surface. P.W.= 584µm

Fig.74. Infrapatellar fat pad, dog 1. 50µm section. Top: damaged fat cell membrane. Arrow: flat SLC's. Arrowhead: teardrop SLC's and processes. P.W.= 99µm



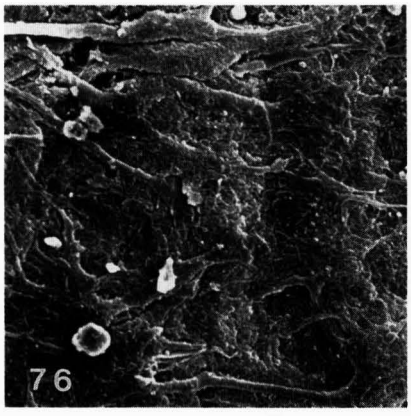
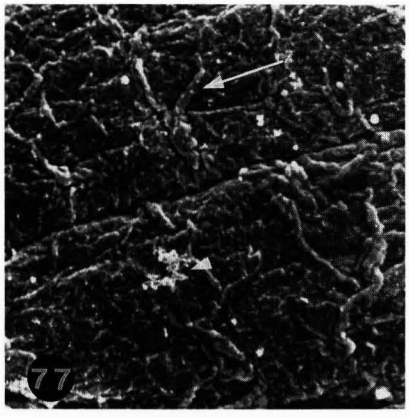


Fig.75. ACL surface, rat. Arrow: SLC's(-processes). 'Striation' in length axis of ACL. P.W.= 79µm

Fig.76. ACL surface, sheep. Continuous layer of SLC's. Processes clearly visible. P.W.= 61µm



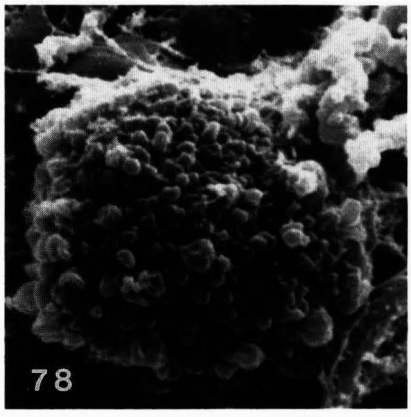
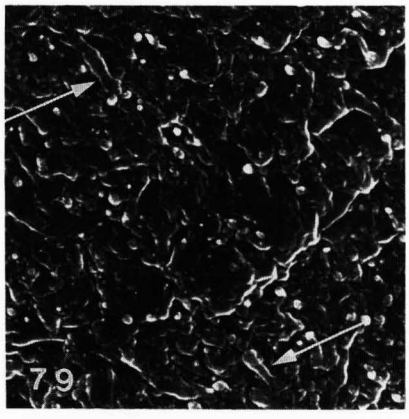


Fig.77. ACL surface, dwarf goat. Groove in length axis of ACL. Arrow: cell processes. Arrowhead: debris. P.W.= 45µm

Fig.78. PCL, human. Cauliflower-like cell. P.W.= 13µm



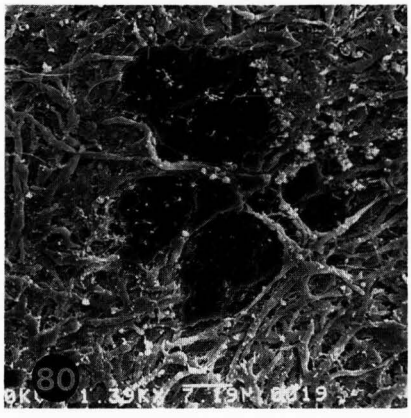


Fig.79. ACL surface, pig. Near synovial fold. Scaly appearance. Many globular protrusions. Arrow: processes. P.W.= 47µm

Fig.80. PCL surface, human. Elongated slender SLC's forming a network, surrounding large flat (dark) SLC's. P.W.= 59µm

Infrapatellar fat pad. As in the peripatellar ridges two principal morphological appearances are seen, clearly related to the composition of the subintima.

When fat cell profiles are pre-eminently visible - as is the case when the subintima is either absent or very thin - the fungoid manifestation of the SLC's is seen in dog 1 (Figs.1,63-66) and man (Figs.2,67,68); see for comparison with the peripatellar ridge Figs.50.51,52,61,55. In the dog free running 'fibrils' are observed in between several fat cells; they sometimes form open woven nets and at several spots seem to branch without changing diameters (Fig.63).

The other main morphological appearance is characterized by cauliflower-like SLC's. These are separated from the adipose subsynovium by a subintima which obscures the surface contours of the fat cells (Figs.69-74,93-95). See also Fig.56 for comparison.

The pig's fat pad surface is noteworthy: its flaky pastry-like appearance (Figs.69,70) was not encountered in any other species.

Anterior/posterior cruciate ligament. The SLC's in the rat (Fig.75), rabbit and dwarf goat are orientated in the longitudinal axis of the anterior cruciate ligament (ACL); in the other species there seems to be no preference of direction.

In sheep (Fig.76), rabbit, rat, dwarf goat (Fig.77) and man branching elongated cells with processes exist; except in the dwarf goat polygonal cells also are in evidence. In man sporadic cauliflower-like cells are seen (Fig.78).

The dimensions of the SLC's differ per species.

In the pig the synovial surface is composed of irregular cells from which processes seem to project. In places where the synovial lining from the ACL is continuous with that of overlying synovial folds its appearance is like that of the surface of the folds: the SLC's show a scale-like arrangement (Fig.79) which gets more flush with the surface as they lie further away from the folds. At the SLC surfaces many globular protrusions are in evidence.

The SLC's of the human posterior cruciate ligament (PCL) show two kinds of appearance (Fig.80). Predominant is the elongated slender one. The other is a polygonal one; they lie in between the elongated ones and are larger and much darker.

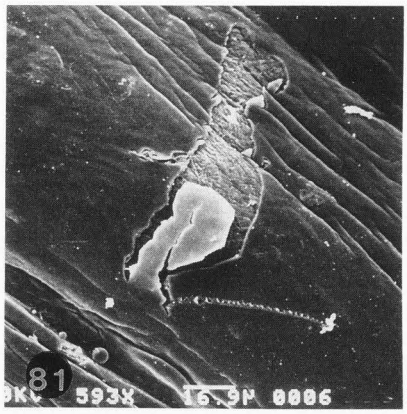
Except in some locations in the rat (Fig.81) the intimal surface is irregular because of wrinkles, folds and protrusions.

The synovial intima of the dogs' ACL consists mainly of a network of slender processes. Sometimes larger structures (20 x 25µm) are seen (Fig.82).

In most species (Figs.77,82,83) the SLC's form a continuous layer; in the rabbit in some locations the SLC's have borders with a sawtooth appearance as if they are shrunken (Fig.84). From the surface of the rabbits ACL rises a fold which is covered by elongated SLC's which occasionally branch or show processes (Fig.85).

In the rat, the sheep, the rabbit, dog 1 and man the subintima consists of a network of fibrils. In dog 1, in some places near section planes this network is not visible and instead a honeycomb-like fibrous layer consisting of thick (2µm) fibres is observed (Fig.86). Beneath the subintimal network in the human specimen lies another one, which has a braided appearance which consists of flat groups of fibrils which are arranged in a bundle-like fashion (Fig.87).

Transmission electron microscopy The specimens which in the process of preparation were embedded in paraffin and subsequently deparaffinised with toluene showed a complete absence of stained membranes; the fibrous structures did not seem affected. The same observations were made in specimens prepared from SEM-specimens which had not been imbedded in paraffin. However, it was very well possible to assess the presence of rough endoplasmatic reticulum (rER) because of the clearly visible arrays of ribosomes, and of large vacuoles as well. Mitochondria seemed to be discernable because of their shape and density and their often being located between rER laminae. Golgi complexes obviously were unrecognizable, as well as micro-pinocytotic vesicles. Secondary lysosomes were presumed to be identifiable because of their often dense and irregularly granulated contents and roundish shape. An occasional centriole was seen. The locations of pores in the nuclear envelope were recognizable as bright spots interrupting the chromatin layer surrounding the nuclear matrix.



