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Synovitis of Familial Mediterranean Fever

A Histologic and Ultrastructural Study

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Summary. The microscopic and ultrastructural features seen in the synovium of twelve patients affected by the protracted arthritis variety of FMF is described. It would appear that the small vessels of the synovial membrane are the principle target organ in this articular pathology. Intra articular injections of colloidal radioactive gold, or hydrocortisone, do not alter the histologic and ultrastructural appearance of the affected synovia. Neither do these findings change in the regenerated synovia, growing after a surgical synovectomy. The implications of these findings are discussed.

Key words: Synovia — Arthritis — Blood vessel wall.

Familial Mediterranean Fever (FMF), sometimes termed Periodic Disease or Recurrent Polyserositis was first described by Reinmann (1948) as a periodically recurrent fever chacterised by abdominal pain and arthralgia. It is a genetic disorder of autosomal recessive inheritance, with complete penetration. This syndrome which has long been recognised around the Mediterranean Basin, is now seen in other areas of the world due to the expansion of travel facilities. Heller, and his associates who studied this disorder extensively, attributed it to an inborn error of metabolism and grouped it with the heriditary amyloidoses (Heller, Sohar and Sherf, 1958; Shahin, Sohar and Dalith, 1960; Heller *et al.*, 1966).

Joint involvement constitutes a cardinal feature of this syndrome, 25 per cent of these patients having their first joint symptoms at the age of 1–5 years, while a total of 50 per cent of them show signs of joint involvement before their tenth year (Heller *et al.*, 1966). Joint pain is more common than joint swelling. The episodes of arthralgia are recurrent, and may affect any of the major synovial joints as a transient, or protracted attack. The sequelae of these episodes vary from complete recovery of the affected joint, to contractures, destruction of the joint cartilage and permanent loss of function in protracted cases.

Simultaneous involvement of two joints is rare, but some patients may develop an acute attack in one joint, while the previously affected one has not yet completely recovered.

The orthopaedic management consists mainly of conservative measures, i.e. splinting and physiotherapy. Reconstructive surgical procedures are attempted only in late cases of severely affected joint.

It is the purpose of this communication to describe the detailed histologic and electron microscopic findings of the affected synovium in treated and untreated patients with Familial Mediterranean Fever pointing out rather persistent structural changes.

Material

Fresh synovial tissues were obtained during surgery from a total of twelve patients. Nine of them underwent synovectomy and three underwent arthroplasties. These patients were affected by the more severe and protracted form of joint involvement in Familial Mediterranean Fever, and they underwent a total of fourteen operations (one patient being operated on three times). In one patient the synovium was a regenerated specimen, the original synovium having been excised at the time of a previous cup arthroplasty. Three patients had intra-articular injections into the affected joints prior to surgery.

Two of them had intra-articular injections of colloidal radioactive gold, 10 mCi. per injection (Secific activity 2–10 mCi/mg. Radiochemical Centre, Amersham, England, Code GCP IP); the particle size was 10–20 nm, (1 nm = 10 Å). One of them had the injection 6 years prior to surgery, while the other had it four months prior to surgery. A third patient had three repeated intraarticular injections of hydrocortisone into her knee, the last injection being given 1 year prior to surgery. All three of them, ultimately underwent synovectomy.

Methods

The excised synovial tissues were fixed in 10% formaline, embedded in paraffin and sections were cut at 5μ. These were stained with Hematoxylin and Eosin. Samples for electron microscopy were fixed in 2% osmium tetroxide buffered with 0.1 M sodium cacodylate (pH = 7.6). After rapid dehydration (Bencsome and Tsutsumi, 1970) the tissues were embedded in Epon 812, cut on a Porter-Blum ultratome and mounted on uncovered copper grids. The sections were viewed unstained or lightly stained with uranylacetate using an accelerating voltage of 40 kV for better contrast. For further improvement of contrast the photographic plates were slightly overexposed and overdeveloped. The specimens were examined in a Philips 300 electron microscope.

