THE UPTAKE OF IRON IN RABBIT SYNOVIAL TISSUE FOLLOWING INTRA-ARTICULAR INJECTION OF IRON DEXTRAN

A Light and Electron Microscope Study

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ABSTRACT

Iron dextran (molecular weight 7,000) diffuses rapidly from the joint cavity through the synovium, along lymphatics and extracellular tissue spaces; articular cartilage is impermeable to iron dextran. There is also rapid cellular uptake by synovial lining cells, particularly of the vacuolar type; endoplasmic reticulum–containing lining cells rarely take up iron dextran. Cellular uptake is probably effected by pseudopodial folds projecting from the cell surface and enclosing extracellular material. Cells containing iron may degenerate and be ingested by phagocytes, and this may account for the concentration of iron in a smaller proportion of cells on or below the synovial surface in the later stages. At 6 to 18 hours after injection there is a mild inflammatory reaction and some synovial proliferation; from this stage onwards intracellular iron occurs in the form of haemosiderin. Granules of haemosiderin are present in the synovium 3 months after injection and possibly longer.

INTRODUCTION

The synovial membrane of the normal knee joint of the rabbit appears as a glistening, translucent layer less than 1 mm thick which lines the joint cavity and which is loosely or firmly bound to subjacent structures forming the joint capsule. Its general features closely resemble those of human synovial tissue as described for example by Barnett, Davies, and MacConaill (4). Histologically the membrane can be seen to consist of a finely fibrous tissue containing a prominent vascular bed. At the surface bordering the joint cavity there is an apparently discontinuous layer of cells (the synovial intimal or lining cells), the cytoplasm of which is often not clearly delineated by light microscopy from the neighbouring fibrous tissue. Apart from their position at the synovial surface, synovial cells are not readily distinguished morphologically from other connective tissue cells in the membrane but several workers have shown that intimal cells possess cytochemical properties and enzymatic activities which differentiate them from the fibrocytes of the stroma of the synovial membrane and subjacent capsular tissues (9, 20, 21, 31).

The ultrastructure of synovial intimal cells has been studied by a number of workers (3, 13, 25, 26). Barland, Novikoff, and Hamerman (3) ob-

served that human synovial intimal cells could be classified on the basis of their ultrastructural morphology into two main cell types (or into two states of functional activity of a single cell type), the commoner cell being characterised by many vacuoles and filopodial projections of the cytoplasm and the other by an abundant endoplasmic reticulum and an absence of vacuoles. We have confirmed that healthy rabbit synovium presents a similar picture (12) and we shall report a detailed study of this material later. The vacuolar cells are irregular in outline and the vacuoles (up to 1 micron in diameter) are mostly clear; a few enclose varying amounts of amorphous material. Cells with an abundant endoplasmic reticulum are smoother in outline and those which lie in the deeper aspects of the intimal layer may possess elongated cytoplasmic processes extending to and spreading out at the synovial surface. Both types of cell possess numerous micropinocytotic vesicles. In rabbit synovium wide gaps of intercellular material may occur between cells; in the surface region this material is either amorphous or consists of fine unstriated filaments less than 100 A in diameter; in the deeper regions the intercellular material is predominantly collagen. Contrary to the observations described by Langer and Huth (25) we have no evidence of any form of basement membrane below the intimal layer.

Synovial membrane has two main functions: (a) the production of synovial fluid components and (b) absorption from the joint cavity (Rynearson, 34). Synovial fluid hyaluronate is produced in synovial tissue cultures (8, 18, 22, 24, 35, 37) and there is some evidence suggesting that it is the intimal cells which are responsible for synthesis (20). Active turnover of other components of synovial fluid is also likely to occur and mechanisms must certainly exist for the removal of joint debris and foreign materials from the joint cavity.

The role of intimal cells in absorptive processes remains uncertain. Key (23) injected 50 per cent india ink into rabbit knee joints and maintained that most of the carbon was absorbed by macrophages; these passed freely through the synovial layer to settle finally in the subsynovial tissue. He also noted that a small amount of finely divided carbon was taken up by the synovial intimal cells themselves. Adkins and Davies (1) reported that true solutions injected into the rabbit knee joint were removed in a few hours via capillaries and lymphatics (mainly the former) whereas larger col-

loidal particles, such as india ink and colloidal mercuric sulphide, did not readily enter either channel apart from small quantities which passed via lymphatics to regional lymph nodes. These authors did not report uptake of either true solutions or colloidal particles by synovial intimal cells, although diffusion into subsynovial tissue was observed. The prompt removal of serum proteins from the knee joint into the lymphatic system and the blood stream has been demonstrated by serological tests (5) and by radioactive tracer techniques (33).

The division of intimal cells into two morphological types (or states of activity) may well be associated with the two functions attributed to synovial tissue; the present investigation was undertaken to study the absorptive function and its relation to the structural differentiation of the two cell types existing in the intimal layer. Iron, in the form of iron dextran complex, was injected into the knee joints of rabbits so that the subsequent fate of the iron could be followed by light and electron microscopy. Iron dextran is readily visible in the electron microscope and it is known, from experiments with subcutaneously injected iron dextran, that the complex is taken up by cells and converted to ferritin (27). Our results indicate that iron dextran (of average molecular weight 7000) passes rapidly both into and between synovial intimal cells; it will be shown that considerable cellular uptake occurs in intimal cells of the vacuolar type, and that intracellular iron is still present in the synovium three months after injection.

MATERIALS AND METHODS

Iron dextran complex (Imferon) from Benger Laboratories, Holmes Chapel, Cheshire, England, containing 5 per cent w/v Fe and 20 per cent w/v dextran and having a weight-average molecular weight of 6500 to 7600 was used (2, 17). Dutch rabbits weighing about 2 kg were anesthetised with about 1.0 ml intravenously injected Nembutal with or without additional open ether; 0.5 ml iron dextran complex was then injected through the patellar ligament into each knee joint. Animals were sacrificed at various intervals of time after iron dextran injection (½, 2, 6, and 18 hours; and 2, 4, 14, 30, and 90 days) by asphyxiation under Nembutal anesthesia, the limb being raised to produce a relatively bloodless field during excision of the synovial specimen. In the first experimental series, involving 9 animals (one at each time point), tissue from one joint was taken for electron microscopy and the other joint was used for

histology. In a second series, using the same number of animals at the same time points, only histological studies were made. In this series the amount of iron dextran injected into individual knee joints was sometimes varied, 0.2 ml being injected into one joint while 0.5 ml was injected into the other; no significant qualitative differences were observed. Three animals were injected with 0.9 per cent saline and sacrificed after ½ hour, 6 hours, and 3 days. In no instance did secondary infection occur.

