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Association of Experimental Chronic Arthritis with the Persistence of Group A Streptococcal Cell Walls in the Articular Tissue

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A single injection of isolated fragments of group A streptococcal cell walls into the joints of rabbits stimulated an initial acute reaction which was followed by a prolonged inflammatory process. The chronic process was characterized by hyperplasia of the synovial cells, diffuse infiltration of the villi by macrophages, and focal collections of lymphocytes in the stroma of the villi. These histological changes were similar to those seen in the early stages of rheumatoid arthritis. Antibodies specific for the mucopeptide and group-specific C polysaccharide antigens of group A streptococcal cell walls were labeled with either fluorescein or ^{125}I, and were used to demonstrate antigen in the synovial tissues. The antigens persisted within macrophages for at least 5 weeks. Their presence correlated with the evolution of the chronic inflammatory process.

The observation, that a single intradermal injection of a sterile extract of sonically disrupted group A streptococcal cells induces a prolonged remittent and intermittent multinodular lesion of dermal connective tissue of rabbits (3, 9), led to a series of studies to define the nature of the toxic moiety responsible for this reaction, and to determine what role this material might play in streptococcal disease. The toxic material is a carbohydrate-mucopeptide complex from cell-wall fragments of a limited range of particle sizes (1, 7). Utilization of methods that show the localization of cell-wall fragments in tissue has demonstrated their association with the evolution of the chronic and recurrent inflammatory process in the skin of rabbits (6). The concept, that fragments of group A streptococcal cell walls may be able to act as durable toxic material, localize in the heart, and induce the sequelae of streptococcal infection, is supported by studies which demonstrated that intraperitoneal injection of extract containing this toxic moiety induced a carditis in mice. The cardiac lesions, in terms of their distribution and histological features, were similar to the cardiac lesions of rheumatic fever (2).

In addition to the experimental models mentioned above, a model of chronic arthritis has been produced in the rabbit. This report describes the histopathology of the joint lesion and its relationship to the persistence and localization of group A streptococcal cell-wall material.

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MATERIALS AND METHODS

Animals. New Zealand white rabbits weighing 2.0 to 3.0 kg were obtained from a single local breeder.

Cell-wall fragments and injection of animals. The preparation of isolated cell-wall fragments and crude sonic extracts from group A, type 3, strain D58 streptococcus and group D, strain F-24 streptococcus has been described (6, 8). All of the preparations were filtered through Millipore HA membranes (Millipore Corp., Bedford, Mass.) and sterility was checked by streaking 0.1 ml onto sheep blood-agar plates. Careful attention was given to aseptic technique. One group of 36 rabbits was injected in the right knee-joint with 0.2 ml of a suspension of group A cell-wall fragments containing 114 μg of rhamnose or approximately 400 μg of cell-wall material. The left knee-joint was injected with an identical concentration of group D cell-wall fragments.

A second group of 12 rabbits received the same materials in a higher concentration; 1 ml of the group A cell-wall fragments containing 1,300 μg of rhamnose was injected into the right knee-joint, and 1 ml of group D cell-wall fragments containing 1,500 μg of rhamnose was injected into the left joint.

A third group of 12 rabbits was injected with the crude sonic extracts of group A and group D cells; 1 ml of group A extract containing 850 μg of rhamnose and a similar amount of the group D extract

were injected into the right and left knee-joints, respectively.

Animals were sacrificed at intervals from 3 hr to 9 weeks after injection. Sections for histological examination were fixed in 10% Formalin. Paraffin-embedded sections were stained with hematoxylin and eosin.

Persistence and localization of cell-wall antigens in joint tissue. Specifically purified antibodies against mucopeptide and polysaccharide antigens from group A streptococci were labeled with either ^{125}I or fluorescein (6). Serial sections of the above-described Formalin-fixed tissue were cut at 4 μ and stained with hematoxylin and eosin, or Giemsa, or with the labeled antibody. Controls consisted of staining with normal labeled globulin, or blocking with unlabeled antibody prior to the application of the labeled antibody. Another control group consisted of tissue from the left knee-joints which had been injected with group D streptococcal cell walls. The details of the fluorescence microscopy and autoradiography procedures have been presented (6). Paraffin-embedded sections were suitable for fluorescent antibody studies, if they were deparaffinized in xylol within 1 month after fixation.