Results

Macroscopic Findings. At operation the synovium was usually found to be thickened and oedematous. The joint cavity was filled with a colourless mucinous jelly-like exudate. In the most advanced cases of joint destruction, there were extensive fibrous adhesions, the articular cartilage being destroyed. The gold-injected synovia showed a bluish grey colouring resulting from aggregation of colloidal gold particles (Gosselin, 1956). The regenerated synovia was very fibrotic while the steroid injected joint showed no specific changes.

Histology

The changes observed were on the whole similar in all the examined cases regardless of the treatment given. They consisted of:

(a) increased cellularity and proliferation of small blood vessels in the synovial and subsynovial tissues (Fig. 1).

(b) focal infiltration with inflammatory cells which included polymorphonuclear cells, plasma cells, lymphocytes, histiocytes and fibroblasts and

(c) hypertrophy of the synovial lining cells (Fig. 1). Proliferation of synovial lining cells appeared to be less prominent than in other forms of arthritis, but in some areas three or more layers of lining cells were seen.

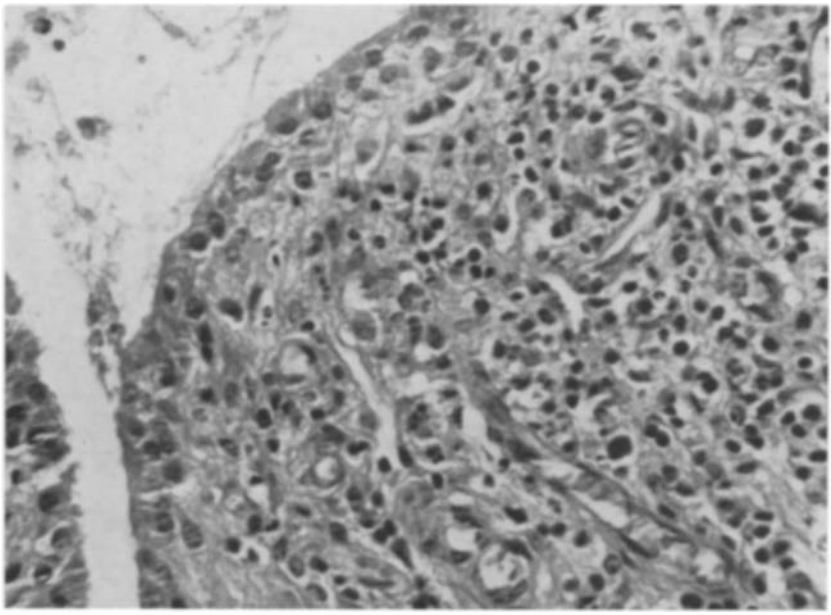

Fig. 1. Synovial membrane showing the cellular infiltration and small blood vessel proliferation. The infiltrate is mainly mononuclear but some polymorphs are also present (H and E $\times$ 415)

