# Peroxidase Arthritis

## *I. An Immunologically Mediated Inflammatory Response with Ultrastructural Cytochemical Localization of Antigen and Specific Antibody.*

Richard C. Graham, Jr, MD and Sarajayne Limpert Shannon

**Synovitis was produced in rabbits by daily intra-articular injections of the heterologous antigen horseradish peroxidase. The resulting "peroxidase arthritis" resembled rheumatoid arthritis histologically. Many of the subsynovial plasma cells, plasmablasts and immunoblasts contained specific antibody to horseradish peroxidase; the remainder appeared to contain immunoglobulins of other specificities. Peroxidase arthritis has unique advantages for the study of the cellular and subcellular events in the pathogenesis of the local immune inflammatory response to heterologous antigen. Antigen and specific antibody can be localized precisely by ultrastructural cytochemical technics. The reaction can be terminated at any stage, permitting observation of the early events in its pathogenesis. (Am J Pathol 67:69–94, 1972)**

THERE IS MUCH EVIDENCE suggesting that immunologic mechanisms are involved in the pathogenesis of rheumatoid arthritis. Immunoglobulins,[1] complement[2] and extranuclear nucleoprotein[3] have been identified by immunofluorescence in rheumatoid synovial membranes. Complement levels are reduced in rheumatoid synovial fluids[4] and rheumatoid synovial fluid leukocytes contain characteristic cytoplasmic inclusions in which immunoglobulins, complement and nucleoprotein have been identified.[3,5,6] Finally, rheumatoid arthritis frequently is accompanied by the presence of rheumatoid factor, a macroglobulin with antibody specificity toward IgG.[7]

The antigenic stimulus for the immunologic phenomena accompanying rheumatoid arthritis is unknown. Accumulating evidence suggests that an infectious agent may be involved.[8–10] It has been suggested that the pathogenesis of rheumatoid inflammation might depend on the host immunologic response to such a microbial antigen. Chronicity could be explained either by persistence of the heterologous antigen[8] or by an autoimmune reaction to antigens made available by the initial inflammatory response.[11]

From the Department of Medicine, Case Western Reserve University, University Hospitals of Cleveland, Cleveland, Ohio 44106.

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Address reprint requests to Dr. Richard C. Graham, Jr.

In the studies described in this series of reports, repeated intra-articular injections of antigen were employed to study the synovial response to prolonged local antigenic stimulation. In the resulting synovitis, which resembles rheumatoid arthritis histologically, local antibody synthesis occurs within the synovial inflammatory infiltrate. When the antigen used is horseradish peroxidase (HRPO), both antigen [12] and specific antibody [13] can be localized precisely by ultrastructural cytochemical technics. In this report, the general morphologic and immunologic characteristics of "peroxidase arthritis" are described.

## Materials and Methods

### Procedure and Injection Schedules

Nineteen female albino rabbits were used in the following procedures. All animals were anesthetized by titration with sodium thiamylal (Parke-Davis, Detroit, Mich) and both knees were shaved and cleaned with 70% ethanol. In fourteen rabbits, the knees were injected intraarticularly with 2 mg horseradish peroxidase (HRPO) (Sigma Chemical Company, Type VI, RZ 3.0) in 0.5 ml 0.15 M NaCl. The left knees were injected with 0.5 ml of a 1:30 dilution of autologous serum in 0.15 M NaCl. In the remaining 5 rabbits, both right and left knees were injected with 2 mg HRPO in 0.5 ml 0.15 M NaCl. All solutions were sterilized by Millipore filtration prior to injection. In early experiments, a variety of dosages and intervals between injection were used. It was found that daily injections using 2 mg per knee produced the desired reaction most consistently (see Table 1).

Two rabbits were given 14 daily intra-articular injections of 2 mg bovine serum albumin (BSA) in 0.5 ml 0.15 M NaCl. Procedures were otherwise identical to those described for the HRPO-injected rabbits.

All rabbits were sacrificed by an overdose of sodium thiamylal 24 hours after the last injection of HRPO. Both of the knee joints, spleens, popliteal lymph nodes and kidneys were removed. All tissues were fixed for 4 hours in 2.5% glutaraldehyde and 2% formaldehyde in 0.05 M phosphate buffer, pH 7.4.[14] The tissues were washed overnight in 0.05 M phosphate buffer, pH 7.4.

### Localization of Antigen (HRPO) and Antibody (anti-HRPO)

The fixed synovial membrane and underlying tissue was carefully removed from the connective tissue medial and lateral to the patella and cut into strips approximately 1 mm in thickness. These tissues were divided into two portions.

One portion was incubated according to the method described by Graham and Karnovsky [12] for the detection of peroxidase activity. The tissues were preincubated in 5 mg of 3,3'-diaminobenzidine (Sigma Chemical Co, St Louis) in 10 ml of 0.05 M Tris-HCl buffer, pH 7.4, for 25 minutes prior to the addition of 0.1 ml 1% $H_2O_2$. The tissues were incubated in the complete medium for 10–15 minutes. They then were washed three times in distilled water.

The other portion of tissue was treated according to the method of Leduc *et al* [13] for the detection of anti-HRPO activity. Both whole-tissue strips and 40-μ frozen sections were gently agitated in 5–10 ml of 0.05 M phosphate buffer, pH 7.4, containing 0.5 mg/ml HRPO (type VI) for 2 hours at 4 C. They then were washed and refixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer,

Table I—Injection Schedules, Serum Antibody Titers and Intensity of Reaction in Rabbits Studied

| Rabbit | Dose of HRPO (mg/injection) | Knee injected* | Interval† | Duration (days) | Serum PHA | Antibody‡ precipitin | Intensity of reaction§ |
|---|---|---|---|---|---|---|---|
| I-A | 1 | R | 2 | 18 | 320 | 2 | + |
| I-B | 1 | R | 2 | 18 | 320 | 0 | + |
| II-A | 1 | R | 4 | 18 | 320 | 0 | + |
| II-B | 1 | R | 4 | 18 | 40 | 0 | + |
| III-A | 1 | R | 6 | 18 | 160 | 0 | + |
| III-B | 1 | R | 6 | 18 | 40 | 0 | + |
| V-A | 2 | R | 1 | 19 | 1280 | 8 | 3+ |
| V-B | 2 | R | 1 | 19 | 640 | 2 | |
| VI-B | 1 | R | 1 | 15 | 320 | Undil | 2+ |
| VI-C | 1 | R | 1 | 15 | 320 | 4 | 2+ |
| B-8 | 1 | R | 1 | 12 | 80 | 0 | 2+ |
| VII-A | 2 | R | 1 | 35 | 5120 | 8 | 4+ |
| VII-B | 2 | R | 1 | 35 | 1280 | 4 | 3+ |
| VIII-A | 2 | B | 1 | 15 | 1280 | 4 | 3+ |
| IX-A | 2 | B | 1 | 9 | 40 | 0 | 2+ |
| IX-B | 2 | B | 1 | 9 | 20 | 0 | 2+ |
| 12 | 2 | B | 1 | 15 | 320 | | 3+ |
| 16 | 2 | B | 1 | 37 | 10240 | 8 | 4+ |
| 1-F | | | | | 163840 | 16 | |

* R, right knee; B, both knees.

† 1, daily; 2, every other day; 4, every fourth day; 6, every sixth day.

‡ Reciprocal of titer. PHA, passive hemagglutination

§ Intensity of reaction is expressed in arbitrary units with 4+ representing maximal intensity.

¶ Reference serum from animal immunized with HRPO in complete Freund's adjuvant in conventional manner.

pH 7.4, for 15 minutes. They were washed again in buffer before incubation for peroxidase activity, as described above, for 30 minutes. Incubation was followed by three washes in distilled water.

Both portions of tissue were additionally fixed in 1% $OsO_4$ in 0.05 M phosphate buffer, pH 7.4, for 1 hour, washed in 0.1 M sodium maleate buffer, pH 5.2, and stained with 2% uranyl acetate in 0.05 M sodium maleate buffer, pH 5.2, for 2 hours. After this, the tissues were dehydrated in ethanol and embedded in Araldite. Thick sections (0.5–1 μ) were cut, stained with toluidine blue and examined by light microscopy to determine appropriate areas for thin sectioning. Thin sections were cut with glass knives on Sorvall MT-1 or MT-2 ultramicrotomes, stained with uranyl acetate and lead citrate and examined in an AEI EM-6B electron microscope.

### Light Microscopy

Articular cartilage and adjacent synovial tissue from the knee joints of rabbits receiving daily HRPO injections for 35 or 37 days were taken and fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.05 M phosphate buffer, pH 7.4, overnight. The tissues were washed for several hours, dehydrated and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (H&E).

Synovial fluids from the knee joints of all rabbits were aspirated into heparinized plastic syringes. Smears were made directly from the syringe to achieve maximum preservation of the cells. The smears were stained with tetrachrome.

#### Serologic Studies

Both the passive hemagglutination test and Ouchterlony precipitin test were used for the detection of circulating antibody. For use in the passive hemagglutination test, HRPO was coupled to fresh sheep erythrocytes with glutaraldehyde according to the method of Avrameas *et al.*[16] Passive hemagglutination tests were carried out in 100 × 75 mm glass test tubes by a standard technic.