Light Microscopy

For light microscopical examination the joint was opened by a parapatellar incision and the cavity was rinsed out with saline. The anterior part of the joint capsule, including the patella and attached ligaments, was then removed and the synovial surface briefly washed with a stream of saline. When both knees were available the whole specimen from one knee was immersed in 50 per cent ethanol containing 0.4 per cent acridine (36), the specimen from the other knee being fixed in Veronal-buffered 4 per cent aqueous formaldehyde (pH 7.2). When only one knee was available the specimen was divided between the two fixatives. Fixation was continued for at least 2 days. Blocks were prepared from the suprapatellar fat pad and also from the lateral or medial part of the capsule above the joint line; the results from both situations were essentially the same. Specimens were dehydrated in ethanol and embedded in low-viscosity nitrocellulose and wax. Sections, 2 to 5 microns thick, were stained routinely with haemalum and eosin and for iron by Perls' method as modified by Lison and by Bunting (30), using neutral red as a counterstain.

During fixation in aqueous formaldehyde tissue stained with iron dextran gradually paled as the iron leached out from the tissues (Fig. 1). This did not occur during fixation in acridine-ethanol which precipitates iron dextran from solution. Thus only insoluble (bound) iron complex was detectable in formalin-fixed specimens whereas both colloidal iron dextran and bound iron complex could be localised in acridine-ethanol fixed material. Hereinafter iron-positive material detectable only in acridine-ethanol fixed tissue is referred to as "free iron"; iron-positive material detectable in both acridine-ethanol and formalin fixed tissue will be called "bound iron." Free iron was not easily detected in haemalum-eosin stained sections though it could sometimes be recognized as a finely granular, greyish deposit, especially in extracellular sites; bound iron in haemalum-eosin stained sections had the typical golden brown colour and morphological appearance of haemosiderin.

Electron Microscopy

Immediately after sacrifice the exposed synovium was flooded rapidly with several changes of fresh fixative at 0°C to 4°C and tissue was removed from an area lateral or medial to the patella above the joint line. Specimens from other locations were occasionally selected for examination but all the descriptions given in this paper refer to tissue from the parapatellar region. The excised tissue was immersed in a few drops of fixative, sliced into small pieces and transferred to further fixative. The fixative was ice-cold 1 per cent solution of osmium tetroxide in Veronal-acetate buffer, pH 7.2 to 7.4 (28), made isotonic with sodium chloride. After fixation for 1 hour the tissue was dehydrated in ethanol. Most specimens were stained for 1 hour in 1 per cent phosphotungstic acid in ethanol at this stage; the electron micrographs of Figs. 5 to 15 are all from stained material. Embedding was carried out in Araldite according to Glauert and Glauert (16), but using longer impregnation times and polymerization at 48°C for 3 to 4 days. Care was necessary to avoid mechanical damage of the synovial surface during the preparative procedures.

No attempt was made to orientate the specimen in the block (as suggested by Coulter, 13), blocks being selected in which part of the specimen occurred in a suitable orientation. Trimming of blocks was facilitated by special methods which are described in a separate paper (11). Thin sections were cut on a Huxley mechanical advance ultramicrotome, using a glass knife, and were mounted on carbon-filmed grids (7, 10). Sections were examined in a Siemens Elmiskop I operating at 80 kv, or in a Philips EM 75 at 75 kv.

A few thicker sections were examined at very low electron optical magnifications (down to 150 X) in the Siemens Elmiskop I, using the objective lens as the only imaging lens and with an operating voltage of 40 kv. This technique, which enabled relatively large areas to be recorded in one micrograph at a resolution better than that obtainable by light microscopy, is described in more detail separately (11). The section shown in Fig. 5 was examined in this way.

OBSERVATIONS

General

The intra-articular injection of 0.2 to 0.5 ml of iron dextran caused some slight distention of the joint. At ½ hour later the para-articular ligaments and the fascial sheaths covering muscles were faintly tinged brown; this feature may be particularly prominent in the rabbit knee because of the intra-articular location of the extensor digitorum longus tendon and its covering synovial sheath (Adkins and Davies, 1). Para-articular staining of collagenous tissue persisted as long as

the synovial fluid remained deeply discoloured; i. e., for about 4 days. On opening the joint and washing away free fluid the synovial membrane was visibly stained a brownish colour. This colour, which was apparent ½ hour after injection, deepened to a maximum at about 6 to 18 hours and then gradually became less intense. In contrast the articular cartilage remained unstained throughout.

A few hours after the injection of iron dextran a transitory mild synovial inflammation commenced. In what follows it is convenient therefore to consider our observations in three phases; (a) the pre-inflammatory phase (up to about 6 hours after injection) (b) the inflammatory phase, (18 hours to 48 hours) and (c) the recovery phase. Histologically only free iron (see Materials and Methods, Light Microscopy) was detected in the pre-inflammatory phase whereas only bound iron was present in the recovery phase; both free and bound iron occurred in the inflammatory phase. These observations are summarised in Table I.

Pre-Inflammatory Phase

LIGHT MICROSCOPY

Throughout this early phase the collagenous intercellular stroma in the synovium and the reticulum of subsynovial adipose tissue were stained with iron (Fig. 2). The cytoplasm of synovial cells was neither abundant nor clearly delineated and ½ hour after injection it was difficult to be sure if iron was present intracellularly. By 2 hours the synovial cells had enlarged to some extent and free iron could be detected in some cells as a faint diffuse staining although many cells appeared to contain no iron. By 6 hours the synovium was oedematous, indicating the onset of inflammation, and iron was present in many synovial cells. Throughout this phase a finely granular deposit of iron, more prominent in some areas than others, was found at the surface of the lining layer. Iron could not be detected in the matrix of articular cartilage in this phase, nor could it be detected at any subsequent stage. No evidence of haemorrhage into the synovial tissues or synovial cavity was encountered.

