# TREATMENT OF EXPERIMENTAL EROSIVE ARTHRITIS IN RATS BY INJECTION OF THE MURALYTIC ENZYME MUTANOLYSIN

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A single intraperitoneal injection of rats with an aqueous suspension of peptidoglycan-polysaccharide polymers derived from group A streptococci (PG-APS)[1] or from certain other bacterial species, results in acute joint inflammation that is followed by a chronic, recurrent, erosive arthritis (1–4). The histologic and radiologic features of this experimental disease resemble those of human rheumatoid arthritis. The clinical course is characterized by repeated remissions and exacerbations, and eventual progression to ankylosis, fibrosis, and loss of function (1, 5). The severity and course of arthritis is influenced by the sex and genetic background of the rat (3, 6). The persistence of cell wall in chronically inflammed tissues is important in PG-APS-induced arthritis and in the various other animal models of chronic inflammation induced by PG-APS (1, 7–11).

The size of the PG-APS polymers is important in determining the course and severity of arthritis in the rat model (12, 13). Differential centrifugation of sonicated cell wall preparations has been used to obtain families of PG-APS with different average molecular weight and arthropathic potential (12). The smaller fragments, which have an average molecular weight of $5.3 \times 10^6$, induce a severe acute arthritis but relatively little chronic disease. Fragments of intermediate size, with an average molecular weight of $5.0 \times 10^7$, cause moderate acute and severe chronic arthritis. Recently, it has been demonstrated that PG-APS can be degraded in vitro with mutanolysin (M-1), or phage-associated lysin, to fragments of a molecular weight $<5.0 \times 10^6$. When injected intraperitoneally or intravenously into rats, these enzymatic digests cannot induce arthritis; however, they cause a rapid, transient edema of the limbs (13).

Mutanolysin is a muramidase derived from *Streptomyces globisporus*. It has been used to digest cell walls from a number of bacterial species (14–17), since it is specific for the $\beta$ 1-4 linkage of *N*-acetylmuramyl-*N*-acetyl glucosamine in the glycan backbone of peptidoglycan. The finding that PG-APS digested in vitro with mutanolysin loses its arthropathic potential prompted us to investigate the

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[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; HE, hematoxylin and eosin; IEF, isoelectric focusing; PBS, phosphate-buffered saline; PG-APS, group A streptococcal peptidoglycan-polysaccharide complex.

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in vivo effect of this enzyme on PG-APS-induced arthritis. We found that a single intravenous injection of mutanolysin, even when given at the peak of acute joint inflammation, prevented the development of recurrent, chronic erosive arthritis.

## Materials and Methods

*Preparation of Cell Wall.* Purified cell walls were prepared from group A, type 3, strain D-58 streptococci as previously described (12). Briefly, streptococci were grown in Todd Hewitt broth (BBL Microbiology Systems, Cockeysville, MD), harvested, washed in sterile phosphate-buffered saline (PBS), and disrupted in a Braun MSK shaker (Bronwill Scientific Inc., Rochester, NY). Intact cells were removed by centrifugation at 2,000 *g* for 30 min. Cell walls were pelleted by centrifugation at 10,000 *g* for 30 min and further purified by sequential treatment with RNase, trypsin, and papain, followed by chloroform-methanol extraction. The cell walls were then washed with water, dialysed against water, and lyophilized. The lyophilized cell walls were resuspended to 20 mg/ml in PBS and sonicated for 70 min in a Branson sonifier (Branson Sonic Power Co., Danbury, CT) using a 1/2 inch probe in a sealed 40-ml stainless steel cup that was cooled with a water jacket. The sonicated cell wall was centrifuged at 10,000 *g* for 30 min, and the supernatant collected for injection into rats.

*Mutanolysin Preparations.* The purified M-1 fraction of mutanolysin and crude mutanolysin were obtained from K. Yokagawa (Dianippon Pharmaceuticals, Ltd., Osaka, Japan) or purchased from Miles Laboratories, Inc., Elkhart, IN. The purity of the mutanolysin preparations was tested by analytical isoelectric focusing (IEF) using 5% polyacrylamide gels, Servalytes 2-11 (Serva Biochemicals, Heidelberg, Federal Republic of Germany), and an LKB multiphor flat-bed apparatus (LKB Produkter, Bromma, Sweden). The protein standards were Serva protein test mixture 9 (Serva Biochemicals). When the gels were focused at a constant power of 4 W for 2 h, purified mutanolysin showed a single cathodal migrating band, while crude mutanolysin showed at least five major bands. When an IEF gel was run under the same conditions for 70 min, to avoid gradient drift and to increase resolution in the alkaline region of the gel, an additional band was seen in the purified mutanolysin preparations (Fig. 1).

Proteolytic activity has been reported to co-migrate with M-1 on IEF gels, but it can be separated from the muramidase by ion exchange chromatography on CM Sephadex, which was the procedure involved in the purification of the mutanolysin used in this study (14, 15, 18). Proteolytic activity in the mutanolysin preparations was measured using the Azocoll assay (Calbiochem-Behring Corp., La Jolla, CA), as described by Siegel et al. (18). The purified M-1 preparations had no detectable proteolytic activity, while the crude mutanolysin preparation was heavily contaminated.

Mutanolysin was also purified in our laboratory from crude enzyme by preparative IEF in a granulated gel bed (Ultradex; LKB Produkter) on an LKB multiphor flat-bed apparatus. The procedure followed LKB application note 198, except that Servalytes 3-10 (Serva Biochemicals) were used in place of LKB ampholines. The mutanolysin purified by IEF was free of proteolytic activity and possessed the same in vivo effect on PG-APS-induced arthritis, when administered immediately after cell wall, as M-1 purified by the method of Yokagawa et al. (14, 15). Immediately before injection into rats, mutanolysin preparations were solubilized by sonication for 30 s using a 9 kcycle sonic oscillator (Raytheon Co., Waltham, MA).