Serial dilutions of sera were assayed for precipitating antibodies by double diffusion in agar gel according to the standard Ouchterlony technic.

"Enzyme immunoelectrophoresis"[17] was carried out on sera from 5 different animals. Standard immunoelectrophoretic patterns were developed in agarose gel with goat anti-rabbit whole serum (kindly supplied by Dr. Abram Stavitsky). Slides were then washed at room temperature for 3 days in 0.15 M phosphate-buffered saline, pH 7.2, before incubation overnight at room temperature in a solution of 0.1 mg HRPO/ml in phosphate-buffered saline. The slides were then washed for 5 days at 4 C in repeated changes of 0.15 M NaCl to remove unprecipitated HRPO. Finally, they were reacted by the Graham-Karnovsky technic (see above) to reveal sites of peroxidase activity.

## Results

### Controls

Joints receiving 12 to 35 daily injections of diluted autologous serum appeared entirely normal upon both gross and microscopic examination (Fig 1). No peroxidase activity was detectable in these tissues.

### Peroxidase Arthritis

#### Gross Observations

*After 12–16 Daily HRPO Injections.* The external appearance of the joints was not striking. In most rabbits, external evidence of mild swelling of joints was evident. Upon opening the joints, the synovial tissue appeared markedly thickened and hyperemic. The articular cartilages showed no apparent abnormality. The synovial fluid, which was increased in amount, was turbid and quite viscous.

*After 35 or 37 Daily Injections of HRPO.* The joints were markedly swollen. The appearance of the synovial tissue was similar to that described above, except that synovial thickening was much more marked. Inflamed synovial tissue partially obscured the surface of the articular cartilages. The synovial fluid resembled that seen after the shorter series of injections, except that it was present in larger amounts.

#### Cellular Composition of Synovial Fluid

Examination of tetrachrome-stained smears of synovial fluid by light

microscopy revealed the cellular composition to be similar after 12–37 daily HRPO injections. Of the cells, 75% or more were polymorphonuclear leukocytes. The remainder included lymphocytes, macrophages and cells that appeared to be detached synovial cells.

### Light and Electron Microscopic Histopathology

*After 12–16 Daily HRPO Injections.* The histologic appearance of the inflamed synovial tissue was qualitatively similar in all rabbits examined. The magnitude of the reaction varied somewhat from animal to animal, tending to be greater in those rabbits given 2 mg HRPO in each daily injection, and in those injected with HRPO in both knees (Table 1). Within a given joint, the magnitude of the reaction varied considerably in synovial tissues taken from different areas of the joint.

The synovial lining cells were increased in number, with the lining layer varying from two to five cells in thickness (Fig 2). Most of the synovial lining cells were highly irregular in shape. Their surfaces displayed an extensive and complex network of pseudopods, which was most prominent on the aspect of the cell facing the synovial cavity. Mitotic figures frequently were observed (Fig 3). When viewed by light microscopy, numerous brown granules of reaction product were seen in the cytoplasm of most of the synovial lining cells. A few of the synovial cells contained only scattered granules of reaction product. When examined by electron microscopy, the majority of the synovial cells were seen to contain numerous cytoplasmic vacuoles, many of which contained peroxidatic reaction product (Fig 3). Other vacuoles enclosed ferritin, cellular debris or entire dead and degenerating cells (Fig 4). In a small minority of synovial lining cells, there were few scattered small vacuoles containing reaction product, and the cell surface displayed fewer and less complex pseudopods. Rough-surfaced endoplasmic reticulum was abundant in these cells (Fig 5). Occasional lymphocytes, plasma cells and polymorphonuclear leukocytes were seen within the synovial lining layer. Fibrin frequently was present in the intercellular spaces between the synovial lining cells.

The subsynovial tissues were infiltrated with a variable number of inflammatory cells. In many areas, this infiltrate was quite extensive, with the involved tissue being 1 mm or more in thickness. The infiltrate was composed of numerous plasma cells, lymphoid cells and macrophages, moderate numbers of fibroblasts and polymorphonuclear leukocytes and occasional eosinophils (Fig 2).

Electron microscopy revealed the majority of the plasma cells to be typical mature plasma cells, with numerous, regularly spaced stacks of

rough-surfaced endoplasmic reticulum (Fig 6). The nuclei were round and eccentrically placed, with peripheral condensations of chromatin. Smaller numbers of plasmablasts, distinguished by their prominent nucleoli and the less regular orientation of their rough-surfaced endoplasmic reticulum, were observed. Plasmablasts in mitosis occasionally were seen.

A spectrum of lymphoid cells was present (Fig 7 and 8). Some were typical small lymphocytes, with condensed nuclear chromatin and scant cytoplasm containing few organelles. Others had more abundant cytoplasm containing variable numbers of free polyribosomes (Fig 8). Still others had less condensed nuclear chromatin and numerous free polyribosomes in their cytoplasm (Fig 8). Such cells have been referred to by others as "activated" lymphocytes.[18] Moderate numbers of "immunoblasts" were observed. The nuclei of these cells had prominent nucleoli and highly dispersed chromatin, and the cytoplasm contained abundant free polyribosomes and a few profiles of rough-surfaced endoplasmic reticulum (Fig 8 and 9). Such cells frequently were seen in mitosis (Fig 10).

The plasma cells and lymphoid cells often were aggregated in clusters and were more numerous in the deeper part of the infiltrate. The clusters, which frequently were found near venules, varied in their cellular composition. In some, lymphoid cells predominated (Fig 7). Others were composed mainly of plasma cells (Fig 6). In still others, a mixture of plasma cells and lymphoid cells was observed.

Plasma cells and lymphoid cells were closely associated with each other and with macrophages. These cellular interrelationships, which were quite varied and complex, will be described in detail in a subsequent paper.

Polymorphonuclear leukocytes most often were seen in the superficial part of the infiltrate, immediately beneath the synovial lining cells. Macrophages and fibroblasts were found throughout the infiltrate.

Striking alterations were seen in the larger venules situated in the deeper subsynovial tissue, and in some of the smaller superficial vessels as well. The endothelial cells, which were increased in number, bulged into the vascular lumen, giving the endothelium a pseudocolumnar appearance. Such vessels were the site of emigration of lymphoid cells, monocytes and polymorphonuclear leukocytes. Fenestrated capillaries rarely were observed in the fully developed arthritis. The development of these changes, as well as the mechanism of cellular emigration, will be described in detail in a subsequent communication.

*After 35–37 Daily Injections of HRPO.* The appearance of the syn-

ovial tissue was qualitatively similar to that observed after the shorter series of injections. The cellular infiltrate, however, was much more extensive. Examination of paraffin sections stained with hematoxylin and eosin revealed necrosis of cartilage at sites of contiguity with the inflammatory infiltrate (Fig 11).

Electron microscopy showed that the synovial cells, which were greatly increased in number, had numerous cytoplasmic vacuoles variously containing HRPO, cellular debris, ferritin or lipid (Fig 12). Lymphoid cells and occasional polymorphonuclear leukocytes were found among the synovial cells.

The subsynovial infiltrate contained more cells per unit area than was the case after shorter series of injections (Fig 13 and 14). The cellular composition of the infiltrate resembled that observed after fewer injections. Mature and immature plasma cells, lymphoblasts and the various types of lymphocytes all still were present. Fibroblasts were numerous among bundles of collagen which apparently were newly formed (Fig 15). Scattered throughout the infiltrate were many macrophages, the cytoplasmic vacuoles of which variously contained HRPO, ferritin, cellular debris or lipid (Fig 16). In some of the macrophages, lipid-containing vacuoles were strikingly numerous. Extensive fibrin deposition was observed both in the synovial lining layer and in the subsynovial tissue (Fig 12). The great majority of the small blood vessels were of the pseudocolumnar type described above. Fenestrated capillaries rarely were seen.

### Cytochemical Demonstration of Specific Antiperoxidase Antibody

Study of synovial tissue from rabbits receiving 12–37 daily intra-articular injections of HRPO revealed similar findings, and these will be reported together. The method used was that of Leduc and her associates,[14] in which aldehyde-fixed tissue is incubated with antigen (HRPO). The HRPO, which binds to anti-HRPO in antibody-synthesizing cells, serves as a cytochemical marker indicating the location of the antibody. In peroxidase arthritis, many but not all of the plasma cells and plasmablasts contained anti-HRPO in the rough-surfaced endoplasmic reticulum (Fig 17). In some of the plasma cells and all of the plasmablasts with anti-HRPO activity, this activity was localized in the perinuclear space as well (Fig 17 and 18). Some of the immunoblasts had anti-HRPO activity in the perinuclear space and in scattered profiles of rough-surfaced endoplasmic reticulum (Fig 19). Specific antibody was observed both in blasts undergoing mitosis and in those that were not (Fig 19). Both cells with (Fig 20) and without anti-HRPO

activity were observed in association with macrophages. Differentiation of plasma cells from lymphoplasmacytes, recently described by Avrameas and Leduc,[17] was not possible since we did not study tissues fixed only in formaldehyde.