ELECTRON MICROSCOPY

The predominant cells in the preinflammatory phase were those containing numerous vacuoles, round or oval in outline and up to 1 micron in diameter (Figs. 5 and 6). Not only were these larger and more abundant than the vacuoles oc-

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Figs. 1 to 4 are photomicrographs; the remaining figures are electron micrographs.

FIGURE 1   Anterior part of the rabbit knee joint; ½ hour after injection the synovium is heavily stained with iron dextran in specimens fixed in acridine-ethanol (A) but shows negligible staining after fixation in formalin (F); on the 5th day synovial staining is much less and differences resulting from the two methods of fixation are only just discernible. × 1.5.

FIGURE 2   2 hours after injection of iron dextran and fixation in acridine-ethanol, the synovium shows iron staining of the stroma of cellular surface layers, the adipose reticular tissue and collagenous strands.

2 a, stained with Perls' iron and neutral red. × 480.

2 b, neighbouring section stained with Perls' iron only (i.e. without the neutral red counterstain) showing that the staining of the intercellular stroma is due to reaction with iron. × 480.

FIGURE 3   18 hours after iron dextran injection there is diffuse iron staining of the cytoplasm of some synovial cells and the oedematous synovial stroma and the supporting tissue of subjacent fat (in which occasional polymorphs can be seen). In addition some cells at the surface and others in the deeper fat contain densely staining spherical "haemosiderin-type" granules (arrows). Some shedding of surface cells can be seen. Acridine-ethanol fixation; Perls' iron and neutral red staining. × 480.

FIGURE 4   3 months after iron dextran: iron staining is largely restricted to the cytoplasm of large cells lying just beneath the surface (arrows) and in a smaller number of similar cells in the deeper adipose tissue; the supporting framework of this adispose tissue is unstained. Acridine-ethanol fixation; Perls' iron and neutral red staining. × 480.

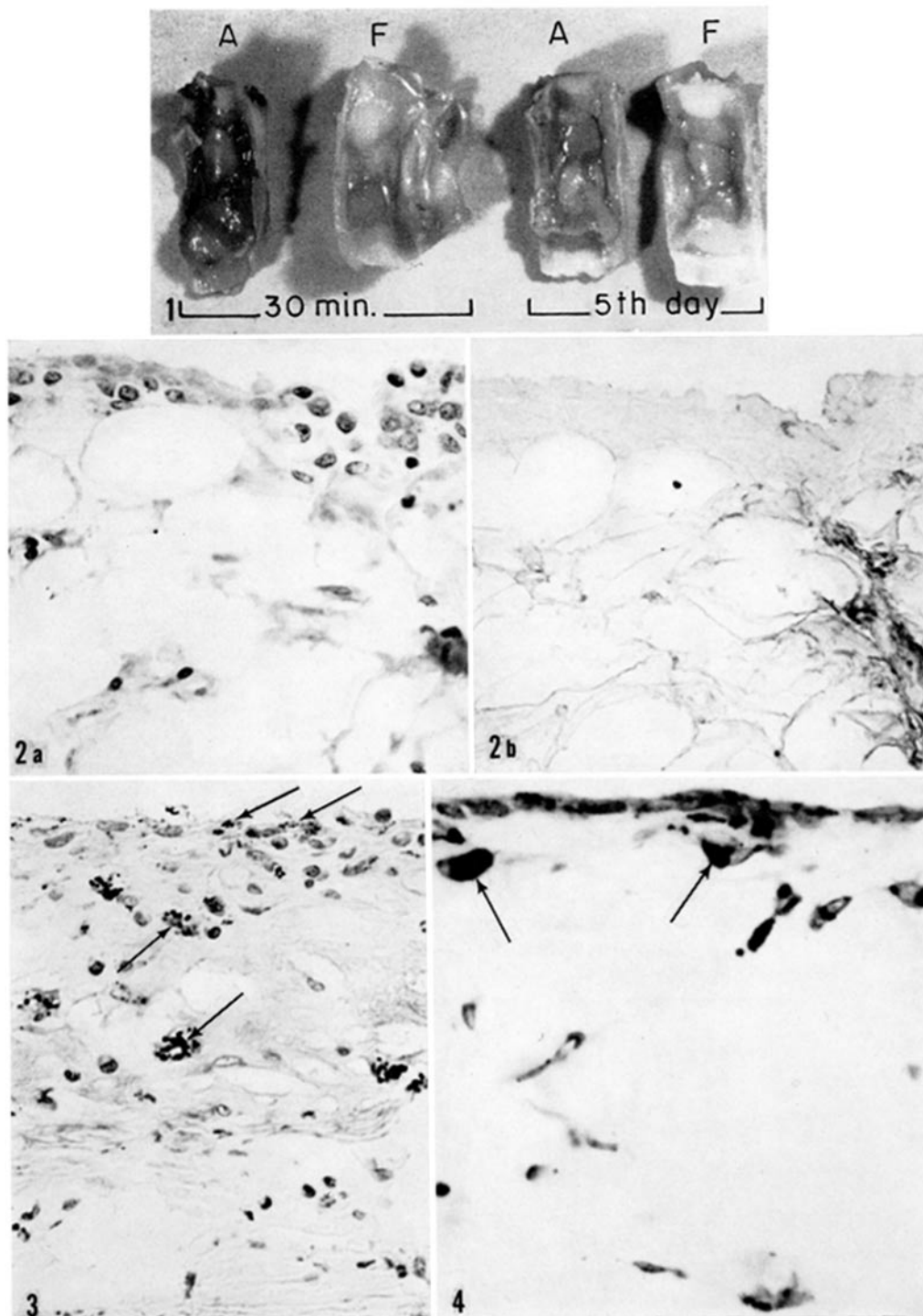J. BALL, J. A. CHAPMAN, AND K. D. MUIRDEN   *Uptake of Iron by Rabbit Synovial Tissue*   355

Phase	Macroscopic	Histological				
		Free iron			Bound iron	
	Iron dextran in joint fluid	Adipose reticulum	Synovial intercellular stroma	Synovial cells	Synovial cells	Sub-synovial phagocytes
Preinflammatory phase (up to 6 hrs.)	++	++	++	± → +	−	−
Inflammatory phase (18 hrs. and 2 days)	+	+	+	some + some −	mainly +	+
Recovery phase (from 4 days onwards)	± → −	± → −	± → −	−	some + some −	++

++, strong reaction for iron.
+, moderate reaction for iron.
±, weak or doubtful reaction for iron.
−, no iron detected.

curring in vacuolated cells of normal synovial membrane but they contained loosely packed particulate and finely filamentous material, either scattered throughout a vacuole or located around its inner edge. In Fig. 8 (of a vacuolar cell ½ hour after injection) this material is seen to include numbers of electron opaque particles and to show the characteristic appearance of iron dextran (27, 32). A smaller number of electron-opaque particles, also assumed to be iron dextran, occurred in the extravacuolar cytoplasm and in scattered form in small irregular vacuoles just below the cell surface. The appearance after 2 hours was similar but a higher proportion of vacuoles possessed material distributed throughout the vacuole and not merely located around the inner edge.