RESULTS

Observation of the joints in the gross revealed an increase in the transverse diameter of 2.5 to 5 mm, which appeared to result from soft-tissue swelling. Maximal swelling was observed 2 days after injection, and the process subsided over a 4 to 5 day period. No recurrence of swelling was observed. There was no significant difference in the extent or duration of the joint enlargement produced by the different inocula.

Histopathological study of joint tissue. The initial microscopic changes consisted of an acute inflammatory response with infiltration of the synovial tissue by heterophils, focal ulceration of the superficial layers of the synovial membrane, and accumulation of a fibrino-purulent exudate in the joint space. These early changes were similar in joints injected with the various inocula. Two weeks after injection, tissue sections of the joints injected with group D preparations showed areas of fibroplasia in the synovial tissue, with scattered foci of macrophages and lymphocytes; 6 weeks after injection, these tissues appeared normal except for focal scarring of the villi.

In contrast, study of the joints injected with extracts of group A organisms revealed that the initial acute exudative inflammatory process was replaced by a chronic reaction which was most marked 2 or 3 weeks after injection. The process appeared to subside slowly. Minimal evidence of inflammation was observed in the tissues collected 9 weeks after injection. No essential difference was noted in the character or extent of the inflammatory process observed in the joints injected with the three different inocula containing material from group A streptococcal cells, although the concentration of rhamnose injections varied from 114 to 1,300. The chronic reaction was characterized by marked hypertrophy of the villi; hyperplasia of the synovial lining cells; and diffuse infiltration of the stroma of the villi by macrophages, many of which had pale staining cytoplasm and a few of which contained several nuclei arranged around the periphery of the cytoplasm. From 4 to 6 weeks after injection, focal accumulations of lymphocytes were observed in the stroma of the villi. Tissues collected 2 to 4 weeks after injection of the components of group A cells, in addition to showing the chronic reaction, exhibited scattered foci of acute inflammation in the synovial tissue, with collections of heterophils that suggested an exacerbation of the inflammatory process. The major histological features of the reactions observed are illustrated in Fig. 1–4.

Localization of cell-wall antigen. Studies of the sections stained with fluorescein- or ^{125}I-labeled antibody demonstrated persistence of group A cell-wall antigens in the tissue for 5 weeks after injection. From 24 hr to 5 weeks after injection, the labeled antibody was localized almost exclusively within macrophages in the stroma of the synovial membrane and villi. These cells corresponded to the large macrophages, some of which were multinucleate, and which had pale staining cytoplasm as described above. The fluorescent material within these cells was granular in appearance. All of the controls were negative. These results are illustrated in Fig. 5–16.

DISCUSSION

The studies reported here are based on the concept that fragments of group A streptococcal cell walls may act as durable toxic material which is able to stimulate a remittent and intermittent prolonged inflammatory reaction. The specific objectives of the experiments were to determine the histological character of the inflammatory response produced by a single intra-articular injection of this toxic material, and to relate this response to the persistence of the cell-wall fragments in the synovial tissue. The microscopic features of the response included an early, acute exudative reaction which was observed in animals injected with group D and group A streptococcal cellular components. This reaction was followed by healing in the animals receiving group D material. In the animals receiving the material from group A cells, the acute reaction was followed by a proliferative and infiltrative process. A feature of the chronic reaction was the development of