The presence of plasma cells without antiperoxidase antibody was unlikely to have been an artifact caused by poor penetration of HRPO during the Leduc procedure, or by poor penetration of the cytochemical reagents. Plasma cells without specific antibody frequently were seen at the very edge of the tissue strip. Such cells also were seen immediately adjacent to plasma cells that unequivocally had specific antibody. Finally, when frozen sections were used, penetration appeared essentially complete, and cells with and without antiperoxidase activity were found throughout the entire thickness of the tissue.

### Detection of Antiperoxidase Antibody in Serum

Specific antibody was detectable both by double diffusion in agar and by passive hemagglutination as shown in Table I. Titers varied from animal to animal, and were higher in rabbits receiving larger doses of HRPO in each injection, and in those receiving the greatest number of injections (see Table I). The titers obtained were considerably lower than those obtained in rabbits immunized with HRPO in complete Freund's adjuvant by conventional technics (Table 1).

### Immunoglobulin Classes of Serum Antiperoxidase Antibody

Sera were analyzed by the enzyme immunoelectrophoresis technic described by Avrameas and Leduc.[17] This method depends upon the ability of specific antiperoxidase antibody in the immunoglobulin precipitin lines to bind HRPO. The bound HRPO is identified by the peroxidatic oxidation of 3,3′-diaminobenzidine, which stains the relevant precipitin lines brown.

In sera from rabbits given 16 daily intra-articular injections of HRPO, anti-HRPO activity was detectable in both IgG and IgM precipitin lines (Fig 21). Sera from rabbits given 35 or 37 daily HRPO injections had detectable anti-HRPO activity only in the IgG precipitin line (Fig 21).

### Effect of Repeated Intra-Articular Injection of Bovine Serum Albumin (BSA)

Rabbits receiving 14 daily intra-articular injections of BSA developed an arthritis similar to that evoked by an equivalent number of HRPO injections. Study by both light and electron microscopy revealed no important morphologic differences between the reactions evoked by the two antigens, although that elicited by BSA was somewhat greater in

magnitude. No antiperoxidase antibody was found in plasma cells or lymphoid cells in BSA arthritis when studied by the Leduc technic. Sera from the rabbits with BSA arthritis contained precipitating antibody against BSA when assayed by double diffusion in agar.

## Discussion

Peroxidase arthritis presents unique opportunities for the study of the synovial inflammatory response to repeated local antigenic stimulation. Its most important advantage is that antigen and specific antibody can be localized precisely by electron microscopy. In addition, with a given dose of antigen and number of injections, the changes produced are reproducible, so that the development of the reaction can be studied easily at any stage. Although giving repeated intra-articular injections to rabbits perhaps is somewhat inconvenient, with experience the injections can be done easily and quickly. Moreover, autologous serum controls have established that the trauma of repeated injection *per se* causes no significant change in the synovial tissues.

Despite the unique methodologic advantages of the peroxidase arthritis model, its ultimate value depends upon the relevance of observations made with it to the pathogenesis of human disease. In this regard, the morphologic and immunologic similarities between the synovitis of rheumatoid arthritis and that of peroxidase arthritis are of some interest. Whether these similarities reflect similar pathogenic mechanisms is, of course, as yet unknown. The histopathologic changes in the human rheumatoid synovial membrane have been well described at both the light [19,20] and electron [21,22] microscopic levels. There is proliferation of synovial lining cells, accompanied by subsynovial infiltration with lymphocytes, plasma cells, macrophages, fibroblasts and a few polymorphonuclear leukocytes. The cells of the subsynovial infiltrate frequently are found in focal aggregates resembling lymphoid follicles. As the disease progresses, inflammatory tissue frequently encroaches on adjacent cartilage and bone, resulting in necrosis of these tissues.

On purely morphologic grounds, the similarities between peroxidase arthritis and rheumatoid arthritis are striking. In both, the proliferation of synovial lining cells is accompanied by greatly augmented synovial endocytic activity. In peroxidase arthritis, synovial endocytosis of large quantities of specific antigen occurs; whether a similar phenomenon occurs in rheumatoid arthritis of course must await the identification of a specific etiologic agent.

The cellular populations of the subsynovial infiltrates in peroxidase arthritis and rheumatoid arthritis are quite similar. Although in peroxi-

dase arthritis there is a definite tendency for the cells to aggregate in follicle-like clusters, the process in general is more diffuse than usually is observed in rheumatoid arthritis. This difference perhaps reflects the greater duration of the usual rheumatoid process before histologic examination is carried out. Like rheumatoid arthritis, peroxidase arthritis is associated with necrosis of cartilage if the reaction is sufficiently prolonged.

In both peroxidase arthritis and rheumatoid arthritis, polymorphonuclear leukocytes make up but a small percentage of the inflammatory infiltrate. In contrast, they are abundant in the synovial fluid. The reason for this discrepancy is not entirely clear. Presumably, the polymorphonuclear leukocytes, after emigrating from subsynovial blood vessels, migrate quite rapidly through the synovial membrane into the synovial cavity. Rapid migration of these cells in the same direction could reflect the presence of a chemotactic stimulus. Possibly, chemotactic factors are released in the synovial fluid through the activation of complement by antigen-antibody complexes.[23]

The endothelial changes and associated interactions of lymphocytes with endothelium that occur in peroxidase arthritis are quite complex and will be discussed in detail in a subsequent paper. Whether significant endothelial changes occur in rheumatoid arthritis is disputed.[24] However, rheumatoid synovial tissues must be studied when they have reached a relatively more advanced stage than the peroxidase arthritis lesions. In this respect, it is of interest that in peroxidase arthritis the endothelial changes reach their peak at 12–16 days and then recede, despite continued intensification of the inflammatory response.

Synthesis of immunoglobulins by cells in the synovial inflammatory infiltrate occurs in both rheumatoid arthritis and peroxidase arthritis. With the exception of rheumatoid factor, the antibody specificity of the immunoglobulins produced in the rheumatoid synovial membrane is unknown. In peroxidase arthritis, some of the synovial immunocytes have been shown to produce anti-HRPO. It is likely, however, that immunoglobulins of other specificities are produced as well, since many of the subsynovial plasma cells are without anti-HRPO activity when examined with the Leduc technic. The nature of the immunoglobulins produced by these cells is as yet unknown. The interesting possibility that at least some of them may produce autoantibodies is being investigated in our laboratory.

There has been much interest in the possibility that an infectious agent may provide the initial antigenic stimulus in rheumatoid arthritis.[25] It has been shown that membranes of *Mycoplasma fermentans* inhibited

leukocyte migration in 67% of patients with rheumatoid arthritis, but had no effect in patients with osteoarthritis or normal controls.[10] It is of interest that certain species of *Mycoplasma* cause an arthritis in swine that resembles rheumatoid arthritis both histologically and serologically.[25,26] Further evidence in support of the presence of an infectious agent in rheumatoid arthritis has come from the demonstration that a slurry from rheumatoid synovial tissue causes swelling and reddening of extremities when injected into mice.[9] These changes were transmissible to the second and third generations of mice without further injection.

The peroxidase arthritis reaction provides a model of a synovial immune inflammatory reaction in response to prolonged stimulation by a heterologous antigen. Comparison with a response to an infectious agent obviously is inexact; the response to a soluble protein antigen may differ from the response to a microbial antigen, which probably is membrane bound. Moreover, the intermittent introduction of antigen can only approximate the stimulus presented by a persistent microorganism, to which the host presumably is continuously exposed. Nevertheless, it seems quite possible that the approximation provided by the model is close enough that in the synovial tissue the mechanisms of response are similar.

Experimental arthritis in animals has been produced in numerous ways. The literature before 1960 has been reviewed well by Gardner.[27] Subsequent interesting models include adjuvant arthritis, produced by the intradermal injection of complete Freund's adjuvant in certain strains of rats.[28,29] Histologically, the arthritis somewhat resembles rheumatoid arthritis and in some rats becomes chronic and is characterized by exacerbations and remissions. Its pathogenesis is uncertain, but may involve an immunologic response to mycobacterial antigens. Another interesting form of experimental arthritis has been produced by immunizing rabbits with heterologous fibrin in Freund's adjuvant, followed by injection of fibrin into a joint.[30] The resulting arthritis resembles rheumatoid arthritis histologically in some respects, and may persist for months without further injection of antigen. Other antigenic proteins can substitute for fibrin in this model.[11] It has been suggested that persistence of inflammation in this model depends upon an autoimmune reaction to products of the initial immune inflammatory response.[11]

Peroxidase arthritis lacks the prominent systemic manifestations and the chronicity characteristic of rheumatoid arthritis, and for the study of these aspects, another of the models would seem more appropriate. Further, for quantitative studies in which a large sample is required,

such as evaluation of anti-inflammatory or immunosuppressive drugs, one of the other models would be more practical. On the other hand, peroxidase arthritis appears to have unique advantages for the study of the cellular and subcellular events in the pathogenesis of this type of immune inflammatory response. Antigen and antibody can be localized by both light and electron microscopy, and the reaction can be controlled so that any stage of its development can be readily studied. Morphologic information obtained can be correlated with the results of conventional immunologic technics.