Even at low electron optical magnifications a conspicuous feature of the early phase was the abnormal abundance of irregular pseudopodial processes protruding from intimal cells into the synovial cavity and into the extracellular space below the synovial surface (Fig. 5). These pseudopodia were invariably associated with vacuolar type cells and were found in several forms. In the simplest a single fold of cytoplasm extended for several microns from the cell surface (left side of Fig. 7); in others the cell surface was thrown into a complex series of coalescing folds apparently enclosing a small region of previously extracellular spaces (right side of Fig. 7). Electron-opaque particles were sometimes observed in these enclosed regions. The largest pseudopodial processes enclosed rounded vacuoles up to 1 micron in diameter and usually containing a small amount of electron opaque material (Figs. 5 and 6); these vacuoles were identical in appearance with those occurring in the body of the vacuolar cell.

A characteristic feature of both vacuolated and

FIGURE 5 Low magnification electron micrograph of a crypt in the synovial membrane, ½ hour after iron dextran injection, showing vacuolar cells (*v*) and pseudopodial processes (*p*), frequently containing vacuoles. × 1,500.

FIGURE 6 A typical vacuolar cell from the same specimen as that of Fig. 5. The vacuoles contain varying amounts of finely divided material. In one place (arrow), two vacuoles show evidence of coalescence (or, less probably, division). Numerous pseudopodia (*p*) occur, some containing vacuoles (*p₁*). The joint cavity (*jc*) and collagen fibrils (*c*) are shown. × 12,000.

FIGURE 7 Pseudopodial processes extending from the cytoplasm of a vacuolar cell of the same ½ hour specimen. Some electron-opaque particulate matter (arrows) is in contact with the cytoplasmic membrane in what appears to be a newly-formed vacuole. × 40,000.
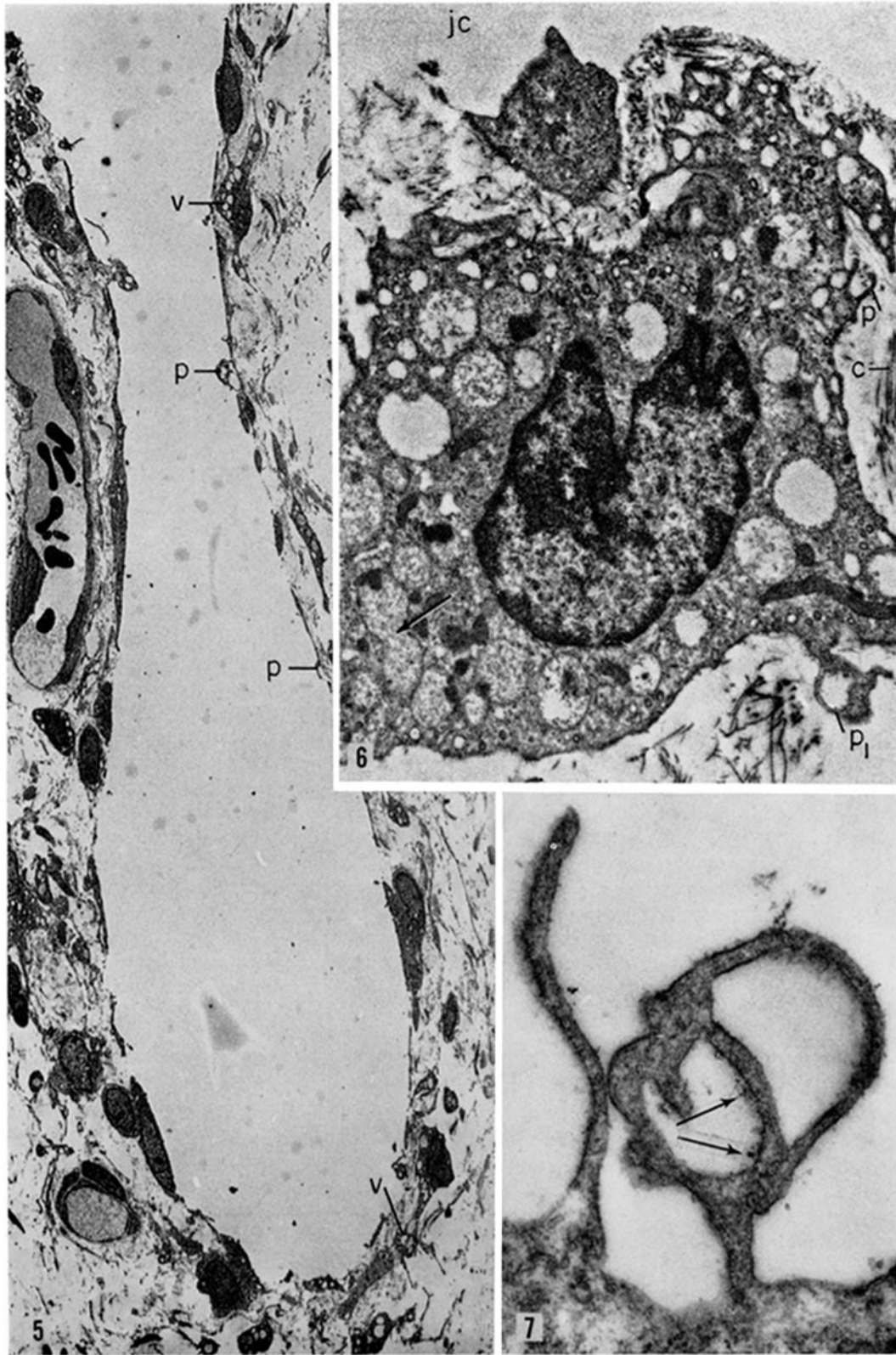
non-vacuolated normal synovial intimal cells is the presence of numerous micropinocytotic vesicles distributed throughout the peripheral regions of cells and frequently showing continuity with the cytoplasmic membrane (3, 12, 13). After iron dextran injection electron-opaque particles were occasionally detected in these vesicles; interconnections between them and iron-containing vacuoles were either infrequent or non-existent.

