Chronic Self-Perpetuating Arthritis Induced in Rabbits by a Cell-Free Extract of Group A Streptococci (37479)

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(Introduced by T. N. Harris)

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Several experimental models of acute and chronic arthritis in the rabbit induced by group A streptococci and some of their products have recently been described. These involved the intra-articular injections of streptolysin S (1–3), cell-wall components (3, 4), extracellular products (5), cell-sensitizing antigen (6), and L-forms (7). Arthritis has also been produced by the use of immune complexes derived from streptococcal extracellular products and rabbit antibodies (8). The experimentally induced joint lesions were characterized by the rapid development of inflammatory exudates rich in polymorphonuclear and mononuclear leucocytes, by the proliferation of the synovial cells, by the appearance of "pseudo-lymphatic follicles" and the accumulation of large amounts of fluid. Some of the experimentally induced lesions previously described were self limited, and a complete recovery usually occurred 5–9 weeks following the onset of inflammatory changes. In the case of the arthritic lesions induced by injections of moderate amounts of cell-wall components (4), there was parallelism between the persistence of cell wall components in the tissues and the perpetuation of the inflammatory reactions.

The present report describes a model of self-perpetuating arthritis in the rabbit knee joint induced by the intra-articular injection of large amounts of streptococcal cell wall components. The histological lesions of this experimental model resemble the findings of human rheumatoid arthritis.

Materials and Methods. Group A type 4 streptococci were cultivated overnight in brain–heart infusion broth (Difco). The washed cells were adjusted to 1,000 Klett units (at 540 nm) and disintegrated in a Mickle cell disintegrator using Ballotini No. 14 glass beads (4). The cell walls and unbroken cells were removed by centrifugation at 1000*g* and the supernatants were further centrifuged at 36,000*g*. The cell free extract (CFE) was then concentrated by per vaporation and filtered through a Millipore filter (0.22 μm). The total amount of rhamnose was determined by the method of Dische and Shettles (9). Four daily injections of 0.25 ml each of CFE containing 3000 μg/ml rhamnose were given intra-articularly into the right knee joint of 44 rabbits weighing 1–2 kg. Normal saline was injected into the left knee joint of each animal. Leukocyte counts and differentials were made on the blood of the animals at the time of the first injection and at sacrifice. Smears of synovial effusions were stained with Giemsa stain. Sterility of the effusions was checked by inoculation of the joint exudates or of pieces of inflamed tissues into brain–heart infusion broth and by streaking on blood agar plates. Sera of the animals were analyzed for streptococcal cell-sensitizing antibodies (6) and for rheumatoid factor, using latex fixation and sheep agglutination tests. The severity of the joint lesions was evaluated by measuring the circumference of the knee joints and by measuring the amounts of texture of the joint fluid. Pairs of rabbits were sacrificed every 24 hr for the first 7 days. Two additional animals were sacrificed on the 14th day and the rest of

the animals at 2-week intervals up to 3 months. Pieces of the synovia, joint capsule, bone, articular cartilage and regional lymph nodes were examined histologically. Pieces of synovia were processed for electron microscopy. The fixation was in 5% glutaraldehyde with 0.1 *M* cacodylate buffer. This was postosmicated, dehydrated in graded alcohols and in propylene oxide and embedded in Epon 812. Thin sections were cut onto 300 mesh uncovered grids, stained with uranyl acetate and lead citrate. The electron microscope used was a Phillips 300.

Results. In the 44 knee joints injected 4 times with 0.25 ml saline no pathological changes were detected macroscopically or microscopically. In the 44 knee joints injected with CFE progressive arthritic lesions developed. Soon after the second injection, the joint became swollen, warm, red, and tender. The animal limped on the affected extremity. Up to 1 ml viscous, cloudy serous fluid was found in all the injected joints. Following the third injection the joint swelling increased further, becoming maximal 3 days later. It persisted for another week and then decreased, but the joint circumferences remained 10% larger than the control level for the rest of the experimental period.

