ARTHRITIS IN RATS AFTER SYSTEMIC INJECTION OF STREPTOCOCCAL CELLS OR CELL WALLS*

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Several unique pathogenic properties of streptococcal cell wall components were made known by a series of studies of a prolonged remittent and intermittent multinodular inflammatory lesion of dermal connective tissue in rabbits. This chronic process was induced by a single injection of a sterile aqueous suspension of cell wall fragments and continued for as long as 3 mo (1). The pathogenesis of this experimental model has been subjected to detailed analysis and several reviews of these studies have been published (2–4). The cell wall structure responsible for this chronic inflammatory reaction is the peptidoglycan-C polysaccharide complex (3). Such complexes resist biodegradation (5, 6) and their persistence in tissues correlates with the prolonged inflammatory reaction (7–9). Page et al., (10, 11) reproduced the chronic inflammatory lesion of dermal connective tissue in rabbits.

Extension of these studies has led to the development of a model of pancarditis in mice (8, 12) and models of arthritis in rabbits and rats (9, 13–15). The model of arthritis in rabbits is induced by direct injection of cell wall fragments into the knee joint (9). The model of arthritis in rats is induced by an intraperitoneal injection of an aqueous suspension of cell wall preparations and has been described in abstract form (14, 15). This report includes a detailed description of the arthritis in rats induced with whole cell sonicate of group A streptococci; presents evidence that the toxic moiety is a peptidoglycan-polysaccharide fragment of cell wall; compares the arthropathic properties of whole cell sonicates of several species of streptococci; and describes the ability of whole, killed cells to induce arthritis in rats.

Materials and Methods

Animals. Female Sprague-Dawley rats (Zivic-Miller Laboratory, Allison Park, Pa.), weighing ≅ 100 g, were used in all experiments.

Bacterial Cultures. The following organisms were tested: group A, type 3 streptococcus, strain D58; group B streptococcus (ATCC 12386); group C streptococcus (ATCC 12388); group D streptococcus, strain F24; group H streptococcus, strain Challis; *Streptococcus mitis* (ATCC 6249); *S. pneumoniae,* type 3; and *S. pneumoniae,* type 19. The growth of streptococci and preparation

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of a sterile extract of sonically disrupted cells has been reported (1). All the organisms used in these studies were grown and treated in the same manner, except as noted. A lyophilized culture was inoculated into 10 ml of Todd-Hewitt broth (BBL, Div. of BioQuest, Cockeysville, Md.) and incubated for 6 h at 37°C. This was innoculated into 2.4 liters of broth, and after 18 h, the cells were centrifuged at 7,000 rpm (8,000 *g*) in the GSA rotor for 20 min at 4°C in the RC-2 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The cells were washed three times with sterile phosphate-buffered saline (PBS)[1] pH 7.0.

Whole Cell Sonicate. The washed, packed cells were resuspended in 35 ml of PBS, and then subjected to 90 min of ultrasonic vibration in a Branson sonifier (model S125, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) at maximal power. The disrupted cell material was sterilized by filtration through a series of Millipore filters (Millipore Corp., Bedford, Mass.) 3.0, 1.2, and 0.45 μm in succession. Sterility was tested by inoculating 0.1 ml of filtered sonic extract on a sheep blood agar plate. Rhamnose content of the cell wall material was determined according to Dische and Shettles (16). The sonicates of the two strains of *S. pneumoniae* and the strain of *S. mitis* were standardized by adjusting their turbidity to that of a standardized whole cell sonicate of group A streptococci by use of a Klett-Summerson colorimeter (Klett Manufacturing Co., Inc., New York, N. Y.). The material prepared as outlined above is referred to as whole cell sonicate.

Preparation of Fractions of Group A Streptococcal Cells. Three fractions of group A cells were prepared as follows. The washed, packed cells were resuspended to ≅15% suspension (wet wt/vol) in pH 7.0 PBS. An equal volume of no. 12 ballotini glass beads and one drop of tributyl phosphate were added and the cells were broken by treatment in a Braun shaker (Bronwill Scientific, Rochester, N. Y.) for 3 min with flowing CO_2 to keep the temperature close to freezing. The beads were allowed to settle, the supernate was removed and after centrifugation at 10,000 *g* for 30 min, the supernate was removed and is referred to as the crude membrane-cytoplasmic fraction. The top layer of sediment was carefully washed off from a lower layer of unbroken cells with PBS. The top layer of cell walls was washed four times with PBS with complete dispersion obtained in each resuspension by treatment in a 9 kcycle sonic oscillator (Raytheon Co., Waltham, Mass.) for 30 s. They were then washed three times with deionized H_2O and lyophilized. This is referred to as crude cell wall preparation. The crude cell wall preparation was resuspended in PBS (10 mg/ml) and treated with 0.025% ribonuclease at 37°C for 4 h. They were washed once with PBS and treated with 0.025% trypsin at 37°C for 4 h. After washing twice with PBS and three times with deionized H_2O, they were lyophilized. This preparation is referred to as purified cell walls. All procedures were conducted aseptically and the stock enzyme solutions were filtered through a Millipore 0.22 μm filter before adding to the cell walls. To prepare small fragments of cell walls, the lyophilized purified cell walls were suspended in 35 ml of PBS and subjected to ultrasonic vibration for 60 min, as described above under preparation of whole-cell sonicate, and then filtered through 1.2- and 0.45-μm sterile Millipore filters. The rhamnose value was then determined on the sample. This preparation is referred to as a sonicate of purified cell walls or cell wall fragments. Sterility was confirmed by culturing 0.1 ml on sheep blood agar plates.

Preparation of Heat-Killed Streptococcal Cells. Culture of groups A, B, and C streptococci were grown and harvested as described above. The washed, packed cells from 2.4 liters of Todd-Hewitt broth were resuspended in 35 ml of PBS and heated at 60°C or 30 min. Sterility was determined by streaking 0.1 ml of the undiluted suspension onto sheep blood agar plates. The heat-killed streptococci were standardized by their rhamnose content.

Method of Scoring the Arthritis. Rats were examined two or more times during the 1st day after inoculation and then daily for the duration of each study. The severity of the disease was graded using a scale of 0–4 for each extremity. The score was based on the number of joints involved, the severity and extent of the erythema and edema of the periarticular tissues, and the enlargement, distortion, or ankylosis of the joints. The method used is similar to that developed by Wood et al., (17) for scoring adjuvant arthritis, with the exception that lesions of the skin or tails did not occur in the model under study. The maximum total score that a rat might receive in our system is 16. The joints scored were those of each extremity distal to the knees and elbows.