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A preliminary report of a portion of this work has been presented in abstract form.[31]

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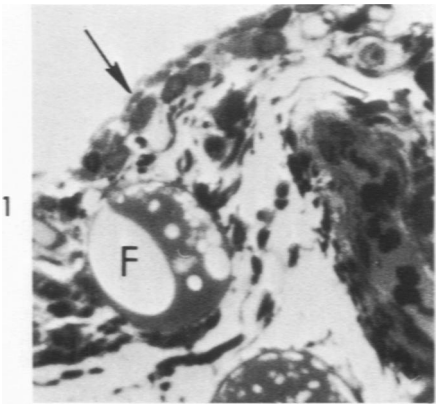
## Legends for Figures

**Fig 1**—Synovial membrane from rabbit control knee joint given 35 daily injections of diluted autologous serum. The synovial lining cells *(arrow)* are normal in number and appearance. No inflammatory cells are seen around the small blood vessel or elsewhere in the subsynovial connective tissues. Two fat cells *(F)* are present (light micrograph, toluidine blue, × 300).

**Fig 2**—Synovial membrane and subsynovial infiltrate from rabbit given 16 daily injections of HRPO. The number of synovial lining cells *(arrow)* is increased. The subsynovial connective tissue contains many macrophages, plasma cells, lymphocytes and fibroblasts (light micrograph, toluidine blue, × 440).

**Fig 3**—Synovial cell from a rabbit receiving 15 daily injections of HRPO. Numerous cell processes and pseudopods are seen extending from the plasma membrane. Many vesicles and vacuoles containing reaction product are present in the cytoplasm. The clumped nuclear chromatin and incomplete nuclear membrane suggest the cell is in an early phase of mitosis (electron micrograph, × 9000).

**Fig 4**—Synovial lining cell from rabbit receiving 12 daily HRPO injections. The large cytoplasmic vacuole contains the remains of a degenerating cell. Two smaller vacuoles contain electron-opaque peroxidatic reaction product (electron micrograph, × 16,000).

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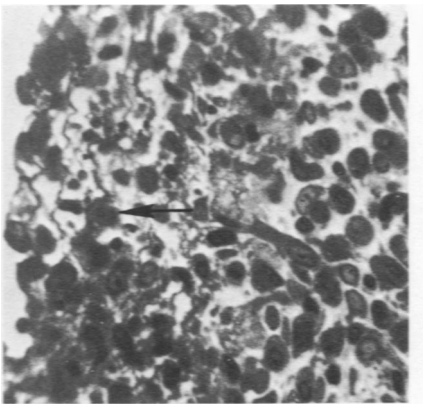

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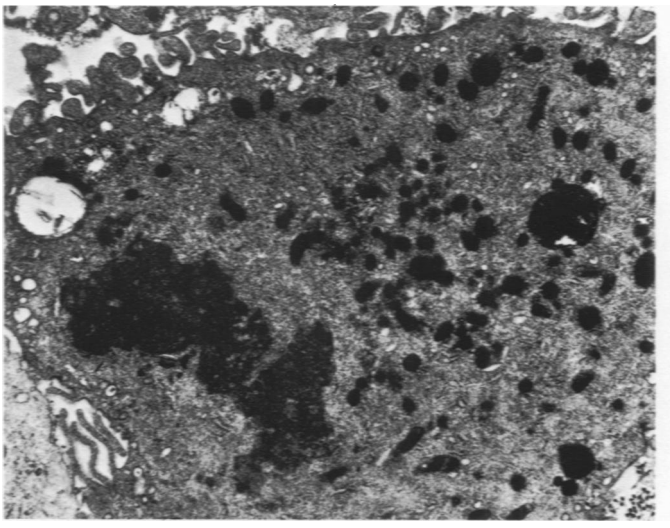

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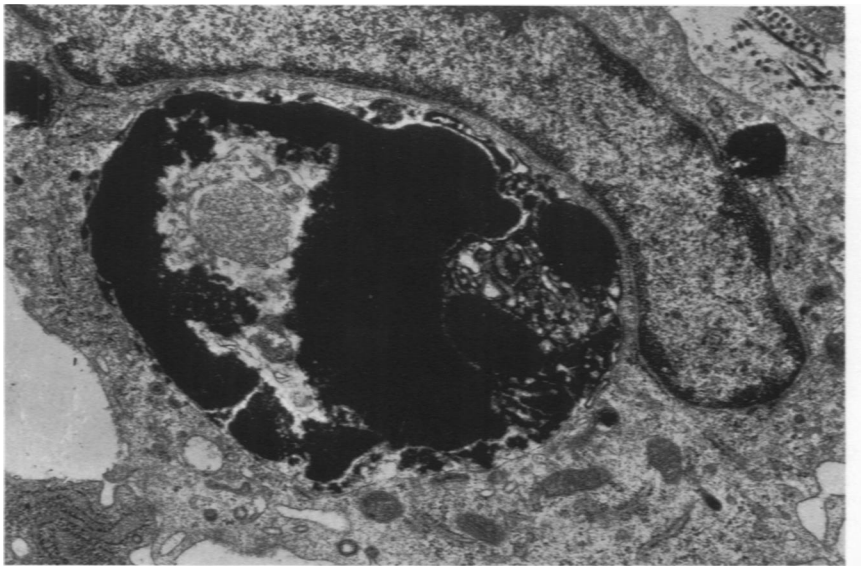

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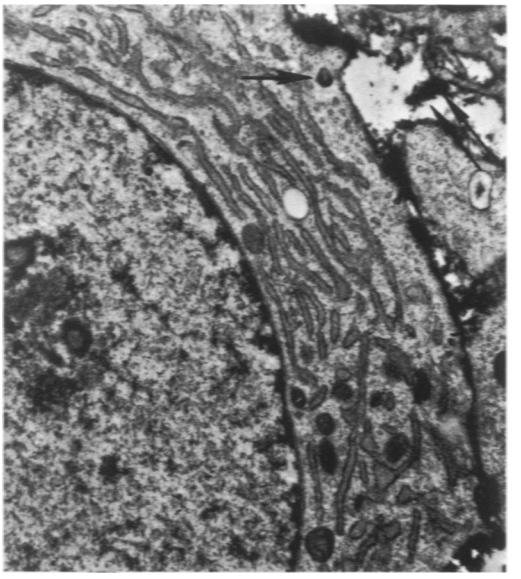


**Fig 5**—Synovial lining cell from rabbit receiving 16 daily HRPO injections. In contrast to the majority of synovial cells, this cell contains only a few cytoplasmic vesicles and vacuoles, and abundant rough-surfaced endoplasmic reticulum. Peroxidatic reaction product, which is abundant outside the cell (*double arrows*), is seen only in occasional small vesicles (*single arrow*) within the cell (electron micrograph, × 20,000).

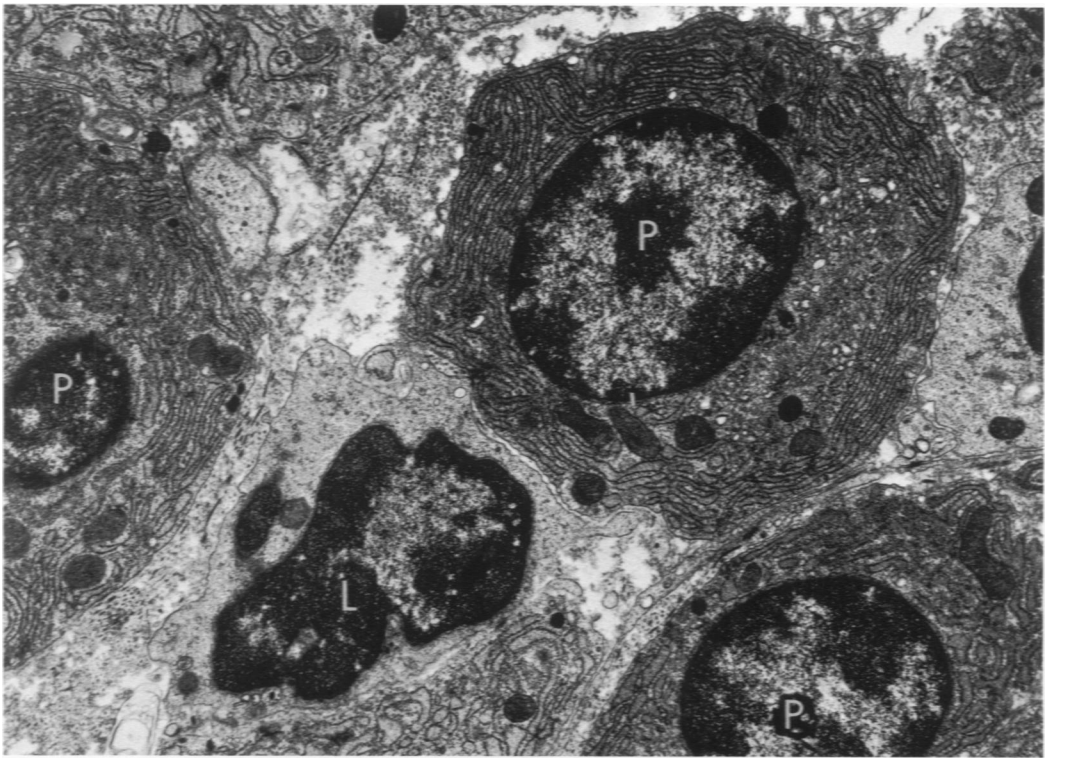



**Fig 6**—Portion of subsynovial infiltrate from rabbit given 16 daily HRPO injections. Mature plasma cells contained typical stacks of rough-surfaced endoplasmic reticulum and peripherally condensed nuclear chromatin. A medium lymphocyte (*L*), closely opposed to a plasma cell (*P*) contains scant cytoplasmic organelles and condensed nuclear chromatin. Collagen fibers of varying orientation lie between the cells (electron micrograph, × 10,000).