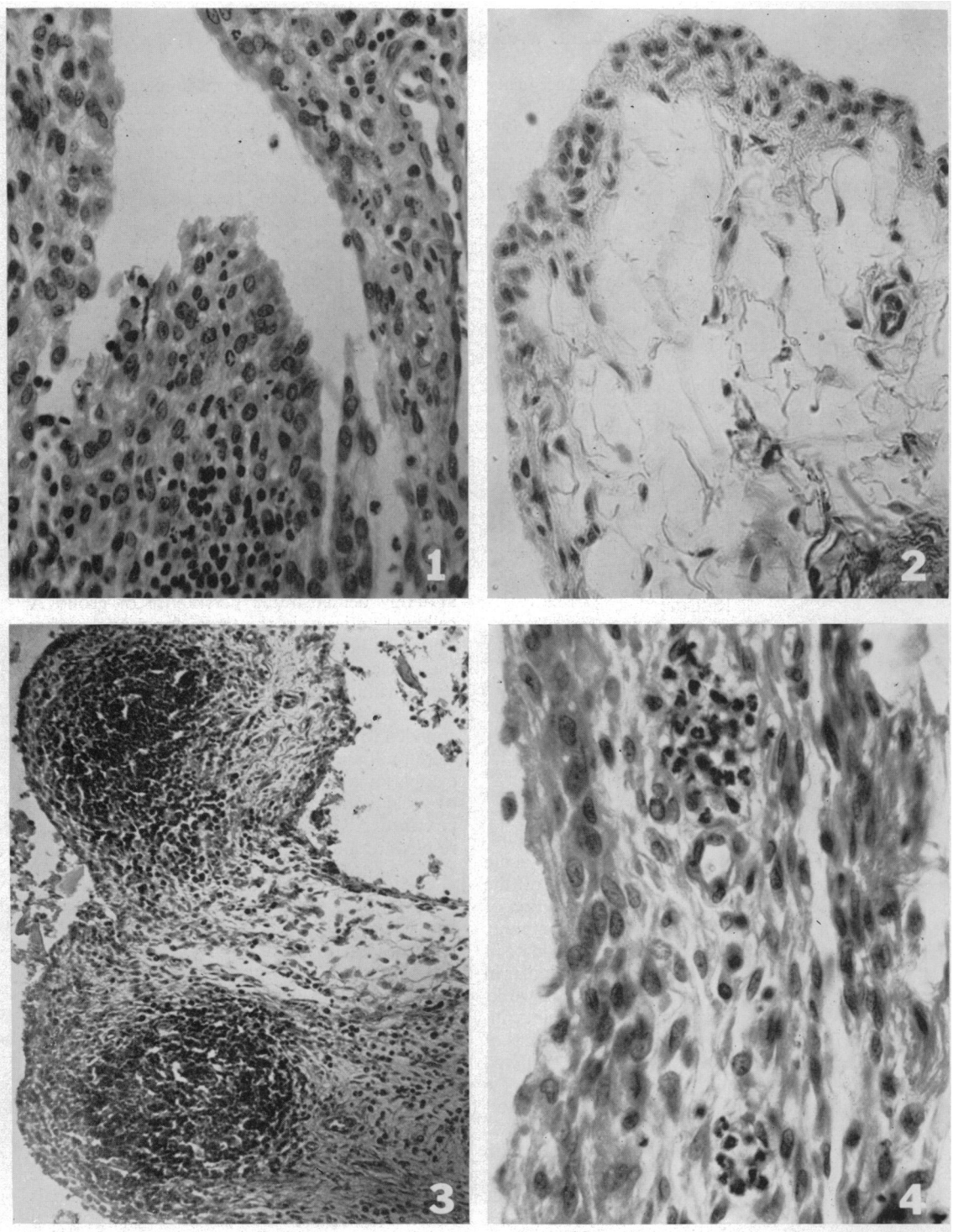
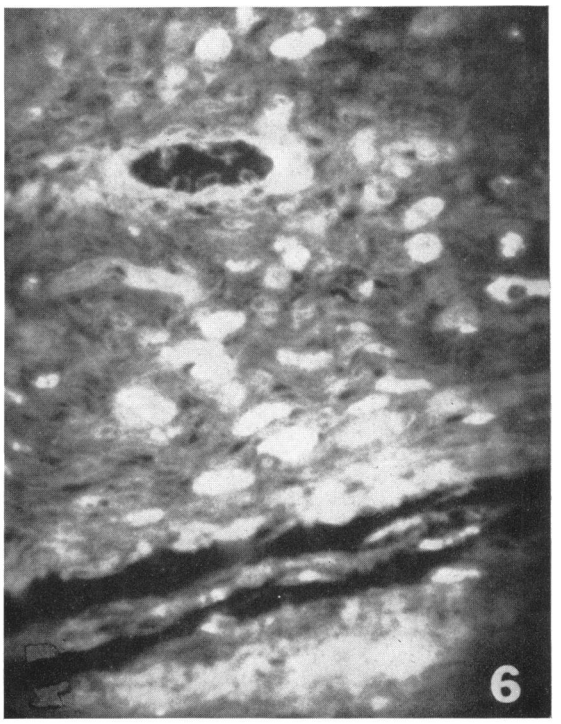