Animals sacrificed 3–7 days following the last injection of CFE showed severe synovitis with an exudate consisting mainly of heterophilic granulocytes, and of lymphocytes which sometimes formed pseudofollicles (Fig. 1). Electron microscopic examination of synovia taken at this stage showed proliferation of synovial cells, infiltration with polymorphonuclear and mononuclear cells and appearance of new capillaries (Figs. 2, 3). No fibrinoid was seen. None of the samples yielded any bacterial growth. Electron microscopic examination of the CFE alone revealed amorphous material and very small quantities of membrane-like structures.

Three to six weeks after the last injection the synovial cell proliferation increased (Fig. 4), B cells predominating (Fig. 5). The numerous capillaries seen in the lesions appeared to be composed of light and dark endothelial cells which had sharp papillary projections. At later periods, the proliferation of B cells was even more marked although intermediated and A cells, too, were encountered. Granulocytes were seen on

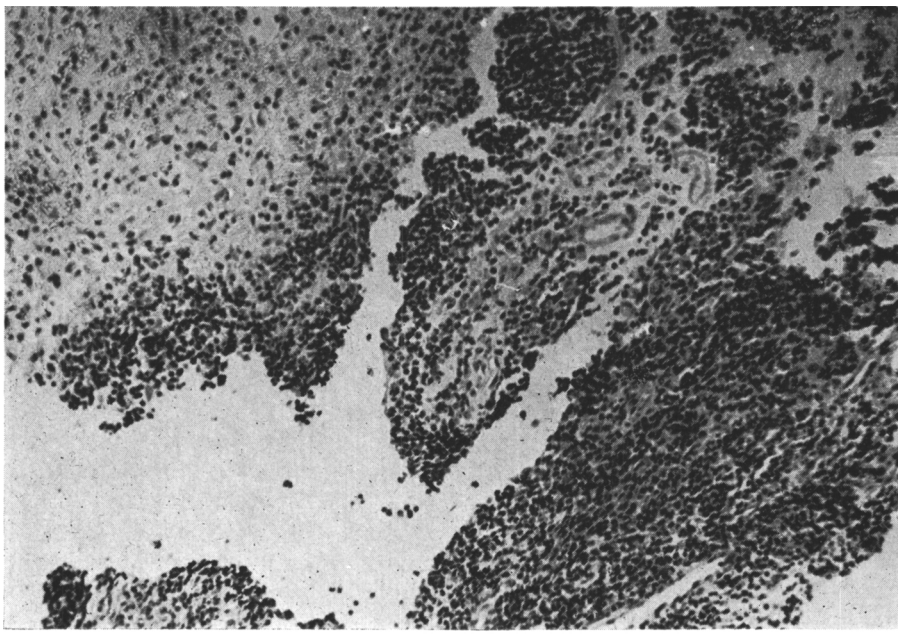

FIG. 1. Synovial inflammatory infiltrate three days after the last injection of cell free extract (Hematoxylin and eosin × 42).

