Iron in the Synovial Membrane in Rheumatoid Arthritis and Other Joint Diseases*

By

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The most constant pathological feature of rheumatoid arthritis is involvement of the synovial membrane. The lesions lack specificity and vary from site to site even in one joint, but generally they show exudation and cell proliferation. The exudates consist of fibrin and inflammatory cells including plasma cells and lymphocytes. Synovial lining cells become larger and more numerous and blood vessel proliferation is prominent. Less commonly reported are giant cells and haemosiderin deposits within the synovial tissue (Sokoloff, 1966).

In recent years the fine structure of the normal synovium in man and animals has been described and this has been followed by descriptions of the ultrastructure of rheumatoid synovial tissue. Barland, Novikoff, and Hamerman (1964) have described the presence of large cytoplasmic granules considered to be lysosomes in the Type A lining cell in rheumatoid arthritis. This feature has been confirmed by Hirohata and Kobayashi (1964) and by Norton and Ziff (1966). Recently the presence of ferritin molecules in many of these complex cytoplasmic granules and also scattered throughout the cell cytoplasm in the Type A lining cell has been described (Muirden, 1966). In continuing the latter studies, particles with the molecular dimensions of ferritin have been identified in fourteen out of fifteen open biopsies of synovium from rheumatoid patients. The consistency of this finding on electron microscopy indicated the need to survey larger areas of synovium than is possible with the electron microscope. The purpose of the present study was to examine with the light microscope rheumatoid synovium removed at open operation, using Prussian blue staining to demonstrate the distribution of iron. Samples of both normal synovium and synovium removed during surgery for other joint diseases have been examined in the same way. In a subsequent paper (Senator, Muirden, and Balazs, 1968), the concentration of iron measured chemically after ashing samples of synovial membrane will be presented.

Materials

Synovial tissue and fluid was obtained from 23 patients (17 female, 6 male) with rheumatoid arthritis (Table I, opposite) presenting for joint surgery at the Royal Melbourne Hospital.

All these patients satisfied standard diagnostic criteria for "classical" or "definite" rheumatoid arthritis (Ropes, 1959). Their ages ranged from 35 to 71 years.

Synovial tissue was also obtained from nine patients undergoing surgical procedures for joint pathology other than rheumatoid arthritis, and post mortem from the knee joints of five patients with no previous history of joint disease (Table II, opposite).

Methods

The tissue obtained at operation was immediately washed free of blood clot with chilled, iron-free, isotonic saline. The tissue to be used for histological studies was then fixed in neutral, phosphate-buffered, isotonic 10 per cent. formalin. When a considerable amount of tissue was available macroscopically differing areas of synovium were selected for study. However, where procedures were carried out on the hip or on the small joints of the extremities, frequently only one block of tissue could be sectioned. The formalin-free material was dehydrated with ethanol, cleared in benzene or chloroform, and embedded in paraffin. Sections were cut on a Leitz rotary microtome to a thickness of from 3 to 5μ.

Routine haematoxylin and eosin (H and E) staining was carried out on the second and fifth sections of a series of six cut from each block of tissue. The remaining four sections were stained for iron by the Prussian blue method (immersion in a fresh mixture of 2 per cent. potassium ferrocyanide and 2 per cent. hydrochloric acid for 20 minutes) and counterstained with safranin O.

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IRON IN SYNOVIAL MEMBRANE

23 PATIENTS WITH RHEUMATOID ARTHRITIS: HISTOLOGICAL DISTRIBUTION OF IRON

<table>
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<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Disease Duration (yrs)</th>
<th>Joint Examined</th>
<th>Haemosiderin in H and E Sections</th>
<th>Intimal Cells</th>
<th>Superficial Stroma</th>
<th>Deep Stroma</th>
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<td>46</td>
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<td>Knee</td>
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*Proximal interphalangeal joint. †Metacarpophalangeal joint. ‡Metatarsophalangeal joint.