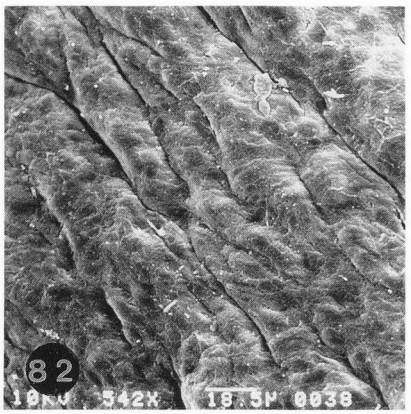
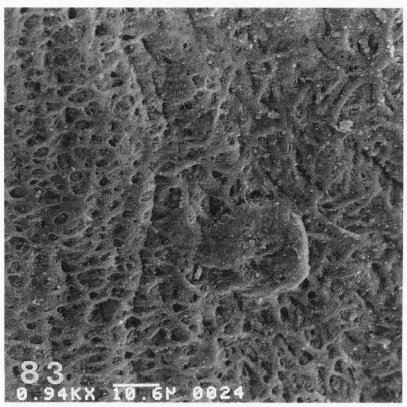


Fig.81. ACL surface, rat. Smooth surface continuous with that shown in fig.75. Fibrillous layer in defect.
P.W.= 139µm

Fig.82. ACL surface, dog 1. Continuous surface, mostly cell-processes.
P.W.= 47µm



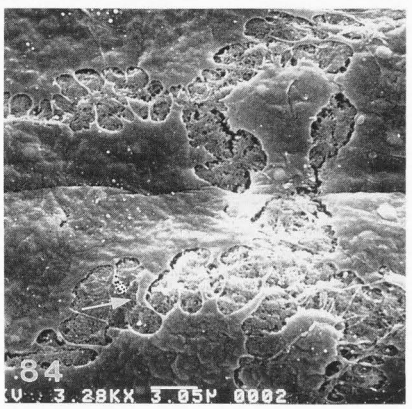
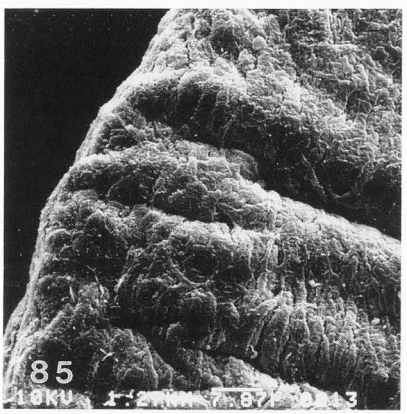


Fig.83. ACL surface, rabbit. Continuous intima.
P.W.= 152µm

Fig.84. ACL surface, rabbit. Shrunken SLC's, 'sawteeth' (arrow) between cell borders.
P.W.= 25µm



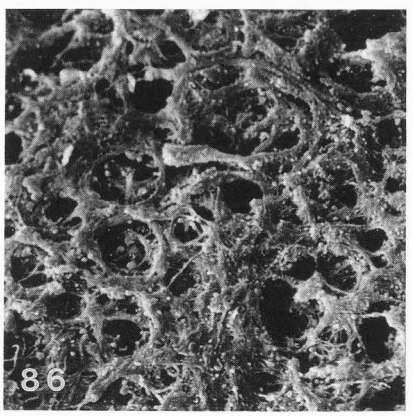


Fig.85. ACL, rabbit. Surface of synovial fold, covered by elongated SLC's.
P.W.= 65µm

Fig.86. ACL, dog 1. Surface layer accidentally removed. Coarse fibrous honeycomb-like surface exposed.
P.W.= 42µm

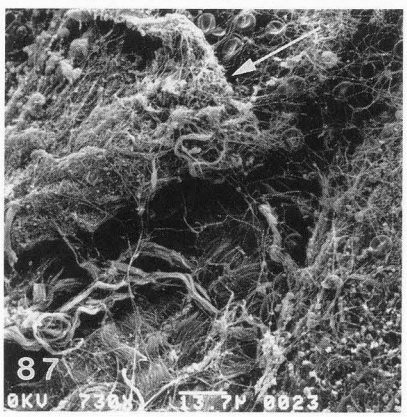
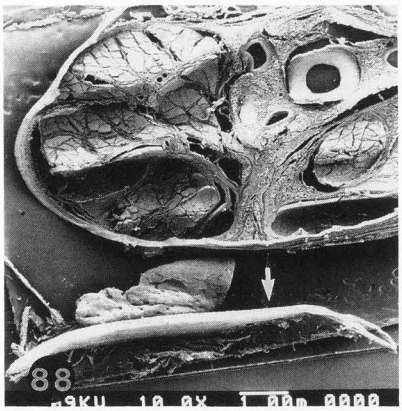


Fig.87. PCL surface, human. Damaged surface; synovial flap lifted off (arrow). Braided fibrous network now visible. Fibrin strands entrapping red blood cells are present as well.
P.W.= 113µm

Fig.90. Tendon sheath, dog 2. Section from specimen shown in fig.89. (TEM only). Synovium 1-2 cell layers thick. The surface is often lined by cell processes. Asterisk: tendon sheath wall. P.W.= 92µm

Fig.91. Tendon sheath, dog 2. From section shown in fig.90. (TEM only). SLC, possibly B-type. Many wide rER cisternae.
P.W.= 13µm

Fig.92. Tendon sheath, dog 2. From section shown in fig.90. (TEM only). Fibroblast-like cell with prominent cytoskeleton of intermediate filaments. P.W.= 13µm



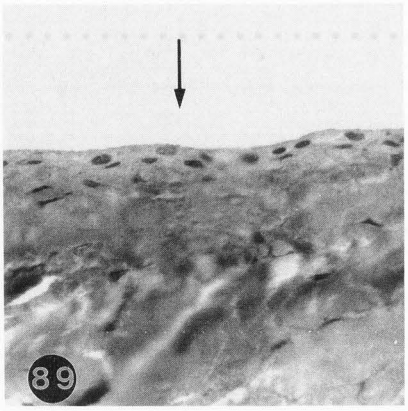
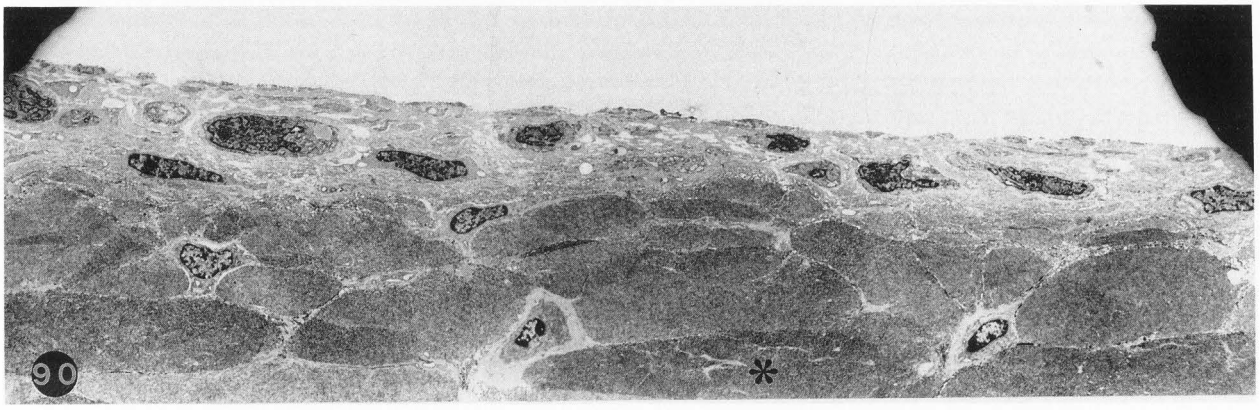
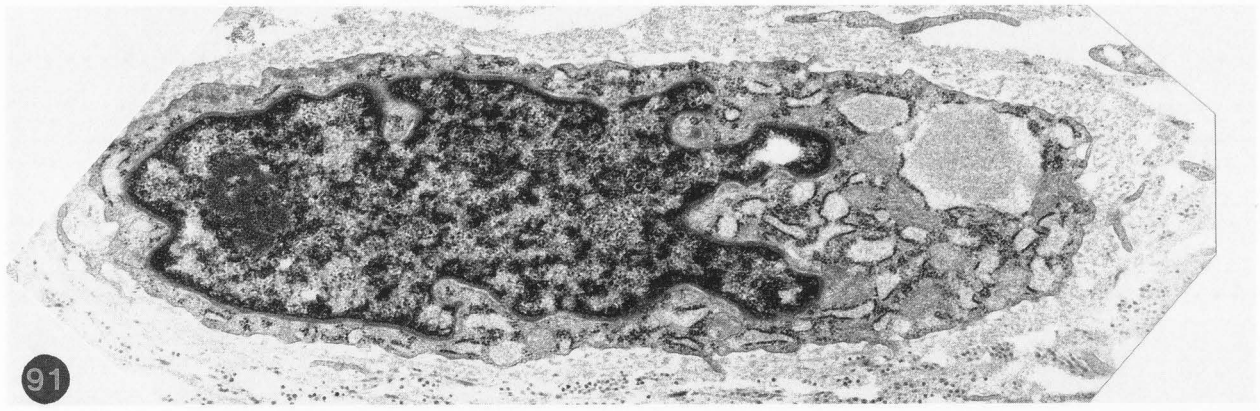