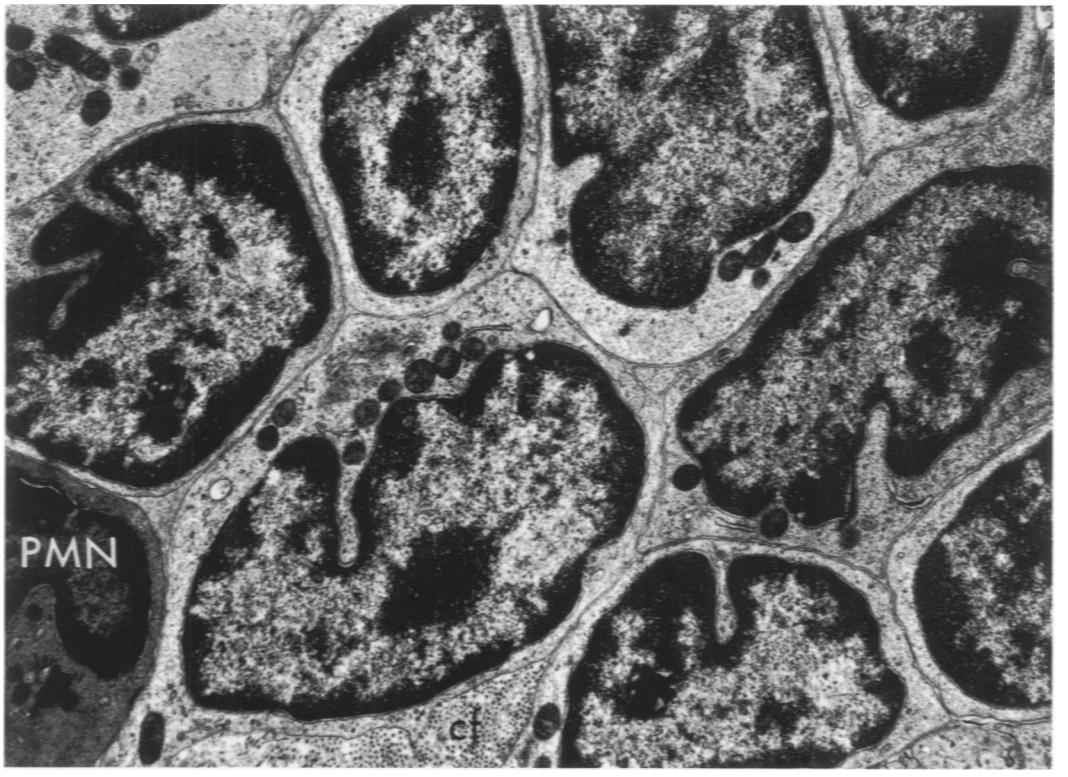



**Fig 7**—Cluster of lymphocytes in the subsynovial infiltrate from a rabbit receiving 16 daily HRPO injections. The cells are typical of small to medium lymphocytes, with condensed nuclear chromatin and scant cytoplasm containing few organelles. The lymphocytes are closely apposed with very little intercellular space intervening. A bundle of collagen fibers (*cf*) and a portion of a polymorphonuclear leukocyte (*PMN*) are at the lower left (electron micrograph, × 10,000).

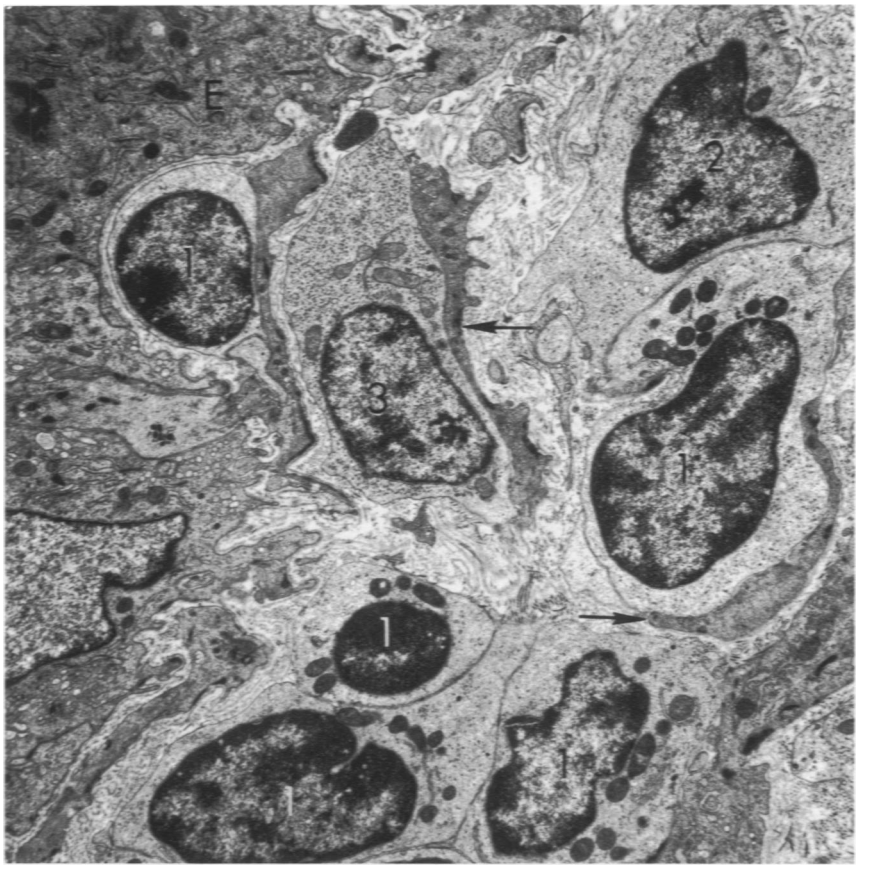
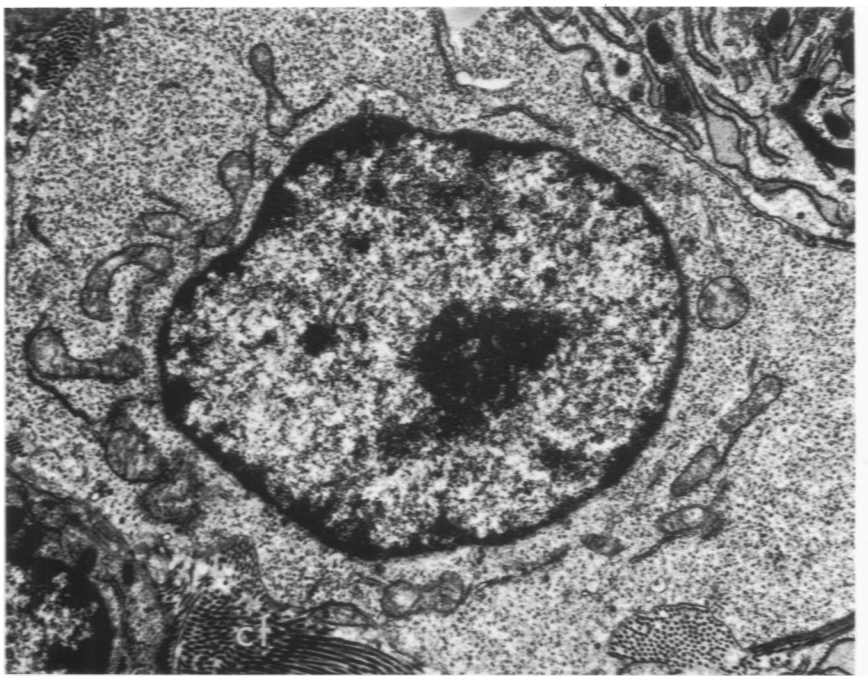



**Fig 8**—Perivascular lymphoid cells from subsynovial tissue of rabbit given 16 daily HRPO injections. The albuminal aspect of the endothelium (*E*) is at the left. Small to medium lymphocytes (*1*) have condensed nuclear chromatin and bland cytoplasm. A large lymphocyte (*2*) has a prominent nucleolus and moderate numbers of free polyribosomes in the cytoplasm. An immunoblast (*3*) has a prominent nucleolus, dispersed nuclear chromatin, and abundant free polyribosomes. A single profile of rough-surfaced endoplasmic reticulum is evident. Slender processes of pericytes (*arrows*) are interposed between the lymphoid cells (electron micrograph, × 6000).

**Fig 9**—Subsynovial immunoblast from animal killed after 12 daily injections of HRPO. Note the prominent nucleolus, dispersed nuclear chromatin, abundant free polyribosomes and scant elements of rough-surfaced endoplasmic reticulum. Collagen fibers (*cf*) are seen in the interstitial space (electron micrograph, × 14,000).

