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THE PRODUCTION OF OSTEOMYELITIS IN RATS¹

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Rodet (1) in 1884 was the first to produce, by the intravenous injection of staphylococci into young rabbits, the suppurative necrotic lesions of bone resembling those of hematogenous osteomyelitis in man. Since then, other workers have employed rabbits, with considerable success, for studies of this disease. The dog has also been used, (2, 3, 4), but the results have been somewhat less satisfactory. Since small laboratory animals offer special advantages for the study of osteomyelitis, it seemed desirable to investigate the production of this disease in a relatively cheap and available animal, the rat.

Several years ago, in this laboratory, Mr. Paul Prager was able to produce experimental osteomyelitis in the tibia of the white rat. His technique was to insert a piece of No. 50 white cotton thread into the bone marrow, and then inject into the area a culture of *Staphylococcus aureus*. This paper deals with further studies and technical modifications of Prager's method.

The organism used in these experiments was a strain of *Staphylococcus aureus* which had been isolated from a patient with chronic hematogenous osteomyelitis. It was maintained on blood agar slants and stored between growth periods for as long as 6 months without losing infectivity. All cultures used as inoculums were prepared by transferring organisms from a blood agar slant to 0.1% glucose proteose peptone broth (Difco), and incubating at 37 C. for 24 hours.

The rats employed in these experiments were of the Wistar strain. Preparatory to inoculation, the animals were anesthetized by the parenteral injection of nembutal (40 mg. per kg. of body weight). For producing trauma of the bone or local inoculation, the hair on the leg was clipped, the skin swabbed with iodine, and a 1-cm. incision made over the medial aspect of the proximal portion of the tibia. The periosteum was scraped from the tibia, and a hole 1 to 2 mm. in diameter was pierced with a small forceps or scissors through the compact layer into the bone marrow. Following inoculation, the skin was sutured with cotton thread. The skin incisions healed rapidly, as a rule.

A number of variations in procedures used for the production of osteomyelitis were made:

Method 1: Rats were inoculated by injecting 0.1 ml. of culture deep into the marrow cavity.

Method 2: A 2- to 3-cm. length of No. 50 white cotton thread, saturated with the broth culture, was inserted deep into the marrow. This was followed by the injection of 0.1 ml. of culture into the marrow.

Method 3: A wisp of cotton, soaked with the culture, was inserted into the bone with the aid of a small curved forceps. As in method number 2, this was followed by the injection of 0.1 ml. of the inoculum.

¹A preliminary report of this investigation was presented at the East Lansing meeting of the Michigan Branch of the Society of American Bacteriologists, June 1, 1945.

Method 4: About 0.1 ml. of warm 1.5% agar gel was injected into the marrow cavity. A few minutes were allowed to elapse, after which 0.1 ml. of the culture was injected into the agar mass. Dochez (5) used agar to protect streptococci against the defenses of the horse in inoculations made for the purpose of producing antiserum.

Method 5: One-tenth ml. of a 10% suspension of gastric mucin,² sterilized by autoclaving, was injected into the tibial lesion. An injection of 0.1 ml. of the culture was then made.

Method 6: One-tenth ml. of xylene, followed by the same quantity of culture, was injected into the marrow cavity.

Method 7: This was the same as method 6, except that cotton soaked in culture, as described in method 3, was inserted after the injection of the xylene, and before the injection of the culture.

Method 8: One-tenth ml. of the inoculum was injected into one of the tail veins. No local trauma was made.

Method 9: The rats were given an intravenous inoculation of culture as described above. Within 30 minutes, an opening was made into the marrow of the tibia, but no local inoculation of culture was given.

Method 10: The animals received 0.1 ml. of the culture intravenously, as well as 0.1 ml. locally into the marrow cavity of the tibia.

Method 11: Intravenous inoculation of culture was followed by the injection of 0.1 ml. of a 10% solution of sodium morrhuate³ into the bone marrow.

Method 12: This procedure was similar to Method 11, except that the tibial lesion was treated with xylene prior to being injected with the culture.

Following inoculation, the rats were observed for 3 or 4 weeks, at which time they were anesthetized, the site of inoculation reopened, and observations made as to the presence of subcutaneous abscesses, pus, necrotic bone, and the size of the aperture in the bone. A culture was made from the center of the inoculated area, in 0.1% glucose proteose peptone broth. If growth occurred, the organisms were stained by Gram's method and examined. If the aperture of the bone lesion was 3 mm. or more in diameter, contained pus, and yielded gram-positive cocci on culture, a diagnosis of osteomyelitis was made. To verify this conclusion, sections of affected bones from 14 rats, judged to have osteomyelitis, were sent to the Department of Pathology, where the diagnosis was confirmed by microscopic examination.