By 6 hours the intimal cells had become swollen and numbers of polymorphonuclear leucocytes were present. The enlarged vacuolar cells possessed increased numbers of vacuoles, ranging up to 2 microns in diameter and containing a high concentration of filamentous material studded with electron-opaque particles (Fig. 9). Little evidence on the mechanism of concentration of iron in vacuoles could be adduced although in rare instances (as in Fig. 6) vacuoles appeared to be undergoing coalescence (or, less likely, division).

Throughout the early phase, endoplasmic reticulum–containing cells were seldom noted. When such cells were observed, the membranes of the endoplasmic reticulum were poorly defined and limited in extent. Large vacuoles were absent and relatively few electron-opaque particles could be detected.

The almost complete absence of electron-opaque particles of iron dextran in contact with the outer surface of the synovial membrane is in contrast with the histological picture of deposits of iron on the synovial surface. It must be presumed that free iron dextran at the surface is easily removed during the preparative techniques for electron microscopy. Electron-opaque particles were observed in intercellular spaces below the synovial surface, particularly in association with collagen fibrils, but the over-all concentration of iron in such extracellular situations was much less than that found intracellularly.

Inflammatory Phase

LIGHT MICROSCOPY

18 hours after injection the synovial layer was thickened and more cellular. Capillaries were dilated and numbers of polymorphonuclear leucocytes, whose concentration varied from place to place, had invaded the synovium. For the first time traces of bound iron were detected in synovial cells and in subsynovial phagocytes (Table I). Most of the iron, however, occurred in the free form, distributed as in earlier specimens (Fig. 3). By 2 days there was less free iron and more bound iron. Bound iron closely resembled haemosiderin; it possessed a golden colour in sections stained with haemalum and eosin and it was weakly positive in PAS stained sections. Neither free nor bound iron was detected in polymorphonuclear leucocytes. During this phase some iron-containing cells could be seen lying loosely attached to the synovial surface as though in the process of being shed (Fig. 3).

ELECTRON MICROSCOPY

Vacuolated cells containing electron-opaque particulate matter formed a much smaller proportion of the cell population at the inflammatory phase. By 18 hours after injection, particulate matter in vacuoles showed a further increase in concentration but many cells also contained dense granules of closely packed particulate matter. These granules, $\frac{1}{2}$ to 1 micron in diameter, were usually surrounded by an osmiophilic membrane. In all these vacuolated cells small numbers of electron-opaque particles also occurred freely in the extra-vacuolar cytoplasm. Non-vacuolated cells containing a well defined endoplasmic reticulum were not commonly encountered and these cells were usually free of electron-opaque particles.

FIGURE 8   Part of a vacuolar intimal cell $\frac{1}{2}$ hour after injection, showing electron opaque material (arrows) diffusely scattered in vacuoles. The cytoplasmic membrane (cm) is shown. × 65,000.

FIGURE 9   6 hours after injection the vacuoles possess a higher concentration of electron-opaque particulate material. Similar electron-opaque particles occur in the extra-vacuolar cytoplasm but at lower concentration. The cytoplasmic membrane is indistinct due to oblique sectioning. × 60,000.