**Fig 10**—Subsynovial cells from rabbit given 16 daily HRPO injections. An immunoblast ($I_1$) is in mitosis. A similar cell ($I_2$) recently has divided. A macrophage (*M*) is closely associated with the two immunoblasts (electron micrograph, × 10,000).

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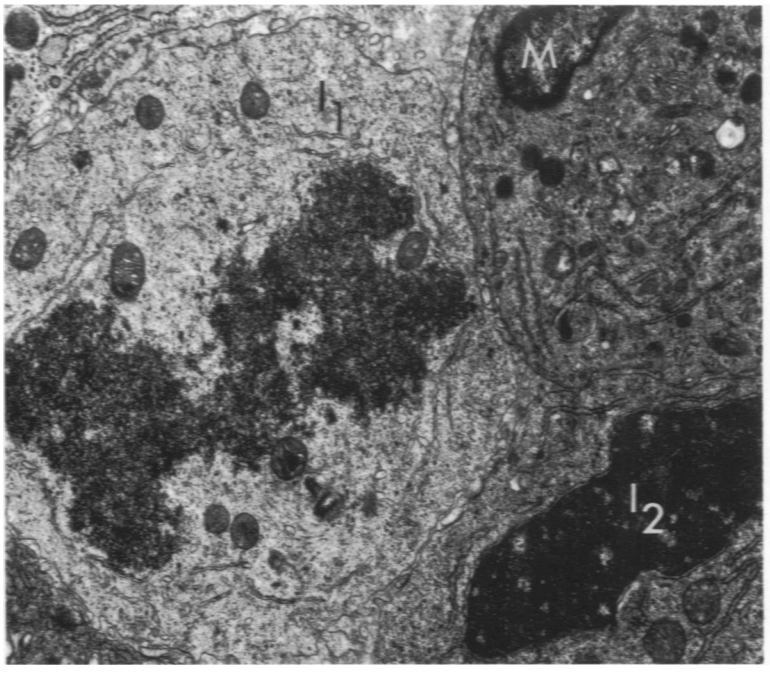


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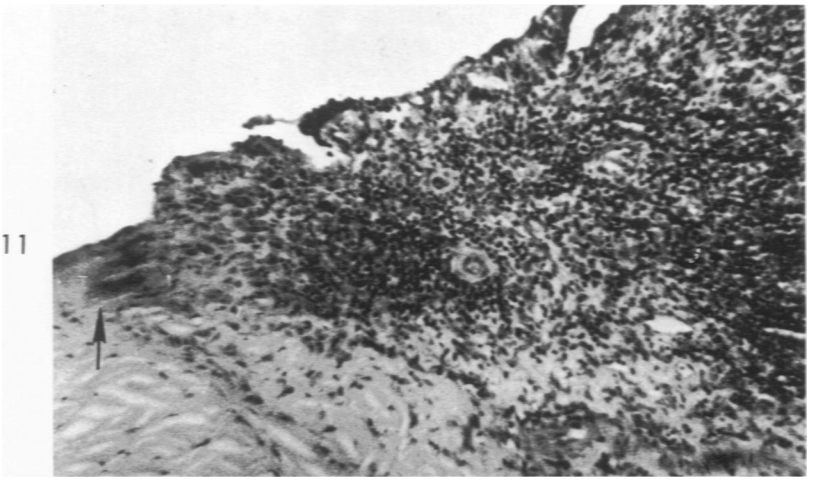


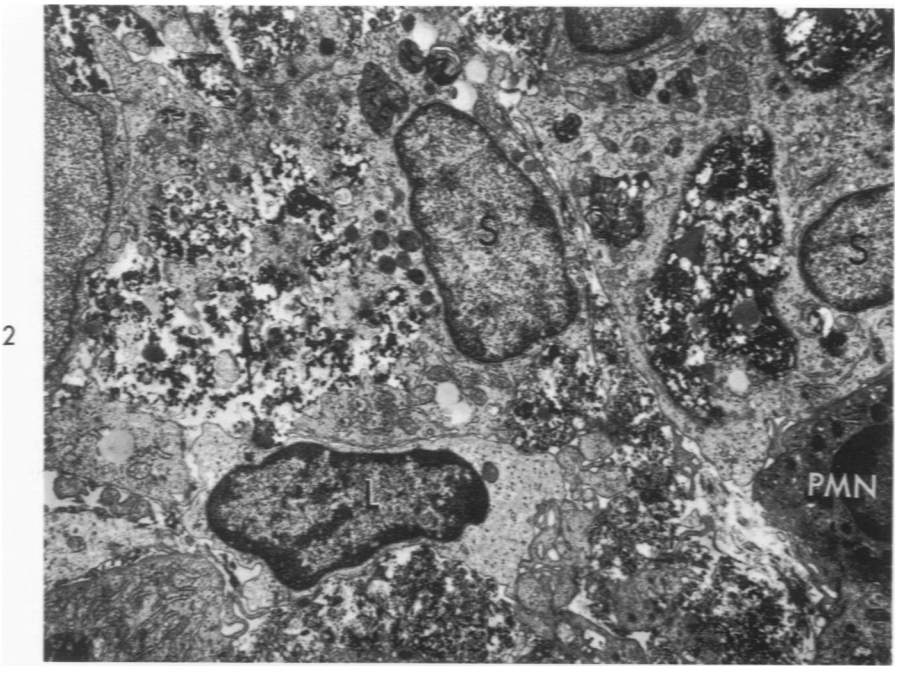
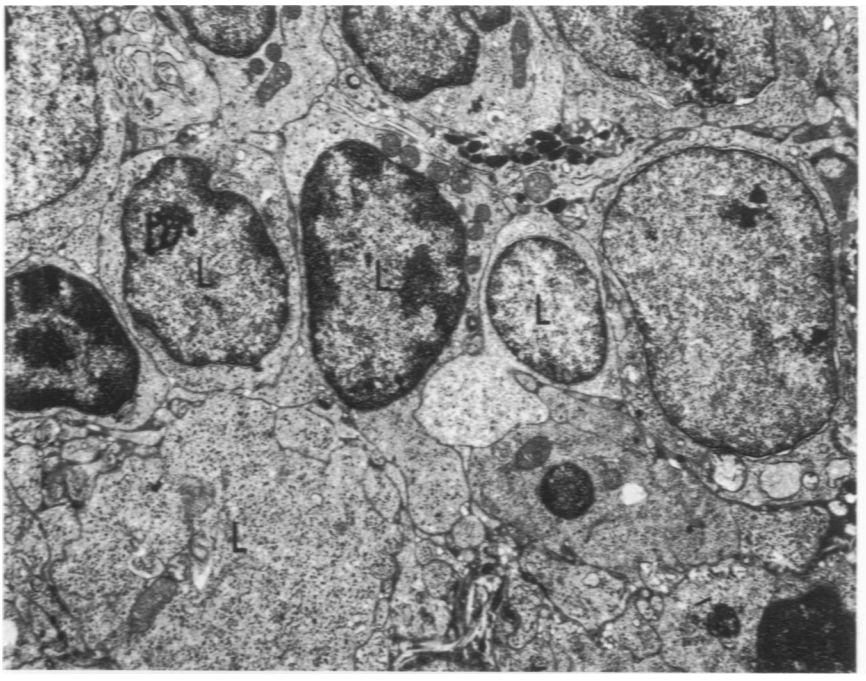



**Fig 11**—Inflammatory synovial tissue and cartilage from rabbit given 35 daily HRPO injections. The dense inflammatory infiltrate consists primarily of lymphocytes and macrophages. The altered staining characteristics of the cartilage adjacent to the inflammatory infiltrate (*arrow*) reflect necrosis of cartilage (light micrograph, H&E, × 120). **Fig 12**—A portion of the synovial membrane from an animal sacrificed after 37 daily injections. The synovial cells (*S*) appear highly active with large vacuoles containing cellular debris and other dense material. Extracellular fibrin and precipitated protein are present in abundance between the cells (*arrow*). A lymphocyte (*L*) and polymorphonuclear leukocyte (*PMN*) are seen within the synovial lining layer (electron micrograph, × 5000).

