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ARTHRITIS IN RATS CAUSED BY PLEUROPNEUMONIA-LIKE MICRO-ORGANISMS AND THE RELATIONSHIP OF SIMILAR ORGANISMS TO HUMAN RHEUMATISM

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The etiology of rheumatoid arthritis has remained obscure in spite of considerable investigation. The difficulty of its elucidation is increased by the fact that no experimental animal has yet been found in which it is possible to reproduce the disease or in which a strictly comparable disease has occurred spontaneously. Until comparatively recent, all attempts to produce arthritis in animals by the injection of micro-organisms, either directly into joints, into the blood stream or by the establishment of foci elsewhere, have led to the development of joint disease which bears only slight resemblance to the typical changes of rheumatoid arthritis. The animal arthritis produced by bacteria is an acute process, usually markedly purulent, which may go on to a chronic state in which the late stages of the inflammatory process suggest, to a certain extent, the pathological changes found in rheumatoid arthritis, but the animal disease lacks many of the outstanding features of the human, particularly the phenomenon of recurrences, and also destruction of cartilage under a granulation tissue pannus.

Recently there have appeared reports of spontaneous arthritis occurring in rats and mice, which have given new impetus to attempts to find an animal disease which resembles more closely rheumatoid arthritis and also to investigate any possible relationship between causative agents of the animal diseases and human rheumatism. Collier[1] described a polyarthritis in wild rats which he was able to transmit to laboratory rats and to propagate by the transfer of infectious material from the affected animals; cultural attempts were originally reported to be negative. Details concerning the pathology of the lesions are meager in Collier's publications. Findlay et al.[2] described a similar disease observed by them in England, from the lesions of which an organism of the pleuropneumonia group was isolated. More recently Klieneberger[3] has reported the isolation of probably the same organism from rats infected with material obtained from Collier, and Beeuwkes,[4] working in Collier's laboratory, confirmed this finding. Warren[5] in this country has also studied a similar disease.

Sabin[6] described a proliferative, chronic arthritis in white mice having many resemblances to rheumatoid arthritis, also caused by organisms of the pleuropneumonia group. It should be noted that Sabin's strains were obtained from normal mice, and that a spontaneous mouse disease was not observed.

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1. (a) Geneesk. tijdschr. v. Nederl.-Indie **78:** 2846, 1938; (b) J. Path. & Bact. **48:** 579, 1939; (c) Ztschr. f. Immunitätsforsch. u. exper. Therap. **95:** 132, 1939.

2. Lancet **2:** 7, 1939.

3. J. Hyg. **40:** 204, 1940.

4. Geneesk. tijdschr. v. Nederl.-Indië **80:** 1823, 1940; J. Infect. Dis. **70:** 1, 1942.

5. Proc. Third Internat. Cong. Microbiol, New York, 1940, 180.

6. Science **89:** 228, 1939; J. Bact. **39:** 1940.

Klieneberger[3] reported spontaneous arthritis in mice from which she obtained a pleuropneumonia-like micro-organism; details of its relationship to other strains are as yet not available.

These observations, together with the fact that arthritis is a frequent complication of other animal diseases known to be caused by organisms of this group (bovine pleuropneumonia, mastitis of sheep and goats), stimulated interest in the possibility that related organisms might be concerned in human arthritis. However, the few reports available at present do not support the idea that members of the pleuropneumonia group of micro-organisms are causally related to rheumatoid arthritis or to rheumatic fever in man. Sabin[7] and Sabin and Johnson[8] have published results of attempts to isolate pleuropneumonia-like organisms from cases of human rheumatic disease; in all instances the results were negative. Similar findings were reported by Eagles, Sullivan, Angevine, and Swift, at the Third International Congress for Microbiology in 1939.

It is the purpose of this paper to report the isolation of pleuropneumonia-like micro-organisms from spontaneous rat arthritis, the production of experimental arthritis in rats together with a description of the pathology of the disease and to summarize the attempts made in this laboratory to isolate similar organisms from patients suffering from rheumatoid arthritis.