TABLE II

14 NON-RHEUMATOID JOINTS: DIAGNOSIS AND PRESENCE OF IRON

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Joint Examined</th>
<th>Diagnosis</th>
<th>Haemosiderin in H and E Sections</th>
<th>Prussian Blue Staining</th>
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<td>24</td>
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<td>26</td>
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<td>37</td>
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<td>Autopsy—normal joint</td>
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<td>37</td>
<td>F</td>
<td>52</td>
<td>Knee</td>
<td>Autopsy—normal joint</td>
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Where large iron deposits were detected by this method, further sections were cut from different parts of the corresponding tissue block, and stained in the same way. Each block of tissue was evaluated with respect to its iron content in:

(a) Synovial surface (intimal) cells;
(b) The area of stroma immediately subjacent to the intima;
(c) The deeply-situated connective tissue stroma.

In these three regions a semi-quantitative grading of the iron content was made, ranging from 0 denoting no iron present, through + for small and patchy or diffuse but faintly staining deposits, up to +++++ signifying extensive and dense collections. The presence of the following features was also noted:

(a) Golden brown haemosiderin granules visible in H and E sections;
(b) Giant cells containing stainable iron.

Results

(A) Rheumatoid Arthritis (Cases 1 to 23)
The characteristic histological features of rheumatoid arthritis were present in the tissue obtained from all but one case. In the tissue obtained from Case 12 there was no obvious intimal proliferation, and here the only evidence of an inflammatory
process was the presence of scattered collections of lymphocytes and a mild, generalized increase in fibrous connective tissue.

The H and E sections showed that in about one-third of the cases many multinuclear giant cells were present in the connective tissue immediately beneath the intima. Under high magnification small golden-brown haemosiderin granules were seen in the peripheral cytoplasm of some of these giant cells. Haemosiderin granules also appeared in the sub-intimal areas within, or close to, inflammatory cell follicles, but the largest collections of haemosiderin occurred in the deeper stroma in association with increased amounts of fibrous tissue.

The extent and distribution of iron deposits was only fully appreciated in the Prussian blue stained sections (Fig. 1). In most biopsies synovial intimal cells showed in places faint blue staining. In some areas the majority of surface cells stained for iron, whilst elsewhere in the same joint iron was absent from many or all of the surface cells. In one patient (Case 1) intimal-cell iron proved to be the most prominent type of deposition in the tissues (Fig. 2, opposite).

Prussian blue staining was far more prominent in the stroma subjacent to the intimal layer and it occurred here in all but Case 12. In this zone iron was commonly found within the cytoplasm of macrophages which were often perivascular. Here, multinuclear giant cells were invariably noted to contain iron. Within the giant cells staining was evenly distributed except in three cases (6, 13, and 19), where discrete granules of iron-positive material were concentrated in the peripheral cytoplasm, leaving a central zone which occasionally stained faintly and evenly for iron (Fig. 3, opposite).

In Case 6 many multinuclear giant cells were seen to compose the synovial surface layer, but these were apparently iron free.

As with the haemosiderin collections noted in H and E stained sections, the largest iron deposits were seen in the deeper stroma, although these deposits were less frequent than in the subintimal zone. Enormous areas of dark blue to black stained material representing aggregates of extra-cellular iron were not uncommon.

In Cases 1 and 20 positive Prussian blue staining of collagen fibres was seen, but H and E stained

![Fig. 1.—Case 10, rheumatoid arthritis. Prussian blue stained tissue showing three areas of iron deposition. The surface layer of intimal cells (A) shows faint iron staining. The vascular stroma beneath the intima (B) is the area which most constantly contains iron. Large deposits of extra-cellular iron are seen in the deeper stroma (C) around which the fibrous tissue is very dense. × 150.](http://ard.bmj.com/fig1.jpg)
Fig. 2.—Case 1, rheumatoid arthritis. Prussian blue staining. The intimal cell layer is convoluted but not greatly thickened. Fine haemosiderin granules are scattered throughout the cells of the intimal layer and also in the subintimal macrophages. $\times 240$.

Fig. 3.—Case 13, rheumatoid arthritis. H and E staining. A multi-nuclear giant cell amongst inflammatory cells in the subintimal stroma is seen to contain small haemosiderin granules in the peripheral cytoplasm. Prussian blue staining confirms the iron content of these granules and reveals that the central zone of cytoplasm stains faintly and evenly for iron. $\times 500$. 