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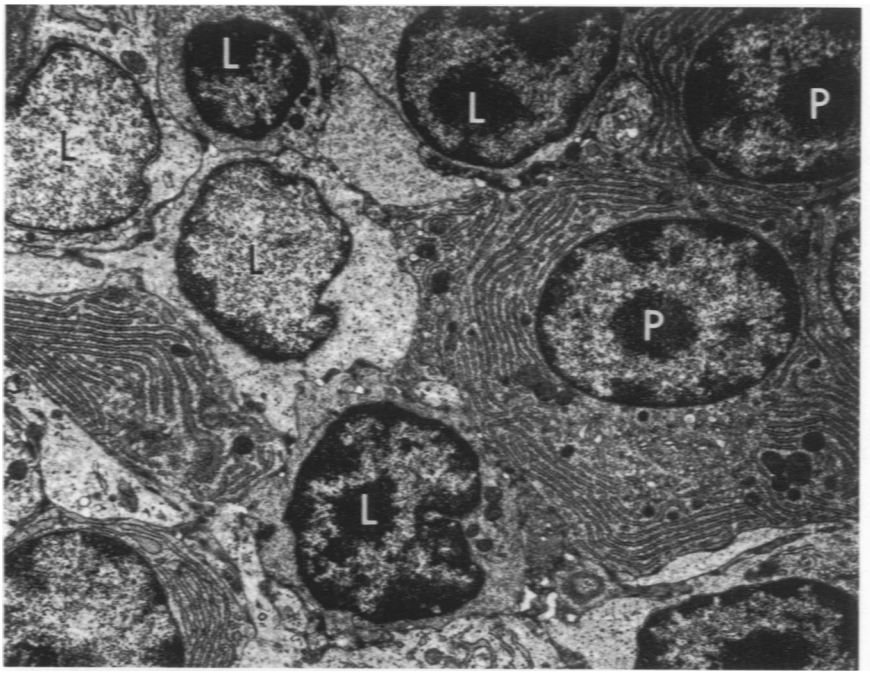


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**Fig 13**—Subsynovial infiltrate from rabbit killed after 37 daily HRPO injections. A spectrum of lymphoid cells (*L*) is present. There are several small to medium lymphocytes, with condensed nuclear chromatin and scant cytoplasm containing few organelles. Other lymphoid cells have more dispersed nuclear chromatin and moderate numbers of cytoplasmic free polyribosomes. The cells are closely packed, with little intercellular space intervening (× 6000). **Fig 14**—Subsynovial infiltrate from rabbit receiving 37 daily HRPO injections. Portions of several plasma cells (*P*) and a variety of lymphoid cells (*L*) are visible. As in Fig 13, very little intercellular space is present between adjacent cells (× 6000).

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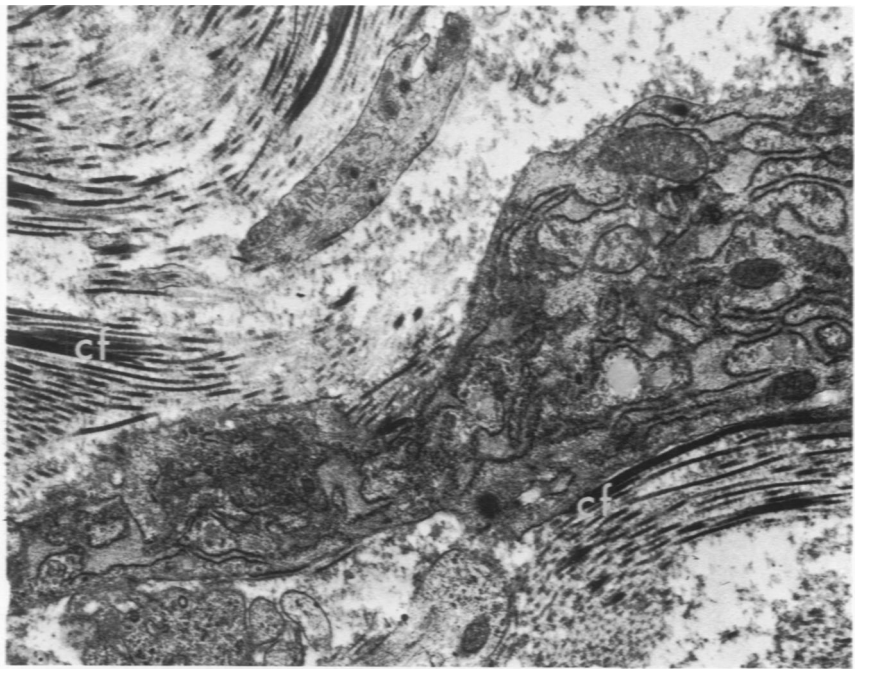


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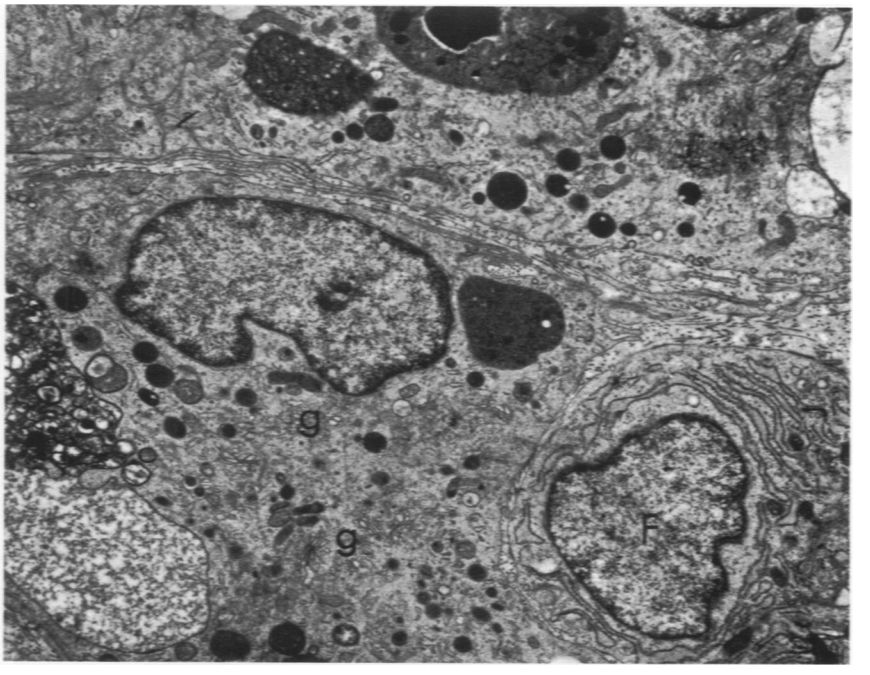
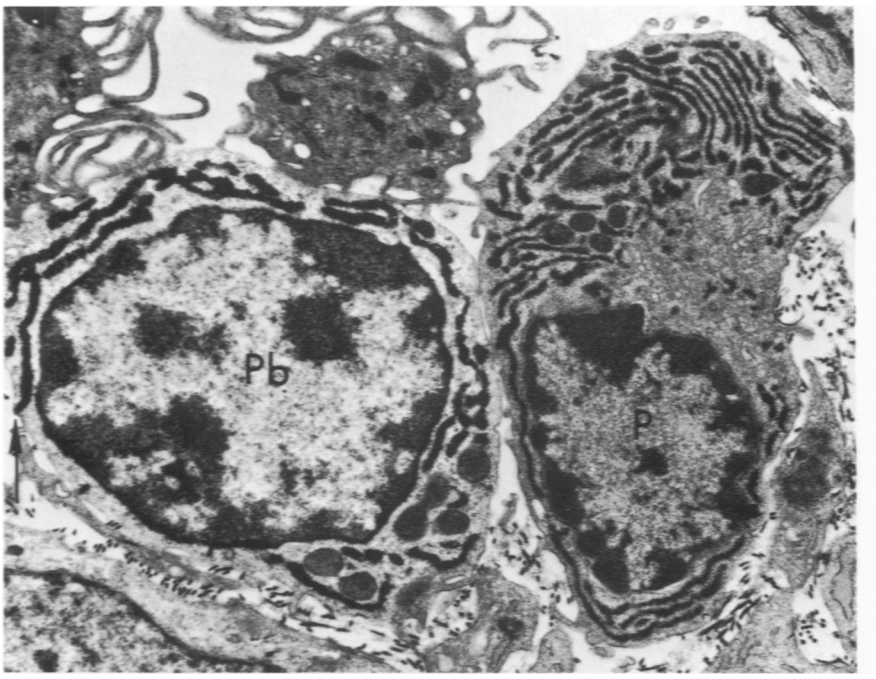



**Fig 15**—Fibroblast in subsynovial infiltrate of rabbit given 37 daily HRPO injections. The dilated rough-surfaced endoplasmic reticulum contains granular material which presumably is precipitated protein. Numerous collagen fibers (*cf*) surround the fibroblast (× 16,000). **Fig 16**—Macrophages from subsynovial tissue of rabbit given 35 daily HRPO injections. Numerous cytoplasmic vesicles and vacuoles variously contain cellular debris or amorphous material of varying electron opaqueness. The Golgi complex (*g*) is well developed. The cell at the right (*F*) with the extensive rough-surfaced endoplasmic reticulum probably is a fibroblast (electron micrograph, × 10,000).