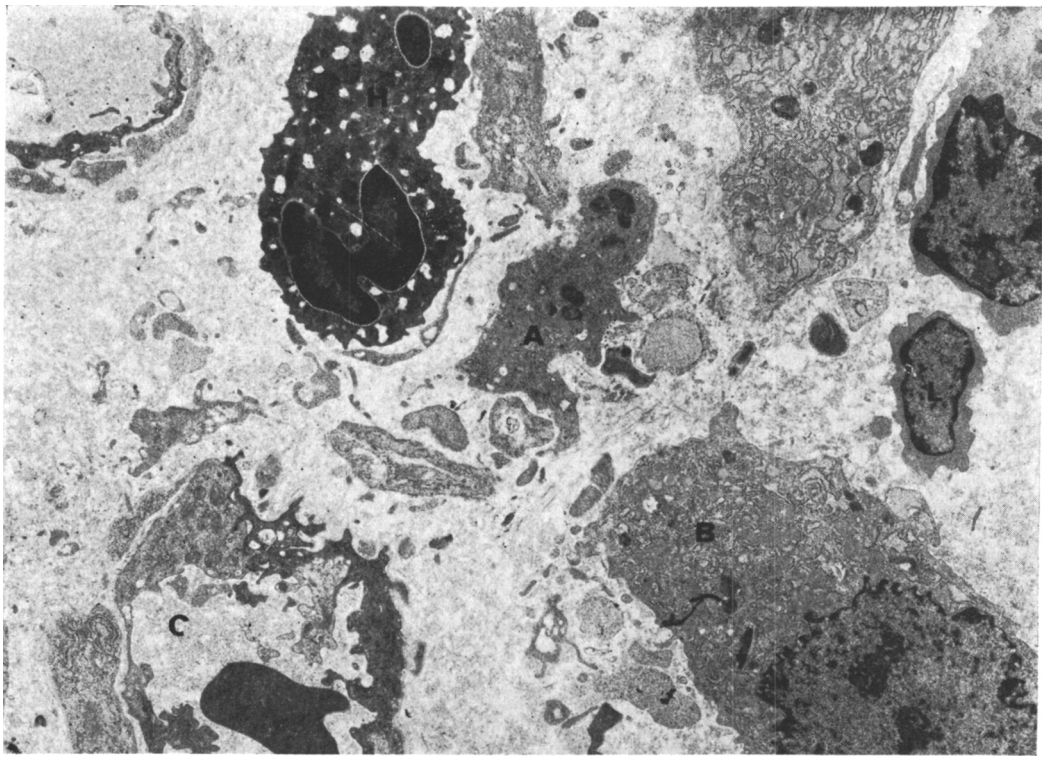

FIG. 2. Electron micrograph of synovia showing phagocytic A cells (A), synovial B cells, rich in rough endoplasmic reticulum (B), small and large lymphocytic cells (L), and polymorphonuclear heterophils (H). The capillaries (C) have lighter and darker endothelial cells with papillary projections into the lumen. The matrix is granular and contains many cell processes and debris (× 6000).

occasion while lymphocytes and fibroblasts were frequent (Fig. 6). In no case could parts of the CFE be identified as such on electron microscopic examination.

Damage to the articular cartilage, in the form of diffuse ulcerations, new bone formation and regions of pannus were evident (Fig. 7). Animals sacrificed during the 10th week had severe erosions of the articular cartilage. In some of the animals with these chronic lesions, penetration of fibrous tissue into the bone marrow cavity resembling chronic osteomyelitis was found. Only very few animals had a positive test for rheumatoid factor. Neither was there any correlation between the development of chronic arthritis and the serum titer of antistreptococcal antibodies. The development of arthritis was accompanied by an increase in total white cell count which reached a peak of 30,000 after three weeks. The differential counts shifted from a predominant granulocytic pattern at the beginning of the experiment to predominant mononuclear patterns afterwards.

Discussion. The data presented indicate that a chronic self-perpetuating arthritis characterized by persisting exudation, severe cartilagenous lesions and pannus formation developed in the knee joint of rabbits injected intra-articularly with large amounts of streptococcal cell-wall components.

This model differs in several aspects from that described originally by Schwab *et al.* (4) who employed a single intra-articular injection of cell-free streptococcal extract which contained 700 μg rhamnose. The arthritis which developed was self-limited and healed within 7–9 weeks. No evidence of pannus formation or cartilagenous involvement was found, while in our model the activity of the lesions leading to pannus formation per-