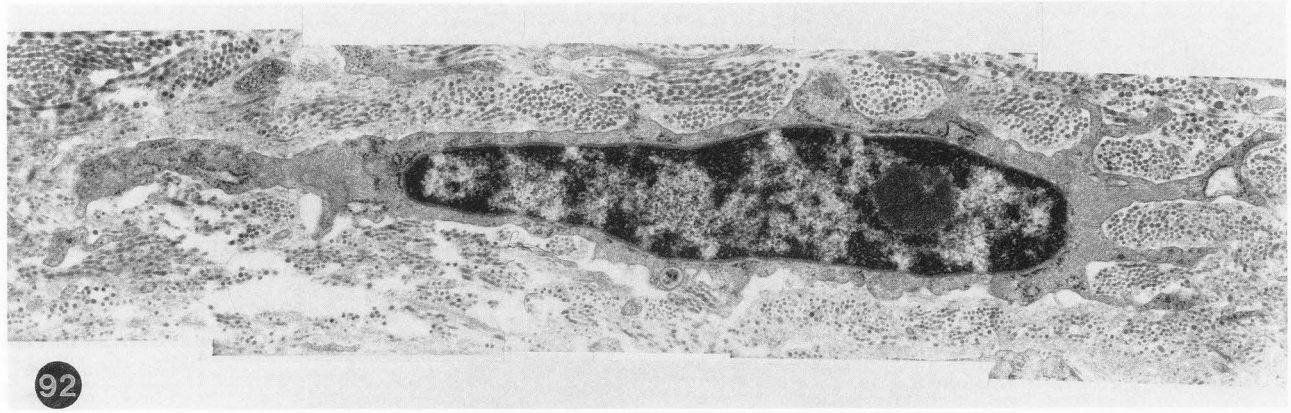
Fig.88. Tendon and tendon sheath, dog 2. 50μm section via paraffin. Arrow: dissected and studied sheath wall. Asterisk: tendon. Arrowhead top right: artery. P.W.= 8.2mm.

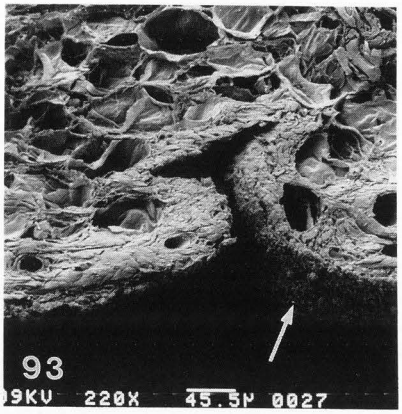
Fig.89. Tendon sheath, dog 2. TEM only, from specimen shown fig.88. Arrow indicates synovial surface. Stain: toluidin blue. P.W.= 100μm

Figs.90-92, see previous page for legends









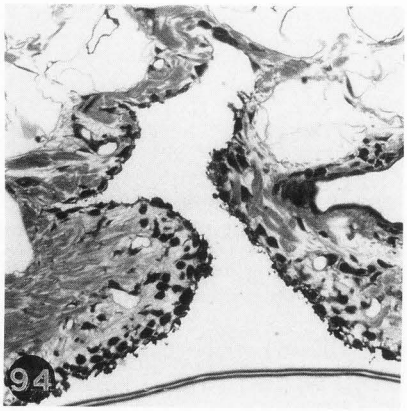
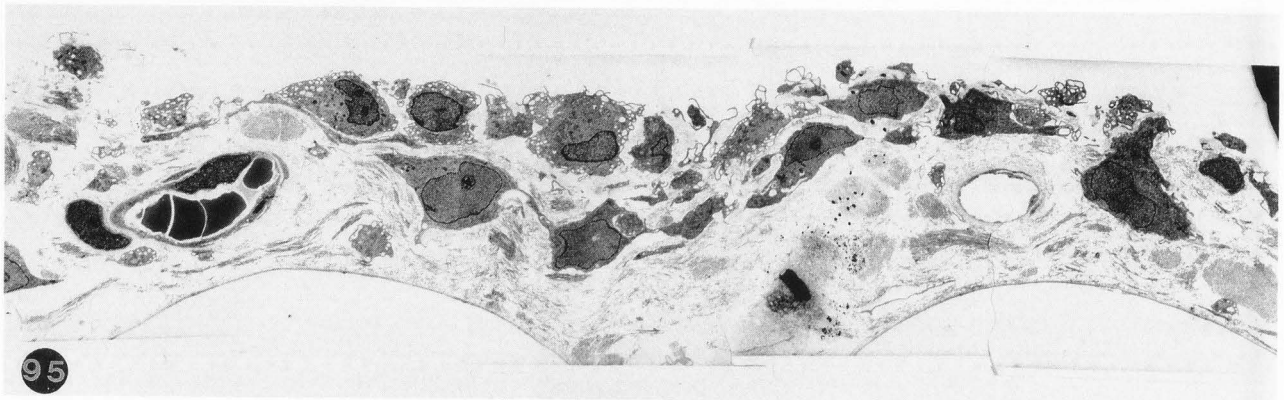
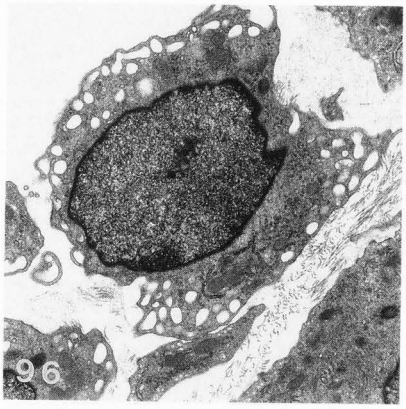


Fig.93. Infrapatellar fat pad, dog 2. 50µm section via paraffin. Arrow: synovial intima. P.W.= 375µm

Fig.94. Infrapatellar fat pad, dog 2. Section made from specimen shown in fig.93. Stain: hematoxylin eosin.
P.W.= 180µm

Fig.95. Infrapatellar fat pad, dog 2. TEM only. Section from same specimen as shown in figs.93,94. P.W.= 135µm





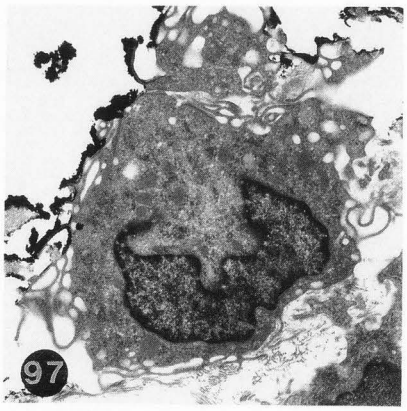
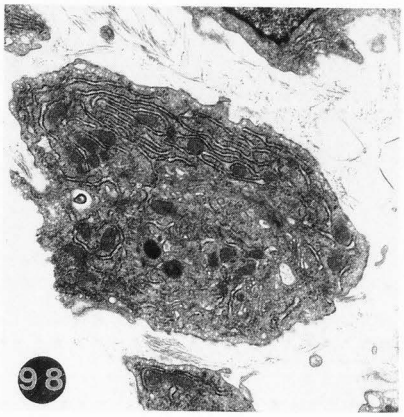


