Adjuvant Arthritis Induced by *Corynebacterium rubrum*[1] (34459)

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The pathogenesis of adjuvant-induced arthritis—an experimental disease produced in rats by intracutaneous injection of heat-killed mycobacteria or Nocardia (1, 2), or some fraction of mycobacteria (2, 3) in an oil emulsion—is still obscure. The disease has been related to hypersensitivity and dissemination of tubercle bacilli (4), viral infection (5), and autoimmune processes (6). Previous publications have reported failure of all attempts to induce the disease by injecting microorganisms other than mycobacteria (7).

The finding of a substitute for mycobacteria in the induction of the disease may help to understand the pathogenesis and the responsible chemical factor of this form of arthritis. *Corynebacterium rubrum*—a recently described gram-positive nonpathogenic bacterium (8)—has already demonstrated, like mycobacteria, an adjuvant activity on humoral antibodies and delayed hypersensitivity (9), and on the induction of allergic encephalomyelitis (10), and orchitis (11) in guinea pigs.

The present study shows that *Corynebacterium rubrum* can induce arthritis in rats.

Materials and Methods. Animals. Female rats (Sprague-Dawley), weighing approximately 150 g, female guinea pigs (Hartley strain), weighing 400 g, and female mice (Swiss Webster), weighing 30 g were used.

Injection of bacteria. Except as specified otherwise, 6 mg of autoclaved lyophilized bacteria were homogenized in 1 ml of heavy mineral oil (E. R. Squibb and Sons, New York, New York). In animals under ether anesthesia, 0.1 ml of the suspension was injected intracutaneously into the base of the tail (12).

Quantitation of arthritis. Animals were observed daily. At every examination the involvement of each extremity was graded 0 to 5, depending upon the degree of swelling and redness (5). Involvement of the tail was not scored. Thus the maximal score per animal was 20.

Culture of Corynebacterium rubrum. *Corynebacterium rubrum* (American type culture collection, Rockville, Maryland), was grown for 7 days at 37° in Erlenmeyer flasks containing approximately 250 ml of brain–heart infusion, and 1.5 maltose. The bacteria were washed four times with sterile physiological saline, autoclaved, washed five times with distilled water, and then lyophilized.

Human *Mycobacterium tuberculosis* (heat-killed strain N Pm/De and C) were donated by Lederle Laboratores (Pearl River, New York).

At time of sacrifice, usually 30 days after the injection of bacteria, tissues were fixed in formalin, bones were decalcified. Paraffin-embedded tissues were stained with hematoxylin and eosin.

Results. Injection of an emulsion of *Corynebacterium rubrum* and mineral oil induces a severe arthritis, which usually begins 8–10 days after the injection, and is most pronounced in the hind legs. The ankle, tarsal, and metatarsal joints are red, swollen, and painful, causing the animals to reduce their movements to a minimum. The tail may be beaded. Alterations of the eye, ears, and other mucosae are not observed. In animals sacrificed 3 months after the beginning of the lesions, arthritis still persisted and resulted in ankylosis. The appearance and course of the disease is similar to that observed in rats

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injected with mycobacteria.

The induction of arthritis by *Corynebacterium rubrum* is dose-dependent (Table I). Six hundred gamma per animal is the optimal amount, 300 gamma is less effective, and 100 gamma or smaller amounts are completely ineffective.

Injection of a suspension of *Corynebacterium rubrum* in guinea pigs and mice fails to induce any joint alteration.

Histologically the lesions of *Corynebacterium rubrum*-treated rats are similar to those