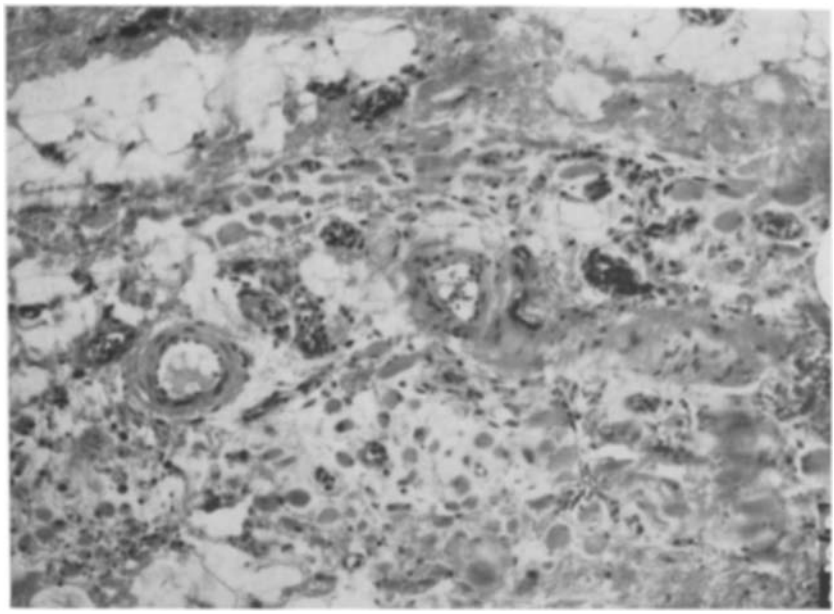

Fig. 2. Muscle showing atrophy, loss of peripheal nucleii and increased vascularity. As in the synovia the blood vessels here show perivenular cellularity and arteriolar thickening. (H and E $\times$ 170)

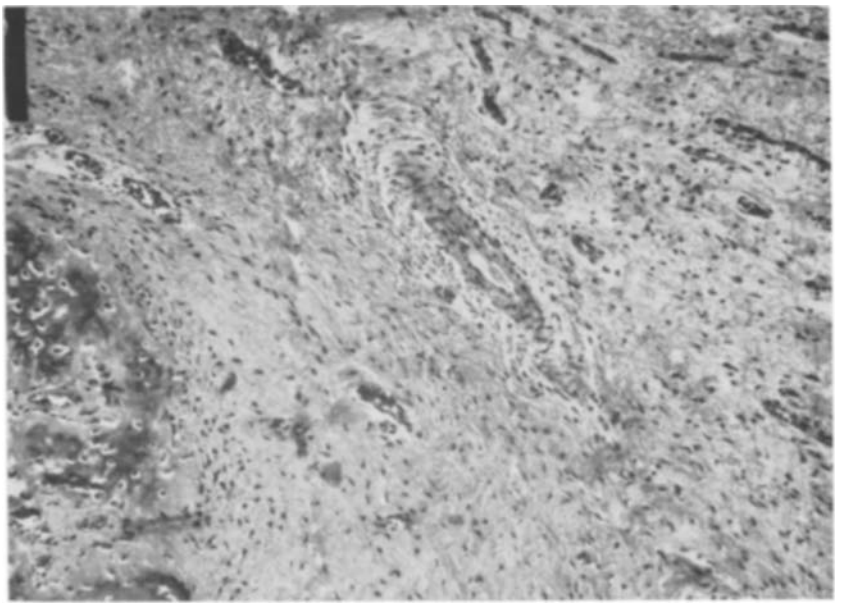

Fig. 3. Vascular fibrous tissue, invading reactive bone (left). The joint cavity is totally occluded, and no remnant of the articular cartilage is to be seen (H and E × 115)

The subsynovial layers contained large cells resembling the synovial lining cells, macrophages and many small blood vessels. In the deeper layers of the synovium there was more collagen, and less cellularity. Many small blood vessels were still present, including thick-walled arterioles and thin-walled venules Around the venules there was often a cellular accumulation resembling lymphorrhagies. Two of the biopsies included skeletal muscle taken at a distance from the musculo tendinous junction (Fig. 2). The myofibres were variable in size and often obviously atrophic. In between them there was an increased amount of fibrous tissue and fat. Small blood vessels were numerous and often showed the same changes as were noted in the vessels of the synovial layer. Nerve fibres were not found. In one knee joint specimen, fibrous tissue could be seen invading reactive bone, with no cartilage interposition (Fig. 3), while other cases showed definite pannus formation. Fibrin was often seen on the surface and between the synovial lining cells.

