HOMOGRAFTS OF BONE TISSUE STERILIZED BY FORMALIN VAPOR

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In some experiments pieces of bone tissue treated with formalin vapor were transplanted into rabbits, while in others grafts prepared with full aseptic precautions were used. Bone fragments of both types were conserved by freezing. Histological investigation showed that the fate of the grafts in the recipient was the same: they were replaced by the recipient's bone tissue.

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The preparation of bone tissue under aseptic conditions is frequently an intricate procedure requiring a specially equipped room and trained staff, and for this reason the possibility of carrying out this procedure without observance of the rules of asepsis with subsequent sterilization of the bone tissue by γ-rays [4-6], β-propiolactone [2, 3, 7], and ethylene oxide [1, 8] has been studied. However, sterilization of tissues by these methods is not always possible. The object of this investigation was to study the antibacterial action of formalin vapor, to determine whether this can be used to sterilize bone tissue, and to study the fate of bone homografts prepared in this way.

EXPERIMENTAL METHOD

The antibacterial properties of formalin vapor were studied in three series of experiments in which fragments of rabbit bone tissue were infected with a bacterial flora.*

In series I (32 experiments) bone fragments were infected with a suspension of pure cultures of microorganisms (Staphylococcus, Bacillus pyocyaneus, Escherichia coli, and spore-bearing bacteria), in physiological saline. The concentration of bacterial cells in the suspension was 500,000/ml. Exposure of the bone fragments to the bacterial suspension lasted 5 min. In series II (6 experiments) infection was carried out with a suspension of a combination of bacterial cultures in physiological saline. The concentration of each species (Staphylococcus, B. pyocyaneus, Enterococcus) was 500,000 bacterial cells/ml suspension. Exposure of the bone fragments to the bacterial suspension lasted 30 min. In series III (50 experiments) during preparation of the bone tissue the fragments were infected with a random microflora without observance of the rules of asepsis.

The infected bone fragments were placed in an airtight glass vessel and treated with formalin vapor at 20-23°. After treatment with formalin vapor (for between 15 min and 24 h) they were transferred to tubes of meat-peptone broth and incubated at 37° for 10 days. Infected bone fragments untreated with formalin vapor acted as control. If growth occurred in the tubes films were made, stained by Gram's method, and examined under the microscope to identify the microorganisms.

To study the fate of bone homografts sterilized by formalin vapor, standard fragments of bone tissue measuring 12-13 mm were prepared without observance of the rules of asepsis, and treated with formalin vapor for 60 min at 20-23°, after which they were frozen at between −25 and −30°. The fragments were homografted into full-thickness transperiosteal defects in the radii of rabbits (15 experiments). Burn homografts obtained under sterile conditions and conserved by freezing in the same way but without treatment with formalin vapor (10 experiments) were used as the control. The animals were sacrificed two

*The antibacterial properties of formalin vapor were investigated in the microbiological laboratory (Head, V. M. Mel'nikova) of this Institute.

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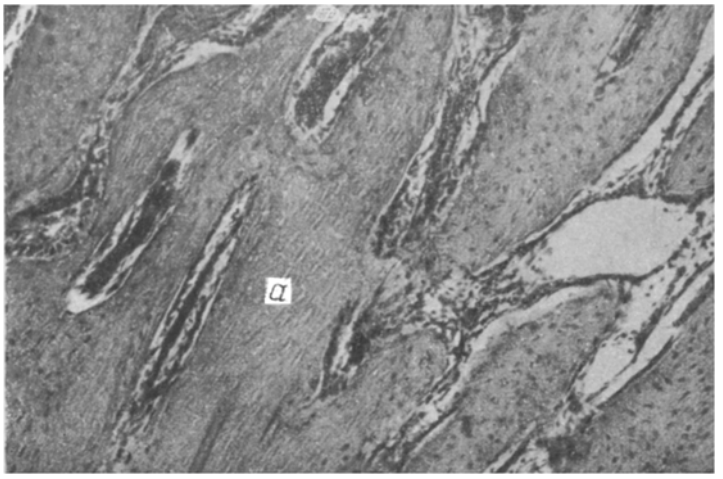

Fig. 1. Fragment of bone homograft sterilized with formalin vapor (a). Three months after transplantation. Hematoxylin-eosin, 105 ×.