Fig.96. Infrapatellar fat pad, dog 2. From section shown in fig.95. (TEM only). A-type SLC.
P.W.= 9µm

Fig.97. Infrapatellar fat pad, dog 2. From section shown in fig.93. (Via paraffin). A-type SLC. Gold-palladium coating is clearly visible.
P.W.= 9µm



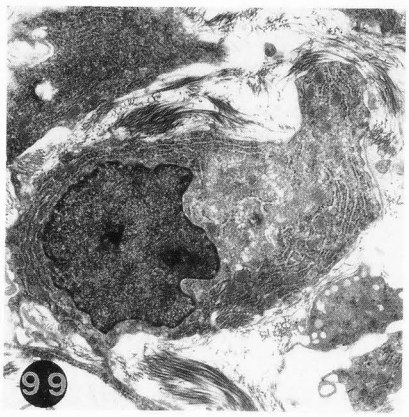
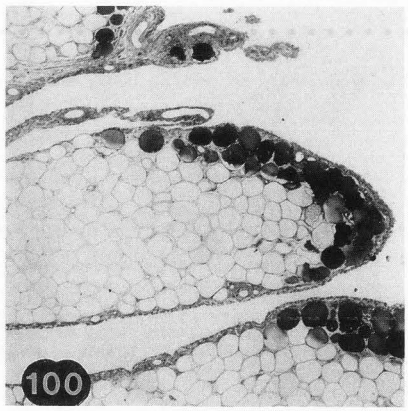
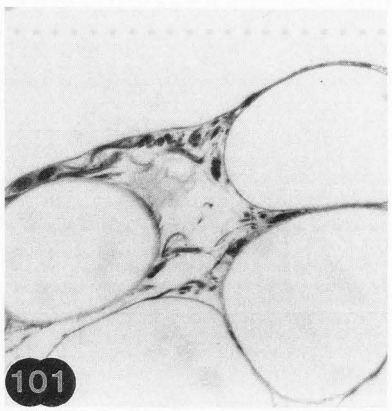


Fig.98. Infrapatellar fat pad, dog 2. From section shown in fig.95. (TEM only). B-type SLC.
P.W.= 9µm

Fig.99. Infrapatellar fat pad, dog 2. From section shown in fig.93. (Via paraffin). B-type SLC.
P.W.= 9µm





Figs.100 and 101. Infrapatellar fat pad, dog 2. From specimen shown in Figs. 93,94. (TEM only). Stain: toluidin blue. P.W. = 1.2 mm (Fig. 100); and = 400 μm (Fig. 101).

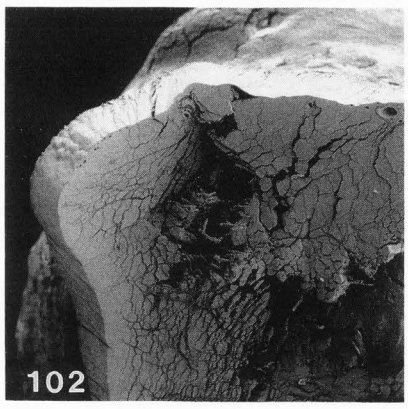


Fig.102. ACL, dog 2. 50 μm section via paraffin. The subintima is relatively richly vascularized (see Fig. 103). P.W. = 3.7mm.

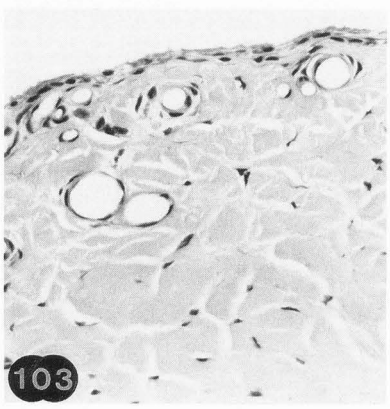
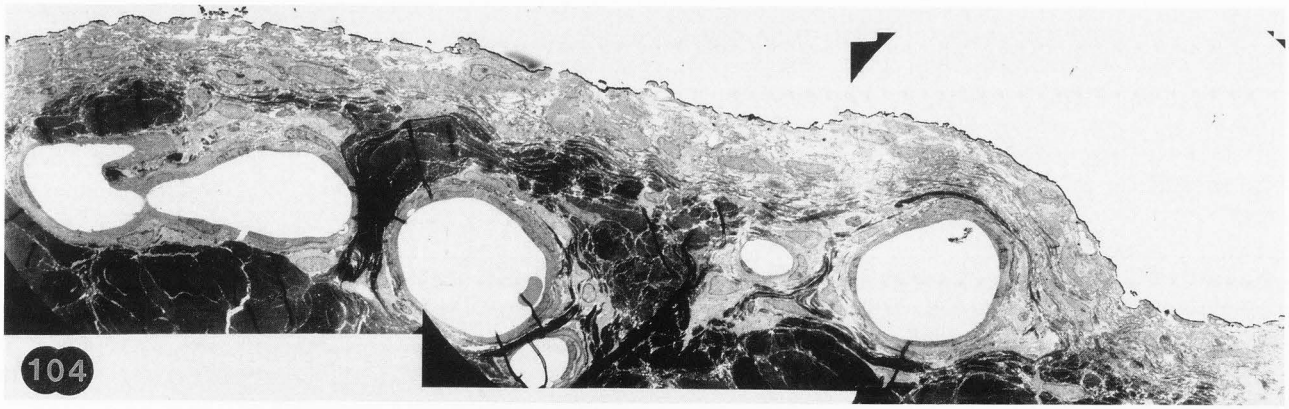
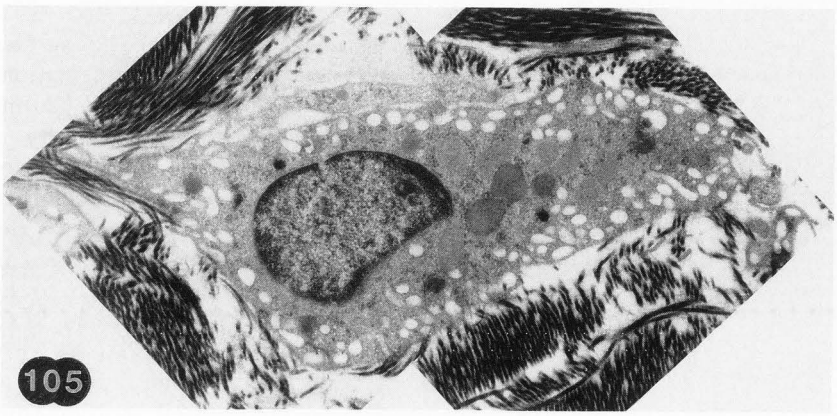
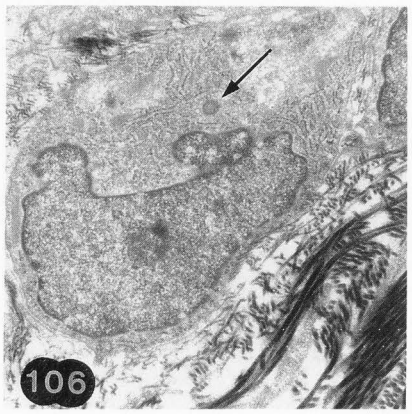


Fig.103. ACL, dog 2. Section adjacent to Fig. 102. Stain: toluidin blue. P.W. = 175μm.

Figs.104 - 106 (below). ACL, dog. 2. From section shown in Fig.102. (Via paraffin). In **Fig.104** the sputtered surface is evident; beneath it many elongated and spindle-shaped nuclei are present (P.W. = 200μm). **Fig.105** shows A-type-like cell (P.W. = 13μm). **Fig.106** shows B-type cell, arrow points to centriole-like structure (P.W. = 5.2 μm).