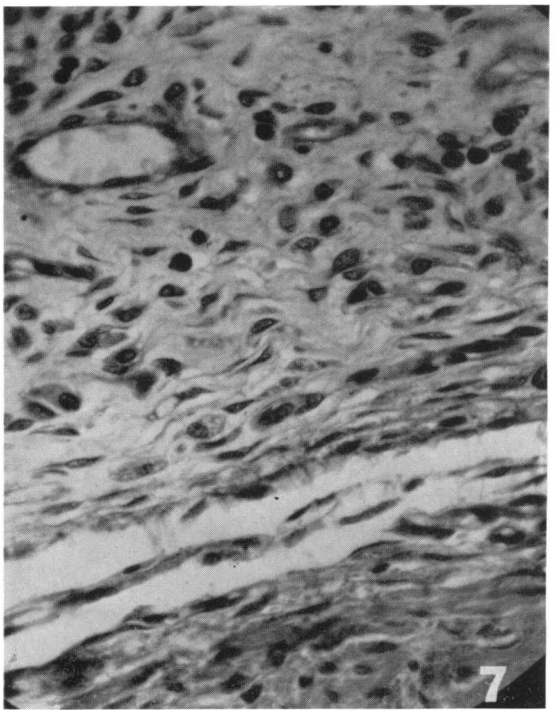
FIG. 1. *Synovial tissue 2 weeks after intra-articular injection of 1 ml of crude extract of group A streptococcal cells. Hyperplasia of the synovial cells and infiltration of the stroma with mononuclear cells is illustrated. Hematoxylin and eosin stain. × 340.*

FIG. 2. *Synovial tissue 3 weeks after intra-articular injection of 1 ml of group D cell-wall fragments. Relatively little evidence of inflammation is present. Hematoxylin and eosin stain. × 130.*

FIG. 3. *Villus 3 weeks after intra-articular injection of 0.2 ml of group A cell-wall fragments. Aggregates of lymphoid tissue resembling Allison-Ghormley bodies are present. Hematoxylin and eosin stain. × 130.*

FIG. 4. *Synovial tissue 2 weeks after intra-articular injection of 1 ml of group A cell wall fragments. Foci of heterophils are present in the stroma. Hematoxylin and eosin stain. × 560.*





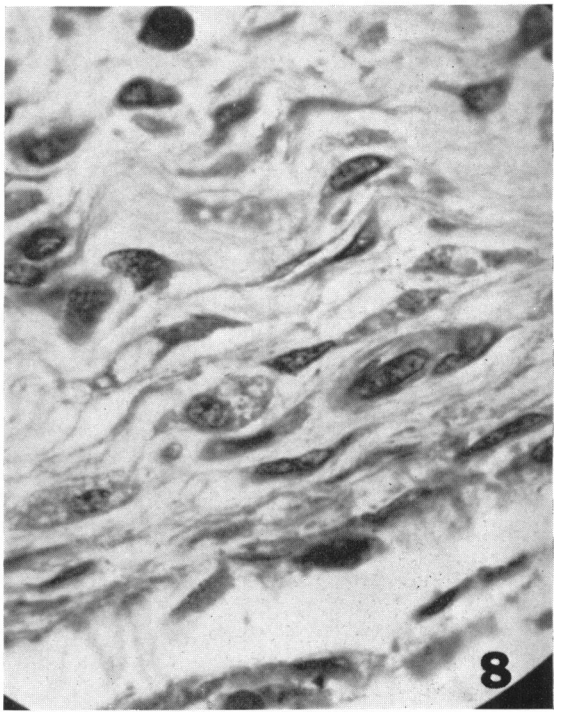


FIG. 5–8. *Synovial tissue 4 weeks after intra-articular injection of group A streptococcal cell-wall fragments. (Fig. 5) Hyperplasia of the synovial cells and infiltration of the stroma with mononuclear cells are illustrated. Giemsa stain. × 100. (Fig. 6) Section with fluorescein-labeled anti-group A polysaccharide. Higher magnification of the area around vessel in upper right quadrant of Fig. 5. × 420. (Fig. 7) Same area of same section shown in Fig. 6 after staining with Giemsa stain. This illustrates the type of cell in which antigen is found. × 420. (Fig. 8) Higher magnification of central area in Fig. 7. Antigen is found exclusively in macrophages. Giemsa stain. × 970.*