Immunofluorescence. Unconjugated and fluorescein-conjugated rabbit antiserum specific for group A, C, or D streptococci, fluorescein-conjugated normal rabbit globulin, and lissamine rhodamine-conjugated normal bovine serum were purchased from Difco Laboratories Detroit,

[1] *Abbreviations used in this paper:* PBS, phosphate-buffered saline; rh, rhamnose.

Mich. These materials were utilized according to the methods of Smith et al. (18). Impression smears of tissues from rats injected with group A whole cell sonicate were air dried and fixed in absolute methanol for 10 min. The fixed smears were stained in a humid chamber for 30 min with fluorescein-conjugated rabbit antiserum and washed with PBS. Controls included: smears from control animals stained as outlined above, smears from test animals stained with fluorescein-conjugated normal rabbit serum, smears from test animals stained with fluorescein-conjugated rabbit antiserum specific for group C or D streptococci, and smears from test animals stained first with unconjugated group A-specific antibody followed by staining with fluorescein-conjugated group A-specific antibody. In the test preparations cell wall antigen occurred as large, brilliant, apple green fluorescent particles. The control preparations were negative for particles with this appearance.

Histologic Studies. Animals were sacrificed at various intervals after inoculation. Specimens were fixed in 10% formalin and paraffin sections were stained with Weigert's hematoxylin and alcoholic eosin. The joint specimens were decalcified in formic acid-sodium citrate solution after fixation for 5–14 days.

Results

Course of the Arthritis. The initial experiment was designed to study the gross features, course, and duration of the arthiritis induced by a single intraperitoneal injection of whole cell sonicate of group A streptococci. Each of 35 rats was given a dose equivalent to 60 μg rhamnose (rh) per g body wt. Each of 12 control animals was given an equal amount of whole cell sonicate of group D streptococci and each of six rats was injected with 5 ml of sterile PBS. The animals were observed for 130 days.

All of the rats injected with whole cell sonicate of group A streptococci developed arthritis with involvement of multiple joints. By the 2nd day after injection, all the rats in this group showed two or more hot, red, swollen joints. The involvement of joints was in decreasing order of frequency: ankle, wrist, interphalangeal, tarsophalangeal, carpophalangeal, and the joints of tarsus and carpus bone. The knee, hip, elbow, and shoulder joints showed no changes upon general inspection. The general features of the changes observed in the overall inspection are shown in Fig. 1. The course of the disease followed three general patterns (Fig. 2).

PATTERN 1. 4 of the 35 test animals (11%) were observed to have minimal swelling and erythema of at least two and not more than four joints which subsided completely by the 4th day after injection.

PATTERN 2. 15 of the 35 test animals (43%) exhibited two to four complete remissions followed by recurrences. The course of the disease in one rat will be described in detail. 15 h after injection, erythema and swelling were noted around an interphalangeal joint of the left front paw and around two interphalangeal joints of the right front paw. 35 h after injection there was acute inflammation of additional interphalangeal joints of the front paws, carpophalangeal joints bilaterally, tarsophalangeal joints bilaterally, and the ankle joints bilaterally. On the 3rd day, a total of 35 joints were involved by the acute inflammatory process. The reaction then decreased and by the 6th day only the ankle and wrist joints were inflamed. By the 8th day, only the two ankle joints were still inflamed. From the 15th to the 19th day, all the joints were normal in appearance. The first recurrence was noted on the 23rd day; this involved several interphalangeal joints of the right front paw. By the 29th day, this mild recurrence had subsided except for minimal involvement of one interphalangeal joint of the right front paw. An exacerbation of the process occurred on

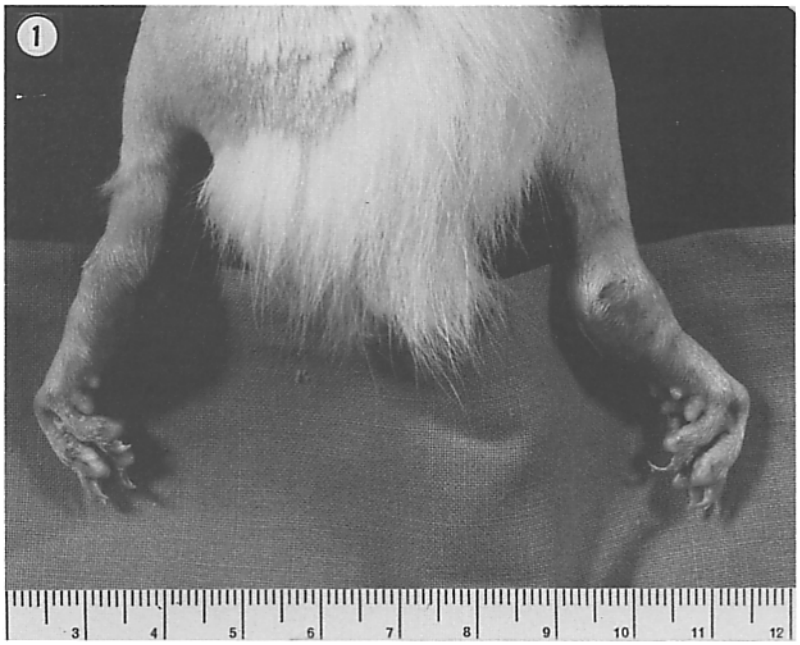

FIG. 1. Hind paws of rat photographed 2 mo after injection of whole cell sonicate of group A streptococci (60 μg rh/g b.w.).

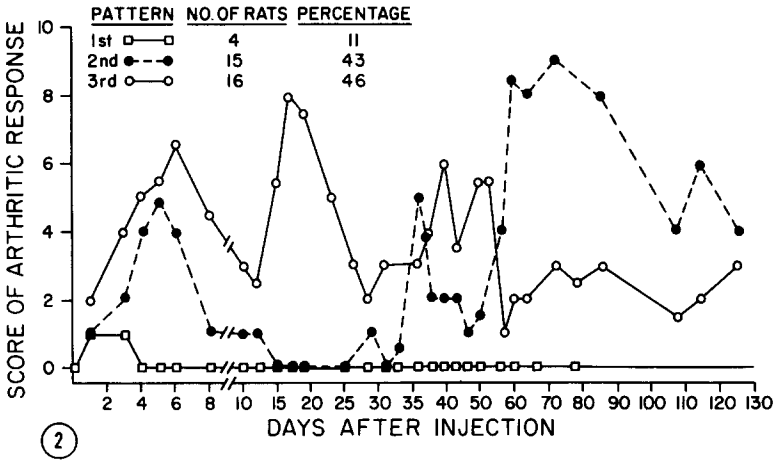

FIG. 2. Plot of the scores of articular reactions of three rats selected to demonstrate the three patterns of disease observed after the injection of whole cell sonicate group A streptococci.

the 23rd day and by the 64th day, 20 joints were extensively involved. The process continued with partial remission and additional exacerbations of individual joints with the most severe involvement being that of the ankle and wrist joints. By the 86th day, the ankle and wrist joints were actively inflamed as were six interphalangeal joints. By the 108th day of the process, the inflammation had subsided to a score of four. On 112th day it increased to a score

of six. By the 130th day the score was four. Both of the ankle and wrist joints were deformed and ankylosed when the experiment was terminated 130 days after injection of the rats.