The nuclei of A-cells and B-cells often show distinct difference in chromatin structure. Nuclei of A-cells display a heterochromatin of a coarse unevenly distributed and lumpy character as well as a thick, irregularly deposited layer of condensed chromatin lining the inner nuclear envelope. B-cells have nuclei which show a much more diffusely dispersed euchromatin of a fine granular character; the layer of condensed chromatin lining the inside of the nuclear envelope is quite thin and regular.

This observation is of obvious relevance for the identification of A- and B-type cells. We did find one comment on this interesting phenomenon in the literature (Mitchell and Blackwell '68).

Notwithstanding the feasibility to identify the general composition of synovium as well as differences between individual cells in the deparaffinised specimens, most data presented were based on specimens which were prepared for TEM straight from fixative as described above in "Preparation for TEM only".

As the main purpose of the TEM observations is to demonstrate that the SEM inspected surfaces indeed are synovial linings, and occasionally to assess whether a SEM determined distinction concerning A- and B-types held, it is hardly necessary to report on those in full detail. The most significant findings are presented in Table 4. and are substantiated by Figs.88-106, of which Figs.90-92, 95-99, and 104-106 represent TEM. Any further significances will be dealt with in the Discussion section.

Light microscopy

As LM, in combination with TEM, was employed in order to demonstrate the synovial nature of the surfaces inspected with SEM, its description shall be limited to those structures which were subjected to serial observations.

Tendon and tendon sheath, dog 2.

Because the LM characteristics of the synovial lining of both structures appeared to be essentially similar, a single description will represent both. See Fig.89.

The subsynovium is composed of bundles of dense collagen, on which the synovium rests. In between the subsynovium and the synovial intima an irregular layer of dense collagen bundles, sometimes of smaller diameter, is apparent. This layer is not delimitable from the subsynovium and might well be part of it. In it fibroblast-like cells are apparent as well as some small vessels and capillaries, those also being present in deeper parts of the subsynovium.

The intima consists of 1-2, sometimes 3 layers of elongated, ovoid or polygonal SLC's which each contain a flat, spindle-shaped or ovoid nucleus of varying density and size. Sometimes they lie close together, almost on top of each other or in neat lines. In several nuclei a nucleolus is identifiable. Over small distances (1-3 nuclear lengths) no nuclei may be in evidence, cell borders however are not distinguishable so such spots may well contain cellular processes.

The lining of the intimal folds which connect the synovium of the tendon sheath with that of the tendon consists of SLC's which often have a more roundish shape. Its subintima is composed of loose areolar connective tissue, containing vessels. Beneath it clusters of adipose tissue may be observed.

Anterior cruciate ligament, dog 2. The subsynovium consists of bundles of dense collagen and seems to merge with the synovium inconspicuously. The only places where the presence of a subintimal layer is assumed is in those regions where small vessels and capillaries lie directly beneath the surface (Fig.103). The composition of the intima varies with the presence of underlying vessels. If none are in evidence the SLC's may be seen to lie in 1-2 layers, irregularly spaced, having elongated, ovoid or sometimes polygonal shaped nuclei of various densities, in which nucleoli may be recognized. In places where vessels are present SLC's of similar shapes occur and may form as much as 4-5 layers. Occasionally capillaries are observed to lie immediately beneath a single or two partially overlapping SLC's. At high magnification bright spots may be observed in the cytoplasm of some lining cells, either peripherally or juxtanuclearly.

Infrapatellar fat pad, dog 2. The subsynovium is composed of fat cells. The surface of the structure shows folds, lobes, indentations (Fig.100). At most locations the synovial intima is separated from the fat pad proper by a subintima of varying diameter, consisting of loose collagen fibres and bundles which are orientated in a random fashion. In the subintima vessels of various diameters are in evidence. Occasionally capillaries are seen to be separated from the joint space by only one SLC. At several locations the subintima may be very thin or non-existent and SLC's may appear to rest directly upon the individual collagenous

TABLE 4. Short account of TEM acquired data of synovial lining as observed in specimens from dog 2.

SYNOVIUM	TENDON & SHEATH from fixative	FAT PAD from fixative	CRUCIATE[2)] via paraffin
Thickness synovium:	5 - 10µm	15 - 32µm	7 - 30µm
Intimal cell layers:	1 - 2	1 - 2	1 - 2
Intima: fibrils (Ø 20nm-2µm) :	25 - 45nm	45nm	21 - 31nm
fibres (Ø 2-100µm) :	2 - 3µm	≥ 6x3µm	-
bundles (Ø > 100µm) :	-	-	-
Subintima: fibrils:	+	+	30nm
fibres :	+	≥ 9x4µm	-
bundles :	-	-	-
cells :	fibroblasts, processi	scanty B, processi	B > A, processi
bloodvessels :	-	5 - 7µm Ø	5 - 20µm Ø
Subsynovium :	fibrous	adipose	fibrous

CELLS:	A-type	B-type	A-type	B-type	A-type	B-type
A/B ratio :	-	+ + +	+ +	+ +	+	+ + +
Size (µm) :		8-12 x 2-3	13 x 6; 8 Ø	12 x 6	11 x 4	15-20 x 3
Processi :		+	+ + +	+	+ +	+
Nucleus: form :		O - L	O - P	E - P	E - P	E - P
chromatin :		H ≈ Eu	much H	much Eu	much H	Eu
nucleoli :		1	1 - 2	1	1	1 - 2
rER:		much	some, short	much	some, short	much
cisternae :		wide	narrow	focally dilated	narrow	narrow
Polyribosomes :		mostly in Pr.	+	+	+	rare
Mitochondria:		O - E	several	O - E, many	some	O - E, many
location :		near rER	no preference.	every-where	no preference.	near rER
Golgi apparatus		-	4 in 1 cell spread	+ + juxta nuclear	?	?
location :						
stacks :			4	4 - 9		
Vacuoles :		-	+ + +	scanty	+ + +	-
Lysosomes :		some	+ +	some	+	some
Micro-pinocytotic vesiculae :		several	+	+++ some coated	some, coated?	?
Microtubuli (25nm diam.) :	-	-	-	-	-	
Filaments :		many	+	+	-	-

E = elongated
O = oval(oid)
L = lobulated
P = polygonal

H = heterochromatin
Eu = euchromatin
Pr = processi

network of fat cell surfaces (Fig.101). In those zones the fat cell contours often lend a bumpy appearance to the intimal surface.

The SLC's are irregularly spaced. They form layers of 1-3, even 4 cells thick. Many SLC's have a roundish, ovoid or polygonal shape, the superficial ones often protruding into the joint space. Their nuclei show various shapes and 1-2 nucleoli. At high magnifications intracellular bright spots may be observed.

Discussion

Preliminary remarks.

Almost all research on synovium concerns ultrastructure and function of SLC's, related to its role in rheumatoid diseases. The ontogenesis of synovial cells is a subject of attention as well. The morphology of the surface of normal synovial intima, however, does not seem to enjoy such a warm interest; in the world-literature hardly any reports at all on tendons, tendon sheaths, cruciate ligaments, joint capsules and intra-articular fat pads are available.

The importance of SEM as a method for assessing the morphology of synovial surfaces lies in the simple and fast way in which it can be effectuated, and in the considerable size of the area which can be observed. Furthermore, acquaintance with the three-dimensional structure of synovial lining can deepen the insight in pathological changes of its microstructure.

Unless there is a distinct coherence with the morphology of the synovial surface functional, ultrastructural and ontogenetic aspects of SLC's will not be dealt with in this discussion.

SEM observations in the literature.

Most publications on SEM-observations of synovium date from the early SEM days (Fujita et al. 1968; Gryfe et al. 1969; Redler and Zimny 1970; Woodward et al. 1971; Wysocki and Brinkhous 1972; Hayashi 1976; Shively and Van Sickle 1977); Fujita et al. (1968) claim to be the first to have studied synovium with SEM. Sometimes reports were presented by research workers who apparently did not have much experience in judging biological SEM-specimens (for example as indicated by comments from Ghadially following Schmidt and Mackay, 1982). Ghadially (1983, p.26) states that personal experience leads him to believe that SEM of synovium is hardly feasible because of "formidable technical problems" in the preparation of such specimens; he refers to only three out of - at that time - at least nine available sources in the literature and might have found encouragement in the results of some of these other authors.