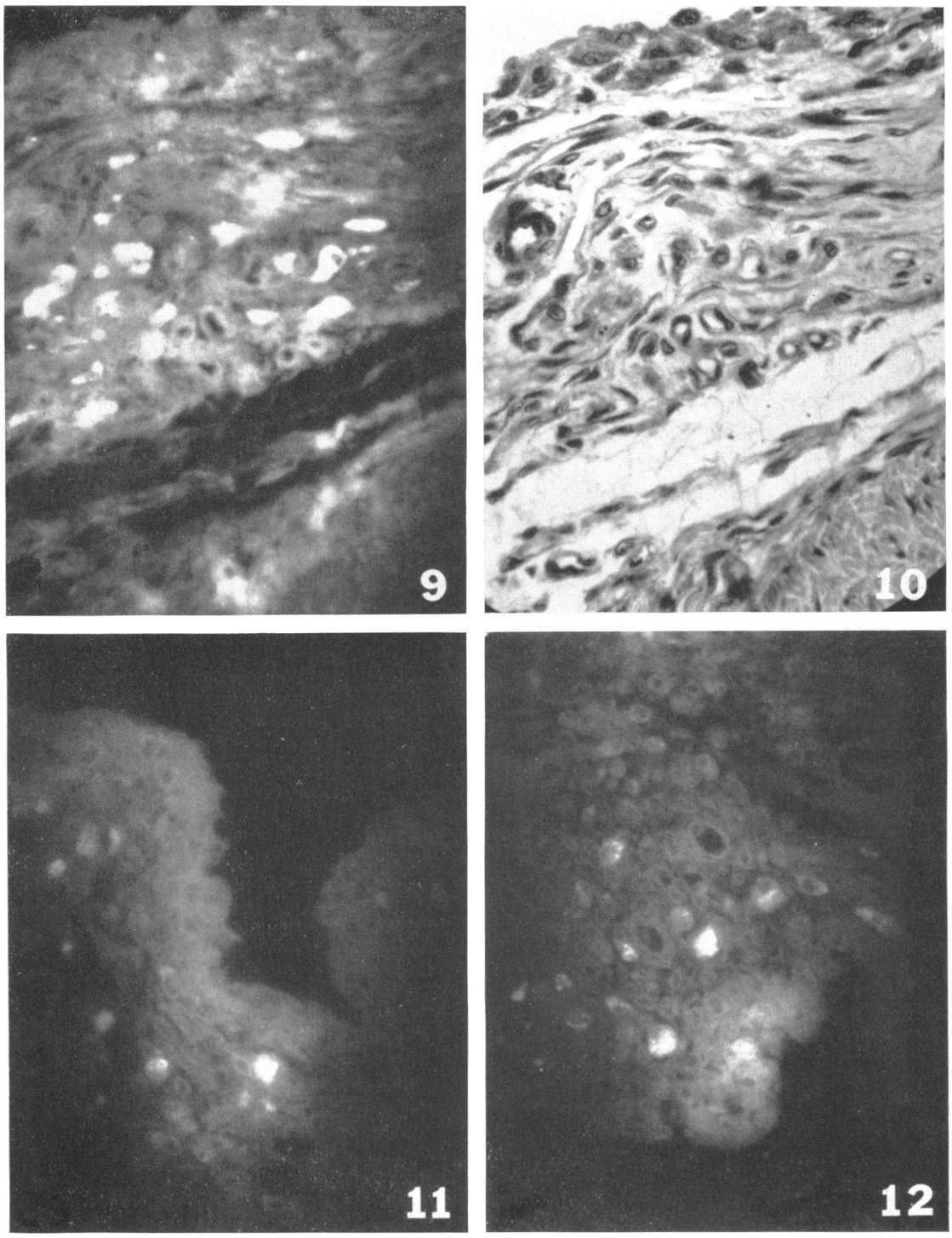


FIG. 9–10. *Discrete localization of group A streptococcal cell-wall antigen within macrophages in the stroma of a villus 4 weeks after intra-articular injection of group A cell-wall fragments. (Fig. 9) Stained with fluorescein-labeled anti-group A polysaccharide. × 420. (Fig. 10) Giemsa stain of same section as shown in Fig. 9. × 420.*