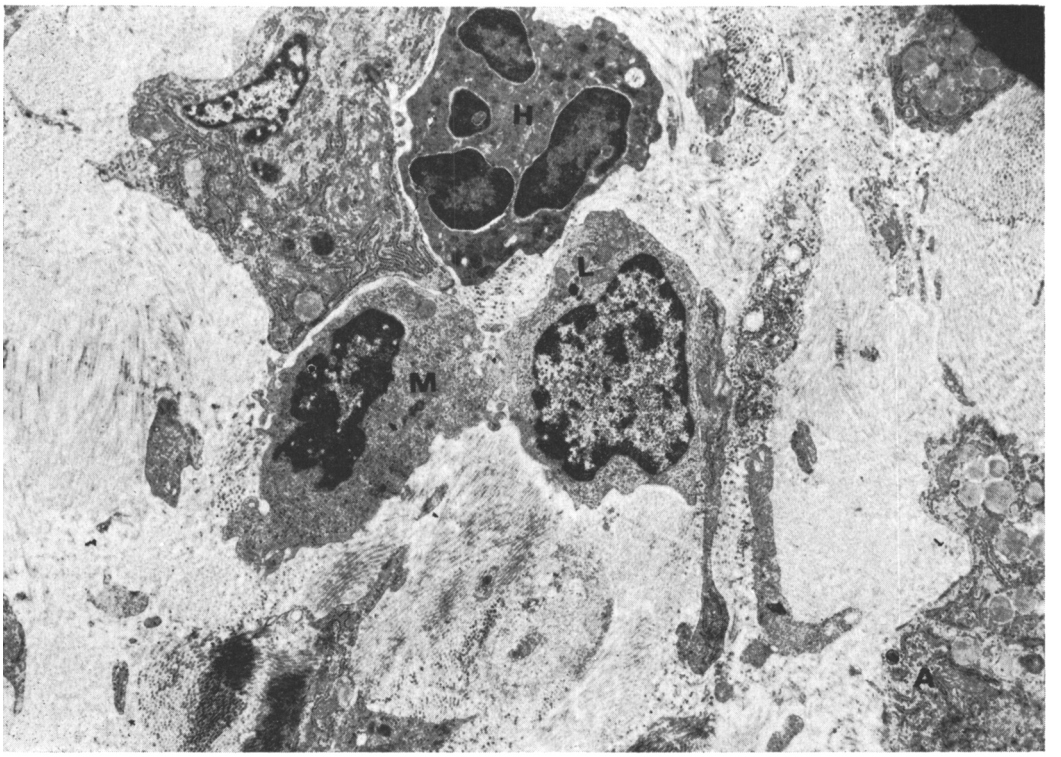

FIG. 3. A typical group of cells infiltrating synovia. H = heterophil polymorph. M = cell in mitosis. L = activated lymphocyte. A = phagocytic A cell containing lipid droplets. The matrix is rich in collagen fibrils (× 5250).

sisted for many months. The histological appearance in the model presented here is also similar to the arthritis produced in rabbits by injections of streptococci, staphylococci and pneumococci (10). Moreover, similar results were obtained following intra-articular injections of immune complexes derived from streptococcal extra-cellular products (devoid of streptolysin S) (5), following the injection of streptolysin S (1, 2) or of streptococcal L-forms (7).

The fact that different streptococcal products can induce comparable histological lesions does not mean that they have a common pathway of pathogenesis. The mechanism by which streptococcal cell wall components produce arthritis is not fully understood. Schwab *et al.* (4) suggested that there is a connection between the perpetuation of the chronic lesions and the persistence of the undegraded streptococcal cell wall within macrophages at the inflammatory site. It is reasonable to suppose that in our model the presence in the joints of overwhelming amounts of cell-wall components initiated a toxic irritation of the synovial cells with resulting self-perpetuating arthritis. The persistence of the streptococcal products as shown by fluorescent antibody techniques has already been reported by Schwab *et al.* (4). Other experiments with mice (11) have shown that ^{14}C-labeled group A streptococci persisted for several months in chronic granulomatous lesions in muscles pointing towards a possible inefficiency of the leucocyte enzymes responsible for the cleavage of the C-polysaccharide mucopeptide linkages, thus hindering the split of the mucopeptide by lysozyme (4).

