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The Effect of Polymethylmethacrylate and Antibiotic Combinations on Bacterial Viability

AN *IN VITRO* AND PRELIMINARY *IN VIVO* STUDY*

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ABSTRACT: **Polymethylmethacrylate itself has a slight, long-term bacteriostatic effect on the growth of staphylococci, but while polymerizing the heat of polymerization and possibly the elaboration of monomer can be bactericidal to many pathogenic organisms. Twelve antibiotics incorporated into polymethylmethacrylate cement retained their antibacterial activity for long periods in dry storage, but the antibiotics leached out of polymethylmethacrylate quite rapidly *in vitro* and *in vivo*. No antibiotic could be found after thirty-seven days in treated cement pellets placed in a rabbit thigh.**

Failure of total joint replacement is often due to infection, or to loosening that may in turn be due to occult infection. In the great majority of total joint replacements polymethylmethacrylate is employed as a filler to provide mechanical fixation of the prosthetic components to bone.

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Early authors were concerned about the sterility of the methacrylate powder and liquid monomer. Most authors thought that the powder required sterilization [5,10,13]. It has been shown that the liquid monomer sterilizes cultures of *Staphylococcus aureus*, *Escherichia coli*, beta-hemolytic streptococci, and *Staphylococcus pyogenes* within twenty minutes [5,12,13], but Robinson and Macalister found that this was not necessarily true. *Bacillus subtilis* can survive in monomer up to twenty-four hours and *Bacillus cereus*, up to fourteen days [5,12]. For these reasons it has been recommended that only presterilized materials be used [13]. A review of the literature revealed no studies of the long-term effects of solidified polymethylmethacrylate on the viability of micro-organisms.

Dutton noted the ease with which the dough mix of polymerizing cement could be contaminated, but Charnley was unable to culture organisms from the cement he used and believed that bacteria in the acrylic cement are not responsible for late infections in total hip arthroplasty.

Polymethylmethacrylate polymerizes with an exothermic reaction, and at the bone-cement interface in

total hip replacements [8] the temperature approaches 70 degrees centigrade. We found that in the center of a sixty-gram bolus temperatures as high as 97 degrees centigrade are reached, and monomer is also elaborated. The separate effects of the monomer and high temperature on bacterial viability have not been assessed.

Polymethylmethacrylate has been shown to be a network connecting microscopic spherules of polymer and it is reasonable to expect that *in vivo* body fluids will penetrate the network, and that substances contained in the interstices will escape into these body fluids. Köning found that monomer is released over a prolonged period of up to fifty-eight weeks.

Buchholz [2,3] and Buchholz and Engelbrecht were the first to recognize that sustained slow release of antibiotics from polymethylmethacrylate might significantly lower the infection rate in total joint-replacement operations. They found that tetracycline added to the cement powder was destroyed during polymerization, but that there was sustained release of penicillin G, erythromycin heptogluconate, and gentamicin sulphate from polymethylmethacrylate for periods of up to six months. They then used two grams of gentamicin per batch of cement in total hip-replacement arthroplasty procedures. Their attempts to recover gentamicin sulphate from the urine of these patients were unsuccessful. From this they concluded that the antibiotic is not released into the systemic circulation in significant amounts. In 1,138 total hip-replacement procedures done without antibiotics mixed in the cement the deep infection rate was 1.2 per cent, whereas in a subsequent series of 1,115 cases with erythromycin and gentamicin mixed into the cement the infection rate was 0.09 per cent. Mixing antibiotics into the cement reduced its resistance to fracture by 10 to 15 per cent.

We are reporting here our investigations undertaken to study the antibacterial effect of cement* alone and in combination with various antibiotics. Concomitant with the laboratory studies, we determined that in forty consecutive total hip-replacement procedures cultures of samples of the liquid monomer, powder polymer, and polymerized product were sterile. Our four experiments were as follows:

1. The effects of cooled, solidified cement pellets on the growth of twelve different strains of bacteria were studied.

2. An *in vitro* study was made of the effects of several antibiotics on a group of bacterial species.

3. The effect of the polymerization of methylmethacrylate on bacterial viability was measured.

4. An *in vitro* study in saline and an *in vivo* study in rabbits were made to assess the rates of release of gentamicin and clindamycin from polymethylmethacrylate.