FIG. 11–12. *Localization of antigen in villi of rabbit joint 2 weeks after injection of group A streptococcal cell-wall fragments. Stained with fluorescein-labeled anti-group A polysaccharide. × 420.*

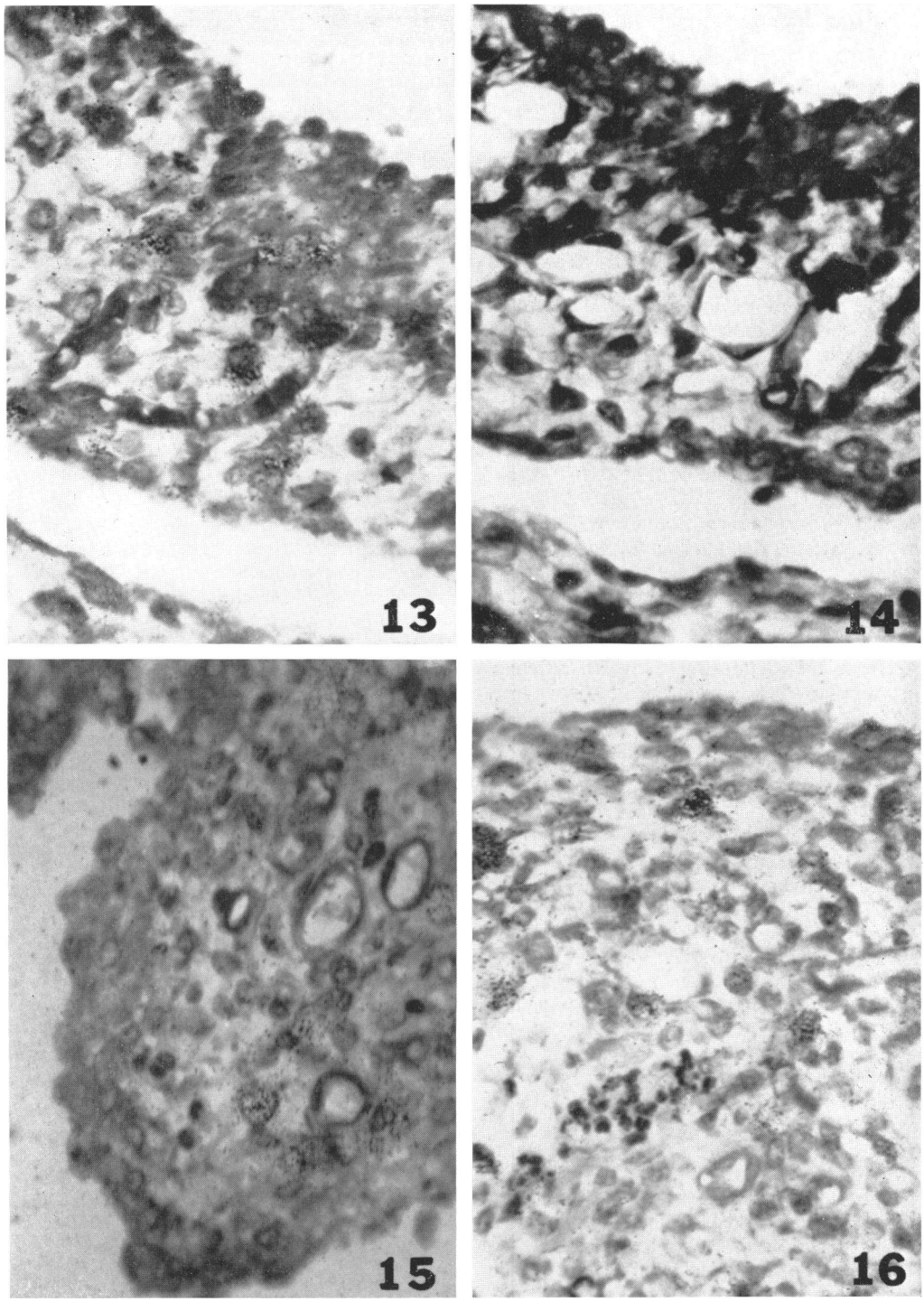


FIG. 13–16. *Rabbit joint 2 weeks after intra-articular injection of group A streptococcal cell-wall fragments. Radioautographs of sections stained with ^{125}I-labeled globulins, followed by Giemsa stain after photographic development. × 420. (Fig. 13) ^{125}I anti-group A polysaccharide. (Fig. 14) Approximately same area in consecutive section of Fig. 13 stained with ^{125}I normal rabbit globulin (diethylaminoethyl chromatography). (Fig. 15) ^{125}I anti-group A polysaccharide, showing intracellular antigen in villus. (Fig. 16) ^{125}I anti-group A polysaccharide, showing focal collection of heterophils in the stroma which is infiltrated with macrophages. Note absence of antigen in heterophils in contrast to labeling of macrophages.*

nodular aggregates of lymphocytes. The inflammatory reaction was most marked at 2 to 3 weeks, with minimal evidence of chronic inflammation and scarring of the villi found at 9 weeks after injection. As late as 6 weeks after inoculation, focal accumulations of heterophils were observed in the stroma of the villi, thus suggesting recurrent acute injury. The evolution of the inflammatory changes correlated with persistence and localization of group A cell-wall antigens in the tissue. Both the mucopeptide and C-polysaccharide cell-wall antigens were associated with the process. After 1 day, and until 5 weeks after injection, the antigens appeared to be localized in macrophages. These observations are similar to those of Ohanian and Schwab, who studied the persistence of these materials in the dermal connective tissue of rabbits (6). Abdulla and Schwab (1) have presented data which suggest that the toxic moiety from cell walls of group A streptococci is the mucopeptide structure, and that the associated polysaccharide masks the reactive sites on the mucopeptide, slowing its reaction with the tissue. It was also suggested that the C-polysaccharide protects the mucopeptide from destruction by tissue muramidases. According to this concept, the recurrent acute lesions, indicated by the focal accumulations of heterophils within the areas of chronic inflammation, result from the periodic release of free mucopeptide as the