The results of attempts to produce osteomyelitis by the various methods outlined above are summarized in Table I. The most successful method appeared to be that of placing a non-absorbable foreign body, such as cotton, saturated with a broth culture of *Staph. aureus*, in the bone marrow. This method produced osteomyelitis in 94% of 81 rats. The use of method number 2, i. e., the insertion of culture-soaked thread into the bone marrow, followed by injection of 0.1 ml. of the culture, produced osteomyelitis in 72% of 60 rats. The addition of an agent, such as xylene, known to inhibit the action of the leukocytes, at least during the first hours following inoculation, did not increase the incidence of bone lesions.

Results following the injection of staphylococci and agar into the bone marrow, in the hope that the agar would interfere with the defenses of the rat, were as satisfactory as those obtained when thread was used as a foreign body. Of thirteen rats so inoculated, 77% developed osteomyelitis. The injection of mucin or xylene along with the bacterial inoculum did not result in any significant increase in the incidence of the disease over that of the group of animals receiving culture

²Lot No. 49799, prepared by the Wilson Laboratories, Division of Wilson and Company, Incorporated, Chicago, Illinois.

³Lot No. 097-S, Burroughs Wellcome and Company, New York.

alone. Staphylococci injected into the bone marrow, with no foreign body, caused osteomyelitis in only 12.5% of 16 animals so inoculated. .

The intravenous injection of staphylococci into rats, with or without trauma to the bone, did not produce the disease.

Since osteomyelitis in man has a tendency to occur more frequently in the young, it seemed advisable to determine the relationship of the age of rats to their susceptibility to experimentally induced osteomyelitis. Rats were divided into two age groups as determined by their weights, the young group averaging 50 grams each and the adult group approximately 250 grams. All were inoculated by method 3. The results are presented in Table II. It will be noted that 85% of fourteen 50-gram animals developed osteomyelitis, while 72% of 14 older rats developed the disease. The difference in incidence of infection is suggestive of a somewhat greater susceptibility of young rats in comparison with older animals.

TABLE I
THE RESULTS OF VARIOUS METHODS FOR THE PRODUCTION OF OSTEOMYELITIS
IN THE TIBIA OF THE WHITE RAT

Method Number and Procedure*	Number of Animals	Percentage of Animals which Developed Osteomyelitis
1. <i>Staph.</i> culture injected into bone.....	16	12.5
2. <i>Staph.</i> and cotton thread in bone.....	60	72
3. <i>Staph.</i> and cotton wisp in bone.....	81	94
4. <i>Staph.</i> and agar in bone.....	13	77
5. <i>Staph.</i> and mucin in bone.....	18	11
6. <i>Staph.</i> and xylene in bone.....	16	19
7. <i>Staph.</i> , cotton and xylene in bone.....	20	85
8. <i>Staph.</i> intravenously.....	9	0
9. <i>Staph.</i> intravenously and tibial trauma.....	9	11
10. <i>Staph.</i> intravenously and in bone.....	13	0
11. <i>Staph.</i> intravenously and sodium morrhuate in bone.....	25	20
12. <i>Staph.</i> intravenously and xylene in bone.....	11	27

*The several methods are described in detail in the text.

TABLE II
THE EFFECT OF THE AGE OF RATS ON THEIR SUSCEPTIBILITY
TO OSTEOMYELITIS

Average Weight of Animals	Number Inoculated	Percentage which Developed Osteomyelitis
50 grams.....	14	85
250 grams.....	14	72

Since the course of osteomyelitis in man is variable, ranging from acute to chronic, the course of the disease in one group of rats was followed for an extended period. Thirty-five rats with osteomyelitis 25 days after inoculation were observed 50 to 65 days longer. Sixty-six per cent of these animals still had gross evidence of the disease, while the remainder had evidence of healed craters in the bone, from which bacteria could not be cultured.

SUMMARY

Several modifications of a method for the production of osteomyelitis in the tibia of the white rat have been evaluated. The insertion into the bone marrow of a cotton wisp impregnated with a broth culture of *Staph. aureus* was found to be the most satisfactory of the various procedures tested. Substitution of a piece of cotton thread or of agar, for the cotton wisp, resulted in a significant though somewhat lower incidence of osteomyelitis. Little success was attained when no foreign body was present. The use of such agents as mucin or xylene did not significantly change the incidence of infection from that obtained when culture alone was injected into the bone.

The susceptibility of young rats appeared to be slightly greater than that of older animals.

The lesions persisted for as long as 75 to 90 days in 66% of 35 animals.

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