Conclusion

The histology of synovitis of Familial Mediterranean Fever showed an absence of the marked lining cell hyperplasia and lymphatic follicles formation usually encountered in rheumatoid arthritis. Nor was there any occlusion of small blood vessels by cells and thrombi as described in several forms of arthritis of recent onset (Schumacher and Kitridou, 1972). Yet, the proliferation of blood vessels in the synovial layer with perivascular lymphorrhagies was a very persistent feature. Giant cells, a feature of villondular synovitis (Willie, 1969) were also

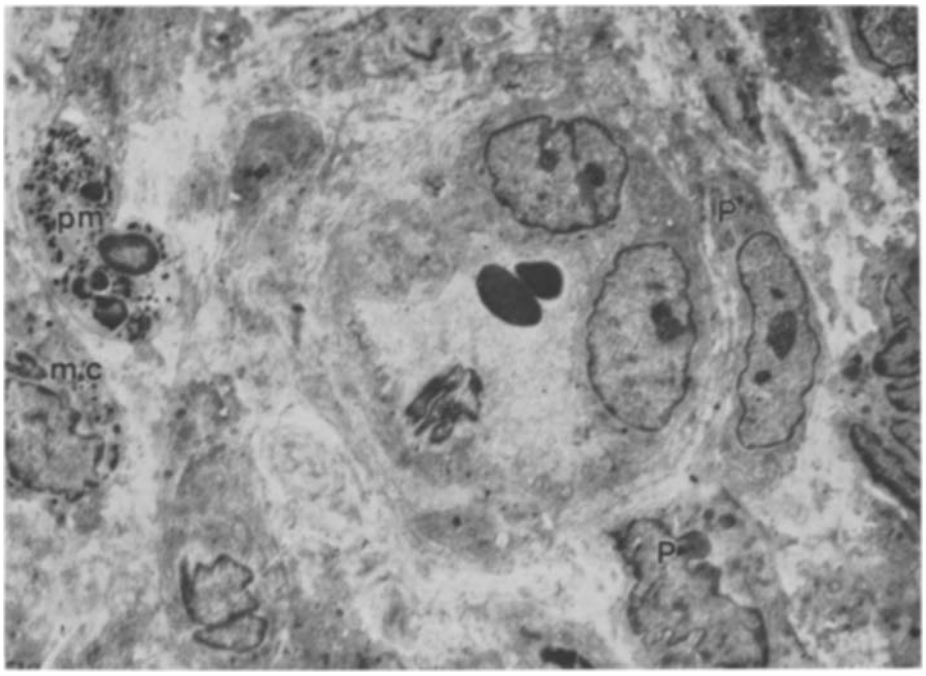

Fig. 4. Small synovial blood vessel and surrounding cells. A polymorph (*pm*), Macrophages (*mc*), a pericyte (*p*) and cells with indented nuclei can be seen (× 3000)

absent. On the other hand, many of the described features are rather similar to the changes often encountered in the synovium of osteoarthrosis.

Ultrastructural Findings

Biopsies were taken from both the superficial and the deep synovial layers. Upon electron microscopic examination, even more than with light microscopy, there was a striking similarity of morphology in all the cases.

The matrix was abundant and made up of amorphous and fibrillar material of varying electron density. In addition to much collagen, there were also fibres of various diameters, devoid of periodicity. The cells encountered varied in kind and size (Fig. 4). There were some polymorphonuclear granulocytes, lymphocytes, plasma cells and larger cells. These latter cells were either fibroblast-like (B-type synovial cells) or macrophage-like (A-type synovial cells). Mixed cell types were also seen. The macrophages had many lysosomes and some contained colloidal gold particles when this element was used (Fig. 5). In two cases macrophages containing intracellular electron dense thick fibres were seen, these fibres sometimes displayed collagen periodicity but often did not (Fig. 6). Fat containing cells were frequent.