*Animals.* Inbred female Lewis rats were obtained from Charles River Breeding Laboratories, Wilmington, MA. They weighed 100–120 g at the time of PG-APS injection. The rats were housed two to three per cage and fed Purina rat chow and water ad libitum.

*Scoring of Edema and Arthritis.* Arthritis was scored on an arbitrary scale of 0–4, as described previously (1), with a maximum score of 16 per rat. The chronic erosive nature of the arthritis was confirmed by x ray (5) and by histological evaluation. The transient edema that appeared in the front and hind limbs of rats given M-1 immediately after PG-APS was estimated by a visual assessment of swelling over the first 10 h postinjection. An arbitrary scale of 0–4 was also used for each limb; therefore, the maximum score per rat

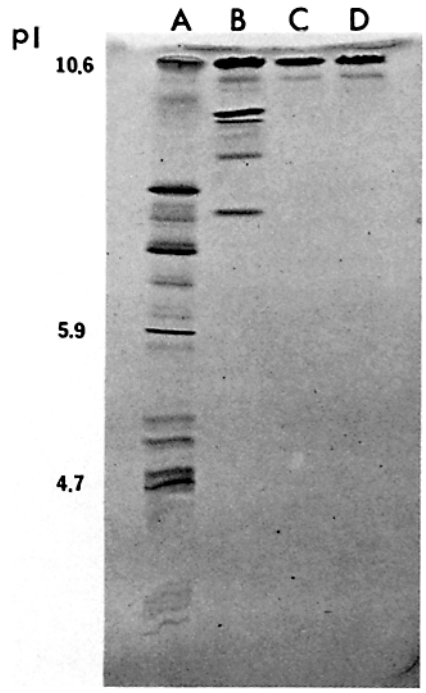


FIGURE 1. IEF of several mutanolysin preparations. 10-μg samples were applied in the acidic region of the gel and allowed to migrate towards the basic region. (*A*) Protein standards with isoelectric points ranging from 3.5 to 10.6. (*B*) Crude mutanolysin. (*C* and *D*) Two different purified preparations of mutanolysin.

was 16. In a previous study (13, 19), using PG-APS digested in vitro with mutanolysin, or purified group A polysaccharide, we found an excellent correlation between clinical scoring and the accumulation of $^{125}$I human serum albumin in the limbs.

*Histology.* Rat tissues were fixed in 10% formalin, and paraffin sections were stained with hematoxylin and eosin (HE) and by periodic acid-Schiff digest method. The tissues of the ankles and hind feet were decalcified in formic acid–sodium citrate solution for 5–14 d after fixation. Tissue sections from two to eight rats from each group were examined.

*Quantitation of Cell Wall in Tissue Extracts.* An enzyme-linked immunosorbent assay (ELISA) modification of the solid phase radioimmunoassay developed by Eisenberg et al. (9) was used to quantitate the amount of PG-APS in rat blood, liver, spleen, and limbs. Briefly, tissue samples were frozen in liquid nitrogen, crushed, and extracted with 0.2 M Tris buffer containing 1% Tween 80 and 5 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO) for 6 h at 37°C. Supernatants of the extracts were added to 96-well microtiter plates previously coated with affinity-purified anti–group A antibody. Biotinylated affinity-purified anti–group A antibody was then added, followed by avidin alkaline phosphatase. The color reaction was developed using *p*-nitrophenyl phosphate substrate (Sigma Chemical Co.) and monitored by an automated Microelisa reader (model MR 580; Dynatech Laboratories, Inc., Alexandria, VA).

*Chemical Analysis.* Cell wall preparations were analyzed by gas-liquid chromatography. *N*-acetyl-muramic acid, *N*-acetyl-glucosamine, rhamnose, and glucose were assayed as their alditol acetates (20). Samples were hydrolyzed under vacuum for 3 h at 100°C with 2 N sulfuric acid before derivatization. Rhamnose was also assayed using the method of Dische and Shettles (21).

## Results

*Mutanolysin Can Prevent PG-APS-induced Arthritis.* In the initial experiment, 18 rats were injected intraperitoneally with an arthropathic dose of PG-APS (20 μg rhamnose/gram body weight) and immediately thereafter injected with 0.4 mg i.v. of mutanolysin. A control group of 18 rats was injected intraperitoneally with PG-APS followed by 0.4 ml of PBS i.v. Another control group of three rats was injected with PBS i.p. and 0.4 mg mutanolysin i.v. The control group injected with PG-APS and PBS developed the typical course of severe acute and chronic arthritis (Fig. 2). In contrast, the rats injected with PG-APS and treated with mutanolysin developed a mild acute arthritis that disappeared within 14 d with no subsequent appearance of inflammation over the period of observation (Fig. 2). The control group receiving only enzyme displayed no evidence of inflammation at any time.

It is important to note that the rats given mutanolysin immediately after PG-APS developed a transient limb edema that reached a peak by 2–3 h and subsided by 9 h (Fig. 2). This response is identical to that previously reported (13) with the injection of fragments of PG-APS generated by in vitro digestion with mutanolysin. This transient edema did not develop in either control group.

