Rev. Inst. Med. trop. São Paulo
6(4):142-149, julho -agôsto, 1964

UDC 616.71-002

CHRONIC EXPERIMENTAL OSTEOMYELITIS

Luiz Gustavo WERTHEIMER (1) e Ivo Vecina MARTIN (2)

SUMMARY

The inoculation of a varying number of *Staphylococcus aureus* which had undergone also a varying number of transfers, has always caused death of the animals, independing of the inoculation route employed. Postmortem examinations showed multiple visceral abscesses and liquefaction of the bone marrow. When previously immunized with staphylococcal anatoxin, guinea-pigs survived inoculation of staphylococci.

Nevertheless, development of osteomyelitis was occasional. It was only by employing the hematogenous route, as well as the direct (intra-osseous) one, that the AA. were able to obtain a picture of osteomyelitis.

The number and virulence of the microorganisms proved to be important factors in the development of experimental osteomyelitis. Thus, inoculation of non-attenuated staphylococci, obtained from a case of experimental osteomyelitis, caused death of the animals notwithstanding their previous immunization by anatoxin. Also, inoculation of massive doses of microoganisms caused death of the previously immunized animals, even after 40 days.

Reinoculation of moderate doses of attenuated staphylococci in cases of chronic experimental osteomyelitis, caused recrudescence of old lesions as well as the appearance of new ones.

INTRODUCTION

Notwithstanding the enormous progress of antibioticotherapy, which has a decisive influence upon treatment and development of osteomyelitis, there are still chronic patients who do not benefit from any treatment and suffer periodical activation of the disease.

In order to explain the persistency of infection and the occurrence of activation in these chronic cases, various factors have been invoked, such as deficiency of natural defences, an unfavourable immuno-allergic condition and the resistance of microorganisms against the antibiotics.

Impressed by the stubborness of some of these cases of chronic infection, we tried the experimental bone infection in the hope of getting some data which might explain clinically observed facts.

EXPERIMENTAL OSTEOMYELITIS

The very first informations concerning the etiopathogeny of the human infection were obtained through experimental osteomyelitis.

RODET [3] was the first to obtain a bone abscess through intravenous injection of *Staphylococcus aureus* in the rabbit. LEXER [2] verified the necessity of employing small doses of microorganisms in order to prolong survival of the animals. In 1921, HOBO [1] proved that the microorganism fixes itself at first at the epiphysis and metaphy-

Presented at the XV Seminar of the Instituto de Medicina Tropical de São Paulo (1962).

(1) Professor of Orthopedics and Traumatology at the Sorocaba School of Medicine, Sorocaba (SP), Brazil.

(2) Assistant in Orthopedics and Traumatology at the Sorocaba School of Medicine, Sorocaba (SP), Brazil.

sis of the long bones, due to the distribution of vessels and also to the paucity of phagocytes in these parts of the bone.

WILENSKY [8] and ROBERTSON apud [4] drew attention, respectively, to the importance of vascular thrombosis and of the *locus minoris resistentiae* in the pathogenesis of osteomyelitis.

Long after the first experiments, difficulties concerning experimental reproduction of osteomyelitis still persisted. Thus, SHIODA [5] observed microscopically bone obscesses in 23% of his inoculated rabbits, but none of these survived beyond 96 hours. THOMPSON & DUBOS [6], in 1938, obtained bone abscesses in 58% of the animals, which they had previously immunized with small doses of microorganisms.

SHEMAN, JANOTA & LEWIN [4] obtained hematogenous osteomyelitis in animals surving for one week the direct inoculation of staphylococci in the bone, after previous injection of muriatic acid in the metaphysis.

WEAVER & TYLER [7] also obtained hematogenous osteomyelitis in 39% of the animals, which they inoculated with controlled doses of staphylococci. They did not observe any influence of trauma upon localization of osteomyelitis.

This brief summary of the experimental observations shows that the AA. who death with the subject have found it difficult to reproduce the picture of osteomyelitis and also that the various factors which influence the localization of the microorganism and the evolution of the disease are still a controversial matter.

MATERIAL AND METHOD

Microorganism: the microorganisms were obtained from a case of chronic osteomyelitis of the humerus, in the Orthopedic Clinics of the Sorocaba School of Medicine.