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sections lacked haemosiderin granules in this situation (Fig. 4).

A faint positive staining reaction for iron was occasionally found in cartilage fragments occurring in the synovial tissue, and, where giant cells were found in proximity to these fragments, positive iron staining was noted in the cytoplasm of these cells.

(B) Haemochromatosis associated with Joint Symptoms (Case 24)

Synovial tissue was obtained from the knee of a patient with haemochromatosis who complained of episodes of pain, swelling, and tenderness of the knee and small joints of the hands for 10 years. At the time of operation the patient’s serum iron was 200 μg./100 ml., while the synovial fluid iron was 86 μg./100 ml.

Histologically the synovial biopsy showed patchy intimal hyperplasia, but no inflammatory cell infiltration. In H and E stained sections, the surface cells contained many haemosiderin granules, and this was confirmed by Prussian blue staining. Small deposits of iron-positive material were detected in the connective tissue stroma, lying free in the connective tissue, and only occasionally in relation to blood vessels (Fig. 5, opposite).

In sections cut from a sample of radio-opaque articular cartilage removed concurrently with the synovial membrane, iron staining of the mature chondrocytes was noted in otherwise normal tissue.

(C) Pigmented Villonodular Synovitis (Case 25)

Synovial tissue was excised from the knee joint of a patient with an 8-year history of recurrent blood-stained effusions in the knee joint following a suspected menisceal tear. At meniscectomy 6 years previously, both the meniscus and synovium were considered macroscopically normal, although the synovial fluid appeared “sero-sanguinous”. There had been no history of joint symptoms elsewhere.

Macroscopically, the excised synovial tissue was strikingly “rusty” and appeared grossly thickened and villous.

Histological examination showed the intima, in many places, to be composed of large polyhedral cells, while in other areas large numbers of multinuclear giant cells were seen. Occasional foam cells containing haemosiderin granules were also scattered in the intima, but these cells occurred more prominently in the subintimal connective tissue stroma.

Fig. 4.—Case 1, rheumatoid arthritis. Prussian blue staining. In addition to iron staining of intimal cells, Prussian blue positive material can be seen along collagen fibres in the stroma. × 120.
where they were often seen in large aggregates. Scattered in the synovial tissue were occasional collections of lymphocytes but none of these contained germinal centres. Dense fibrous tissue was seen in some areas, and a marked increase in the size and number of the venules and capillaries was noted.

Prussian blue staining revealed large haemosiderin deposits mostly in the cytoplasm of foam cells and other macrophages. Smaller deposits were seen lying free in the connective tissue stroma, especially in the more superficial zone. Many polyhedral and a few multinucleated giant cells in the intima contained giant collections of iron (Figs 6 and 7, overleaf).

(D) Miscellaneous Joint Diseases (Cases 26 to 32)

This group consisted of cases of intermittent knee hydrarthrosis, osteo-arthritis of the hip, fractured neck of femur, osteo-chondritis dissecans of the knee, and postradiation necrosis of the femoral head. There was no Prussian blue staining of any of the synovial tissues obtained from this group.

(E) Normal Joints (Cases 33 to 37)

The synovial tissues excised post mortem from the knee joints of the five patients with no previous symptoms of joint disease showed no inflammatory or degenerative change, nor did they show a positive reaction with the Prussian blue stain.

Discussion

Although pathologists are aware that the synovial membrane can appear rusty in rheumatoid arthritis (Collins, 1951) and it has been said that “areas of haemorrhage are quite common as evidenced by the deposits of haemosiderin” (Sokoloff, 1966), the fact that iron deposits are consistently present does not appear to have been widely appreciated. In this study, using open surgical biopsies from 27 joints in 23 patients with rheumatoid arthritis, Prussian blue staining was present in all but one biopsy. In the exception (Case 12) biopsy material processed for electron microscopy contained granules with the appearance of ferritin in some of the surface cells.
Fig. 6.—Case 25, pigmented villo-nodular synovitis. Prussian blue staining. The villous nature of the tissue is clearly shown as are the dense deposits of iron in the connective tissue core of the villi. The paler-stained tissue represents foci of lymphocytes around blood vessels. × 50.