A separate experiment was performed for the histological evaluation of rats injected with an arthropathic dose of PG-APS and immediately thereafter with mutanolysin or PBS. The histological changes observed in the tarsal joints of non–enzyme-treated Lewis rats were similar to the changes previously reported to occur in Sprague-Dawley rats (1, 2), with the exception that the late destruction of subchondral bone and growth of new bone was much more extensive in Lewis rats. When active mutanolysin was injected immediately after PG-APS, the initial exudative and proliferative synovitis observed 3 d after injection was qualitatively similar in the treated and untreated groups (Figs. 3 and 4). At 7 d after injection

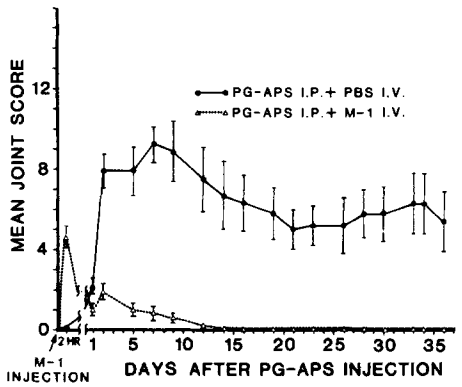


FIGURE 2. The course of arthritis in rats treated with mutanolysin (M-1) intravenously immediately after an intraperitoneal injection of an arthropathic dose of PG-APS. The control group received PG-APS i.p. and 0.4 ml of PBS i.v.; the experimental group received PG-APS i.p. and 0.4 ml M-1 (1 mg/ml) i.v. There were initially 18 rats in each group. Six rats from each group were killed at 2, 5, and 36 d after PG-APS injection for quantitation of cell wall in tissues (Table I). Clinical joint scores (arthritis or edema) in this and subsequent figures are presented as the mean ± SEM. The difference in arthritis scores between groups was significant ($P < 0.01$) at all time points as calculated by the Student's $t$ test.

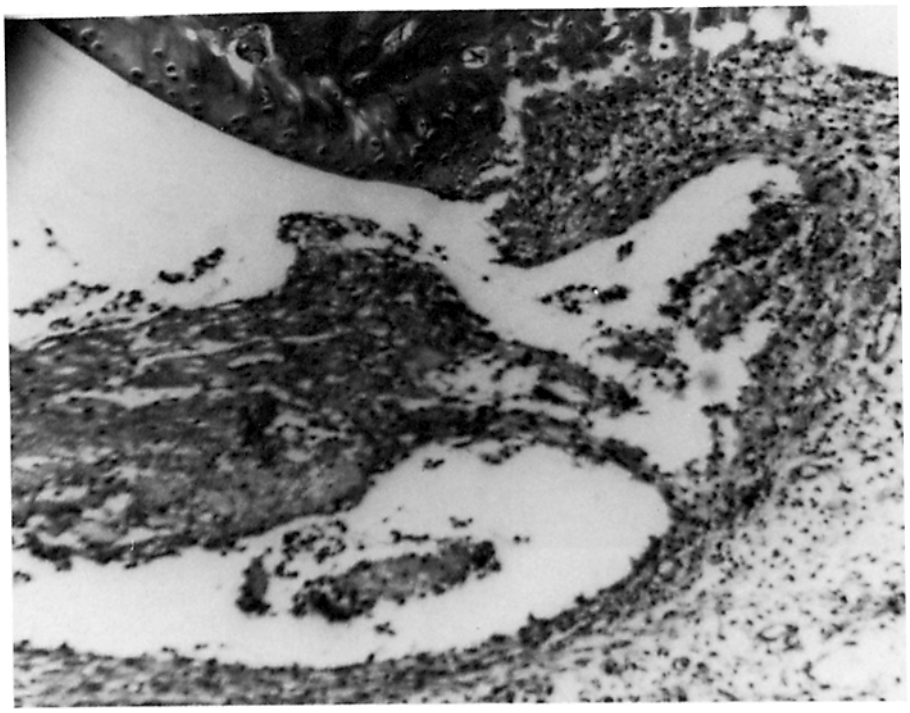

FIGURE 3. Tarsal joint of a control rat that received PG-APS followed immediately by PBS. Tissues were collected after 3 d. An acute exudative synovitis was present. The joint space contained leukocytes and fibrin. HE stained. × 100.

the extent of the acute synovitis was less in the animals receiving mutanolysin. At 14 d after injection of PG-APS the animals given mutanolysin showed very mild synovitis characterized by focal proliferation of synovial lining cells, focal areas of scarring in the synovial stroma, and a small number of mononuclear cells in the synovial tissues. This was in striking contrast to the active synovitis in the non–enzyme-treated control rats. Fig. 5 illustrates a normal-appearing joint tissue from an enzyme-treated rat 41 d after injection of PG-APS. For contrast, the extensive proliferative erosive synovitis observed in the non–enzyme-treated animals at 41 d after PG-APS injection is illustrated in Fig. 6.

*PG-APS Arthritis Is Reversed by Mutanolysin When Administered After Acute Inflammation Has Been Established.* A series of experiments were performed to determine whether mutanolysin could effect the course of arthritis after the initiation of disease. In the first experiment, rats were injected intraperitoneally with an arthropathic dose of PG-APS and randomly divided into two groups. 24 h later, experimental rats were injected with 0.4 mg of mutanolysin, and the control group received 0.4 ml of PBS. At the time of enzyme injection, acute arthritis was just beginning and was relatively mild. Acute arthritis continued to develop in enzyme-treated rats, but was significantly less severe than in control rats (Fig. 7). Joint disease resolved in enzyme-treated rats by day 14, and no evidence of recurrence was observed through day 36. Control rats went on to develop the typical pattern of chronic arthritis.