PATTERN 3. 16 of the 35 animals (46%) showed a similar course to that described as pattern 2, with the exception that the process from the 1st to the 130th day of observation did not subside completely and was characterized by from two to four clear-cut remissions and exacerbations of the inflammatory process. The maximum number of joints involved in a single animal at any one time was 22. The control rats injected with PBS showed no evidence of arthritis. 2 of the 12 rats that were injected with the sonicate of group D streptococci had mild erythema and slight edema surrounding several interphalangeal joints on the 2nd day after injection. This reaction was absent on the 3rd day and no additional evidence of arthritis developed in this group of animals. We have now accumulated results with several hundred rats and many group A cell preparations and the three patterns of response with the dose of 60 μg rh/g body wt. has been consistently observed. With lower doses the distribution of responses shifts toward pattern 1.

Relation of Dose of Whole Cell Sonicate of Group A Streptococci to the Severity and Duration of the Arthritis. Six groups of animals were used. The dose injected varied from 10 to 320 μg rh/g body wt. The animals were observed for 100 days after injection. The data in Table I show a relationship between the dose of sonicate and the duration and severity of the arthritis induced. Included in this table are the 35 rats given 60 μg/g body wt. described in the previous section, in which this dose induced a severe prolonged reaction in 90% of the animals. 10 μg/g body wt. induced a transient mild arthritis in five of the 10 animals tested. With a 20-μg/g dose, all animals developed early joint inflammation and three rats developed recurrent arthritis. At doses above 60 μg, all animals consistently developed chronic remittent disease (patterns 2 and 3). A 320-μg/g body wt. dose injected into each of five rats killed two of the animals after 2–3 days. A dose 260-μg/g body wt. caused no deaths and induced severe and prolonged arthritis in all the rats receiving this dose.

Arthropathic Properties of Whole Cell Sonicates of Selected Streptococcal Species. This experiment was designed to test the ability of whole cell sonicate of streptococci other than group A to induce arthritis in rats. 42 rats were utilized in the study. All animals were observed for 35 days after inoculation. Each animal was injected intraperitoneally with whole cell sonicate containing 80 μg rh/g body wt. or an equivalent amount of whole cell sonicate material. As indicated above, the sonicates of *S. mitis* and *S. pneumoniae* were standardized in terms of turbidity reading, comparing them with a sonicate of group A streptococci containing a known amount of rhamnose. The percentage of rhamnose in the cell wall varies from 19% in groups B and D streptococci to 29% in group A, and is absent in *S. mitis* and *S. pneumoniae*. Therefore, the amount of cell wall in the crude cell extracts injected on the basis of rhamnose can be up to 30% greater in groups B, C, D, and H, compared to group A. With this qualification, we conclude from Table II that animals injected with whole cell sonicates of groups, A, B, C, and H reacted in a similar manner during the period of observation. Three to five of the animals in each group had active

TABLE I
Effects of Dose of Whole Cell Sonicate of Group A Streptococci on Articular Reaction

Dose	No. of animals	No. of animals positive	Disease pattern*		
			1 No. of rats	2 No. of rats	3 No. of rats
μg rh/g body wt					
0	4	0	0	0	0
10	10	5	5	0	0
20	10	10	7	3	0
60	35	35	4	15	16
80	12	12	0	6	6
260	5	5	0	2	3
320	5	3‡	0	0	3

* See Fig. 2.
‡ One animal died on the 2nd and one on the 3rd day after injection.

TABLE II
Arthropathic Properties of Whole Cell Sonicates of Selected Streptococci

Organism	No. of rats injected	No. of rats that developed arthritis	No. of rats with active arthritis 35 days after injection
Group A	6	6	5
Group B	6	6	4
Group C	6	6	4
Group D	6	0	0
Group H	6	6	3
S. mitis	6	6	0
S. pneumoniae, type3	3	0	0
S. pneumoniae, type 19	3	0	0

arthritis at the time the experiment was terminated on day 35. In this experiment, the animals injected with whole cell sonicate of group D organisms or *S. pneumoniae* failed to develop arthritis.

Arthropathic Properties of Heat-Killed Streptococcal Cells. This experiment was designed to determine whether whole, heat-killed streptococcal cells injected intraperitoneally would lead to arthritis in rats (Table III). After a latent period of 7–10 days, five of the rats injected with group B cells developed polyarthritis involving all extremities. Active arthritis was present in these five animals when this part of the experiment was terminated on the 35th day after injection. None of the animals injected with group A or group C organisms had developed arthritis at 35 days after injection. Observation of the animals receiving group C organisms was terminated at this time. Observation of animals injected with group A organisms was continued. 3 of 20 animals injected with group A cells at a 60-μg rh/g body wt. dose developed polyarthritis

TABLE III
Arthropathic Properties of Heat-Killed Streptococcal Cells

Organism	Dose injected intraperitoneally	Incidence of arthritis	Latent period*	Duration of observation
	μg rh/g body wt		*days*	*days*
Group A	60	3/20	57, 91, 91	130
Group A	80	3/10	56, 112, 120	160
Group A	80	2/15	39, 64	93
Group B	80	5/6	7–10	35
Group C	80	0/6	–	35
Controls	0	0/12	–	160

* Interval after injection before joint inflammation was detected in the gross.

after a latent period of 57–91 days. This experiment was terminated after 130 days. 15 rats were injected with group A cells at an 80-μg rh/g body wt. dose. Two of these animals developed polyarthritis after latent periods of 39 and 64 days. This experiment was terminated after 93 days of observation. In an additional group of 10 animals injected with group A cells at an 80-μg rh/g body wt. dose, three rats developed polyarthritis after latent periods between 56 and 120 days. This experiment was terminated 160 days after injection. The polyarthritis which developed followed patterns 2 and 3 as described above and was observed to continue until the experiment was terminated.

Arthropathic Properties of Subfractions of Group A Streptococcal Cells. Three fractions of group A streptococcal cells were injected: (*a*) purified cell walls, not sonicated, (*b*) purified cell wall fragments (sonicate of purified cell walls), (*c*) a crude membrane-cytoplasmic fraction (≅ 10 mg total dry wt per ml PBS). A fourth group of rats received PBS (Table IV). The purified cell walls (not sonicated) produced arthritis in only 1 out of 11 rats with a latent period of 45 days. These results are similar to those obtained with whole, heat-killed group A streptococcal cells. The cell wall fragments produced arthritis in all of 15 rats after a latent period of 1 day. These results are analogous to those obtained with whole cell sonicate. The crude membrane-cytoplasmic fraction produced no arthritis in seven animals and control animals injected with PBS developed no arthritis.