The most common site for iron, revealed by Prussian blue staining, appear to be in the vascular stroma just beneath the proliferated layer of synovial intimal cells. Larger patchy deposits were also frequently seen in the deeper connective tissue stroma. In most cases, the intimal cells showed positive staining in places although this was usually quite faint. This surface staining would correspond to the ferritin deposits seen by the electron microscope in the Type A surface cell (Muirden, 1966). Haemosiderin granules were seen in the connective tissue stroma in routine H and E stained sections in fifteen of the 27 biopsies.

Fine haemosiderin granules were seen in multinuclear giant cells which occurred in one-third of the biopsies. Prussian blue staining outlined these granules or revealed a faint, even blue staining throughout the cytoplasm. Grimley and Sokoloff (1966) have recently noted the frequency of such giant cells in sero-positive rheumatoid arthritis. The presence of iron and the peripheral distribution of the nuclei distinguishes these cells from multinuclear plasma cells or foreign-body giant cells which were also seen occasionally in rheumatoid synovium. Histological, chemical, and ultrastructural evidence suggests that these cells have features in common with the macrophage Type A synovial cell but the mode of formation of the giant cells remain uncertain.

In the fourteen non-rheumatoid biopsies obtained either post mortem from normal joints, or from surgical procedures from other joint diseases, iron was absent histologically except in those from patients with haemochromatosis and villo-nodular synovitis. The accumulation of haemosiderin in the synovium in haemochromatosis has been described by Sheldon (1935), who also found iron deposits in articular cartilage. Collins (1951) noted rusty pigmentation in the synovial membrane but not in cartilage. Cappell, Hutchison, and Jowett (1957) described cases of transfusional siderosis in which the synovial membrane was heavily pigmented, but was not proliferated in contrast to their case with classical haemochromatosis. The finding of iron deposition and synovial proliferation was also noted by Kra, Hollingsworth, and Finch (1965) in a patient who had haemochromatosis with joint symptoms. Schumacher (1964) however, found no proliferation in the pigmented synovium of patients with or without joint symptoms. He was unable to detect
any iron deposition in the articular cartilage. In our patient the cartilage showed iron staining of chondrocytes. Pre-operative x-rays had demonstrated "calcification" in this cartilage and we wondered if the iron deposits could at least partially explain the radio-opacity. However, Bauer and Jeffries (1966) have described a case of haemochromatosis in which the radio-opaque joint cartilage and meniscus contained calcium but not iron.

The occurrence of joint symptoms in approximately 10 per cent. of patients with haemochromatosis has been cited by Finch and Finch (1955), but it is much disputed whether the extensive haemosiderosis seen in the synovial membrane is responsible for the joint symptoms. The histological picture is quite distinct from rheumatoid arthritis in that the proliferation of synovial cells is of minor degree, there is no inflammatory cell infiltrate, and the iron deposits are confined to the surface layer or to the stroma immediately below the surface. Deposits in the deeper connective tissue stroma are not found.

In haemochromatosis, the persistently high serum iron levels could be linked with the heavy deposition of haemosiderin in synovial cells. By contrast, the serum iron in rheumatoid arthritis is characteristically low. Unless the synovial cells have a stronger affinity for iron it is unlikely that circulating transferrin-bound iron would be transferred directly to synovial cells in such quantities. Collins (1951) considered that pigmentation of the synovium will follow continued oozing of blood from the vascular granulation tissue. Evidence from studies of haemarthrosis accords with the idea that the extensive deposits of iron seen in rheumatoid arthritis come from red cells extruded into the synovial fluid and synovial membrane. The pathology of repeated haemarthroses in haemophilia and in experimental haemarthrosis shows obvious similarities in iron distribution. Key (1932) and Collins (1951) have noted hypertrophy of synovial lining cells with deposition of haemosiderin in the lining cells and in macrophages in the sub-intimal stroma in haemophilic arthropathy. Hoaglund (1967) has noted similar changes in the knee joints of dogs after repeated injections of blood.