Further support for the inability of leucocyte extracts to degrade streptococci comes from studies showing that leucocytes which contain hydrolases fail to depolymerize the streptococcal cell walls *in vitro* (12). On

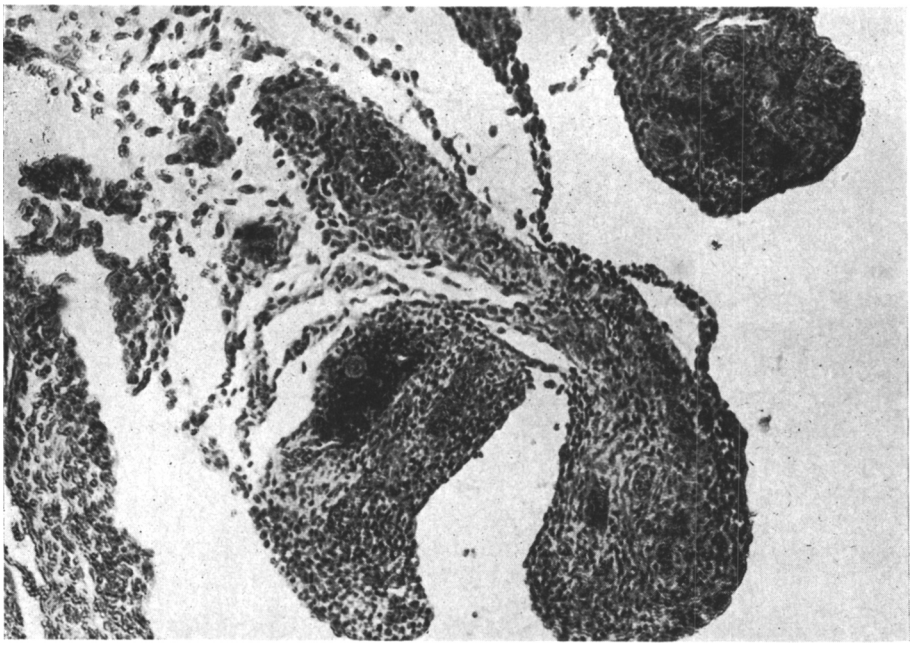

FIG. 4. Subacute synovitis, 5 weeks following injection with cell free extract, showing proliferation of synovial cells and numerous follicle-like aggregates of lymphocytes (Hematoxylin and eosin, × 42).