C-polysaccharide is slowly digested. An enzyme that might initiate the removal of the C-polysaccharide has been identified by Ayoub and Wannamaker (Federation Proc. **26**:581, 1967). These workers reported the identification of a *N*-acetylglucosaminidase in human leukocytes and rabbit macrophages. This suggests that an enzyme which can initiate the degradation of this carbohydrate is present in phagocytic cells. However, knowledge relating to the manner in which streptococcal cells are degraded in the tissues is limited.

The indirect evidence relating group A streptococci to rheumatoid arthritis is not as well documented as that relating these organisms to rheumatic fever or glomerulonephritis. However, the data recently presented by Francois suggest such a relationship (4). Previous attempts to produce an experimental model of rheumatoid arthritis have been reviewed by Gardner (5). These involved the use of living and dead microorganisms, antigen-antibody complexes, and a wide variety of simple and complex chemical materials, many of which are obviously unrelated to the natural disease. The experimental approach presented here utilized a single injection of a defined material derived from an organism for which there is some evidence of a causal relationship to this disease.

The histological picture observed in this model, including the development of nodular aggregates of lymphocytes which resemble Allison-Ghormley bodies, has some of the features of rheumatoid arthritis (11). Since these changes are not considered to be specific for rheumatoid arthritis (10), they do not constitute definite criteria by which one may evaluate an experimental model. However, the findings reported are in keeping with the concept that fragments of group A streptococcal cell walls composed of mucopeptide and C-polysaccharide can act as durable toxic material and play a role in the pathogenesis of rheumatoid arthritis. A direct approach to testing this concept, utilizing labeled specific antibodies to search tissues from cases of rheumatoid arthritis for the presence of bacterial cell-wall antigens in active lesions, is now in progress. Further analysis of the experimental model will be concerned with the detection and possible significance of immunoglobulins with either anti-streptococcal or anti-globulin specificity in the serum or joint-tissue of the animals.

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