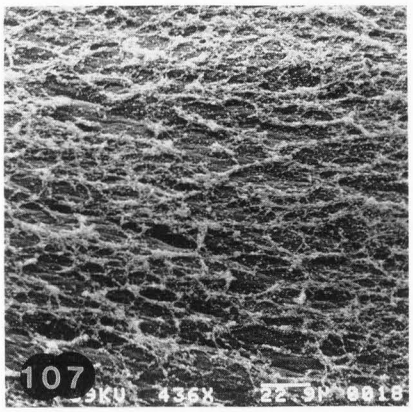
Wysocki and Brinkhous (1972) reported on the morphology of synovial intima which lines different substrata as can be found in the knee joint of the dog. In 1979 Date presented detailed SEM-observations on synovium obtained from childrens' legs which were amputated because of tumors and from rabbit knees. However, he presents details of the surface of SLC's without being explicit about the morphology of the intima in a broader sense; the SEM-observations are not supported by LM or TEM, neither is it clear from which anatomical sites the human specimens derive and no clear distinctions are made between human and rabbit synovium where certain conclusions are concerned.

Cell types. In the literature generally two main types of SLC's are specified: the macrophage-like A-type (also M-or V-type) and the fibroblast-like B-type (also S-, F- or ER-type); in addition an intermediate type (also F-M-, C-, I- or AB-type) is described. The ultrastructure, being a representation of the function of the cells is the basis for these distinctions; no explicit unanimity on the characteristics of the intermediate type exists apart from that they show some of each of those of the A- and B-types (Roy and Ghadially 1967; Wassilev 1970; Krey and Cohen 1973; Ghadially 1980).

In a nutshell, the function of A-cells is, besides the production of hyaluronic acid, phagocytosis; that of the B-cells is protein synthesis, they are capable of phagocytosis as well.

The questions whether these cell-types have a different origin (Watanabe et al. 1974; Linck and Porte 1981; Okada et al. 1981a,b; Edwards et al. 1982; Graabaek 1984; Revell 1989) and whether they represent different functional states of the same cell-type (Davies and Palfrey 1966; Ghadially and Roy 1966; Cutlip and Cheville 1973; Fell et al. 1976; Wassilev et al. 1976; Ghadially 1980; Soren and Waugh 1981; Ghadially 1983; Shiozawa et al. 1983; Stofft and Effendy 1985) still have not been satisfactorily answered (Henderson and Pettipher 1985). Directly related to this are the differing opinions on the existence of an intermediate cell-type: Graabaek (1982) showed in a serial section study of synovium of the rat that the supposed intermediate cells actually were B-cells, something which cannot be visualized by viewing just a few sections. Apparently reluctant to accept the implications of Graabaeks' findings Ghadially (1983, p.26) reacts with an argumentation based on the various functional states of individual cells, displaying a haziness which is in contrast with his campaign against confusing nomenclature (p.67 a.o.).

On the ratio of the different SLC-types several observations exist, in which it is hardly



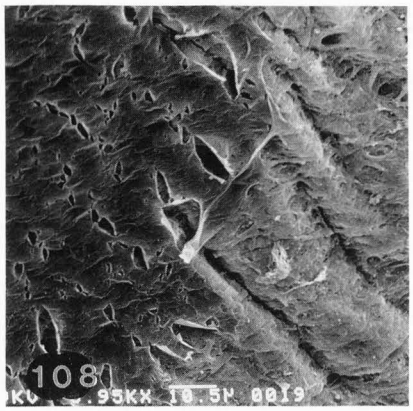


Fig.107. Synovial precipitate on intimal surface.
P.W.= 189µm

Fig.108. Film of synovial fluid precipitate at the bottom of the pigs' tendon sheath's hollow (fig.34).
P.W.= 91µm

possible to detect a broad outline, as there seems to be a lack of uniformity in parameters like species, age, research techniques, anatomical sites. Ghadially (1983, p.27) comments that "all but one report (Wyllie et al. 1964) subscribed to the view that the major cell population in the synovial intima of animals is well endowed (i.e. type B) or fairly well endowed (i.e. type AB) with rER.", which gives the false impression that consensus of opinion on this matter exists. False, because Ghadially fails to refer to several authors who in fact claim the A-type to be predominant: see Table 1. When in the same section (p.26 & 27) Ghadially erroneously refers to two papers (Watanabe et al. 1974, p.288; Fell et al. 1976, p.680) concerning this subject the reliability of his interpretations in this context is severely taxed.

A distinction between A- and B-cells observed with SEM is reported on in only two publications (Wysocki and Brinkhous 1972; Stofft and Effendy 1985); in the latter no clear link with LM or TEM was made.

TEM corroboration of SEM observations.

The way in which the specimens for this study were obtained, fixed and processed was of such a nature that the assumption that the surfaces inspected were those of the synovial lining is an obvious one. To give support to this view specimens of the dog, obtained and processed in a similar fashion as those in the other species, were submitted to serial LM, SEM and TEM inspection. This confirmed that the surface inspected with SEM indeed was the synovial intima; the SLC's exhibited an ultrastructure which complied clearly with those referred in the literature as belonging to A- and B-cells (Table 4.). Consequently the authors conclude that the above mentioned assumption is true and that this holds for all structures from all species as were examined in this study.

Specimen contamination.

Not once was a specimen contaminated with precipitated material to the extent that it impeded the inspection of the entire synovial surface. Only a few times precipitations, of small extent, were observed. Their aspect was reticulate (Fig.87) and probably consisted of fibrin as they were located near cut edges or damaged surface and red blood cells were regularly seen in their proximity. Once a precipitate had such an extent (Fig.107) and was so homogeneous that it may have concerned a precipitate of synovial fluid. Only in a few cases a precipitated film large enough to conceal the underlying synovial surface was found. This was the case in depressions in the surface of the structure concerned, e.g. on the bottom of the hollow in the pig's tendon sheath and in a depression in the pigs tendon (Fig.108). This is contrary to Ghadially's statement that only relatively vigorous washing of the synovial surface would remove enough of the synovial precipitate to enable inspection of the surface, and that this produces so many problems (i.e. artifacts) that it makes the preparation of synovial specimens for SEM almost impossible (Ghadially in comments following Schmidt and Mackay 1982; Ghadially 1983).

Special features.

On the surfaces of several of the studied specimens polyp-like (Figs.21,36,47) and thread-like structures (Fig.37) were observed, and were described in detail. In the surfaces of the tendon sheaths of pig and sheep a hollow and gullies were observed respectively (Figs.34,40), their orientation being parallel and transverse to the longitudinal axis of the sheaths respectively. The doubling of the synovium (Fig.41) which attaches the sheath with the tendon

originates in the gully in the sheep. In the pig's tendon sheath an adipose fold originates from the lateral border of the hollow; a polyp-like structure lies over the opposite border (Figs.34,35,36).

Several tendon sheaths show folds of the synovium (rat, sheep, pig and dog: Figs.27,42,35,9,29 respectively).

The existence of polyp-like structures on the surfaces of tendon and tendon sheath is possibly related to the continuous mechanical stress the synovial intima is subject to: (micro-)traumatic damage of the tendon or tendon sheath surfaces may result in abrasion of (sub-)-intimal fibrous tissue. Thread-like structures may protrude into the synovial cavity and in order to protect the synovium from more damage may become enfolded in SLC's (Fig.37) and eventually phagocytized. If enfolding takes place from the surface towards the free end of the thread this might effect the stalked polyp-like appearance. If enfolding takes place from as well the attached end of the thread as from its free end, as may be possible in case of a thread of some length, then the structure is doubly stalked.

One can only speculate as to the function of the hollow and gullies in the tendon sheaths of pig and sheep. In the sheep it looks as if the doubling of the synovium originates in such a gully; in the pig an adipose intimal fold arises from the side of the hollow. Possibly both hollow and gully function as a reservoir for synovial fluid which is distributed under influence of the mechanical forces which tendon (and adipose fold?) impose on it ('lubrication pits').

Functions of the synovial folds present in several tendon sheaths might be enhancement of lubrication and reduction of friction due to expansion of the synovial surface. Folds which contain one or more layers of fat cells probably have a cushion-effect and reduce mechanical pressure on tendon and tendon sheath, for example in locations over a hypomochlion.