Isolation of Pleuropneumonia-like Organisms and Production of Arthritis in Rats

The organism employed was isolated from the knee joint of a rat being used in hormone studies conducted in the department of obstetrics and gynecology of the medical school of the University of Michigan. The rat, a female, was in a state of continuous oestrus, following treatment with testosterone. Dr. James T. Bradbury, who kindly called our attention to this animal and allowed us the opportunity of examining it, stated that such treatment seemed to favor the development of purulent processes, particularly intra-abdominal abscesses. Arthritis was first observed in the rat in July; autopsy performed August 1 revealed a swollen knee joint and an abscess about the right ovary. The affected knee was two to three times larger than the opposite joint and contained thick, almost caseous pus, from which a pure culture of a pleuropneumonia-like micro-organism was obtained. The same type of organism was seen in mixed culture from the pus obtained from the para-ovarian abscess.

Another rat from the normal stock was observed to have an enlarged ankle and swollen forefeet. These joints were incised and peri-articular edema, rather than a frankly purulent joint was found. What appeared to be the same pleuropneumonia-like organism was isolated in pure culture from the ankle joint of this animal.

Culturally, these strains appeared identical with the type of organism described by Klieneberger[9] as "L 4" which was first obtained from the submaxillary gland of a rat and which was shown[10] to be the causative agent of the suppurative disease of rats described by Woglom and Warren.[11] We also isolated a strain of L 4 from the commonly occurring middle ear infection of rats.

Isolation was accomplished by inoculating aspirated material into tubes of "special medium"[9] consisting of 30%

7. Science **90:** 18, 1939.

8. Proc. Soc. Exper. Biol. & Med. **44:** 565. 1940.

9. J. Hyg. **38:** 458, 1938.

10. Klieneberger, E.: J. Hyg. **39:** 260, 1939.

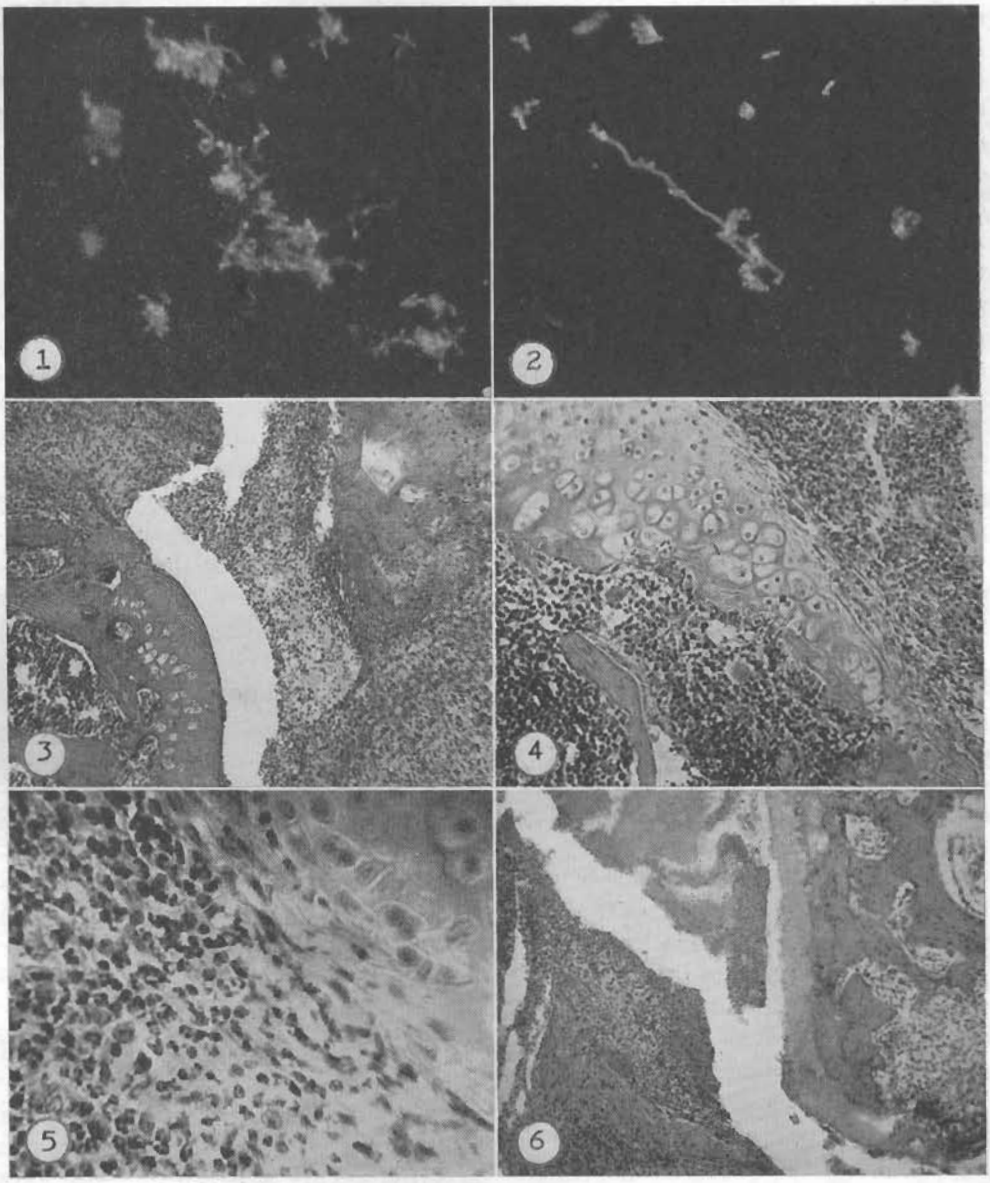
11. Science **87:** 370, 1938; J. Exper. Med. **68:** 513, 1938; J. Hyg. **39:** 266. 1939.