The material was sown on blood-agar plates, as well as on Teague's and Kristensen's media for gram-negative germs and, thus, *Micrococcus pyogenes* (*Staphylococcus pyogenes aureus*) * was isolated. Plasmocoagulase and hemolysis tests were positive. The material was then transferred to glucose broth and, after 24 hours, to simple agar. Another 24 hours later a suspension was made in physiological saline for inoculation. The MacFarland scale was used for counting the organisms present in the inoculum.

Number and virulence of staphylococci: in all experiments we injected 1 ml of a suspension containing from 3×10^8 to $9 \times \times 10^8$ organisms. Some animals were submitted to three consecutive inoculations of 5×10^7 staphylococci each.

Cultures used for the different inoculations had passed through 2 to 43 transfers, and before use the sample's pathogenicity was again tested, by means of repetitive plasmocoagulase and hemolysis tests.

Laboratory animal: 104 inoculations have been performed in guinea-pigs weighting from 300 to 600 grams.

Routes: various routes have been employed — cardiac (46 times), aorto-abdominal (5), femoral artery (30), bone (11), subperiostal and subcutaneous (3), combined bone and femoral (9 animals).

X-Rays: radiographs were made 14 days after inoculation, then on the 3rd and 4th weeks and monthly, thereafer.

A surgical table and an anaesthetic apparatus were specially made for the experiments.

RESULTS

I — EXPERIMENTS WITH NON-IMMUNIZED ANIMALS

A) *Inoculation of staphylococci varying in number and virulence.*

We refer to the simple attenuation of the microorganism due to successive transfers, and no special methods were used to test their virulence.

1) Inoculation of 9×10^8 staphylococci with 2 transfers, by intracardiac route in each of five guinea-pigs: all died. Due to this fact, we proceeded to make repeated transfers of the staphylococcus before the following experiments.

2) Inoculation of 9×10^8 staphylococci with 6 and 14 transfers (17 guinea-pigs). All died.

* Bergey's Manual of Determinative Bacteriology, 7th ed., 1957.

3) Inoculations of 3×10^8 staphylococci with respectively 4, 10 and 23 transfers: the 13 inoculated animals also died.

B) *Other routes of inoculation.*

Injections of 3×10^8 staphylococci/ml. with 23 and 30 transfers, through the abdominal aorta (5 cases), femoral artery (5), and intra-osseous (4) have also caused death of the laboratory animals.

Clinical picture: in this whole series of 54 experiments, the guinea-pigs showed remarkably poor general condition, and high fever; they walked dragging their hind legs and death supervened within 7 days.

X-Rays: no alterations in bone structure were detected.

Gross pathology: on postmortem all the guinea-pigs showed necro-ulcerative lesions of the rectal ampule, as well as multiple abscesses, especially of the liver, spleen and lungs. The bone marrow was liquefied, of a brownish tint, and smears of the medulla were always positive for staphylococci.

Histological examination: in all cases, there was "hypercellularity of the medula, indicative of an intense reactional process", according to the report of the pathologist, Dr. José Donato de Prospero.

II — EXPERIMENTS ON ANIMALS IMMUNIZED WITH STAPHYLOCOCCAL ANATOXIN

Among the lesions found in the preceding series of experiments, in which all guinea-pigs died within the first week our attention has been especially drawn by the necrosis of the rectal ampulla.

We tried a means of fighting the toxin action and proceeded to immunize the guinea-pigs with *staphylococcal anatoxin*, as suggested by Dr. Luiz Ribeiro. Using the subcutaneous route, we injected three doses of staphylococcal anatoxin, 0.25 ml, 0.50 ml and 1 ml, at weekly intervals. The experiments were started fourteen days after the last dose of anatoxin.

A) *Inoculations of the same number of staphylococci of varying virulence by various routes.*

1) Inoculations of 3×10^8 staphylococci with 23 transfers throug intra-cardiac route: for the first time the animals survived having by now been immunized. The six guinea-pigs showed only a poor general condition on the first two days. However, radiographs made weekly did not show any bone alterations and thus we decided to try other routes.

2) Inoculations of 3×10^8 staphylococci with 30 transfers, through sub-cutaneous and sub-periostal routes: no radiological signs of osteomyelitis were observed in the 3 inoculated guinea-pigs.

3) Inoculations of 3×10^8 staphylococci with 23 transfers, directly into the upper extremity of the tibia: for the first time in our experiments, osteomyelitis, was produced, as proved through X-rays (fig. 1) and on "postmortem", with the appearance of sequestrums, rarefaction zones and zones of new bone formation.