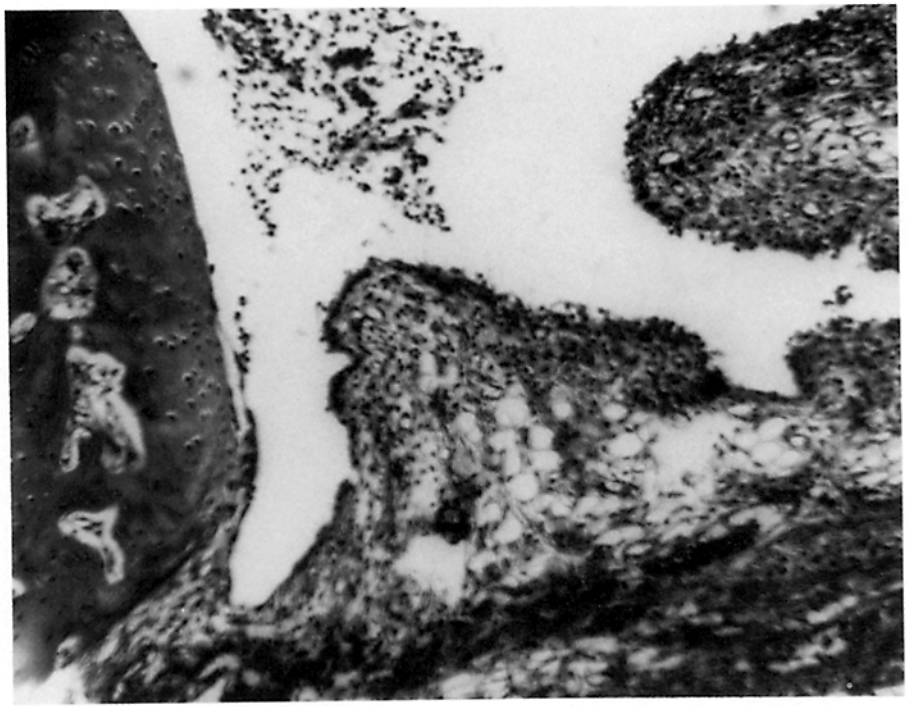

FIGURE 4. Tarsal joint of an experimental rat that received PG-APS followed immediately by mutanolysin. Tissues were collected after 3 d. An acute exudative synovitis was present. The joint space contained leukocytes and fibrin. HE stained. × 100.

In a second experiment, rats were injected with mutanolysin or PBS 3 d after receiving an arthropathic dose of cell wall, which is approximately the time of peak acute inflammation. Joint disease in the enzyme-treated rats was significantly less than in the control group within 48 h after treatment and eventually completely resolved (Fig. 8). Thus, mutanolysin can reverse progression of cell wall–induced arthritis even when administration is delayed until severe joint disease has developed.

Mutanolysin and PBS–treated rats described in Fig. 8 were examined histologically 83 d after PG-APS injection. Rats injected with PG-APS and 3 d later given PBS showed erosive synovitis that had evolved to a stage characterized by widespread destruction of cartilage and bone, extensive growth of new bone with marked distortion of the normal architecture of the tarsal bones, severe scarring of the synovial tissue, and fibrous ankylosis of many of the joints. A few of the joints in each animal were still involved by an acute exudative and proliferative synovitis. Rats injected intraperitoneally with PG-APS and 3 d later given mutanolysin intravenously showed minimal active inflammation at 83 d, which consisted of focal areas of hyperplasia of synovial lining cells. The only indication of previous erosive synovitis were a few small areas where cartilage appeared to be replaced by fibrous tissue at the margin of the joint surfaces and focal areas of scarring in the synovial stroma.

In a third experiment, mutanolysin or PBS was administered to rats 14 d after

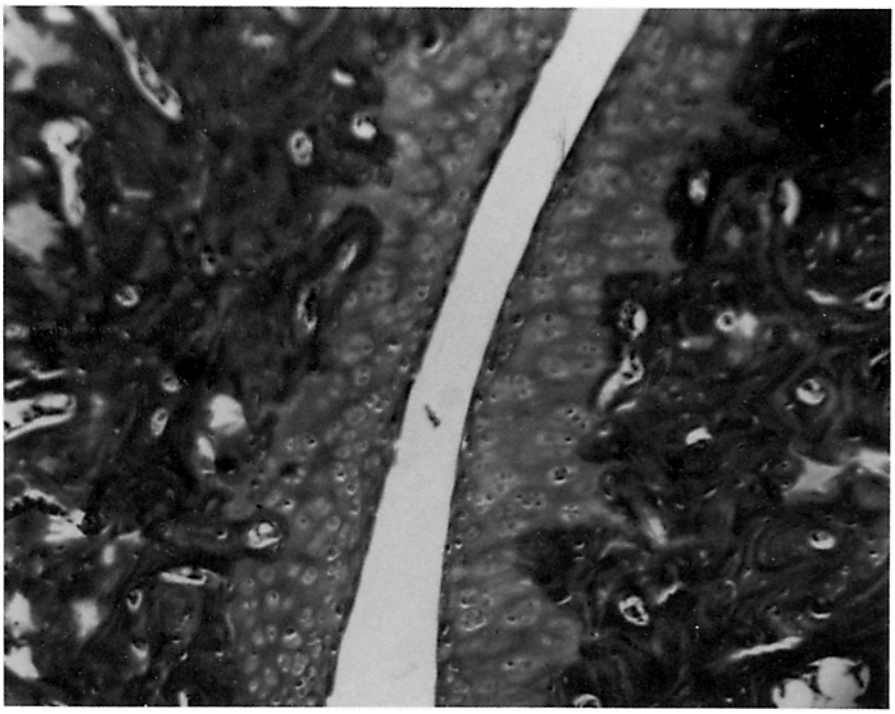

FIGURE 5. Tarsal joint of an experimental rat that received PG-APS followed immediately by mutanolysin. Tissues were collected after 41 d. The joint space contained no exudate and the cartilage was intact. HE stained. × 100.

PG-APS injection, which is the approximate time when the chronic phase of arthritis begins. After a slight initial increase, joint scores of mutanolysin-treated rats declined, and became significantly lower ($P < 0.01$) than those of PBS-treated rats by day 30 after PG-APS injection (Fig. 9). Fig. 10 illustrates the difference in clinical appearance between treated and untreated rats on day 44. Subsequently, only three of the nine mutanolysin-treated rats demonstrated a recurrent episode of chronic joint disease. In two of these three rats, the exacerbation was mild and short-lived. The third rat with recurrent arthritis developed severe joint disease that lasted from day 80 to the end of the experiment (day 127).