FIGURE 10   The synovial surface layer 18 hours after injection. A surface cell shows numerous degenerative foci (df). Endoplasmic reticulum–containing cells (erc) and a blood capillary (bc) with erythrocytes are shown. × 10,000.
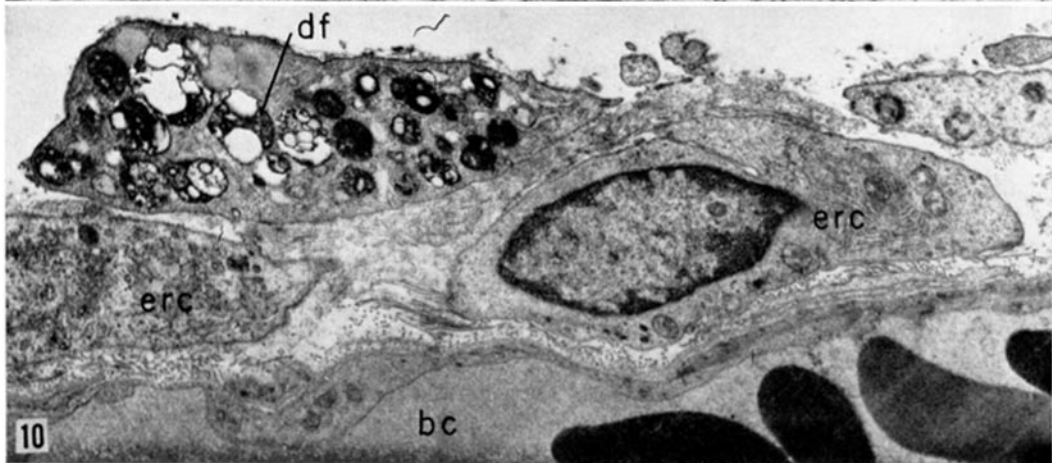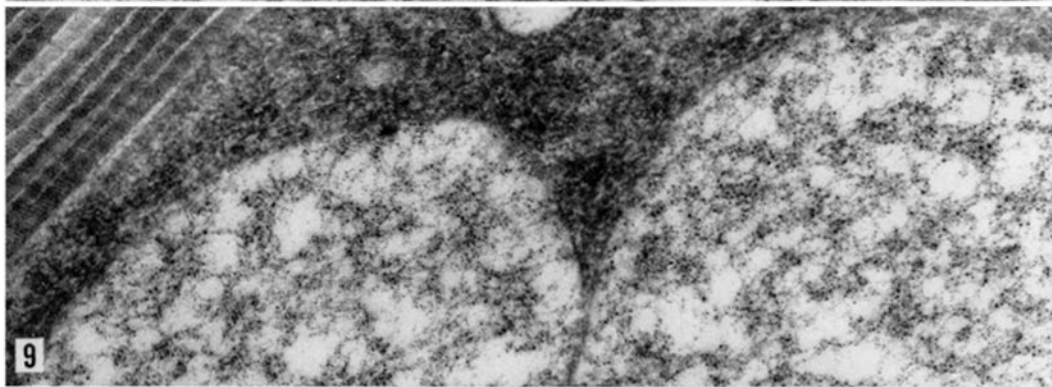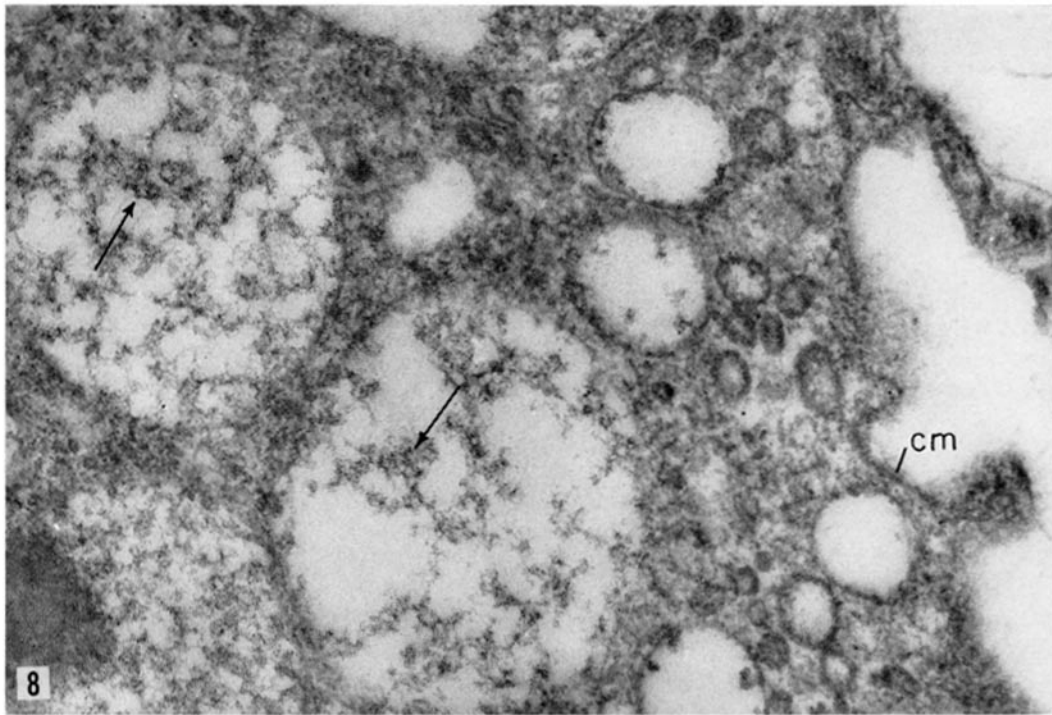
A prominent feature of specimens taken 18 hours after injection was the occurrence, frequently at the synovial surface, of numbers of cells containing round and irregularly shaped inclusions, quite unlike any cytoplasmic constituent encountered in normal synovium or at earlier stages after iron dextran administration (Fig. 10). These inclusions consisted of electron-opaque material in close association with apparently empty vacuoles; although electron-opaque particles were usually present much of the electron-opacity appeared to be non-particulate in origin. When the number of inclusions was small it was often possible to recognise the cell either as a vacuolated intimal cell or as an endoplasmic reticulum–containing cell; similar inclusions occurred in a small proportion of the polymorphonuclear leucocytes. The nature of these inclusions is in some doubt but the available evidence (loss of cytoplasmic detail, vacuolation and increased osmiophilia) points to cytoplasmic degeneration as the most likely explanation. A comparison of surface cells in electron micrographs (such as Fig. 10) and in photomicrographs (Fig. 3) suggests that some of these cells showing evidence of cytoplasmic degeneration may be shed into the joint cavity.

Degenerative foci in cells were widespread in 18-hour specimens and were not entirely confined to surface cells. Abnormally large cells below the synovial surface frequently contained one or more sharply defined bodies similar in structure to the degenerative foci in surface cells but up to 5 microns in diameter (Fig. 11). The nuclei and other cytoplasmic components of these large cells appeared to be normal and it is suggested that the enclosed bodies represent engulfed degenerative

cells or fragments thereof. The surrounding large cells showed prominent pseudopodial processes, pinocytotic vesicles and vacuoles enclosing electron-opaque particulate material. In order to distinguish them from normal phagocytic vacuolar intimal cells it will be convenient to refer to these large subsynovial phagocytic cells as "macrophages."

In accord with the histological observations iron, in the form of electron-opaque particles, could not be found in polymorphonuclear leucocytes. Dense granules in leucocytes were not particulate and differed markedly in appearance from the particulate electron-opaque inclusions in vacuolar intimal cells.

In the later stages of the inflammatory phase (about 2 days after injection) thickening of the synovium was less marked. Macrophages were still present but degenerative inclusions were infrequent. The distribution and appearance of particulate electron-opaque material resembled that at 18 hours. The subsynovial cell population included a small proportion of eosinophilic leucocytes containing distinctive oval granules similar to those described by Gusek (19); these granules appear in Figs. 11 and 12.

Recovery Phase

LIGHT MICROSCOPY

By about the 4th day there was little evidence of inflammation; synovial cells were more numerous and larger than in earlier specimens and occasionally contained mitotic figures. Some evidence of shedding of surface cells into the synovial cavity was also noted. Free iron was barely detectable

FIGURE 11 A large vacuolar cell ("macrophage") at the 18-hour stage, containing what appears to be the engulfed remnant of another cell. An eosinophilic leucocyte (el) with granules is shown. X 12,000.

FIGURE 12 Characteristic oval granules in an eosinophilic leucocyte at the 2-day stage. The nature of the granules is unknown; selected area electron diffraction yielded no diffraction pattern. X 40,000.