Identification of Group A Streptococcal Cell Wall Antigen in Tissues. Each of 36 rats was injected with whole cell sonicate which contained 60 μg rh/g body wt. Two test rats were sacrificed at the following times after injection along with one control animal which had received 3 ml of PBS: 1, 3, and 6 h; 2, 4, 5, 7, 12, 19, 26, 33, 50, 63, 70, 73, 84, 160, and 180 days. Impression smears of liver, kidney, spleen, and synovial tissues of an ankle joint were made from the tissues of each animal immediately after the test and control animals were killed. A few green fluorescent particles were seen in the smears of liver tissue of test animals collected 1 h after injection. 3 h after injection, all tissues of test animals which were studied contained antigen. Appearance of the antigen in

TABLE IV

Arthropathic Properties of Components of Group A Streptococcal Cells

Preparation	Dose injected intraperitoneally	Incidence of arthritis	Latent period*
			days
Cell walls, not sonicated	60 μg rh/g body wt	1/11	45
Cell wall fragments	60 μg rh/g body wt	15/15	1
Crude membrane-cytoplasm fraction	60 mg total dry wt	0/3	–
	30 mg total dry wt	0/4	–
PBS control	5 ml	0/6	–

* All animals were observed for 60 days.

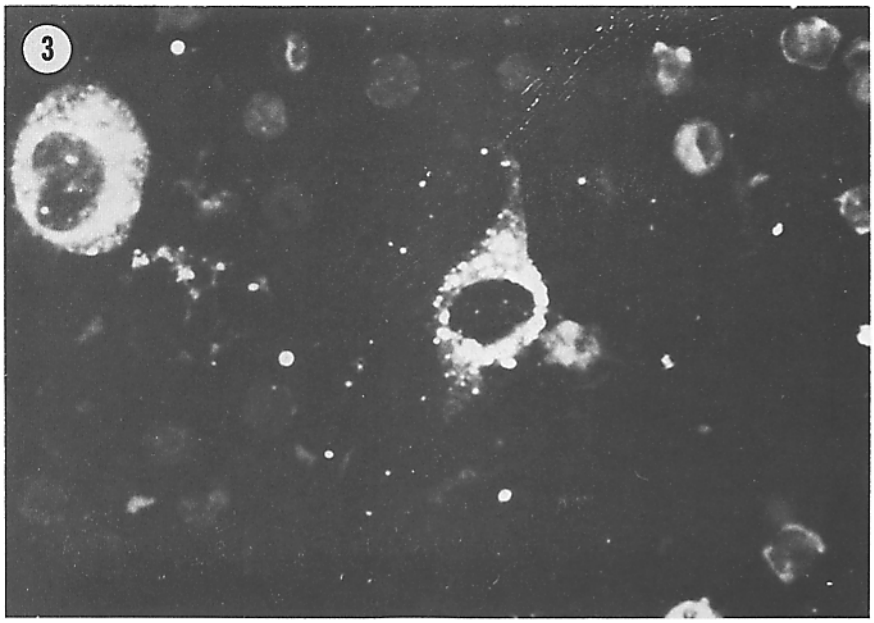

FIG. 3. C-polysaccharide antigen of group A streptococci in direct imprint of articular tissue of ankle joint of rat injected intraperitoneally 48 h earlier with whole cell sonicate of group A streptococci. × 1,100.

smears of the synovial tissue is illustrated in Fig. 3. A similar picture was seen in smears of joint tissue collected at all intervals from 3 h to 63 days after injection. A large proportion of the antigen in the joints has a granular distribution within the cytoplasm of mononuclear cells (Fig. 3). A large quantity of cell wall antigen had accumulated in the spleen by 2 days after injection. Thereafter, there appeared to be a slow decrease in the amount of antigen in the spleen during the study; however, impression smears made 180 days after injection were still positive for C-carbohydrate antigen. Preparations from liver tissue showed moderate amounts of antigen throughout the 180 days of the study. Small amounts of antigen were found in the smears of kidney tissue collected from 3 h to 26 days after injection.

Microscope Pathology. Each of 90 rats was given an intraperitoneal injection

of whole cell sonicate of group A streptococci equivalent to 60 μg rh/g body wt. Each of 30 control animals was given an equal volume (5 ml) of sterile PBS. The test animals were harvested in groups of three along with one control animal at: 5 h after injection; daily for 1 wk; 9 days; and at the following wk after injection: 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 40, and 50. The severity of the arthritis was similar to that observed in the experiment which was designed to study the course of the disease and which is the first study described above. No clinical signs of arthritis were apparent at 5 h after injection. All animals killed between 1 day and 10 wk after injection exhibited active arthritis with scores of activity as defined above in the range of 2–12. Between 11 and 30 wk, one or more of the animals killed showed active arthritis with scores ranging from 1 to 8.5. After 30 wk, with the exception of one animal killed at 36 wk that exhibited a relapse with a score of 3.5, all animals were scored as 0 although each of them showed ankylosis and deformity of several joints. In this experiment no evidence of inflammation was observed in the gross after 36 wk.

In addition to this experiment which was specifically designed to permit histologic studies, tissues of selected rats from all the experiments described above were fixed for histological study at the time the experiments were terminated. These specimens provided tissues from animals with active arthritis as long as 180 days after injection with whole cell sonicate and as long as 160 days after injection with heat-killed whole cells.

Pathology of Heart, Lungs, Kidneys, Liver, Spleen, Lymph Nodes, and Intestinal Tract. Sections of heart, lungs, and kidneys showed no significant histologic changes. The control and test animals showed a moderate degree of bronchitis and pneumonitis in a majority of the sections of the lungs. As early as 2 days after injection of the test animals, aggregates of macrophages with foamy-appearing cytoplasms were seen in sections of the liver and the cytoplasm of the individual Kupffer cells also had a vacuolated appearance. These changes were most marked between 2 and 6 wk after injection, and gradually decreased and were not seen after 12 wk. As early as 5 h after injection, the splenic pulp appeared markedly congested. This was followed as early as 1 day by infiltration of the pulp by macrophages with foamy cytoplasm. Sections collected between 1 day and 2 wk after injection showed focal areas of necrosis in the spleen involving both the follicles and pulp. The sections of the spleen collected between 2 and 14 wk showed a moderate degree of hyperplasia of all elements and focal collections of macrophages with vacuoles in their cytoplasm scattered in the pulp. After 14 wk, until the experiment was terminated, the sections of spleen collected at the intervals listed above showed a moderate degree of hyperplasia of all cellular elements with a small number of focal collections of macrophages with foamy cytoplasm in the pulp. A systematic study of the lymph nodes was not made. However, the peribronchial nodes included in the sections of lung showed accumulations of macrophages with foamy cytoplasm in the sinuses and moderate hyperplasia of all cellular elements in sections collected between 2 and 10 wk, with no significant changes noted after 10 wk.