The fold - or plica - in the tendon sheath of the rat partially separated two tendon slips from each other and so provided them with their own lubrication. The plica is reinforced at its intraluminal border by a rib-like fibre-bundle which runs lengthwise just beneath the subintima, possibly to prevent luxation of the plica from between the tendon slips (Fig.27).

The observations on pores in human synovium as described by Cameron and MacNab (1973) could not be corroborated.

Considerations.

Reports on (normal) synovium of the dwarf goat are not available in the literature. The observations on the synovial lining of tendon, tendon sheath and cruciate ligaments as presented in this paper are first in the field as well. SEM observations in relation to sheep synovium also are presented for the first time; TEM of sheep synovium was reported on, albeit briefly, by Cutlip and Cheville in 1973.

In all species studied the lining of tendon and tendon sheath - and for that matter of infrapatellar fat pad and peripatellar ridges - is composed of a layer of SLC's and, contrary to Cormack's statement (1987), not mostly of collagen. This also applies to the cruciate ligaments which have never been described in this context before. Neither do our observations support those of Key (1932) who states that in the "fibrous type of synovium" as a rule the cells are few in number and on the whole tend to be well embedded in the connective tissue surface and often are covered by a dense layer of collagenic tissue.

Cell form and orientation. The surfaces of synovial lining of structures which are constantly subjected to pressure and friction (tendon, tendon sheath) clearly show similarities in form and structure. The surfaces are flat and the elongated SLC's are orientated in the longitudinal axis of the structure. This orientation may well be the result of the longitudinally directed friction forces acting upon the surfaces, thereby causing the SLC's to orientate themselves in the described manner, as was also observed by Wysocki and Brinkhous (1972) in relation to synovium with a fibrous substratum in the knee joint of a dog. An identical situation is observed in endothelial cells which likewise align themselves under the influence of shear forces. Exceptions are seen in sheep and dwarf goat: on neither specimens of tendon and tendon sheath do the SLC's lie in a preferred direction.

The cruciate ligament which is subject mainly to longitudinally directed traction forces and not to forces which substantially affect its surface in most species has a flat synovial lining without a preferred direction of the SLC's being in evidence. These observations are consistent with the foregoing in the absence of shear forces. In man the synovial intima of the PCL consists mainly of flat elongated SLC's which lie criss-cross like in a network.

Synovial surfaces which are not exposed

to high pressures or severe friction (e.g. infrapatellar fat pad, peripatellar synovium) in most cases have a spatial structure of folds, ridges, lobes, etcetera. The constituent SLC's are often not flat.

The different appearances of the synovial surface of the cruciate ligaments and that of the latter structures may be explained by the assumption that, although both are relatively free from pressure and friction, the adipose subsynovium enables even more reduction of those forces by a 'cushion effect' whereas the fibrous subsynovium does not.

The effects of pressure on the morphology of the synovial surface in rabbit knees was studied by McDonald and Levick (1988). Their findings are in accordance with the observations presented here.: the higher the pressure the flatter the SLC's.(also the number of slender processes increased).

Stated in general the subsynovial structure is a determining factor of the synovial morphology.

Cell features and -types. In or on the synovial intima cells are present which show a resemblance with macrophages. They may have a cauliflower-like appearance because of many cytoplasmic protrusions; in for example the rat such cells may be flat and polygonal with many tiny processes jutting into the joint space. Because of their appearance these cells are supposed to be A-type SLC's. The elongated and branching SLC's often resemble fibroblasts and might well be B-type SLC's.

Both assumptions are supported by TEM observations made in this study in various structures: the A-type cells show many vacuoles and processes whereas the B-type cells contain much rER. See Table 4. for more details. These observations support those made by Wysocki and Brinkhous (1972) who suggested that in the knee of the dog SLC's with prominent processes probably belonged to the A-type.

Nearly all synovial specimens referred to in the literature were obtained from (knee-) joints; about anatomical structures with a fibrous type of subsynovium nothing or hardly anything is known.

The A-cell/B-cell ratio as found in this study in the synovium of fibrous structures shows a distinct advantage in favor of the B-type; in the peripatellar and infrapatellar types of synovium a fair guess based on SEM is hardly possible (except in dog 2). The SEM observations of the specimens from dog 2 are supported by TEM: in the infrapatellar fat pad A-cells are clearly predominant, whereas in the intima of tendon, tendon sheath and ACL the B-types predominate.

The differences in predominance of SLC-types in the various structures might be explained as follows.

Tendons and tendon sheaths are likely to benefit from a production of synovial fluid which is spread all over their touching surfaces in order to prevent damage by reducing friction. This might explain the predominance of B-cells, which supposedly are the manufacturers of amongst others LGP-1 (lubricating glycoprotein), this at least being isolated from synovial joints (Ghadially 1983).

Because of large mechanical loads the knee joint is subject to wear and tear, for the removal of its products an adequate amount of phagocytosing cells is mandatory. This might be the reason for a large amount of A-type SLC's. In relation to the composition of the synovial intima of joints which are or are not subject to large mechanical loads different opinions are put forward: Wassilev et al. (1976) describe a clear increase in the number of A-cells (those being predominant) in the synovium of the rat knee after the animal ran for a long time. In contrast Letizia et al. (1980) indicate that the synovial intima of joints which are normally performing heavy duty (human knee) shows a predominance of B-cells, and that the total amount of SLC's in relation to joints which are normally not heavily burdened (human elbow) and in which mostly A-cells are evident, is smaller. Neither Wassilev et al. nor Letizia et al. indicated from which topographic site their specimens originated and which types of subsynovium were involved.

The sawtooth appearance of the rabbit ACL synovium can be explained as a shrinkage artefact resulting in interrupted cell contact. This might indicate the presence of desmosomes, concurrent with the observations of Ghadially (1988): "As the cells shrink away from each other the points where the cells are firmly bound together by desmosomes are pulled out and present as bridges" (legend to plate 468, p.1112).

The sizes of SLC's are not referred to in many reports and sometimes the reader must take measures from photographs. Even that may be impossible because quite often the magnification as represented on the pictures is

TABLE 5. Sizes of synovial lining cells as reported on in the literature.

Species	Authors	Source	Method	A-cell (μm)	B-cell (μm)	Type? (μm)
Rabbit	Ghadially et al. '66	Knee	TEM			12 x 4[1]
Rabbit	McDonald et al. '88	Knee	SEM			12 x 24
Rabbit	Watanabe et al. '74	Knee	TEM	15-20 (Ø?)		
Human	Castor '60	Knee	LM			8-12 x 6-7
Human	Krstic '84	?	TEM	20 x 10[1]	20 x 10[1]	
Rat	Graabaek '84	Knee	TEM	10-25 x 5-10	5-20 x 5-10	
?	Henderson et al. '85	?	TEM			8-12 x 6-8

1) Measured from prints as no sizes were indicated.

not clearly indicated. Most reports concern synovium from the knee joint; the sizes are on the whole similar to those in the knees of man and rabbit as found in this study (see Tables 5. and 3. respectively).

The sizes of the SLC's on tendons, tendon sheaths and cruciate ligaments cannot be compared with other observations in the literature as those are non-existent.

Subintima. In most structures in most species the synovial lining rests on a layer of fibrils; frequently its arrangement is network-like (Fig.59), also the fibrils may run parallel to each other and sometimes also parallel to those of the anatomic structure. The diameter of the fibrils is small and in general measures 0.1-0.5μm in SEM and 0.03-0.06μm in TEM. In SEM the subintima is in most cases only visible when a defect of the intima exists. Many small fibrils which normally are situated between and just under the SLC's may be absent due to such a defect which then provides a view on a deeper layer in which the fibrils have a larger diameter; rather thick fibres (Ø. 2.4μm) were observed as well. In several specimens the thickness of the fibrous layer ranged from 2.5-4μm.

In those structures in which the subsynovium is of the adipose type the SLC's are located on and in between fat cells from which the intima is separated by a network of thin fibrils which clings to the fat cells like a skin (Fig.109).