A histological study was performed on day 127 on the rats whose clinical scores are shown in Fig. 9. Despite the clinical improvement in the enzyme-treated rats, erosive synovitis that had resulted in synovial scarring and extensive distortion of the architecture of the tarsal bones by abnormal bone growth was equal to that of PBS controls. This was not surprising, however, since by the time enzyme was administered 14 d after PG-APS, the rats had proceeded through the acute phase of arthritis. Acute exudative, proliferative, and active erosive synovitis was observed in three of the enzyme-treated group and four of the untreated group at 127 d.

*Radiographic Data Confirms Clinical Assessment of Arthritis.* Rats injected with PG-APS and treated with mutanolysin or PBS at 0, 3, or 14 d after cell wall

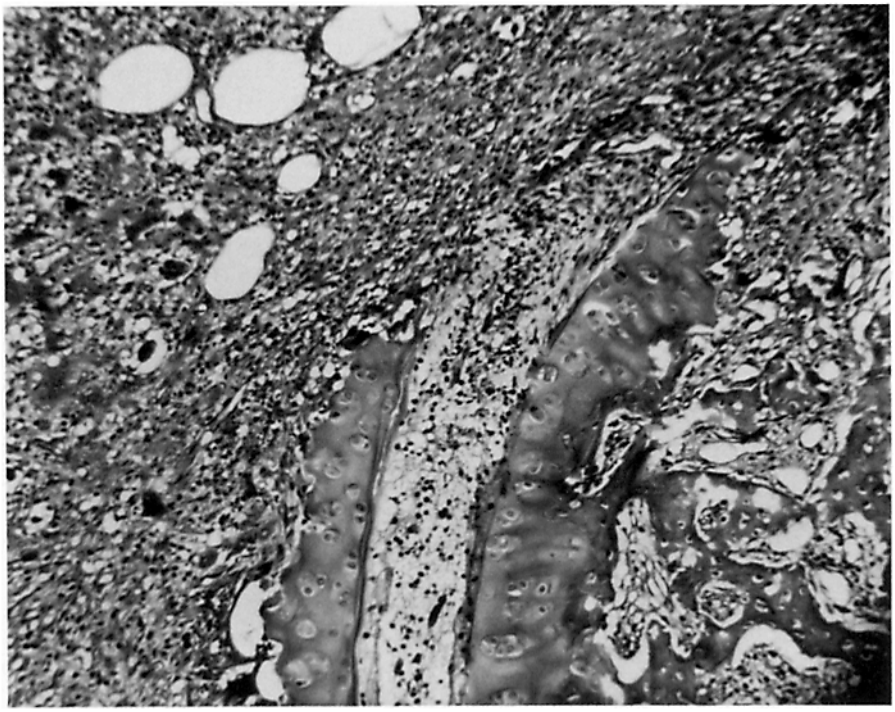

FIGURE 6. Tarsal joint of a control rat that received PG-APS followed immediately by PBS. Tissues were collected after 41 d. Extensive erosion of cartilage and subchondral bone was present. HE stained. × 100.

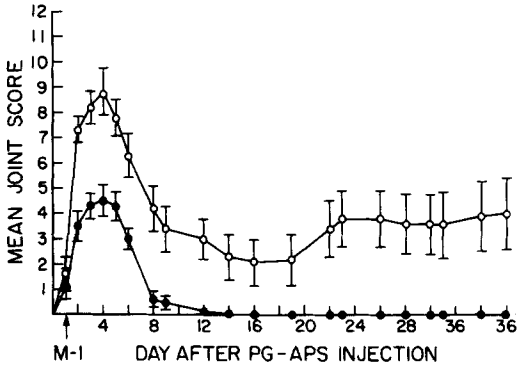


FIGURE 7. Effect of mutanolysin given 24 h after PG-APS on development of acute and chronic arthritis. Two groups of six rats were injected intraperitoneally with an arthropathic dose of PG-APS followed 24 h later by an intravenous injection of PBS (○) or 0.4 mg of M-1 i.v. (●). Statistically significant differences ($P < 0.05$) between the groups were found at all time points after administration of M-1.

injection were x-rayed shortly before the termination of each experiment. Rats treated with mutanolysin up to 3 d after PG-APS injection showed no chronic erosive arthritis, while PBS-treated rats displayed the destructive changes associated with chronic erosive synovitis (5). Those rats that were treated with

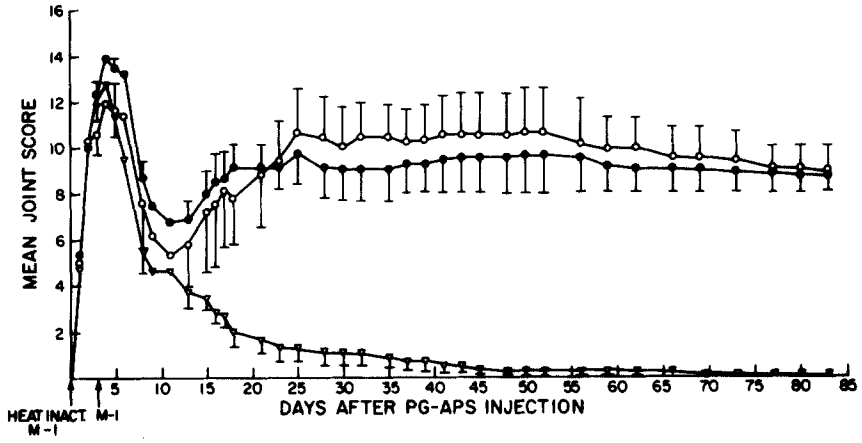


FIGURE 8. Effect of mutanolysin given at the peak of acute arthritis on the further development of disease. Three groups of five rats were injected with an arthropathic dose of PG-APS i.p. on day 0 plus 0.4 ml PBS on day 3 (●); 0.4 mg of M-1 on day 3 (▽); or 0.4 mg of heat-inactivated M-1 on day 0 (○). Statistically significant differences ($P < 0.02$) between PBS-treated and M-1-treated rats were seen at all time points after day 5. The differences between PBS-treated rats and those treated with heat-inactivated M-1 were not significant at any time.