Sections of the liver, spleen, lymph nodes, and kidneys of the control animals showed no significant changes.

Sections of the intestinal tract, spleen, and liver collected from 5 h to 40 wk after injection of the test animals exhibited changes of a peritonitis, associated in the early stages with a fibrinous exudate containing neutrophils and macrophages with foamy cytopasm. In the late stages of the experiment the exudate was replaced by fibrous connective tissue in which foci of macrophages were present.

Joints and Periarticular Tissue. The microscopic change started as an acute exudative inflammatory reaction and evolved into a chronic erosive synovitis which was modified at irregular intervals by recurrence of the acute reaction (Figs. 4–10). Studies of joints which had been inflamed in the gross and which were in a period of remission at the time the animal was killed suggest that an inflamed joint may return to near normal, or the process may lead to total destruction and fibrous ankylosis, depending on the extent of the injury of cartilage and subchondral bone. The microscopic alterations were most severe in the ankle, wrist, interphalangeal, and carpophalangeal joints. As noted above, these joints exhibited the most severe changes on gross inspection. Although the knee and elbow joints showed no changes in the gross, microscopic changes were present (Fig. 7).

The histologic changes observed are divided for descriptive purposes into several catagories. The initial acute exudative reaction began as early as 5 h after injection of the test animals. Each episode of the first and recurrent acute reactions persisted for 10–15 days. It consisted of vascular congestion, edema, fibrin deposit, and infiltration by neutrophils and mononuclear phagocytes. These changes were most extensive in stroma of the synovial membrane; however, they extended into the joint capsule, the periarticular tissue, the tendons, tendon sheaths, muscle bundle and muscle attachments adjacent to the joints, and into the overlying subcutaneous tissue. A feature of this acute reaction was the relatively large amount of fibrin deposited in the connective tissue of the synovial villi and in the joint spaces. Shown in Fig. 4 is a mass of fibrin extending through a break in the synovial lining cells into the joint space. Figs. 4 and 5 show the major features of the acute exudative process. Hyperplasia of the synovial lining cells was associated with the acute inflammatory reaction (Fig. 5). This change appeared as early as 3 days after injection of the test animals and persisted throughout the course of the inflammatory reaction.

Joints that had been inflamed as long as 2 wk at the time they were collected showed the acute exudative reaction to be subsiding, as evidenced by less vascular congestion, edema, fibrin accumulation, and fewer neutrophils in the tissues than were present in sections collected at an earlier stage of the reaction. With some degree of overlap the acute phase was replaced by a chronic erosive synovitis. This phase was characterized by proliferation of the connective tissue and blood vessels of the stroma of the synovial villi and infiltration of the tissue by mononuclear phagocytes and lymphocytes, continued hyperplasia of the synovial lining cells, destruction of cartilage and subchondral bone, and replacement of these tissues by an extension of the vascular connective tissue from the synovial stroma. This vascular connective tissue was infiltrated by a variety of mononuclear cells. The histologic appear-

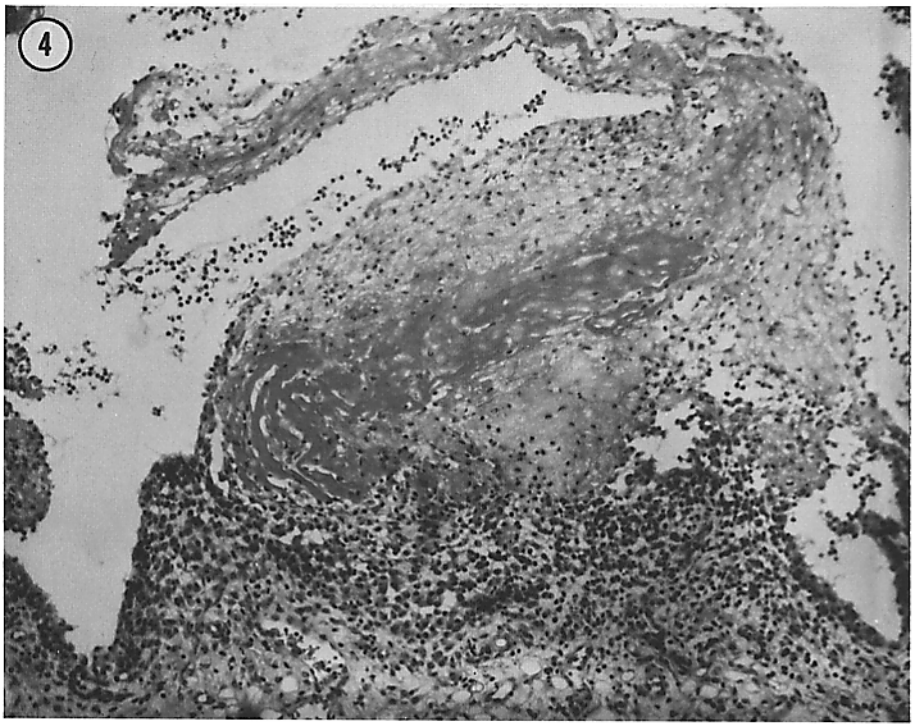

FIG. 4. Histology of an acute exudative reaction in a synovial crypt of an ankle joint 9 days after injection of whole cell sonicate of group A streptococci. A mass of fibrin is attached to the synovial stroma and extends into the joint space. × 100.

ance of the process referred to as chronic erosive synovitis is featured in Figs. 6–10. Figs. 6 and 7 show the replacement of bone and cartilage by vascular connective tissue which contains several types of cells that include fibroblasts, macrophages, and neutrophils. Irregularly shaped islets of osteoid tissue were present surrounding the areas of bone destruction. In these areas there was loss of normal architecture of the bone and cartilage. Pannus formation or extension of the vascular connective tissue over the surface of the articular cartilage was a feature of the erosive process (Fig. 7). Figs. 8 and 9 show the hypertrophy of the synovial villi which was caused by proliferation of the connective tissue, increase in the blood vessels of the stroma, hyperplasia of the synovial lining cells, and infiltration of the connective tissue by mononuclear phagocytes, lymphocytes, and neutrophils. The end stage of the articular disease is shown in Fig. 10. The joint space and the destroyed cartilage and bone is replaced by fibrous tissue in which a small number of mononuclear cells is seen.