At the surface of the peripatellar ridge of the sheep fibrils are seen to cross the joint space from the surface of one fat cell to that of an adjacent one (Fig.61). Sometimes fibrils connect two SLC's or a fat cell with a SLC.

The synovium classified. The fundamental composition of the synovium (intima plus subintima) seems to be constant, irrespective of species and anatomical site. However, the functional demands made of a subsynovial structure - reflected in its architecture - as well as its topographical relationships, induce adaptations of its synovium. These adaptations are reflected in the composition of the subintima and in the morphological features of the intima. Consequently subsynovium and synovium should be considered to form a functional unit.

A classification of the synovium must be consistent with this interrelationship. A classification based on histological descriptions alone offers no insight in this fundamental interrelationship.

To optimize such a classification its terminology should be unequivocal.

A functional classification of synovium is presented in Table 6a. The morphological appearances of synovium in accordance with this classification are listed in Table 6b.

Conclusions.

Interpretation of synovial surfaces with SEM is very well possible. The advantages of that method are based on the fact that it does not require time-consuming techniques, and relatively large specimens can be studied. Another advantage is that SEM specimens may also be used for sunsequent inspection with TEM.

In general three principal appearances of synovium are evident. On structures with a dense fibrous architecture like tendons, tendon sheaths and cruciate ligaments the intimal cells and processes are mostly flat and slender and may tend to orientation in the length-axis of the structure. On the peri- and infrapatellar adipose tissues two principal 'extremes' are

Fig.109. Fat cell from subsynovial cluster in rabbit's tendon sheath's wall (fig.26). Fibrillous network enclosing the cell. P.W.= 50μm

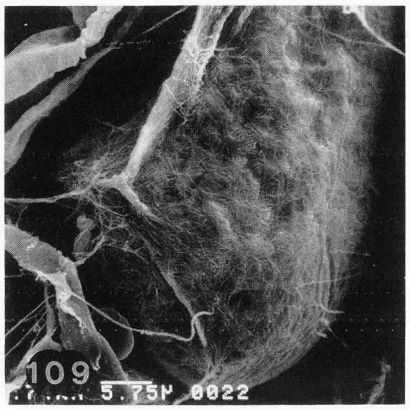


TABLE 6a. Synovium, classification.

Functional unit	Subsynovium	Type of Synovium
Tendon/tendon sheath	Fibrous	Tendinous
Cruciate ligament	Fibrous	Ligamentous
Joint capsule/bursa	Fibrous	Capsular
Fat pad	Adipose	Adipose
(Transition zone	Bone/cartilage	Osseocartilaginous)

TABLE 6b. Synovium, morphological appearances.

		Intimal Morphology		
Type of Synovium	Subintima	Continuity	SLC's	Orientation
Tendinous	Fibrous (dense)	Continuous	Elongated	Longitudinal
Ligamentous	Fibrous (dense)	Continuous	Elongated/polygonal	No preference
Capsular	Fibrous (dense/areolar)	Continuous	Polygonal	No preference
Adipose	Fibrous (dense/areolar) None?	Continuous	Fungoid, cauliflower-like	No preference

seen: one in which the contours of the fat cells are clearly visible with fungoid shaped structures in between, and one in which the fat cell contours are not recognizable and the intima consists of cauliflower-like cells. Transitional forms exist.

Further SEM supported study of the microscopic anatomy of synovial surfaces may deeper insight in tendon-tendon sheath-units and related pathological conditions like (traumatic) tenosynovitis, and in intra-articular lining of biomaterials.

Some overview-papers (Ghadially 1980; Henderson and Pettipher 1985) give the impression that morphology, ultrastructure, function and ontogenesis of the synovium of various anatomical structures in various species fit in an all-embracing framework. Controversies and lack of knowledge concerning the synovium (e.g. on intercellular junctions, on the ontogenesis of SLC-types, on the existence of intermediate SLC-types) still exist. This, and the observations made in this study indicate that - at least for the time being - it is more appropriate to judge synovium of various anatomical structures in different species on their own merits.

In order to optimize communication concerning the synovium an unequivocal nomenclature and consistent classification, as presented in this paper, are mandatory.

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Discussion with Reviewers.

A.Boyde: Do you think the species differences are real? Could they not relate to sampling problems, how many specimens from each species did you look at?

Authors: We investigated 7 species (mostly 1 animal of each) and took samples from 4 anatomical structures (mostly left and right). As the specimens were handled with the utmost care and processed in an identical manner there is no reason to believe that the studied structures are not representative for the individual of the species concerned.

Differences in synovial morphology between various structures do exist; these interstructural differences are consistent in the studied species. As is shown in the paper the synovium has several principal morphological appearances which are considered to be related to the function and topographical relationships of the subsynovium-synovium unit. We think that differences between e.g. measurements of SLC's as found between respectively rats and rabbits, and the other species, are real. To what extent the observed differences between the various species, and between the various structures within one individual, can be contributed to e.g. functional

status cannot be determined from this study and requires further investigation.

So many unanswered questions about synovium exist, that it seems reasonable to advocate prudence before an attempt is made to mold synovium into an all encompassing framework.

One generalizing statement is made: throughout the species investigated the morphological appearance of the synovium consistently varies with place and function of the subsynovium-synovium unit.

A.Boyde: No attempt was made not to fix the synovial fluid in situ. The pictures strongly suggest that the surfaces are varnished with synovial fluid. As the first step in the fixation schedule this reviewer recommends the injection of OsO_4 solution in order to disperse the synovial fluid.

Authors: In some places we did observe a smooth coating of the synovial surface (fig.108). If a varnish of synovial precipitate covers the surface at all, it is so thin that it follows the surface contours as can been seen in some places from TEM sections. In most locations studied with TEM (directly or subsequently from SEM specimens) there was no trace of precipitate at all (fig.95).

We did investigate initial OsO_4 fixation with the aim of preventing any precipitation, as you suggested, The effect was adverse: the surfaces of all specimens - with a 100% reproducibility - were thoroughly contaminated. Onze dank.

A.Boyde: Some of the surface morphology results from the post CPD drying stage.

According to the technical description it is highly likely that the specimens were very much overcoated. Nevertheless, why do many of the illustrations show signs of charging? Too much sample current?

R.Bloebaum: It would be interesting if the authors would comment on the potential artifact that may have been introduced by the processing technique.

Authors: As our methods were standardized and our techniques meticulously carried out, artifacts which did occur are considered to be consistent ones. Taking all this into account, we regard the interpretation of the results as reliable.

It is evident that a certain amount of overcoating occurred; in SEM-specimens subsequently observed with TEM the thickness of the coating ranged between 50 and 130nm. The coating always exactly followed the surface-contours without filling-up spaces or bridging gaps.

Sample currents were never excessively high; the emission bias was always carefully calibrated for 9 respectively 10 kV. A reason for the still occurring charging may be that the SLC's do not form a regular membrane, this possibly facilitating interference with local conductivity.

K.Draenert: More information on the two dogs would be interesting in order to be able to explain the different findings.

Authors: An explanation for the differences between the findings in dogs 1 and 2 may be that dog 2 had more exercise before the specimen of the fat pad was taken out. As a consequence a higher amount of A-cells may have been present, as was described by Wassilev et al. (1976) in the synovium of rat knees after the animal ran for a long time. In dog 1 nor in dog 2 was the entire fat pad studied; as described transitional morphological appearances of synovium exist and the appearances found in the dogs possibly existed in both.

K.Draenert: Bone cells orient their long axis along the collagenous fibre bundles, but there is no friction acting on these cells: this type of elongation is a dynamic phenomenon. Please comment.

Authors: In our paper we do not refer to this dynamic process, and do not use the term "elongation". We use the adjective "elongated" to describe a morphological appearance, i.e. long, slender shaped cells and processes.

An interesting finding is that elongated SLC's which are orientated in the length-axis of e.g. tendon or tendon sheath quite often lie on a fibrillar network which shows no preferred orientation of its constituent fibre structures. This indicates that the orientation of the elongated SLC's is not an active process related to the orientation of fibres as with e.g. bone cells ("elongation"), but is induced by mechanical - frictional - forces caused by movement of the tendon in its sheath, as was discussed in the paper. These two phenomenons are conceptually different.