TABLE 1. Bactericidal Action of Formalin Vapor

Species of microorganism	No. of expts.	Results of expts.		Control
		Exposure (in min)		
		30	60	
Staphylococcus	6	−	−	+
B. pyocyaneus	10	+	−	+
E. coli	8	−	−	+
Spore-bearing bacteria	8	+	−	+

Legend: −growth absent, + growth present.

weeks and 3, 4, 6, and 7 months after homografting. Tissues from the region of homografting were treated by the standard histological method. Celloidin blocks were cut longitudinally. Sections 10-12 μ in thickness were stained with hematoxylin-eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

As Table 1 shows, after exposure of the bone tissue to formalin vapor for 30 min, no growth of Staphylococcus or E. coli was obtained. After exposure for 60 min, no growth of microflora was observed in any experiment. Growth of the corresponding microorganisms was found in the control.

In the experiments of series II, after sterilization for 30 min of bone tissue fragments preliminarily infected by a mixed culture of microorganisms, no growth was obtained in 5 of the 6 tubes. In one tube weak growth of a Gram-positive bacillus was obtained. Growth of the microflora in all tubes was observed in the control.

In series III, each group (10 fragments) of the 50 fragments of bone tissue prepared without observance of aseptic principles was exposed to formalin vapor for a different time (15 or 30 min, 1, 3, and 24 h). In no case was growth of microorganisms obtained. Growth of the microflora occurred in the control in all tubes.

These results show that formalin vapor has a marked antibacterial action. If infected bone tissue was kept in formalin vapor at 20-23° for 1 h, no growth of microflora was observed in any tube.

Clinical observations showed that in every case of homografting of bone tissue sterilized by formalin vapor, healing took place by first intention. Histological investigation of bone homografts sterilized by formalin vapor showed that they were replaced by young bone tissue in the same way as untreated bone homografts. Two weeks after transplantation, grafts sterilized with formalin vapor were intimately joined to the connective tissue bed. Young bone tissue was formed on the surface of the grafts. Three months later the grafts were largely replaced by newly formed bone tissue (Fig. 1).

At this period the grafts consisted of isolated fragments, free from osteocytes, lying among newly formed bone tissue. In the region of grafting of the bone tissue, a partially formed medullary canal could be seen, containing fatty bone marrow and granulation tissue. Areas of the grafts in contact with regenerating bone were largely replaced three months after transplantation, the vascular canals were considerably

widened, and young bone tissue was formed along their walls. Four months after transplantation the grafts consisted of small, separate fragments, free from osteocytes, surrounded by regenerating bone tissue. The medullary canal in the region of grafting contained loose connective tissue, silitary trabeculae of newly formed bone tissue, and granulation tissue. Six months after grafting, formation of the medullary canal continued to progress. The graft in the specimens examined consisted of tiny fragments, free from osteocytes, surrounded by traveculae of newly formed bone tissue. The medullary canals in the region of the graft contained loose connective tissue and fatty bone marrow. After 7 months, solitary, small fragments of graft, free from osteocytes, still occurred among the recipient's bone tissue, which had completely replaced the graft.

Comparison of the histological specimens revealed no significant differences between bone grafts sterilized with formalin vapor and the controls (prepared under sterile conditions); after 2 weeks the grafts of both types were joined with the bed of loose connective tissue and young bone tissue had formed on their surface. After 3 months both types of graft were largely replaced by the recipient's bone tissue. In the later stages substitution of the grafts, both experimental and control, was complete.

Sterilization of bone tissue with formalin vapor after its preparation without observance of the rules of asepsis thus provides a method of obtaining bone tissue which is not inferior in quality to that prepared under aseptic conditions. Sterilization of bone tissue with formalin vapor is economical, it can be carried out anywhere, and it enables bone tissue to be prepared on a large scale without observance of the rules of asepsis.

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