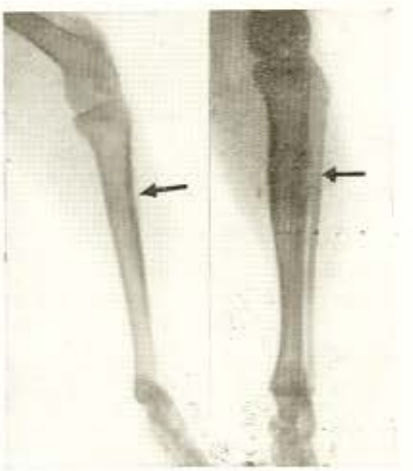

Fig. 1 — Experimental osteomyelitis in tibia of guinea-pigs previously immunized by the staphylococcal anaxin, obtained by direct inoculation in the bone of 3×10^8 staphylococci with 23 transfers; *a* — primary lesions radiographed 28 days after inoculation; *b* — chronic osteomyelitis observed 3 months after inoculation.

4) Inoculations of 3×10^8 staphylococci with 30 transfers, through the femoral artery: two of the inoculated guinea-pigs presented suppurative arthritis of the hip joint, with pathological dislocation and partial destruction of the head of the femur as well as of the acetabulum (fig. 2).

Fig. 2 — Suppurative arthritis of the hip joint: there is a pathologic dislocation with partial destruction of the head of the femur and of the acetabulum. The inoculation of 3×10^8 staphylococci, with 30 transfers, through the right femoral artery, caused development of a suppurative arthritis of the left hip joint.

B) *Local trauma associated with hematogenous inoculations of staphylococci.*

5) Inoculations of 3×10^8 staphylococci with 37 transfers, through the femoral artery, together with trauma of the right tibia: one of the 8 inoculated guinea-pigs showed bone lesions on the left tibia, that is, on the side opposite to the trauma (fig. 3).

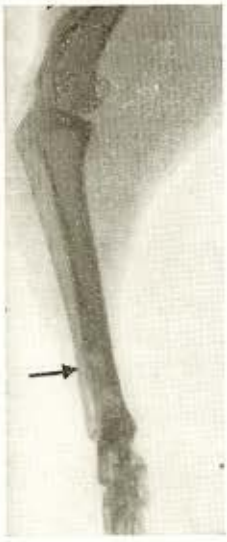

Fig. 3 — Experimental hematogenous osteomyelitis of the left tibia. The inoculation of 3×10^8 staphylococci, with 37 transfers, through the right femoral artery, was made after a previous perforation at the level of the metaphysis of the right tibia. Osteomyelitis occurred in the opposite, non-injured, tibia. The radiograph 5 months after inoculation shows osteolytic lesions in the entire bone.

The trauma was applied at the upper epiphysis of the tibia, by means of a small percutaneous bone perforation with a fine dentist drill.

6) Trauma of the left tibia and inoculation of 3×10^8 staphylococci with 43 transfers, through the right femoral artery: we observed left-sided osteomyelitis in one guinea-pig, right-sided in another, and suppurative arthritis of the right hip in the third one.

Having thus been able to obtain experimental osteomyelitis by direct contamination and by hematogenous route, we tried to ascertain the influence of some factors, besides trauma, which are quite commonly referred to as bearing on the pathogeny and evolution of the disease, such as the degree of virulence of the staphylococcus, the massive doses of infection, the influence of reinfections on an already existing osteomyelitis.

C) *Influence of the pathogenicity of the staphylococcus.*

7) Inoculation in the bone of 3×10^8 non-attenuated staphylococci, isolated from experimental osteomyelitis: caused death of all 3 guinea-pigs, despite their previous immunization by staphylococcal anatoxin.

D) *Influence of massive doses of infection.*

8) Inoculation of 6×10^8 staphylococci with 38 transfers, by two routes: half into the bone and the other half through the right femoral artery; the five animals inoculated died, in spite of previous immunization with staphylococcal anatoxin, but with a diference from the group which was not immunized, that is, when osteoarticular lesions were already present in four of the guinea-pigs, 40 days after inoculation.

9) Inoculations of three consecutive doses of 5×10^7 staphylococci each, in the left tibia, with a 7 day interval, and then another inoculation of 3×10^8 through the right femoral artery, the staphylococci having undergone 43 transfers: in one guinea-pig we observed osteomyelitis at the same side of the bone inoculation, and suppurative arthritis of the opposite hip joint (right); another animal presented osteomyelitis of the right tibia (fig. 5).