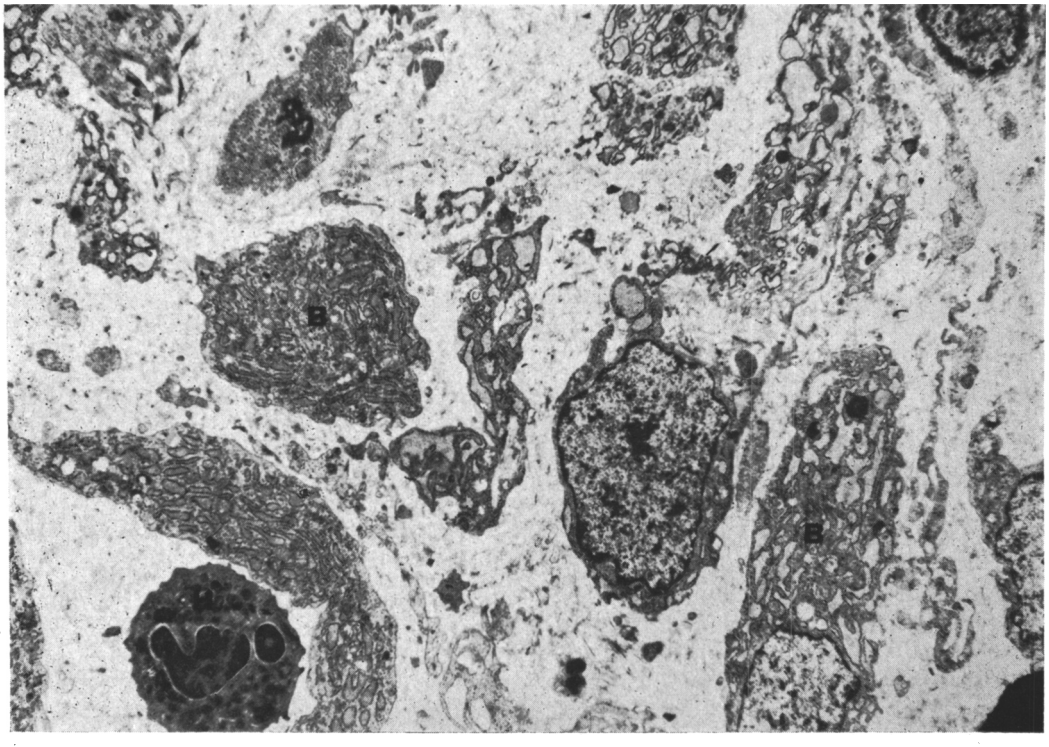

FIG. 5. Six weeks after last cell free extract injection. Numerous fibroblast-like synovial B cells (B) and heterophil polymorphs (H) are seen. In matrix, cell debris and collagen fibrils (× 3250).

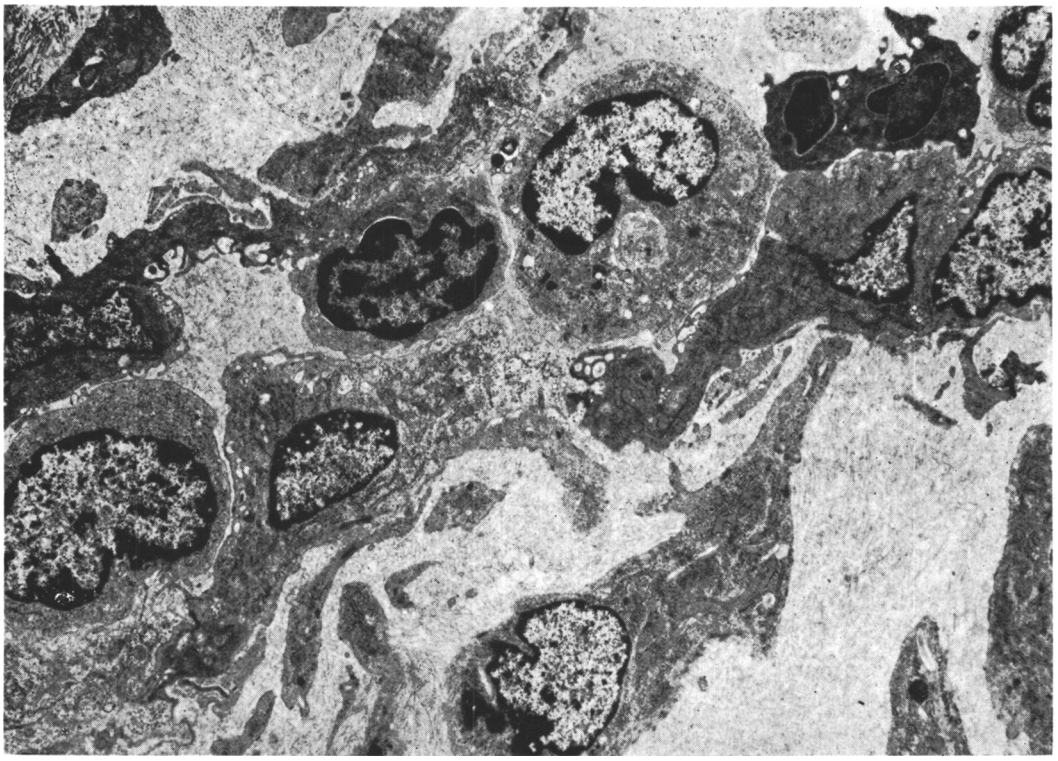

FIG. 6. A synovial vessel, possibly a venule, containing small and large (activated) lymphocytes and heterophils (× 3750).