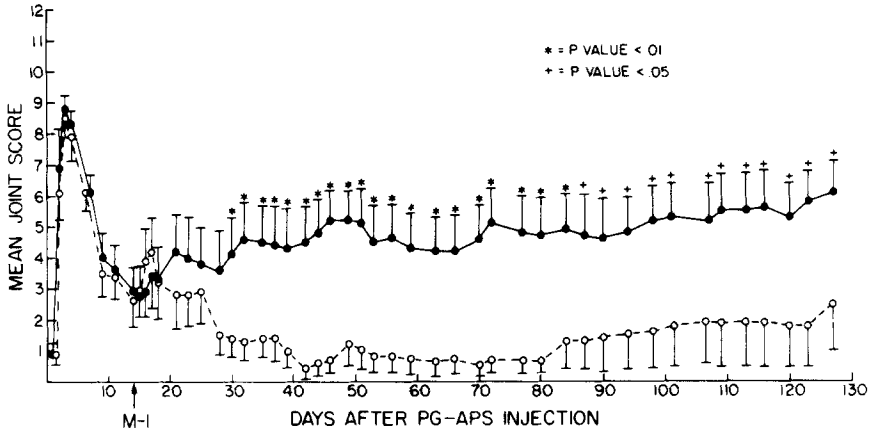


FIGURE 9. Effect of mutanolysin given 14 d after PG-APS on the development of chronic arthritis. A control group of eight rats (●) was given an arthropathic dose of PG-APS i.p. followed 14 d later by an injection of PBS i.v. The experimental group (○) contained nine rats that were injected with an arthropathic dose of PG-APS i.p. and 14 d later given 0.4 mg of M-1 i.v.

mutanolysin 14 d after PG-APS and still developed clinical and histological arthritis also showed evidence of arthritis on x ray.

*Muramidase Activity for Streptococcal Cell Wall Is Required to Modify PG-APS-induced Arthritis.* As part of the experiment in which mutanolysin was given to rats 3 d after PG-APS, 0.4 mg of heat-inactivated mutanolysin (boiled for 10 min) was injected intravenously into a group of rats immediately after the administration of an arthropathic dose of PG-APS. The results presented in Fig. 8 demonstrate that heat-inactivated M-1 does not reduce PG-APS arthritis.