E) *Influence of reinfection on an already existing osteomyelitis.*

10) Inoculations through the femoral artery, of 3×10^8 staphylococci with 30 transfers, in 4 guinea-pigs with experimental osteomyelitis of one year duration: a re-activation of the process was observed (fig. 4).

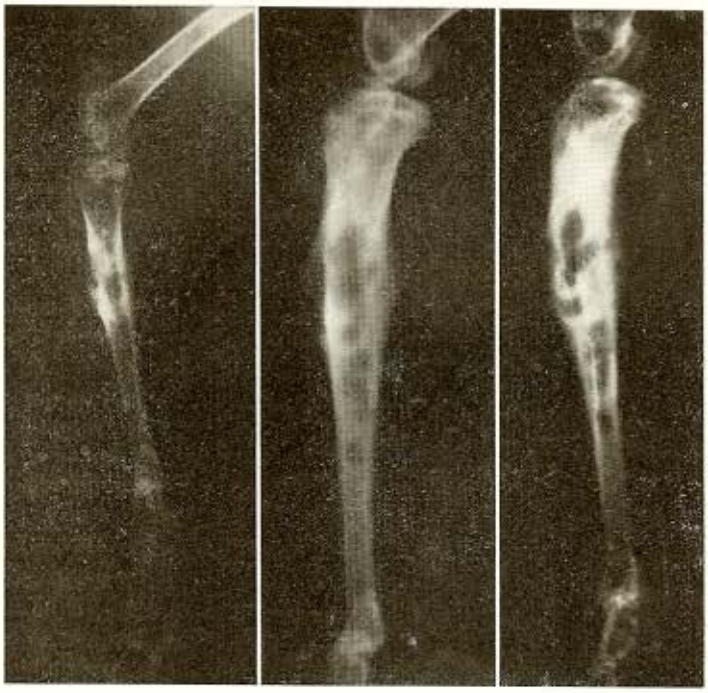

Fig. 4 — Reactivation of experimental osteomyelitis, corresponding to the case of fig. 1. Radiograph made 40 days after reinoculation through the femoral artery with 3×10^8 staphylococci with 30 transfers; *a* — control 40 days after re-inoculation; *b* — control after 9 months of re-inoculation; *c* — 15 months after reinoculation.

Clinical picture: the guinea-pigs with experimental osteomyelitis maintained a good aspect; the affected member was at first swollen, then an abscess developed and was followed by a fistula with drainage of pus, in which the staphylococcus was presente. The fistula persisted for a variable period of time and, in most cases, closed itself spontaneously. During reactivation the fistulae re-opened.

X-Rays: these showed various aspects, according to the phase of the disease. The first signs were observed on the 3rd or 4th week, sometimes even later, and were characterized by one or multiple foci of osteomyelitis, sometimes accompanied by periostal reaction. On subsequent radiographs we were able to find alterations of the form and structure of the bone, and pictures similar to those describe for human osteomyelitis: sequestrums, reabsorption zones (osteolysis), periostal osseous neo-formation (involucrum or sarcophagus), endostal neoformation causing obliteration of the medullary canal, and a greater radiographic opacity.

Radiographs taken after one year showed persistency of the lesions.

Histological examination: this showed an inflammatory process in the medullary spaces, formed by purulent exsudate around the necrotized bone fragments, as well as sequestrums (fig. 6).

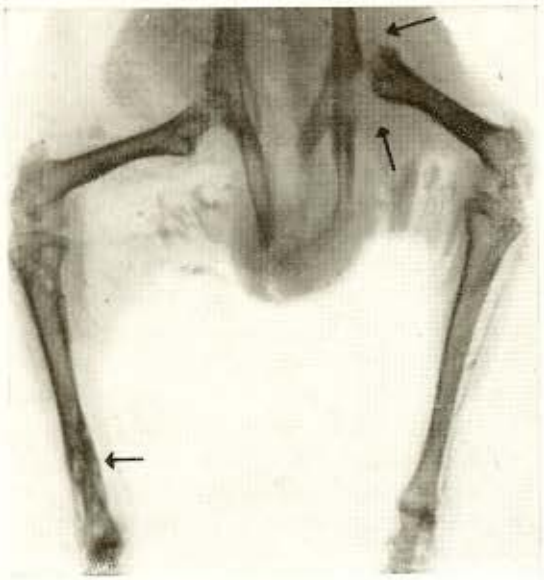

Fig. 5 — Osteomyelitis and suppurative arthritis, obtained through various routes. The inoculation of three consecutive doses of staphylococci in the left tibia, followed by the inoculation of 3×10^8 through the right femoral artery, at weekly interval, caused lesions of osteomyelitis in the left tibia, as well as suppurative arthritis in the hip joint at the opposite side.