ascitic fluid-beef infusion broth, *p*H 8. In this medium, the organism produced a faint clouding in 24 hours, developing to an easily recognized but slight turbidity in 48 hours. Growth was uniform throughout and no clumps were formed. Dark field examination revealed thin filamentous forms (figs. 1 and 2) usually about 1 micron in length, but in young cultures filaments stretching completely across the microscope field have been observed. Branching of the filaments was common to form forked structures. The organism appeared to be very plastic as there was conspicuous waving of the filaments. Cultures on solid medium have been studied and conform to the descriptions given by Klieneberger.

Preliminary experiments were done with a culture several generations from the initial isolation. Young rats weighing approximately 150 gm. were injected under the skin of the abdomen, into the foot-pad, and intracardially. The inoculations into the abdominal wall gave rise only to a local abscess; the rats injected into the foot-pad developed swelling of the inoculated member only, while rats injected directly into the blood stream showed multiple joint involvement. It was, therefore, decided that injection into the blood stream should be the most satisfactory means of producing an arthritis, furthermore, the possibility of local trauma to the joints would thereby be eliminated.

A 48 hour broth culture of the organism in the second passage from its isolation from a swollen joint of one of the preliminary animals was used to inject the majority of the animals studied. Fifteen rats were divided into 3 groups of 5 each; the animals in each group were injected directly into the heart with the same amount of culture. The inoculations consisted of 2 cc., 1 cc. and 0.5 cc. amounts. With the exception of 1 rat which died immediately following the injection, swelling developed in 1 or more joints of all the animals, appearing in 3 or 4 days. Animals were killed at intervals and examined grossly and histologically. Another series of 20 animals was inoculated with organisms isolated 4 days after injection into 1 of the rats of the first series, in varying amounts down to 0.01 cc. of 48 hour culture. There appeared to have been an augmentation of virulence of the strain as the animals appeared sicker, many died, and more generalized pathologic effects were observed. The incidence of arthritis, however, was less, but appeared in animals injected with as little as 0.05 cc. of culture.

Study of the animals which died or were killed led to the following conclusions: This strain of pleuropneumonia-like micro-organism is primarily a "pyogenic agent" as so aptly designated by Woglom and Warren. Following injection into the blood stream, it appears to have a predilection for the joints and readily produces an arthritis. Initially the joints become swollen and edematous, there follows typical purulent inflammation, which is largely periarticular and leads to the development of an abscess which involves all the structures of the joint—both within and around—capsule, synovial membrane, and finally bone and cartilage. One is impressed, however, by the relatively late involvement of the cartilage. Many animals showed a marked exudate within the joint capsule, but with the cartilage apparently uninvolved. However, as the disease progresses, the cartilage is ultimately destroyed, but by a rapid necrotizing process, rather than by a slow destruction beneath a layer of granulation tissue. The bone likewise may become involved in the purulent process and osteomyelitis may occur. The whole joint structure may thus be involved at one time in an acute suppurative proc-



Figs. 1 and 2.—48 hour culture in 30% ascitic fluid—beef infusion broth. Dark field ×1,000.