FIGURE 13 Dense particulate granules of haemosiderin in a cell in 4-day tissue. X 18,000.

FIGURE 14 Electron opaque particles identifiable as ferritin molecules in 14-day tissue. Parts of two haemosiderin granules (hg) and the cytoplasmic membrane (cm) are shown. X 200,000.

FIGURE 15 30 days after injection of iron dextran large iron-containing granules are found in a small number of cells in the subsynovial layer. X 8,000.

el

11

12

13

cm

hg   hg

14   15
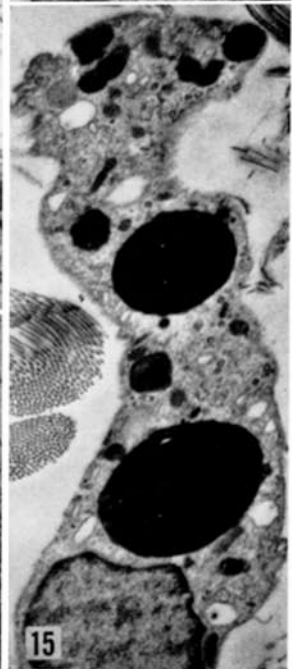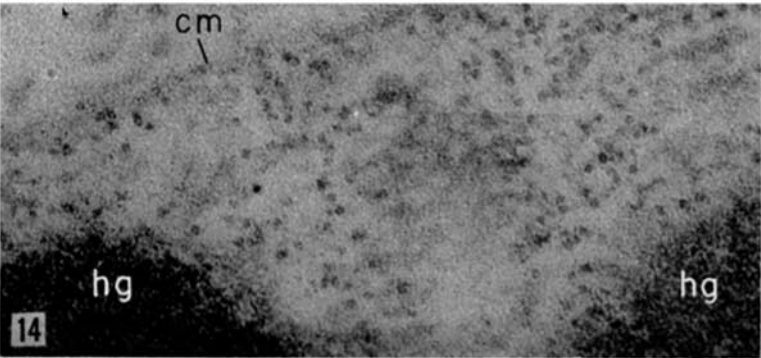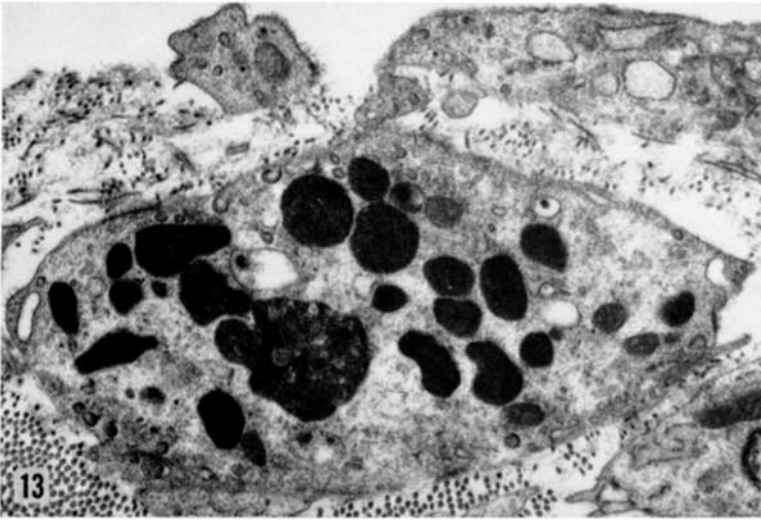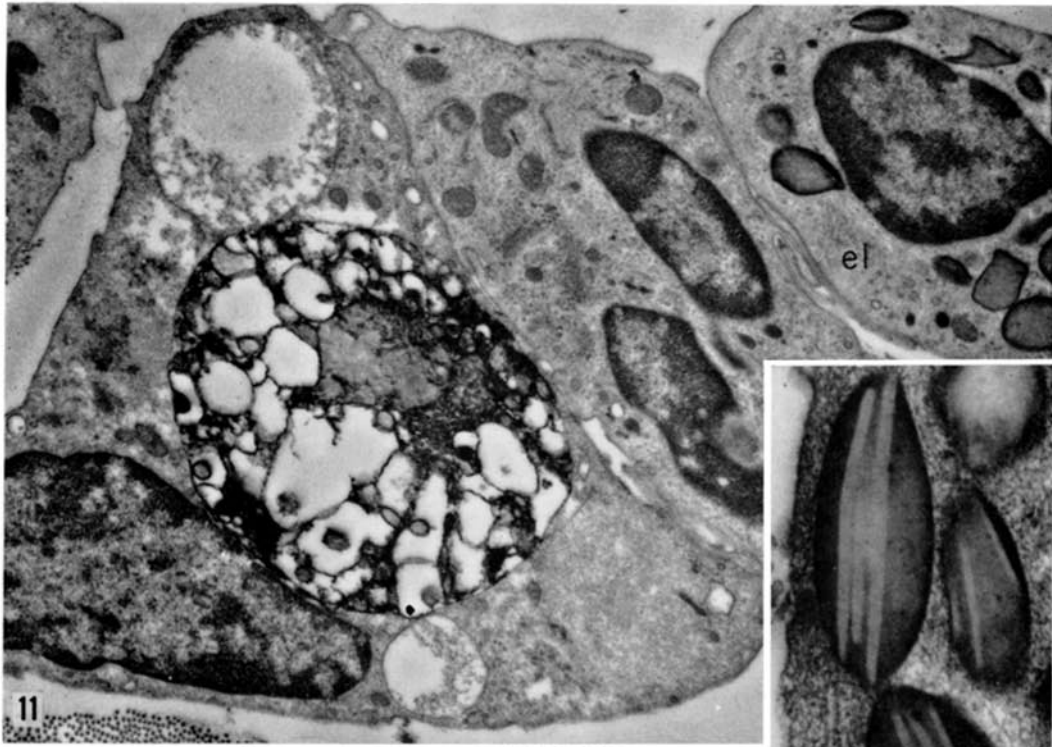
(Table I) and, from 4 days onwards, all the iron was in the bound form (*i.e.* appearances were similar whether specimens were fixed in formalin or in acridine-ethanol). Most of the bound iron was contained in synovial type cells at or just beneath the surface layer. At the synovial surface the iron appeared to be confined to scattered groups of cells. These iron-laden foci in the synovium (and deeper iron-containing phagocytes) were still present 3 months after injection (Fig. 4). Para-articular lymph nodes examined 3 months after injection also contained considerable amounts of iron.