COMMENTS AND CONCLUSIONS

In the first series of our experiments, with non-immunized guinea-pigs, conditions were maintained constant, with variations concerning only the number of inoculated microorganisms, the number of their transfers, and the inoculation routes.

In all this series, death of the animals supervened within 7 days, and we found visceral abscesses, necrotic lesions of the rectum, and liquefaction of the bone marrow. Smears of the latter positive for staphylococci.

A review of the literature on the subject shows that the AA. who tried to obtain experimental osteomyelitis were also confronted with early death of the animals. LEXER [2] tried to prolong their lives by administering small doses, and THOMPSON & DUBOS [6] immunized the animals with a previous injection of small doses of microorganisms. SHIODA [5] obtained bone abscesses in 23% of his animals, but none survived more that 96 hours. SCHEMAN & al. [4] obtained osteomyelitis in animals surviving more than one week.

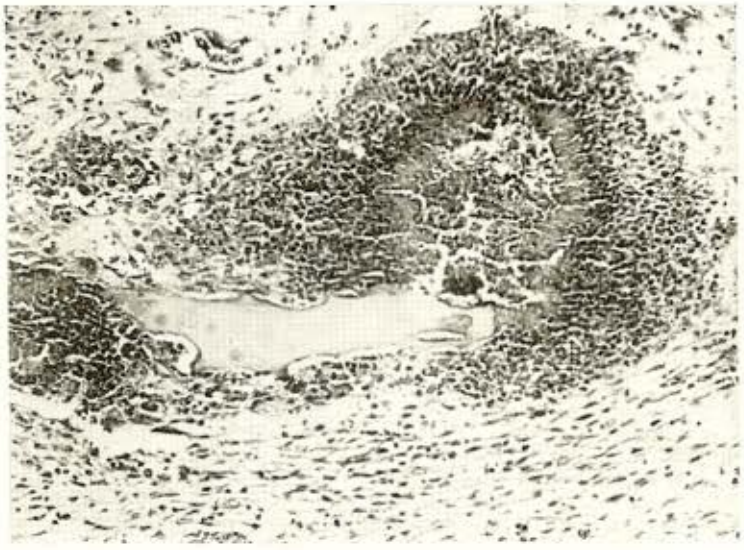

Fig. 6 — Microphotographs of experimental osteomyelitis, corresponding the case in fig. 5. The section shows a sequestrum, surrounded by a purulent exudate; there are peripheric zones of repair, made of fibrous tissue.

It thus becomes evident, from our first series of experiments and from the observations fo the above mentioned AA., that the *toxin* of the staphylococcus has a generalized action which kills the animals, sometimes even on the first or second day after inoculation.

To avoid this early death, it was not sufficient to attenuate the virulence of the staphylococcus through numerous transfers (up to 43) or to use small inocula. Only by protecting the animal through immunisation with staphylococcal anatoxin, were we able to secure their survival paradoxically creating conditions favourable to the development of the disease.

In these immunized animals we were thus able to get experimental osteomyelitis, first by direct bone inoculation of the staphylococcus, and then by the hematogenous route. The clinically evident disease was confirmed through X-rays and histological and biological methods.

We may conclude from these observations that organic defences helped by the anatoxin in our experiments, were a basic factor in protecting the organims against the general toxic action of the staphylococcus. Nevertheless, the resistance which evolves is not always sufficient to completely eliminate the action of the staphylococcus which, favoured by the intercurrence of other factors, may fix itself in a bone and give rise to a process of osteomyelitis.

Vascular thrombosis (WILENSKY [8]), arrangement of the metaphysary vessels and medullary phagocytosis (HOBO [1]), have been reported as factors which might influence fixation and evolution of the infectious bone process.

In our observations, *trauma* did not show any remarkable influence over the inception of osteomyelitis: among 3 guinea-pigs which suffered a left leg trauma after inoculation through the femoral artery, there was only one case of osteomyelitis at the site of the trauma; the two other animals developed osteomyelitis and suppurative arthritis on the opposite limb (group 6). Among another group of 8 guinea-pigs submitted to trauma after injection of staphylococci through the right femoral artery, only one developed osteomyelitis and this was at side opposite to the injured one (group 5). The little influence of trauma is also indicated by the fact that no differences in the localization of the infection were observed between the above mentioned groups and another group of 3 guinea-pigs injected in the same way but not submitted to trauma (group 9).