The precise duration of the articular disease is not made known by these studies. The most severe acute destructive arthritis was observed during the first 10 wk after injection. Between 11 an 30 wk after injection about one-third of the rats killed showed active lesions. After 30 wk only one of the animals killed showed an acute reaction. The histologic changes observed can be

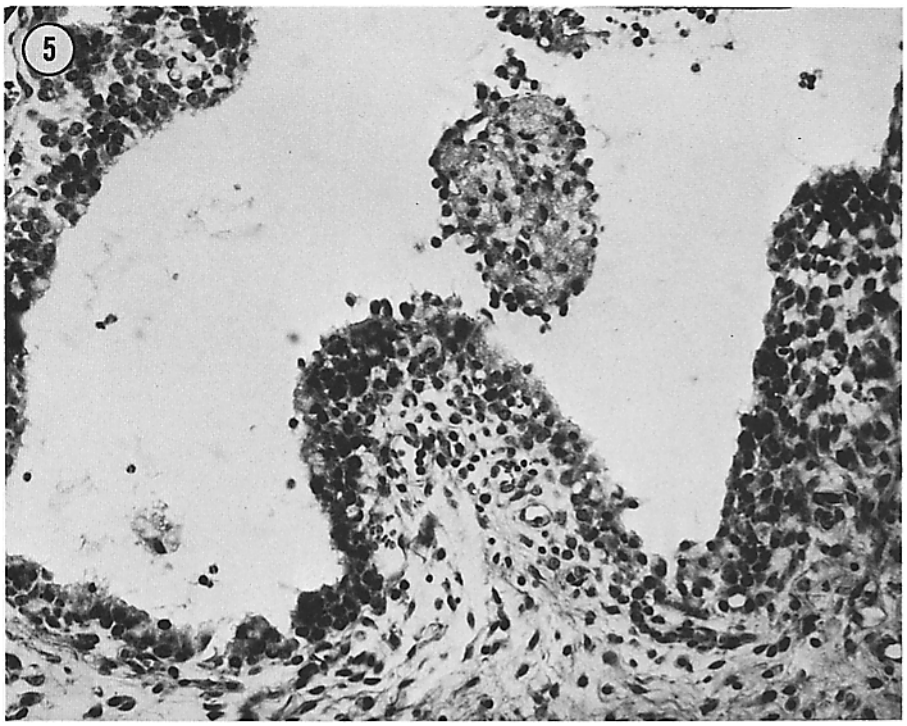

FIG. 5. Histology of an acute exudative reaction in a synovial crypt of an ankle joint 9 days after injection of whole cell sonicate of group A streptococci. Hypertrophy of the synovial villi is associated with hyperplasia of the synoviocytes and the cells of the connective tissue of the stroma, which is infiltrated by lymphocytes, macrophages, and neutrophils. Fibrinous exudate is present in the joint space. × 200.

correlated with the changes observed in the gross. If the joints were harvested at a time when they were swollen and the overlying skin was erythematous for the first time, an acute exudative reaction was present which involved the synovial tissues, joint capsules, periarticular tissues, and extended into the subcutaneous tissues. A similar acute reaction was observed in joints which showed their first involvement in the gross either a few hours or several weeks after injection of the test material. A combination of the acute exudative reaction and the chronic erosive synovitis was seen in joints collected after they had been involved by one or more recurrences of the acute reactions. Destroyed joints and fibrosing ankylosis was observed when joints were collected after the process had continued for 4 or more wk and signs of inflammation had subsided. Sections of the joints collected from the control animals showed no pathologic changes.

Sections were also prepared from tissue collected when the other experiments reported above were terminated. Tissues were collected from animals with active arthritis which had been induced by injection of whole cell sonicate of groups B, C, or H; and heat-killed cells of groups A and B. The histologic changes in these sections were a combination of the acute exudative reaction and the chronic erosive synovitis described above. They were similar to those

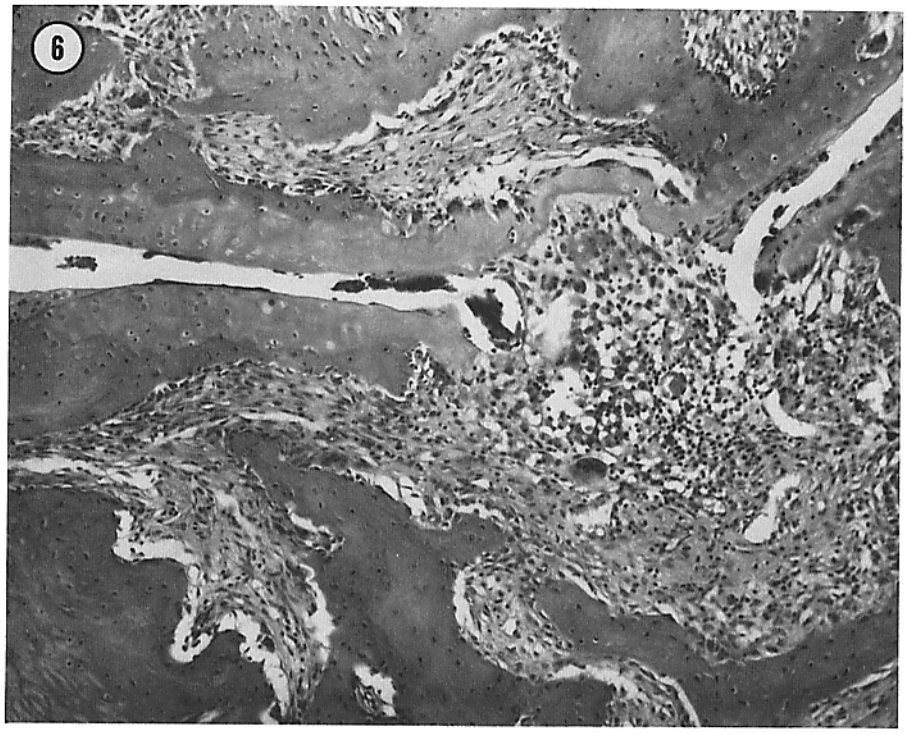

FIG. 6. Histology of erosive synovitis of an ankle joint 16 wk after injection of whole cell sonicate of group A streptococci. Pannus has extended into the joint space and replaced destroyed cartilage and subchondral bone. × 100.

observed in the animals which were injected with whole cell sonicate of group A streptococci, and which were collected at comparable stages in the disease process.

Discussion

Previous reports have demonstrated that a single intraperitoneal injection of an aqueous suspension of cell wall fragments or whole cell sonicate of group A streptococci into mice is followed by the development of a pancarditis with features of rheumatic fever (8, 12). The cardiac lesions are associated with localization of cell wall components within macrophages in the areas of tissue damage (8). The most outstanding feature of the reaction of the Sprague-Dawley rat to a single intraperitoneal injection of whole cell sonicate or cell wall fragments is the development of an arthritis after a short latent period of 15–48 h. This is followed by a prolonged intermittent or remittent course of the arthritis in most animals.