Electron Microscopy

Electron microscopy of tissue 4 days after injection confirms the light-optical findings. Many surface vacuolar cells were free of electron-opaque particles, and endoplasmic reticulum–containing cells were normal in appearance. Iron, which was contained predominantly in a small number of medium or large cells just below the intimal layer and in the deeper aspects of the subsynovial connective tissue, occurred in the form of dense particulate granules (Fig. 13) having all the characteristics of haemosiderin, as defined by Richter (32). Fig. 14 shows parts of two of these granules 14 days after injection; no enveloping membrane surrounds the haemosiderin granules, and electron-opaque particles, now identifiable as ferritin (14, 27, 32), occur freely in the cytoplasm. Fig. 15, from tissue 30 days after injection, shows a typical iron-containing vacuolar cell situated just below the intimal layer and containing very dense granules up to 3 microns in diameter.

DISCUSSION

Our findings with iron dextran amplify the light microscopic observations of Key (23) that synovial intimal cells can take up particulate matter injected into the synovial cavity. Uptake is not limited to a small number of cells, for roughly half the population of intimal cells can take up iron dextran. These cells are all vacuolar in type and little iron dextran is taken up by endoplasmic reticulum–containing cells which are presumably concerned with synthesis. Thus there seems ample evidence that normal synovial intimal cells are inhomogeneous, morphologically and functionally (at least in terms of absorptive capacity).

The method by which vacuolar intimal cells absorb iron dextran remains uncertain. Mecha-

nisms more complex than simple diffusion are likely for several reasons. The iron dextran is not uniformly distributed within the cytoplasm but tends to be concentrated in vacuoles. Secondly there is no doubt that, following the injection of iron dextran, the cytoplasmic membrane of vacuolar cells becomes increasingly irregular in outline owing to the enhanced development of pseudopodia. These pseudopodia take the form of extended cytoplasmic folds, described by Palade (29) as "ruffles," and our observations suggest that membrane-bounded vacuoles may be formed by the coalescence of folds with one another or with the adjacent cell surface. This enveloping of extracellular milieu may be the principal method of uptake of iron dextran by synovial intimal cells. A similar method has been suggested by Gieseking (15) for the uptake of iron by lung alveolar surface cells following the intratracheal injection of finely dispersed colloidal iron. On the other hand, Muir and Golberg (27), from observations of phagocytosis of iron dextran by subcutaneous macrophages, suggest that ingestion is effected by micropinocytosis followed by concentration in cytoplasmic vacuoles. In the present study iron dextran particles were not found in large numbers in micropinocytotic vesicles. However the distinction between these two processes may be more apparent than real for, as Bessis (6) has pointed out, both phenomena imply fundamentally the same mechanism.

Iron dextran is taken up by vacuolar intimal cells within ½ hour of injection, and accumulation continues throughout the preinflammatory phase with increases occurring both in the number of vacuoles and in the iron dextran concentration within vacuoles. Coalescence of vacuoles may take place (Fig. 6) and this, coupled with the selective removal of fluid, may be the method of intracellular concentration.

Between 6 and 18 hours after injection there is evidence that many of the intimal cells become degenerate with large osmiophilic structureless masses (sometimes but not always associated with iron dextran particles) appearing in the cytoplasm. It is possible therefore that iron dextran has cytotoxic properties; it is interesting to note that the appearance of degenerate cytoplasmic foci coincides in time with the earliest signs of the onset of inflammation in the synovium. Further evidence of degenerative changes is provided by the occurrence of large "macrophage-type" cells containing

extensive intracytoplasmic bodies composed of electron-opaque areas interspersed with irregular vacuoles (Fig. 11). These bodies appear to be the remnants of ingested cells or fragments thereof.

Coupled with these changes is an increase in the number of synovial cells. During the whole process of degeneration, proliferation and reconstitution, the iron originally present in a high proportion of intimal cells is concentrated into a few cells on or just beneath the surface layer or in the subsynovial connective tissue. This concentration appears to take place primarily by the ingestion of degenerate iron-containing cells by macrophages. The origin of these macrophages is uncertain but their appearance and location suggest that they may arise from vacuolar intimal cells which have either resisted degeneration or differentiated some time after the injection of iron dextran. Degenerate cells may also be cast off into the synovial cavity, thus giving rise to a kind of intra-articular circulation of iron. This circulation, as well as the prolonged retention of iron by some tissues (Muir and Golberg, 27), may explain the continued presence of iron in a relatively small number of synovial cells for several months; i.e., long after the synovial fluid presents any naked-eye evidence of iron contamination.

Visual inspection of injected joints makes it abundantly clear that cellular uptake is not the only mechanism involved in the removal of iron dextran from the synovial cavity; visible staining of para-articular structures such as tendon sheaths and fascial planes appears within ½ hour of injection and this staining remains until the contents of the synovial cavity are no longer coloured with iron dextran. Provided precautions are taken to fix tissues in solutions which precipitate it, iron dextran can be seen in histological sections in and between synovial cells, on the reticular framework

of subsynovial adipose tissue and staining collagen bundles in the capsular tissues within ½ hour of injection. Thus iron dextran can diffuse freely through the synovium in a water-soluble, probably unchanged form; its presence in para-articular lymph nodes also demonstrates that it passes along lymphatics.

By the time the inflammatory reaction has become established (about 18 hours after injection) the iron dextran appears in an insoluble form, localised almost exclusively within cells and possessing the characteristic histological appearance of haemosiderin. The incidence of this insoluble iron complex coincides with the occurrence of large intracellular electron-opaque granules in electron micrographs. By the recovery phase molecular ferritin was clearly recognisable in the cytoplasm surrounding these granules.

Although the synovial membrane presented naked-eye evidence of intense staining, articular cartilage retained its normal colour throughout the experiment and at no stage was iron detectable histologically within the cartilage matrix. This impermeability of articular cartilage to iron dextran is presumably due to the size of the iron dextran molecule.

The mild transient synovitis induced by iron dextran presented no unusual features. It is however noteworthy that neither light nor electron microscopy revealed iron dextran in infiltrating polymorphs.

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