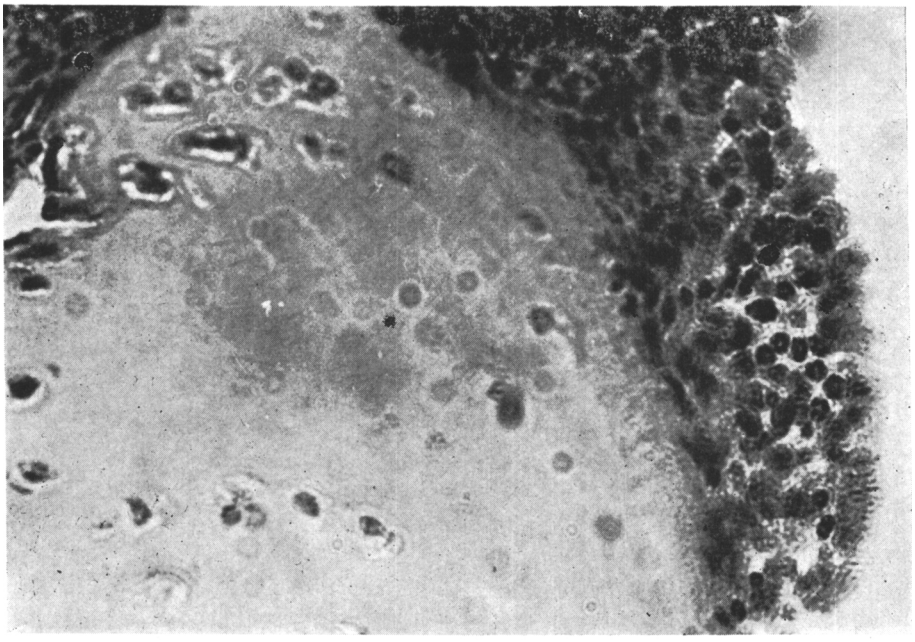

FIG. 7. Six weeks after last cell free extract injection. The articular cartilage is covered by actively expanding pannus (Hematoxylin and eosin, × 120).

the other hand, staphylococcal cells were readily broken down by such lysates. It is thus possible that rheumatoid arthritis in humans may be the result of the localization of undegraded bacterial cell-wall components in joints and that this phenomenon may occur in many other diseases characterized by protracted inflammatory reactions.

An alternative hypothesis which may explain the development of chronic joint lesions is that as with streptolysin S (SLS) (1), the CFE brings about the release of large amounts of lysosomal enzymes from leucocytes which cause tissue damage. However, streptococcal L-forms derived from SLS-less mutant also induced chronic arthritis with pannus formation (7). This shows that lysosomal disruption is not the only important factor in the pathogenesis of chronic lesions. A further possibility is that immune complexes formed between the localized streptococcal products and humoral antibodies or sensitized lymphocytes might be responsible for the perpetuation of the chronic inflammatory process.

The described self-perpetuating arthritis seems to be a model which resembles the histopathological lesions in human rheumatoid arthritis, and may therefore well serve for evaluation of different methods of treatment of this protracted joint disease. Further work along these lines is being carried out.

Summary. Self-perpetuating arthritis was induced in knee joints of rabbits by intra-articular injections of large amounts of cell free extract derived from group A streptococci disintegrated mechanically. The pathological alterations were characterized by synovial lining cell proliferation, polymorphonuclear and mononuclear cell infiltration with the appearance of pseudo-follicles and pannus formation. Electron microscopical proliferation of B cells was predominant. An active inflammatory exudate and numerous new capillaries were also seen. The induced arthritis was self-perpetuating and appears to resemble human rheumatoid arthritis.

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