An interesting finding was the presence of groups of cells surrounded by basement membrane-like material. These resembled the "precapillary structures"

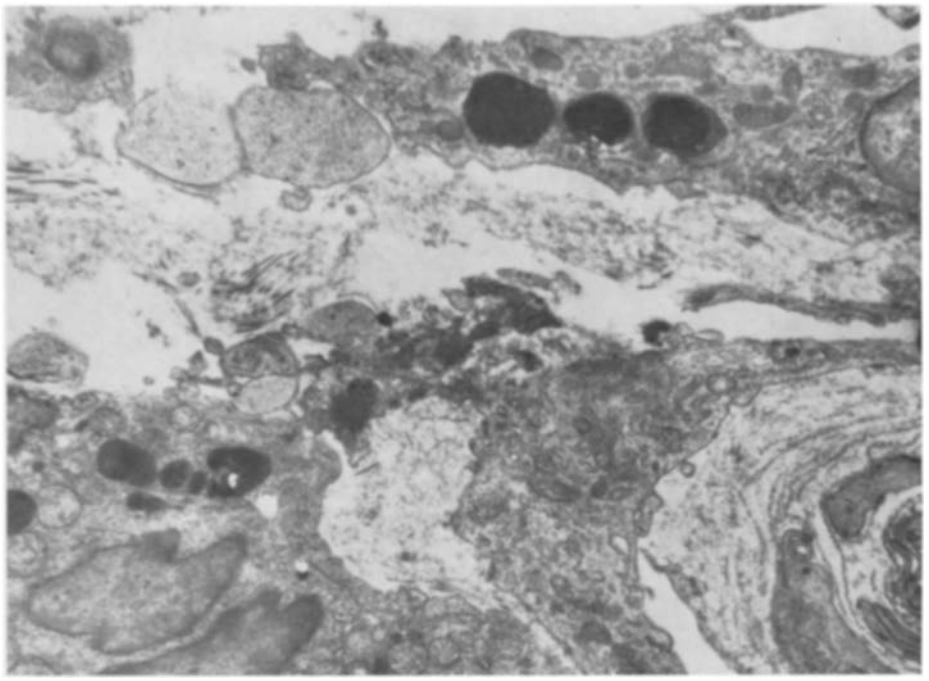

Fig. 5. Synovial tissue from a case treated with intraarticular colloidal gold shows the presence of electron dense particles in secondary lysosomes of macrophages. Note the multilayered basement membrane of small blood vessel at lower right (× 10000)

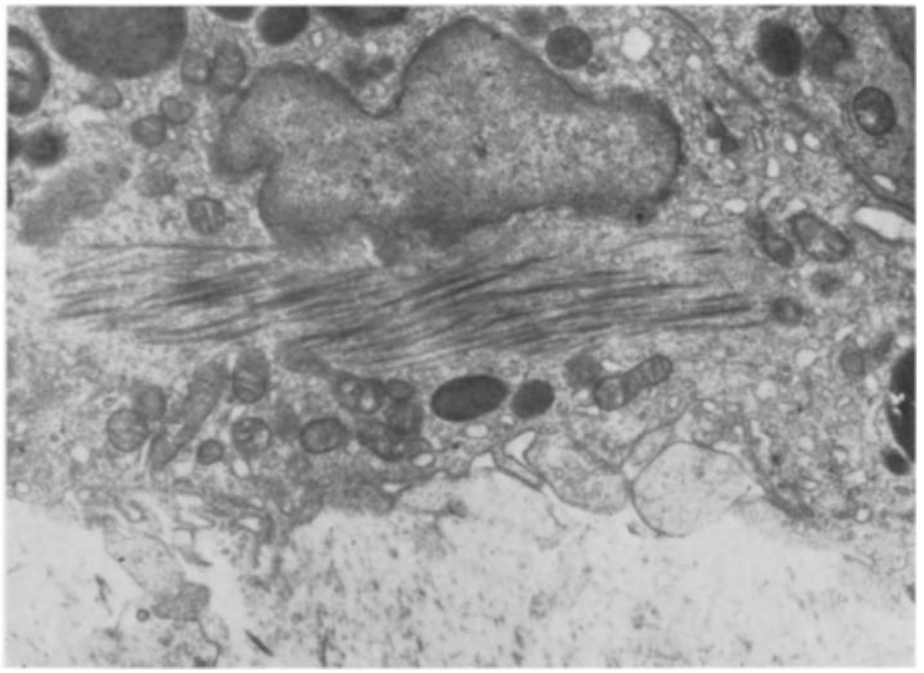

Fig. 6. Macrophage with intracellular collagen fibres. (× 10000)