Fig. 3.—Knee joint of rat killed 4 days after intracardial injection. Acute inflammation, exudate into capsule and at periphery of joint. Articular cartilage well preserved. ×100.

Fig. 4.—Knee joint of rat killed at end of 8 days, following injection. Acute exudate on cartilage. Osteomyelitis. Cartilage well preserved at this stage. ×150.

Fig. 5.—Higher power of same joint as fig. 4 to show character of exudate. ×700.

Fig. 6.—Hind foot of rat injected 10 days previously. Necrotic debris in joint space. Articular cartilage undergoing necrotic destruction.

ess. In this respect, the lesion more closely resembles that seen in pyogenic joint infections of man rather than that of rheumatoid arthritis (figs. 3–6).

Following the acute process in the joint, the abscess may rupture to the

outside, drain, and leave a relatively good function to the joint. In other cases, with more marked destruction, or when the process becomes subacute or chronic, ankylosis occurs.

The rats often showed involvement of structures other than the joints. A rather frequent finding, especially with cultures of exalted virulence, was a purulent pericarditis and epicarditis, probably due to direct infection at the time of cardiac puncture. The livers showed congestion and cloudy swelling. The spleens were enlarged and showed lymphoid hyperplasia—the kidneys occasionally were congested showing early parenchymatous changes. These finding were all consistent with those of a generalized infection. A study of the arthritis produced in mice by Sabin's type B strain and by a serologically related strain isolated in this laboratory from a rat lung has shown that the mouse disease more closely resembles rheumatoid arthritis in its histopathology and thus appears to be an animal syndrome more comparable to the human.

In confirmation of the work of Collier[1b] and Findlay et al.,[12] it was shown that preparations of gold salts which are used in the treatment of rheumatoid arthritis are effective in preventing the rat disease. In a small series of animals studied, it was found that a single injection of 2 mg. of gold sodium thiomalate (myochrysine) given subcutaneously at the time of inoculation of rats with an infective dose of culture, was effective in preventing the development of arthritis in at least 6 of 10 rats; smaller amounts showed less protection. Further studies on the effect of gold salts on arthritis produced in rodents by micro-organisms of this group will be reported in a subsequent publication.

12. Brit. J. Exper. Path. **21:** 13, 1940.

Pleuropneumonia-like Micro-organisms and Rheumatoid Arthritis

Following the announcement of Sabin, and encouraged by the reports of Collier and the British workers on the production of arthritis in rodents by organisms of this group, workers in the field of arthritis research became interested in the possibility of recovering similar organisms from cases of human rheumatism. Using technics which proved entirely satisfactory for the recovery of organisms from infected rats and for the cultivation of other strains of related organisms, attempts were made in this laboratory to incriminate pleuropneumonia-like organisms in human rheumatic disease. Joint fluid aspirated from 19 cases of characteristic rheumatoid arthritis, several of which were represented by more than one specimen, was studied. Tissue obtained by synovial biopsy in 4 cases of rheumatoid arthritis and by excision of subcutaneous nodules in 3 were likewise subjected to culture. On no occasion was any organism related to the pleuropneumonia group obtained. Our negative results are in agreement with those of all others who have reported similar studies.

SUMMARY

Animals infected with organisms of the pleuropneumonia group frequently show arthritis as a complication or as a primary manifestation. A suppurative arthritis occurs spontaneously in rats and has been shown to be caused by an organism of this group—the L 4 type of Klieneberger. The arthritis may occur independently or be but one manifestation of a generalized infection and resembles suppurative arthritis or "septic joint" of humans rather than rheumatoid arthritis. It has not been possible to incriminate organisms of this group as causative agents of human rheumatism.