The histologic changes correlate with the changes observed in the overall inspection. The red, swollen joints show histologic evidence of an acute synovitis and acute inflammatory changes extending into the periarticular tissues. The gross deformities of the joints are associated with microscopic evidence of a chronic erosive synovitis, with extensive destruction of cartilage and subchron-

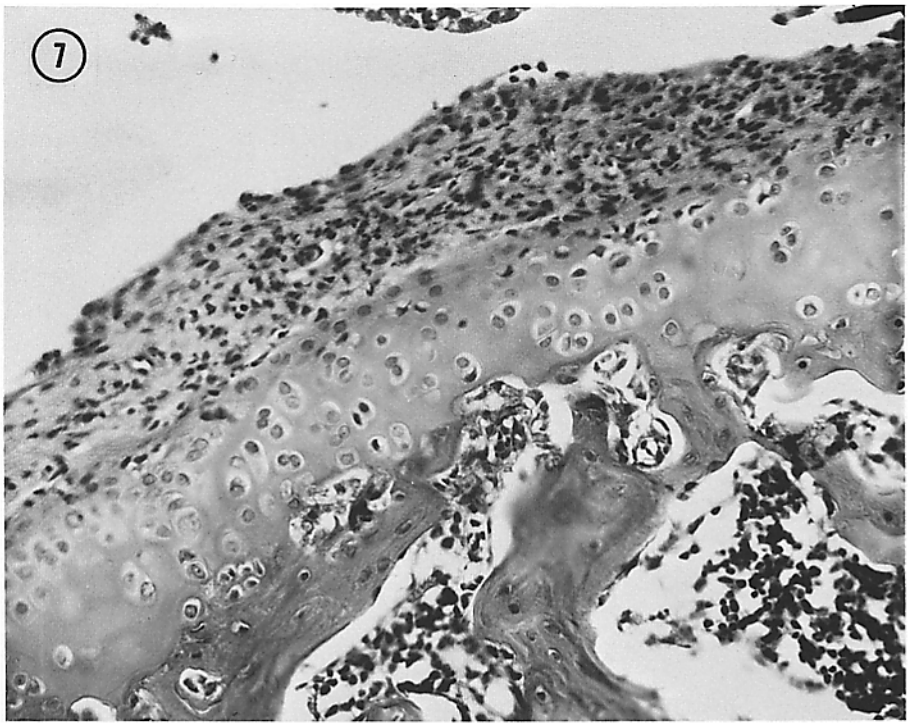

FIG. 7. Photomicrograph of pannus extending over the surface of the articular cartilage of the knee joint in a specimen collected 3 wk after injection of the whole cell sonicate of group A streptococci. × 200.

dral bone, pannus formation, and overgrowth of disorganized islets of osteoid tissue. The cells that infiltrate the stroma of the hypertrophied synovial villi include macrophages, lymphocytes, and neutrophils. Small aggregates of lymphocytes were occassionally noted, but no lymphoid follicles with germinal centers were observed in the synovial tissues.

The histologic changes in the peritoneum, liver, and spleen of the rats are similar to the changes observed in the mouse after a single intraperitoneal injection of whole cell sonicates of group A streptococci (8, 12). The fact that the mouse develops a pancarditis while the rat develops arthritis is the major difference in the two models.

The severity and duration of the arthritis is related to the dose of the material injected. A lethal effect was not produced until a 320-μg rh/g body wt dose was reached. A 260-μg rh/g body wt dose produced no deaths. Sonicates of groups B, C, and H also have arthropathic properties, and are able to induce a prolonged arthritis; *S. mitis* is able to induce an arthritis of relatively short duration and *S. pneumoniae* does not, under the conditions of this experiment, exhibit this property. Sonicates of group D streptococci may have a limited capacity, similar to that of *S. mitis,* to induce an arthritis of short duration.

Groups A and B whole heat-killed cells induce arthritis without fragmentation by artificial means. This is of special interest when one considers the

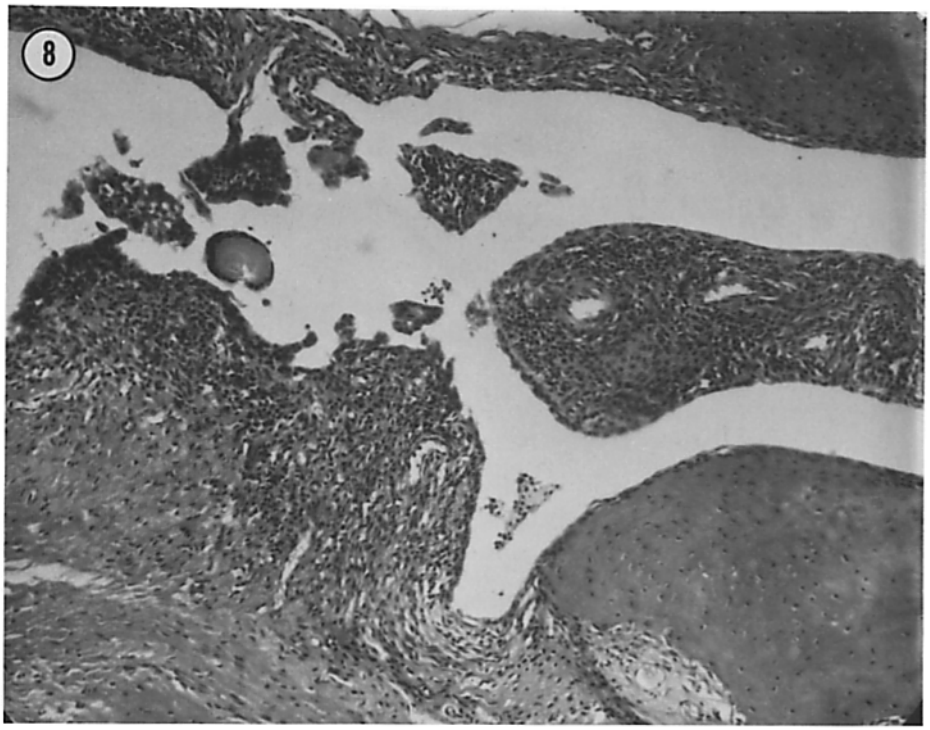

FIG. 8. Histologic section of an ankle joint 16 wk after injection of whole cell sonicate group A streptococci. Hypertrophy of the synovial villi is associated with hyperplasia of vascular connective tissue of the stroma and infiltration of this tissue with mononuclear cells. × 100.

possible relationship of this model to events that might occur under natural conditions. Knowledge of the long latent period observed when whole cells were used is useful in studies attempting to relate human diseases such as rheumatoid arthritis to a previous bacterial infection.