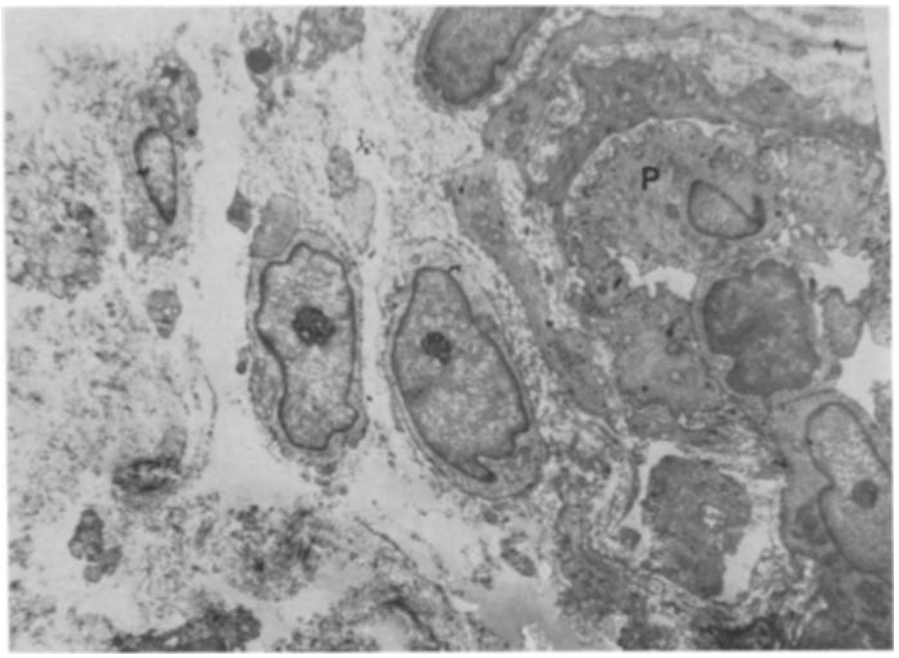

Fig. 7. A collection of cells possibly constituting a developing vessel. Pericyte-like cells are seen in close proximity (*P*) (×3500)

described in experiments with blood vessel cultures in diffusion chambers (Aloisi and Schiaffino, 1970; Aloisi and Schiaffino, 1971). The impression was that transitions occurred from solid "buds" to hollowed vessels through breakdown of parts of cells with incipient lumina formation containing erythrocytes (Figs. 4, 7). The cells in the buds as well as separate, single, ill defined cells and cells obviously endothelial in nature, often contained masses of fine filaments in their cytoplasm.

The basement membrane around some groups of cells, capillaries and other slightly bigger vessels was remarkable. It was very thick and organised in many concentric closely arranged layers, separated by a less dense ground substance (Figs. 4, 5, 7). In places, in between the layers of basement membrane, there were oblong cells, the pericytes, and their cell processes (Fig. 7). The pericytes varied in appearance. Some were simple and small, resembling lymphocytes perhaps, except for a more oblong shape and much paler nucleus with a prominent nucleolus. They were always surrounded by a well-defined basement membrane (Fig. 7). Other pericytes were larger, richer in cytoplasm and in organelles, containing rough endoplasmic reticulum, mitochondria, dense bodies, vesicle, and free ribosomes. They did not resemble mast cells in any way.

The nuclei varied from simple round and oval shapes to complicated, convoluted indented forms. Many cells resembling pericytes were seen some distance from the vessels (Figs. 4, 7).

Discussion

Joint involvement constitutes a cardinal feature of Familial Mediterranean Fever, occurring in up to 70 per cent of the affected patients (Heller *et al.*, 1966). It is accepted that in the protracted form of the disease, the episodes of arthritis last for weeks or months, with marked functional impairment due to a rapid developing muscle atrophy secondary to the synovial involvement (Heller *et al.*, 1966; Ehrlich, 1968). While exploring the joints of patients affected by the more severe and protracted form of the disease, we found extensive intraarticular damage and pannus formation.