The restricted influence of trauma on the etiopathogeny of osteomyelitis, which we report in our experiments, has also been observed by WEAVER & TYLER [7].

Other factors, however, such as *number* and *virulence* of the microorganisms, seem to influence as indicated by the results of our last series of experiments (7, 8, 9 and 10).

Even when previously immunized with anatoxin, guinea-pigs died after inoculation of highly pathogenic non-attenuated staphylococci, isolated from a case of experimental osteomyelitis (group 7).

Also massive doses of staphylococci caused death, 40 days after inoculation, of immunized guinea-pigs, which already presented a picture of osteomyelitis (group 8). Successive reinfections favour development of osteomyelitis, as indicated by results with group 9 in our experiments.

The *contamination route* seems to be also a factor, since we have been unable to get osteomyelitis using the *sub-cutaneous* or *sub-periostal* routes (group 2). The routs which carry the staphylococci more directly to the bone, as the intra-osseous and hematogenous, appear to offer bigger probabilities of fixation of the process in the bone.

Reactivation of existing lesions, and the appearance of new ones, has been observed through inoculation of staphylococci in animals which already had experimental osteomyelitis (group 10).

The summing up of our results indicates the basic importance of the immuno-allergic condition of the organism, the number and virulence of the staphylococci and the contamination route as factors wich influence localization and development of osteomyelitis.

WERTHEIMER, L. G. & MARTIN, I. V. — Chronic experimental osteomyelitis. *Rev. Inst. Med. trop. São Paulo* **6**:142-149, 1964.

RESUMO

Osteomielite crônica experimental

A inoculação de número variável de estafilococos áureos, que sofreram repicagens em número também variável, determinou sempre a morte dos animais, qualquer que fôsse a via empregada. A necropsia revelou abscessos viscerais múltiplos e liquefação da medula óssea. Cobaias imunizadas prèviamente pela anatoxina estafilocócica sobreviveram à inoculação de estafilococos.

Apenas pelas vias hematogênicas e direta (intra-óssea) conseguiu-se o quadro da osteomielite, verificada clínica, radiológica, histológica e microbiològicamente.

O número e a virulência dos microrganismos representam fatôres de importância na instalação e evolução da osteomielite.

A inoculação de estafilococo não-atenuado, obtido de um caso de osteomielite experimental, determinou a morte do animal mesmo imunizado pela anatoxina. Também a inoculação de doses maciças de microrganismos ocasionou a morte de animais imunizados pela anatoxina, embora depois de 40 dias.

A reinoculação de doses moderadas de estafilococos atenuados em casos de osteomielite crônica experimental, já instalada, determinou o re-agravamento das lesões antigas e a origem de outras novas.

REFERENCES

1. HOBO, T. — Zur Pathogenese der akuten haematogenes Osteomyelitis, mit Berücksichtigung des Vitalfärfungslehre. *Acta Scholae med. Univ. imper. Kioto* **4**:1-30, 1921.

2. LEXER, E. — Zur experimentallen Erregung osteomyelitischer Herde. *Arch. klin. Chir.* **48**: 181, 1894.

3. RODET, N. A. — Ètude expérimentale sur l'ostéomyélite infectieuse. *Compt. rend. Soc. Biol.* **99**:569-571, 1884.

4. SCHEMAN, L.; JANOTA, M. & LEWIN, P. — The production of experimental osteomyelitis. *J. Amer. med. Assoc.* **117**:1525-1529, 1941.

5. SHIODA, J. — Osteomyelitis experimental. Acute suppurative osteomyelitis. *Arch. klin. Chir.* **185**:141-163, 1936.

6. THOMPSON, R. H. S. & DUBOS, R. J. — Osteomyelitis experimental. Production in rabbits by intravenous injection of *Staphylococcus aureus*. *J. exper. Med.* **68**:191-206, 1938.

7. WEAVER, J. B. & TYLES, M. W. — Experimental staphylococcemia and hematogenous osteomyelitis. *J. Bone & Joint Surg.* **25**:791-802, 1943.

8. WILENSKY, A. O. — The mechanism and pathogenesis of acute osteomyelitis. *Amer. J. Surg.* **3**:281-289, 1927.

Recebido para publicação em 6 abril 1964.