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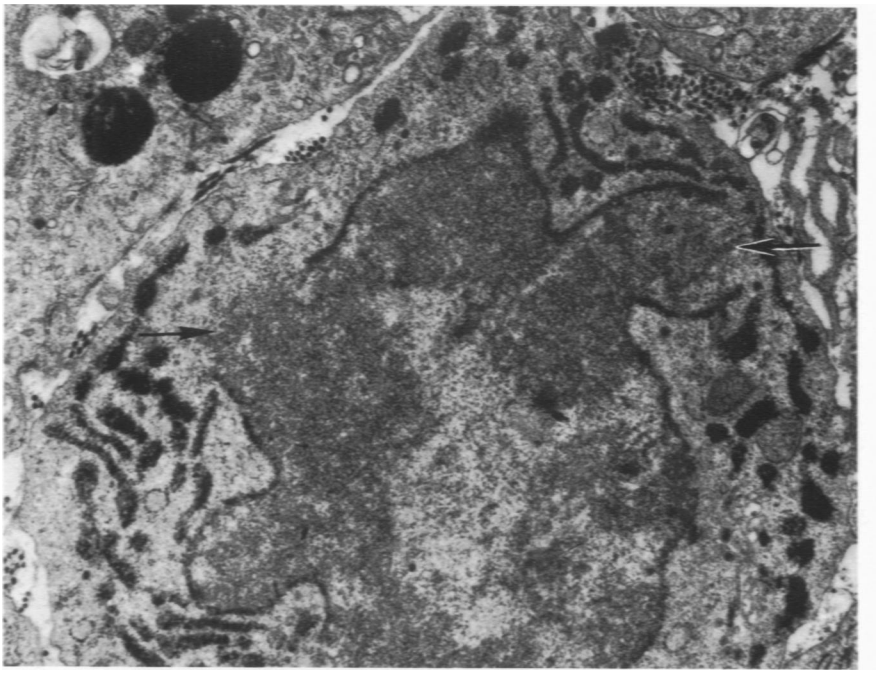

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**Fig 17**—Subsynovial plasma cell (*P*) and plasmablast (*Pb*) from rabbit receiving 15 daily HRPO injections. The procedure of Leduc and Avrameas was used to reveal sites of anti-HRPO activity. Such activity, revealed by peroxidatic reaction product, is present in the rough-surfaced endoplasmic reticulum of both cells. The perinuclear space of the plasmablast also contains anti-HRPO activity. The membrane of one of the profiles of rough-surfaced endoplasmic reticulum appears to be continuous with the plasma membrane of the plasmablast (*arrow*) (electron micrograph, × 12,000). **Fig 18**—Subsynovial plasmablast from rabbit sacrificed after 15 daily HRPO injections. Tissue reacted by method of Leduc and Avrameas to reveal anti-HRPO activity. Such antibody activity is indicated by peroxidatic reaction product in the rough-surfaced endoplasmic reticulum and perinuclear space. The nuclear membrane is discontinuous at several points (*arrows*), suggesting beginning mitosis (electron micrograph, × 15,000).

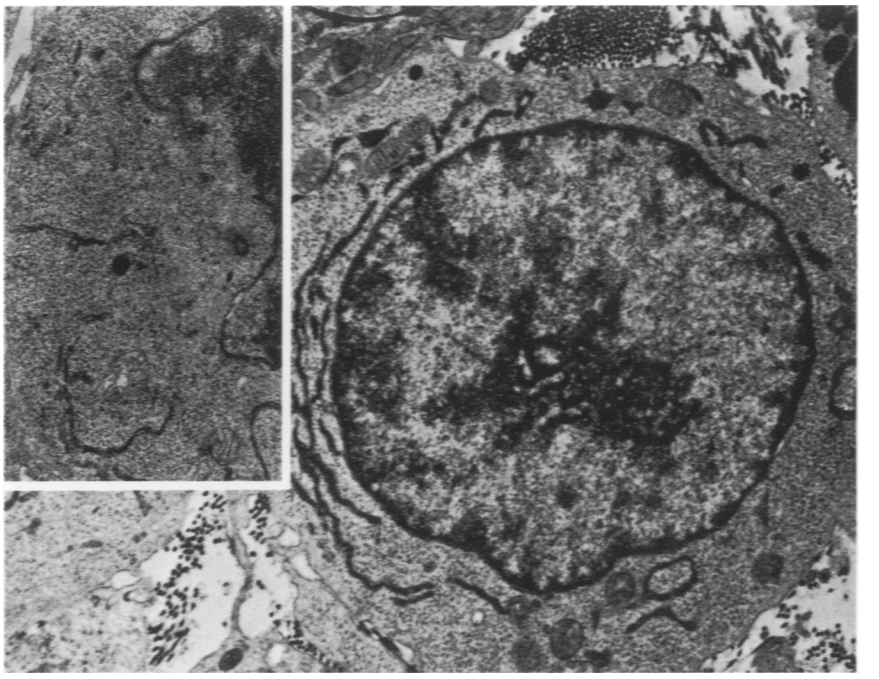
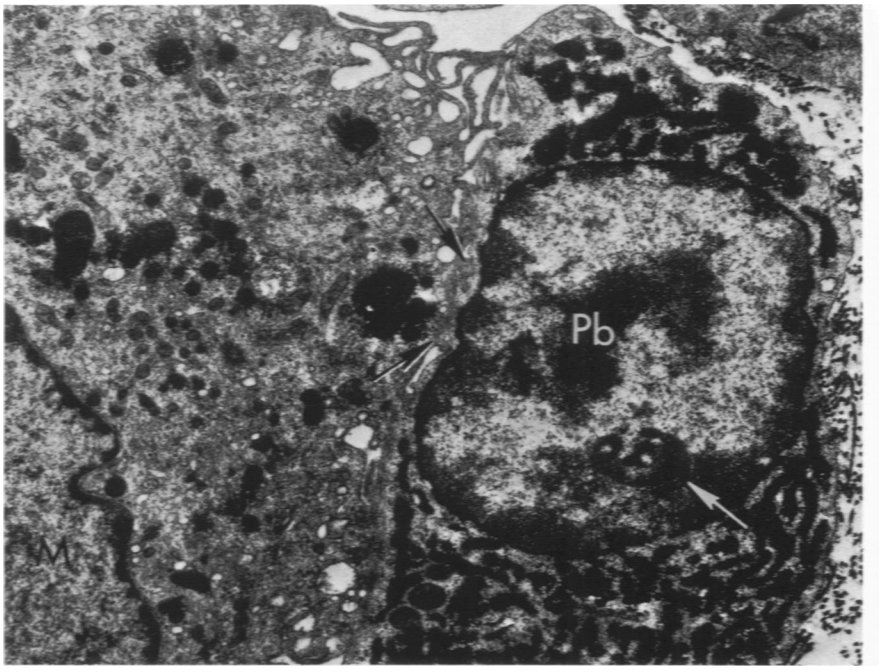


**Fig 19**—Subsynovial immunoblast from rabbit given 15 daily HRPO injections. Technic of Leduc and Avrameas was used to reveal anti-HRPO activity. The cell has a prominent nucleolus and dispersed nuclear chromatin. Several profiles of rough-surfaced endoplasmic reticulum show anti-HRPO activity (electron micrograph, × 10,000). **Inset** shows a similar cell in mitosis (electron micrograph, × 7500).

**Fig 20**—Subsynovial macrophage (*M*) and plasmablast (*Pb*) from rabbit given 15 daily HRPO injections. Anti-HRPO activity revealed by method of Leduc and Avrameas. The macrophage contains many HRPO-containing vesicles and vacuoles. The plasma membrane of the macrophage exhibits a complex network of pseudopods and is very closely associated with the plasma membrane of the plasmablast (*black arrows*). The nucleus of the plasmablast has peripheral condensations of chromatin and a prominent nucleolus (*white arrow*). Anti-HRPO activity is present in the rough-surfaced endoplasmic reticulum and perinuclear space of the plasmablast (electron micrograph, × 10,000). **Fig 21**—Enzyme immunoelectrophoresis in Agarose gel of sera from rabbits receiving 16 (*A*) and 35 (*B*) daily HRPO injections. **Top**—Patterns photographed by reflected light reveal the precipitin lines in which peroxidatic reaction product reveals anti-HRPO activity. With the 16-day serum (*A*), specific antibody activity is present in both *IgG* and *IgM* lines, while with the 35-day serum (*B*), it is confined to the *IgG* line. **Bottom**—Same patterns are photographed by transmitted light to reveal the many precipitin lines without anti-HRPO activity.

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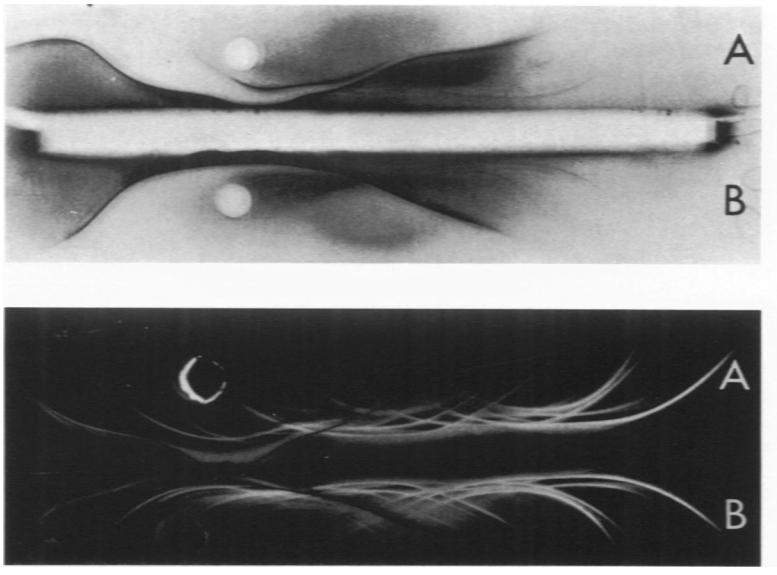


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