The synovial fluid in rheumatoid arthritis is occasionally blood-stained even in the absence of...
trauma. Where there is no macroscopic blood we have noted that the fluid frequently contains appreciable numbers of erythrocytes when examined microscopically. In experimental haemarthrosis, substantial numbers of red cells escape from the joint space into the synovial matrix (Key, 1929). Roy and Ghadially (1966) have recently proved that erythrophagocytosis by synovial cells occurs and we have shown that synovial cells in culture will absorb haemoglobin from the culture medium and transform the iron to ferritin (Muirden, Fraser, and Clarris, 1967). The haemosiderin-laden macrophages seen in the subsynovial stroma in experimental haemarthrosis and rheumatoid arthritis may arise from the phagocytic synovial surface cells, as has been suggested by Roy and Ghadially (1966).

If the synovial iron deposits in rheumatoid arthritis do arise from the continued oozing of blood from the vascular granulation tissue, then this effect could be aggravated by trauma. The joints most likely to be affected by trauma would be the weight-bearing joints, but in a study of haemophilic patients Ghormley and Clegg (1948) noted that, while the knee and ankle were the joints most affected by spontaneous haemarthrosis, involvement of elbow, hand, and wrist joints accounted for 43 per cent. of joint changes. In the present series of rheumatoid patients no difference was found between synovial tissue iron in weight-bearing and non-weight-bearing joints. Nevertheless it is possible that, in the advanced stages of rheumatoid joint disease, any movement of the deformed unstable joint may compress hypertrophied villi or folds of synovial membrane, and so lead to extravasation of blood.

The distribution of iron in pigmented villonodular synovitis shows pathological similarities to rheumatoid synovium as distinct from haemochromatosis. Clinically a characteristic finding is of a sero-sanguinous synovial fluid which could have a bearing on the large amounts of iron in the synovial membrane. However there is some disagreement whether repeated haemarthroses can produce a picture like villo-nodular synovitis (Volz, 1966; Hoaglund, 1967). Other factors would seem to be involved to explain the extensive villous net-work and the lymphocyte collections which are a feature of the pathology of this unusual condition.

Since iron deposition is a constant finding in the synovium of rheumatoid arthritis, does its presence play any part in the pathogenesis of this condition? Iron excess does appear to cause tissue damage in haemochromatosis but the pathology of the synovium in this condition is unlike rheumatoid arthritis. The possibility that incorporation of iron as ferritin in synovial cell lysosomes may cause lysosomal enzyme release and that these enzymes may cause tissue damage has been recently discussed (Muirden, 1966), but as yet there is no proof of this.

Whatever the significance of haemosiderin in relation to the pathogenesis of rheumatoid arthritis, the extensive iron deposits warrant consideration as a factor in the causation of the anaemia of this disease. The aetiology of the anaemia remains uncertain. A common feature is hypoferraemia which is coupled with a rapid plasma clearance of iron (Raymond, Bowie, and Dugan, 1965). These workers have confirmed the work of Freireich, Ross, Bayles, Emerson, and Finch (1957b), which showed that the utilization of exogenous iron from erythrocyte production was unimpaired and that the total daily production of erythrocyte haemoglobin was normal. However, there is some evidence that the metabolism of endogenous iron may be abnormal. A defect in the release of iron derived from effete red cells has been shown in the anaemia of inflammation and infection by Freireich, Miller, Emerson, and Ross (1957a) and by Haurani, Burke, and Martinez (1965). Evidence provided by Weinstein (1959) and Owen and Lawson (1966) has suggested that diminished availability of iron from storage sites in the reticulo-endothelial system also occurs in rheumatoid arthritis. Defective re-utilization of iron from senescent red cells would lead to hypoferraemia and thus to an inadequate supply of iron for red cell production.

If the principal fault in iron metabolism in rheumatoid arthritis lies in the release of iron from storage sites, then one sizeable storage site which may be involved occurs in the synovial membrane. The sequestration of iron in the extensive synovial areas in this disease may result in defective re-utilization of iron and may so be a factor in the production of anaemia. Circumstantial evidence comes from patients with haemochromatosis and joint disease reported by Schumacher (1964) by Kra and others (1965), and by Bauer and Jeffries (1966). In these patients extensive phlebotomy sufficient to reduce the serum iron to normal and deplete the liver and bone marrow failed to affect the synovial membrane which remained heavily infiltrated with iron. It was suggested that synovial iron exchanged poorly with the labile iron pool. The patient with haemochromatosis reported here (Case 24) had also been treated over some years with repeated phlebotomy but at irregular intervals. The absence of a post-treatment liver biopsy for comparison with the extensive synovial iron deposits makes it impossible to draw conclusions from this case.
**IRON IN SYNOVIAL MEMBRANE**