It has been suggested previously (Heller *et al.*, 1966) that in FMF, the joint lesions consist of the development of acute and chronic granulation tissue. This study shows that such an interpretation is still valid, the histology of the affected synovium showing many similarities to the changes found in osteoarthritic synovium, but several additional features should be considered. Blood vessel changes are a prominent feature in FMF arthritis. These changes are similar to those described in synovitis of Reiter's disease (Norton, Lewis and Ziff, 1966), and in synovitis of recent onset caused by different pathologies (Schumacher and Kitridou, 1972). The pathogenesis of this vasculitis is yet to be defined. It is noteworthy that intra-articular injected steroids and colloidal radio-active gold do not seem to affect these vascular changes.

One of the prominent features of the blood vessel changes is the perivascular cellularity. Electron microscopy demonstrated that many of the perivascular cells resemble pericytes. In fact, pericyte like or transitional cells constituted a large part of the cellular elements in the diseased synovia. The origin of the cells in the non-specific stromal and synovial reactions is uncertain. Is it possible that there is a special role for pericytes as progenitors of other cells? Such a role has been suggested in relation to blood vessel wall components (Rhodin, 1968) and even in myogenesis (Van Haelst, 1970; Carlson, 1973), but appears worthy of further study.

An important ultra-structural aspect of the disease consists of the laminar thickening of the vascular basement membrane. Such a change has been described in various conditions such as diabetes (Vracko and Benditt, 1970; Vracko and Benditt, 1972), Reiter's disease (Norton, Lewis and Ziff, 1966), rheumatoid arthritis (Rhodin, 1968) and polyarthritis (Bierther and Wegner, 1971). In these descriptions, the layered basement membrane thickening has been noted but not further commented upon. Recent publications (Vracko and Benditt, 1970; Bierther and Wegner, 1971; Vracko and Benditt, 1972) suggest that lamination of basement membrane material is due to repeated episodes of cell death and replacement, each lamina representing the residue of one cell degeneration, each new cell generation producing only a "normal" complement of basal laminae. The old basement membrane serves as a microskeleton or scaffolding for regenerating cells.

The E/M appearance suggests new blood vessels formation. It has been shown that new blood vessels may form in several ways (Aloisi and Schiaffino, 1970; and 1971). In addition to the classical "loop" outgrowths, proliferation and migration of cells along remaining basement membrane supports may reconstitute

new channels. Another method of capillary genesis is that of lumen formation by "excavation" or degeneration in the centre of a solid collection of mesenchymal cells (Aloisi and Schiaffino, 1970). More cells are added outside and more degeneration may occur inside these "precapillary" structures. Thus, a widening of a new vessel can also take place. It appears to us that what occurs in synovial membranes (and perhaps in other affected tissues) in FMF, is a markedly high small blood vessels turnover by the above mentioned processes.

The occurrence of collagen fibres inside cells, although generally a rare finding, has been previously seen in human arthritis, (Ghadially and Roy, 1969), experimental arthritis (Cullen, 1970), chronic haemophilic arthropathy and scar tissue (Stein, 1974), morphogenesis, post-partum uterus and anuran metamorphosis (Parakkal, 1969). The collagen is usually enclosed in macrophages but has also been noted in endothelial cells, fibroblasts and osteoblasts (Parakkal, 1969). The significance of the above finding in FMF is not clear.

It is difficulty to assess from this study if the findings related to collagen are part of a reparative process or are an integral part of the disease process. The interpretation of finding intracellular collagen will have to wait for a better understanding of collagen metabolism in health and disease.

The findings in this study suggest that the small vessels of the synovial membrane are the principal target organ in joints affected by Familial Mediterranean Fever. This might help to explain the pathophysiology of the clinical picture with its variety of systemic symptoms, its undulating character with remissions and exacerbatious, as well as its sometime severe prognosis.

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