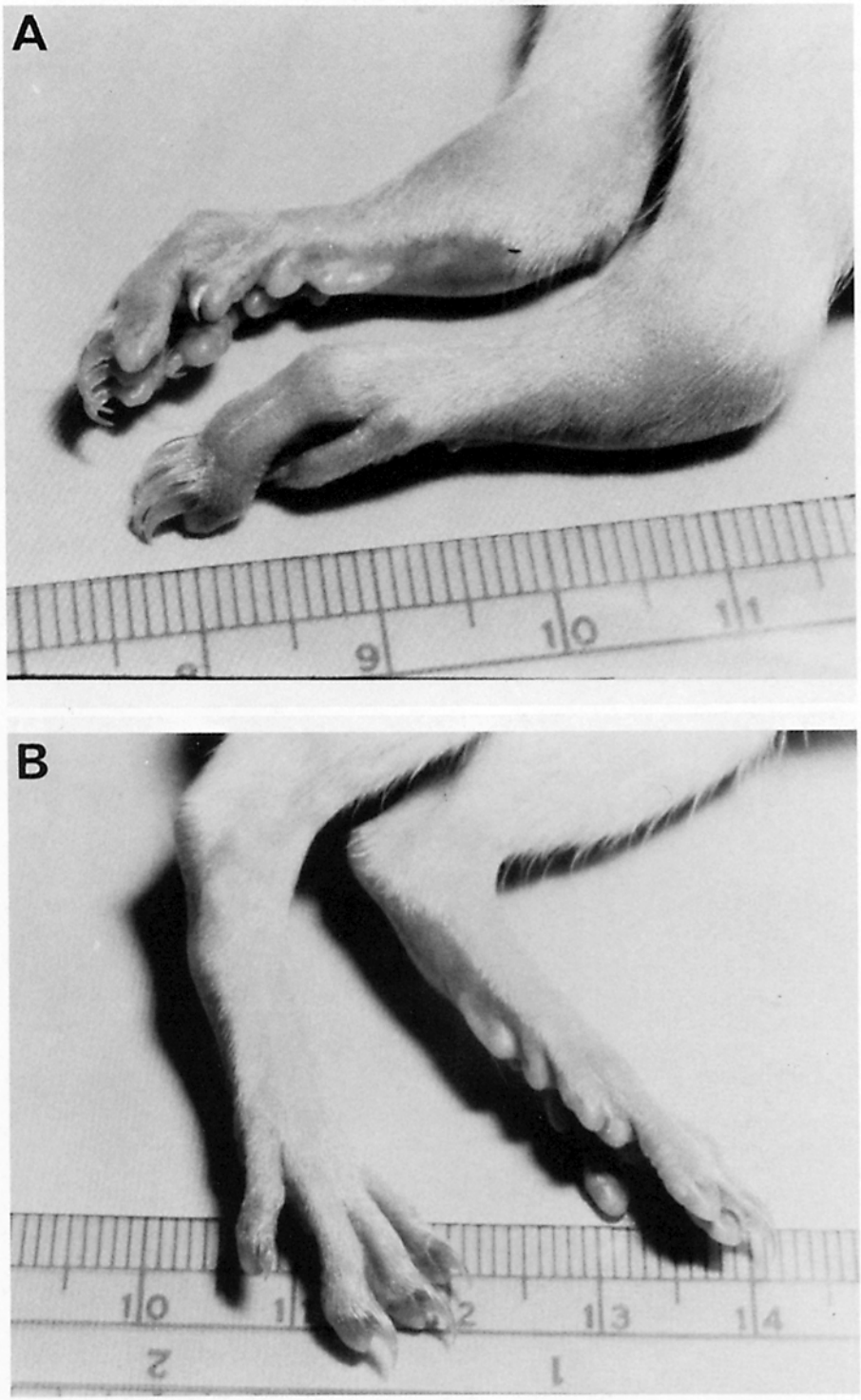


FIGURE 10. (*A*) Hind limbs of a rat with PG-APS-induced arthritis 44 d after PG-APS injection. This rat had a clinical score of 8 and is from the control group (PBS treated) described in Fig. 9. (*B*) Hind limbs of a mutanolysin-treated rat 44 d after PG-APS injection as described for the experimental group in Fig. 9. This rat had a clinical score of 0.

Histological examination confirmed that heat-inactivated mutanolysin had no effect on the development of erosive synovitis.

An additional control experiment was performed to determine if hen egg lysozyme injected in vivo could effect PG-APS-induced arthritis. Hen egg lysozyme is a basic protein with muramidase activity, but it is unable to degrade group A streptococcal cell wall in vitro (22–24). Rats were injected intraperitoneally with an arthropathic dose of PG-APS and randomly divided into two groups. The control group was treated with 0.4 ml of PBS i.v., while the experimental group received 0.4 mg of hen egg lysozyme i.v. The results shown in Fig. 11 demonstrate that treatment of cell wall–injected rats in vivo with lysozyme does not effect the course of arthritis.

*Mutanolysin Can Effect the PG-APS-induced Inflammatory Reaction in Other Tissues.* A histological study of the liver, spleen, and mediastinal lymph nodes was performed on all rats whose joint histology was described above. Rats injected with PG-APS and not treated with enzyme developed granulomatous lesions in the liver, spleen, and mediastinal lymph nodes. The process was extensive in the spleen by day 7 after PG-APS injection and in the liver and lymph nodes by day

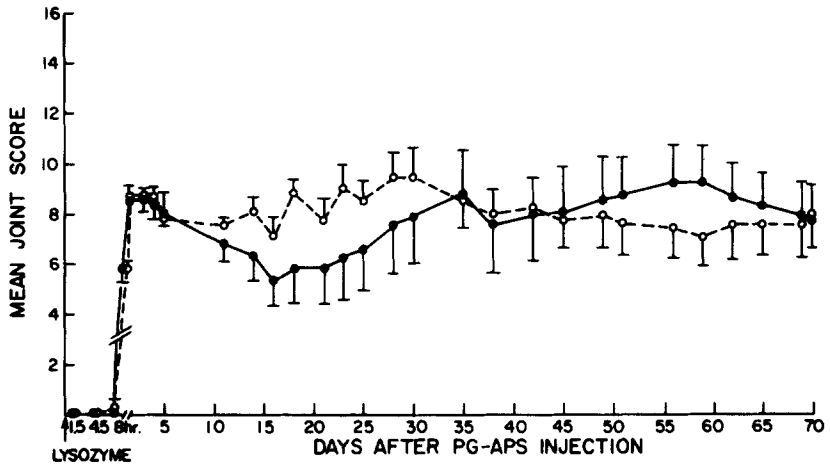


FIGURE 11. Course of arthritis in rats treated with lysozyme intravenously immediately after receiving PG-APS. Two groups of six rats were injected with an arthropathic dose of PG-APS i.p. followed immediately by 0.4 ml PBS i.v. (●) or 0.4 mg hen egg lysozyme i.v. (○). The differences between the two groups were not statistically significant at any time.

TABLE I

*Effect of Mutanolysin on the Distribution of Cell Wall in Rat Tissue*

| | Control (cell wall i.p. plus PBS i.v.) | | | Experimental (cell wall i.p. plus M-1 i.v.) | | |
|---|---|---|---|---|---|---|
| | Day 2 | Day 5 | Day 36 | Day 2 | Day 5 | Day 36 |
| Hind Limbs | 0.70 ± 0.21* | 0.32 ± 0.19 | 0.08 ± 0.06 | 3.7 ± 1.7 | 0.86 ± 0.13 | 0.19 ± 0.07 |
| Spleen | 107 ± 41 | 245 ± 29 | 33 ± 11 | 166 ± 49 | 92 ± 25 | 16 ± 1.7 |
| Liver | 145 ± 25 | 32 ± 11 | ND‡ | 300 ± 189 | 136 ± 37 | 39 ± 24 |
| Blood | 1.7 ± 0.68 | 0.11 ± 0.09 | 0.07 ± 0.01 | 2.3 ± 0.94 | 0.30 ± 0.07 | <0.06 |

* Mean ± SEM of PG-APS in micrograms per gram of tissue; five rats per group.
‡ No data.

14. The initial reaction appeared to be the formation of granulomas consisting of focal collection of macrophages and giant cells associated with the presence of small numbers of neutrophils and lymphocytes. As the collection of inflammatory cells increased in size, the relative number of neutrophils increased in the central areas of the lesions and irregular areas of necrosis developed that became surrounded by concentric layers of fibrous tissue infiltrated with neutrophils and mononuclear cells. In contrast, no such changes were observed in rats treated with active mutanolysin immediately or 3 d after PG-APS injection. Granulomatous lesions did develop in rats treated with mutanolysin 14 d after PG-APS injection. A more detailed description of the histology of this complex inflammatory response and its relationship to the distribution and persistence of PG-APS in the reticuloendothelial tissues of Lewis rats will be reported elsewhere.