**Summary**

Histological examination of the synovial membrane has demonstrated that iron deposits are a constant feature of the pathology of rheumatoid arthritis. Using material from 27 joints in 23 patients with classical or definite disease, Prussian blue positive staining was present in all but one biopsy. Haemosiderin granules were seen in routine haematoxylin and eosin stained sections in fifteen of the 27 biopsies. Iron was also present in the characteristic multinuclear giant cells which occurred in one-third of the biopsies.

In fourteen biopsies from non-rheumatoid joints, iron was absent except in one case of haemochromatosis and in one case of pigmented villo-nodular synovitis.

It is suggested that iron deposits in rheumatoid arthritis arise from continued oozing of blood from the vascular granulation tissue into the synovial cavity.

The possible significance of these findings in relation to the anaemia and pathogenesis of rheumatoid arthritis is discussed.

We are grateful to Dr. R. F. A. Strang for referring many of the cases in this study. The case of haemochromatosis was referred by Dr. J. R. E. Fraser and the case of villo-nodular synovitis by Dr. S. Milazzo of Adelaide. The biopsy material was provided by Messrs. W. Swaney, K. Mills, J. Hueston, O. Deacon, P. Kudelka, D. Ritchie, and M. Wearne of the Royal Melbourne Hospital, and Mr. R. Doig of Prince Henry's Hospital, Melbourne, and for their cooperation we are greatly indebted.

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**REFERENCES**


Le fer dans la membrane synoviale au cours de la polyarthrite rhumatoïde et des autres maladies des articulations

RÉSUMÉ

L'examen histologique de la membrane synoviale a montré que les dépôts de fer représentent un caractère constant de la pathologie de la polyarthrite rhumatoïde. La coloration bleue de Prusse positive fut retrouvée dans tous les cas, sauf un, de biopsies provenant de 27 articulations de 23 malades, atteints de maladie "classique" ou "dénée". On observa des granules d'hémosiderine sur des coupes colorées de façon habituelle à l'hématoxyline et à l'œsine dans 15 des 27 biopsies. Le fer fut aussi retrouvé dans les cellules géantes multinucléées caractéristiques, présentes dans un tiers des biopsies.

Dans 14 cas de biopsies d'articulations non rhumatoïdes on ne trouva de fer que dans un cas d'hémochromatose et un cas de synovite pigmentée villo-nodulaire.

On suggère que les dépôts de fer au cours de la polyarthrite rhumatoïde proviennent du sang venant du tissu de granulation vasculaire et pénétrant dans la cavité synoviale.

On discute la portée de ces résultats à propos de l'anémie et de la pathogénie de la polyarthrite rhumatoïde.

El hierro en la membrana sinovial de enfermos con artritis reumatoide y con otras enfermedades articulares

RESÚMEN

El examen histológico de la membrana sinovial comprobó que depósitos de hierro representan un rasgo constante de la patología de la artritis reumatoide. La coloración al azul de Prusia positiva fue encontrada en todos los casos, menos uno, de biopsias de 27 articulaciones de 23 enfermos con enfermedad "clásica" o "definida". Se observaron gránulos de hemosiderina en cortes colorados de manera habitual con hematoxilina y eosina en 15 de las 27 biopsias. El hierro fue también visto en células gigantes multinucleadas características, presentes en una tercera parte de las biopsias.

En 14 casos de biopsias de articulaciones no reumatoideas el hierro fue encontrado sólo en un caso de hemocromatosis y en un caso de sinovitis pigmentosa villo-nodular.

Se sugiere que los depósitos de hierro en la artritis reumatoide proceden de la sangre que mana persistentemente del tejido de granulación vascular en la cavidad sinovial.

Se discute la importancia de estos resultados en relación con la anemia y la patogénesis de la artritis reumatoide.
Iron in the synovial membrane in rheumatoid arthritis and other joint diseases.

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