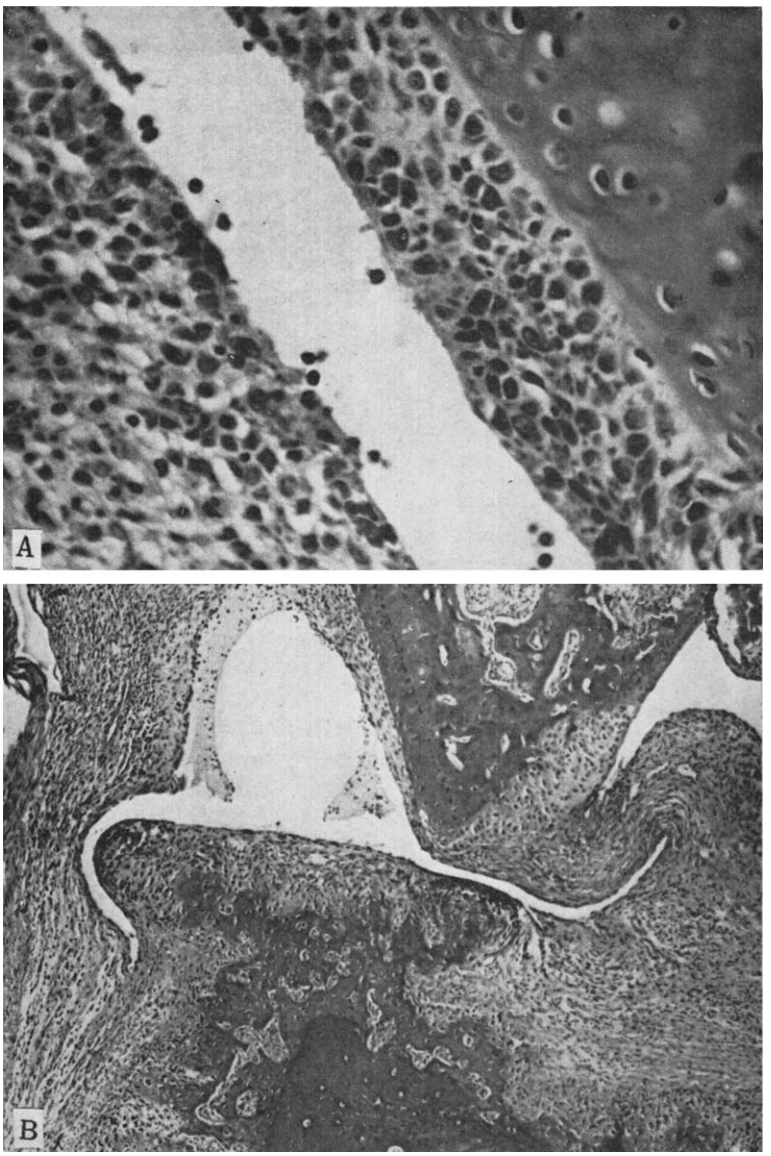

FIG. 1. Tarsal joint of a rat 30 days after injection of *Corynebacterium rubrum* and mineral oil. A. Proliferation of the synovial lining cells with marked mononuclear cell reaction between synovium and articular cartilage. (The exudate in the synovial cavity is minimal.) (Hematoxylin and eosin. ×250). B. Conspicuous proliferation of connective tissue which surrounds the synovial cavity and extends into the parasynovial tissue, subchondral region, and articular cartilage on both sides of the joint. Exudate is present in the joint space. (Hematoxylin and eosin. ×35).

TABLE I. Arthritis in Rats Injected with Various Amounts of *Corynebacterium rubrum* Suspended in Mineral Oil.

Treatment (gamma)	No. of rats	Mean arthritic score 30 days after treatment
Corynebacterium rubrum		
20,000	3	13.0
5,000	3	10.6
600	10	10.8
300	5	6.0
100	5	0
50	5	0
5	5	0
1	5	0
Mineral oil only	12	0
Mycobacterium tuberculosis		
600	6	4.0

animals receiving mycobacteria. Edema of the tissue adjacent to the synovium is accompanied by proliferation of synovial lining cells (Fig. 1A). The synovial space contains fibrin and scattered mononuclear cells with neutrophils. Granulation tissue composed mainly of fibroblasts, histiocytes, scattered mononuclear cells, and neutrophils, is seen in the tissue adjacent to the synovium, joint cartilage, and tendons (Fig. 1B). It even invades the articular cartilage, the periosteum, bones, and bone marrow itself. In the tissue adjacent to the joints, osteoblasts proliferate and new bone forms.

Discussion. A week after injection of *Corynebacterium rubrum* rats develop a severe form of polyarthritis clinically and histologically similar to that induced by *Mycobacterium tuberculosis* (2, 13, 14). Thus, in inducing adjuvant arthritis, *Corynebacterium rubrum* seems to be an effective substitute for *Mycobacterium tuberculosis* (15). Like that of *Mycobacterium tuberculosis* the effect of *Corynebacterium rubrum* seems to be dose-dependent; 300 gamma per animal is the minimal dose able to induce arthritis, 600 gamma the optimal dose. The effect is species-specific, since guinea pigs and mice do not develop arthritis upon injection of the bacterium.

The role of mineral oil on the pathogenesis of the lesion is still unknown, and the pathogenesis of the lesion is even more obscure. The discovery of a nonpathogenic and easily cultivated substitute for mycobacteria may help identify the active factor in this form of arthritis, and contribute to an eventual understanding of this disease, which has some features in common with rheumatoid arthritis of man (13).

Summary. *Corynebacterium rubrum*—a gram-postive nonpathogenic bacterium—induces severe arthritis a week to 10 days after its injection in rats. Production of arthritis is species-specific and dose-dependent, the optimal dose being 600 gamma per animal. Morphologically, the arthritis—similar to adjuvant arthritis induced by mycobacteria—is characterized by granulation tissue invading synovia, periarticular tissue, tendons, articular cartilage, periosteum, and bone spaces. The discovery of a nonpathogenic and easily cultivated substitute for mycobacteria may help to identify the responsible antigen and the mechanism in adjuvant arthritis.

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