*Mutanolysin Treatment Does Not Cause Elimination of PG-APS from the Tissue.* The quantitative distribution of cell wall in rats injected intraperitoneally with PG-APS and immediately injected intravenously with PBS or mutanolysin is shown in Table I. The rats for this experiment are those whose course of arthritis is shown in Fig. 2. On day 2, 5, and 36, rats were sacrificed for assay. The hind limbs, liver, spleen, and blood from enzyme-treated animals contained quantities of PG-APS that equaled or appeared to exceed the amounts found in PBS-treated control rats. Therefore, it is unlikely that mutanolysin causes a rapid elimination of PG-APS from the limbs, liver, and spleen.

## Discussion

The intravenous injection of a single dose of 0.4 mg of the muralytic enzyme mutanolysin profoundly altered the course and severity of PG-APS-induced arthritis, even when the enzyme was given after the inflammation was well established. In rats that received mutanolysin up to 3 d after PG-APS, acute joint inflammation resolved and did not progress into chronic recurrent disease. If treatment with the enzyme was delayed until 14 d after PG-APS, the severity of the recurrent chronic phase of arthritis was significantly reduced by clinical and radiological assessment, although active inflammation was evident in some specimens by histological examination.

We originally hypothesized that the mutanolysin would hasten clearance of tissue-bound PG-APS. However, quantitative ELISA studies on tissue extracts failed to demonstrate that catabolism of PG-APS was significantly enhanced by enzyme treatment. In fact, more PG-APS was detected in the tissues of enzyme-treated rats than in PBS-treated control animals. This difference could be accounted for by increased antibody binding of the enzyme-digested PG-APS in the ELISA assay due to the exposure of more epitopes. This was suggested by in vitro digestion of PG-APS with mutanolysin, which resulted in enhanced ELISA detectability of the same degree as seen in Table I (unpublished observations).

An alternative hypothesis is that mutanolysin modifies cell wall–induced arthritis by degrading PG-APS to polymers that still persist in tissue, but are of a size which is nonarthropathic. Previous studies (12, 13) have demonstrated that the polymer size of PG-APS is important for the production of chronic inflammation. PG-APS degraded in vitro with mutanolysin to fragments with a molec-

ular weight of $<5.0 \times 10^6$ was unable to induce arthritis by systemic injection (13).

The evidence that PG-APS was degraded in vivo by mutanolysin consists of two observations: (*a*) Rats injected intravenously with mutanolysin immediately after an intraperitoneal injection of PG-APS developed a rapid transient edema in their limbs. The kinetics of edema development was the same as that seen in rats injected with PG-APS degraded to a small fragment size by mutanolysin in vitro (13). (*b*) ELISA analysis of sucrose gradient fractions has shown that the proportion of small cell wall fragments in the tissues of mutanolysin-treated rats was greatly increased compared with PBS-treated animals (Janusz, M. J., R. A. Eisenberg, and J. H. Schwab, manuscript in preparation).

An extension of our current hypothesis regarding the reduction of PG-APS-induced arthritis by mutanolysin is that digested PG-APS fragments could interfere with the larger arthropathic cell wall fragments. For example, small nonarthropathic PG-APS fragments may compete with arthropathic PG-APS for binding sites on synovial tissue or soluble proteins such as complement. This hypothesis is attractive, as it would require only a small percentage of the total cell wall injected to be degraded to small fragments in order to suppress arthritis. Also pertinent is recent work (25) demonstrating that muramyl dipeptide (MDP) has antiinflammatory activity in several models of acute inflammation in rats and mice.

It is conceivable that the effect of mutanolysin on PG-APS-induced arthritis is not due to its specific enzymatic activity. However, heat-inactivated mutanolysin did not reduce the severity of arthritis in PG-APS-injected rats. In addition, hen egg lysozyme, which is a cationic muralytic enzyme but is unable to digest the cell wall of group A streptococci, did not reduce PG-APS-induced arthritis. In another model of chronic intermittent inflammation, enzymes have been shown to mediate in vivo suppression of activity. For example (26), incubation of group A cell wall in vitro with the culture supernatant of *Streptomyces albus* results in digestion of the cell wall and a loss of the ability to produce dermal lesions in rabbit skin. In addition, injection of filtrates of *S. albus* or group C streptococcal phage-associated lysin directly into a cell wall injection site in rabbit skin results in a decrease in the severity and duration of dermal lesions (27).

The demonstration that mutanolysin has a profound effect in vivo on PG-APS-induced arthritis may be significant for the study of inflammation, since it provides a unique situation in which the phlogistic agent can be rapidly and specifically inactivated in vivo while the inflammatory response is evolving. The immediate usefulness of mutanolysin is as a tool to dissect various aspects of PG-APS-induced arthritis in the rat. If the model of cell wall–induced arthritis proves to be related to human arthritides, this approach may also be useful in the analysis of the etiology and mechanisms of joint destruction, or even as a therapeutic modality.

## Summary

A single intravenous injection into rats of 0.4 mg of the muralytic enzyme mutanolysin, given as long as 3 d after an arthropathic dose of peptidoglycan-polysaccharide polymers derived from group A streptococci (PG-APS), resulted

in a complete resolution of acute arthritis and the prevention of chronic joint disease. When administration of mutanolysin was delayed until 14 d after the injection of PG-APS, a great reduction in the severity of chronic inflammation was still observed. Quantitation of the amount of PG-APS present in the limbs, spleen, and liver by a solid phase enzyme-linked immunoassay indicated that the tissues of mutanolysin-treated rats contained as much PG-APS as tissues of PBS-treated control rats. In addition, rats treated with mutanolysin immediately after receiving an intraperitoneal injection of PG-APS developed a transient limb edema similar to that seen in rats after the injection of PG-APS digested to a small fragment size in vitro with mutanolysin. We hypothesize that mutanolysin acts in vivo by degrading PG-APS to small fragments that persist but are no longer arthropathic.

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