From the results of previous studies (3, 7, 8) it was postulated that the arthritis was related to the localization of peptidoglycan-polysaccharide complexes in the synovial tissues. The study of subfractions indicates that the toxic activity is associated with the cell wall, whereas the cytoplasmic-membrane fraction produced no arthritis. The search for cell wall antigen demonstrated particulate material containing C-carbohydrate in the synovial tissues as early as 3 h after the injection of sonicate of whole group A cells, and for at least 63 days thereafter.

Our curent hypothesis regarding the pathogenic properties of peptidoglycan-polysaccharide moieties is that they may damage tissue by several mechanisms: (*a*) a primary toxic effect (3), (*b*) activation of a variety of specific immune reactions as a result of their antigenic properties, and (*c*) nonspecific activation of macrophages to become cytotoxic (19) or to release lysosomal enzymes (11) after the ingestion of the nonbiodegradable cell walls. The ability of the peptidoglycan-carbohydrate complex from some bacterial species to resist degradation and/or elimination from the tissues is an important property (7–9).

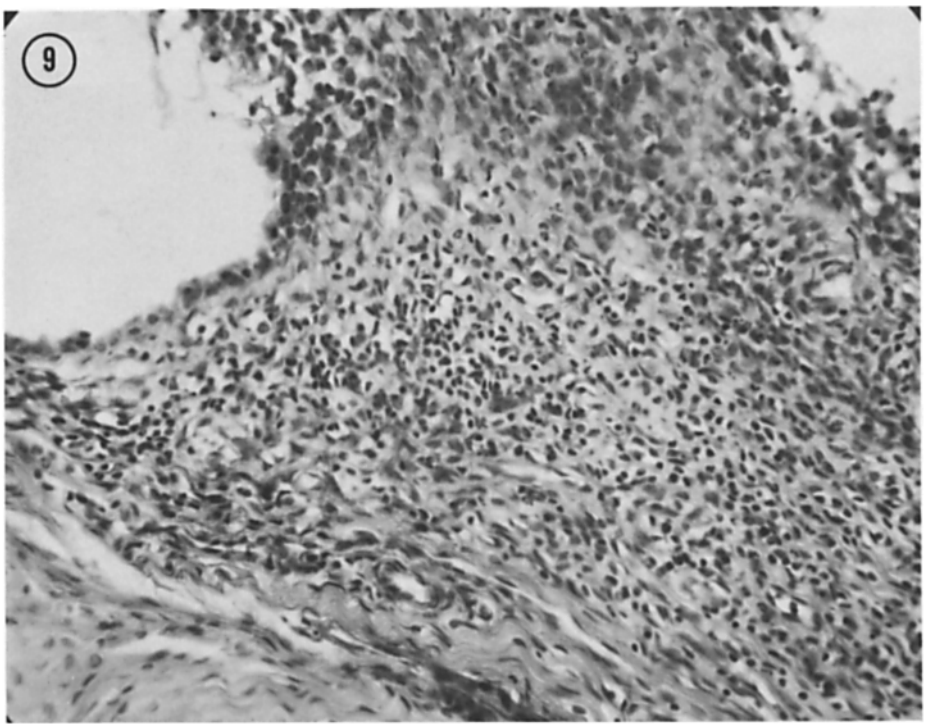

FIG. 9. Fibrosis of the synovial stroma, accumulation of mononuclear cells in the vascular connective tissue, and hyperplasia of the synovial lining cell in a specimen collected 16 wk after injection of whole cell sonicate of group A streptococci. Ankle joint. × 200.

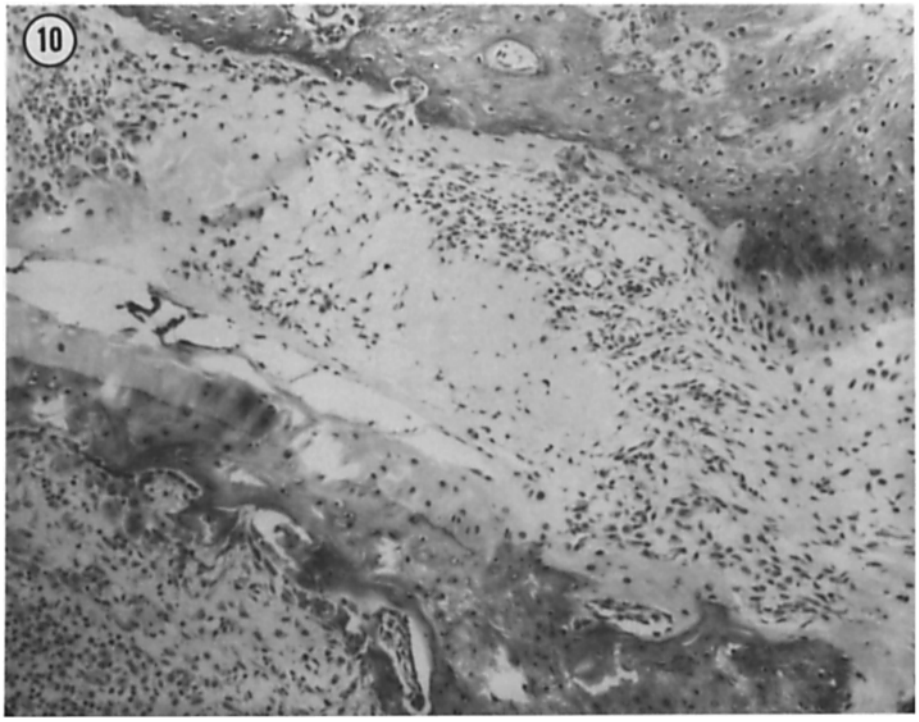

FIG. 10. Ankylosis of a tarsal joint 210 days after injection of whole cell sonicate of group A streptococci. Joint space is filled with fibrous connective tissue. × 100.

Summary

Further investigation of the biological properties of streptococcal cells and their components has produced a model of erosive synovitis in rats. A single intraperitoneal injection of an aqueous suspension of whole cell sonicate of group A streptococci into Sprague-Dawley rats induced an acute arthritis which evolved into a prolonged inflammatory process characterized by several complete or partial remissions, joint deformity, and ankylosis. The toxic moiety is a peptidoglycan-polysaccharide fragment of the cell wall which persists in tissue. Histologic features of the arthritis include an acute exudative phase followed by an erosive synovitis that leads to destruction of cartilage and subchondral bone and fibrous ankylosis of the joints. The arthropathic properties of whole cell sonicates of several species of streptococci are compared along with studies of the ability of heat-killed, whole cells of groups A, B, and C streptococci to induce arthritis in rats. Whole cells induce arthritis after a latent period of 57–120 days when group A cells are injected and 7–10 days when group B cells are tested.

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