* Surgical Simplex P Radiopaque Bone Cement (North Hill Plastics. Ltd., 49 Grayling Road, London N.16, England) was supplied through the courtesy of Howmedica, Inc. It has a liquid component consisting of 97.4 per cent methylmethacrylate monomer, 2.6 per cent N, N-dimethyl-p-toluidine, and seventy-five (plus or minus fifteen) parts per million of hydroquinone. The powder component consists of 75 per cent methylmethacrylate-styrene copolymer, 15 per cent polymethylmethacrylate, and 10 per cent barium sulphate, U.S.P.

Experimental Method

In our first experiment, testing the effects of cement on bacterial growth, standard-sized pellets were produced by mixing commercially available packets of cement under sterile conditions and then placing the liquid mixture into an aluminum mold, producing forty-nine slightly conical pellets per batch, the average weight of each being 0.19 gram (± 15 per cent). Using a technique similar to that described by Bauer and associates, three cool, freshly prepared pellets were placed base down on each of twelve or more Mueller-Hinton plates inoculated with standardized cultures of one of the following organisms: penicillin-resistant *Staphylococcus aureus*, penicillin-sensitive *Staphylococcus aureus*, penicillin-resistant *Staphylococcus epidermidis*, penicillin-sensitive *Staphylococcus epidermidis*, Streptococcus group D (enterococci), alpha-hemolytic Streptococcus not group D, beta-hemolytic Streptococcus group A, Enterobacter group, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Serratia marcescens*. After incubation for twenty-four hours at 37 degrees centigrade, the zones of inhibition of bacterial growth were measured.

In our second experiment, testing antibiotics in the cement, pellets containing antibiotics were prepared. We used powders of all the antibiotics except lincomycin hydrochloride, which was available to us only dissolved in a syrup of benzyl alcohol and water.

The most homogeneous distribution of antibiotic in the cement was achieved by adding the antibiotic powder to the liquid monomer and mixing it well. The dough was manually mixed with a tongue blade and then poured into the mold, where polymerization occurred. The amount of antibiotic employed for each batch of cement was the amount available in the standard commercial package of antibiotic in powder form for injection, for example five grams of cephalothin per sixty-gram batch of cement. The control pellets and the pellets containing the antibiotic were placed on the culture plates as soon as they were cool. In addition, pellets were tested at two hours, six hours, twenty-four hours, and then at approximately ten-day intervals up to 112 days after preparation. The pellets were stored at 37 degrees centigrade in dry sterile Petri dishes.

In our third experiment, testing the effects of polymerization on the bacteria, we made cups of cement by forming the dough about a round-ended metal cylinder. Each cup was five millimeters thick and had at least a one-milliliter capacity, and a standard-sized inoculum of 10,000 organisms per milliliter of *Escherichia coli*, penicillin-resistant *Staphylococcus aureus*, or penicillin-resistant *Staphylococcus epidermidis* was added while the dough was still soft, that is, just prior to the onset of polymerization. We used three cups per organism. Glass tubes were used as controls. After the inocula were placed

in the cups, samples were recovered at thirty minutes and at one, two, and three hours and submitted to a colony count. The study was repeated using two-hour-old cups, which had cooled to the ambient temperature, and two-day-old cups.

In the fourth experiment, to determine whether antibiotics remained active after being incorporated into cement and to measure their ability to be leached out of the cement, we used pellets made as described previously and tested them against these organisms: penicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Staphylococcus epidermidis*, and *Escherichia coli*. Each of the following antibiotics was tested as soon as the pellets cooled and after twenty days of dry storage: potassium penicillin G (five million units per sixty-gram batch of cement), sodium methicillin (four grams), erythromycin gluceptate (one gram), lincomycin hydrochloride (600 milligrams), and sodium nafcillin (500 milligrams). Pellets with polymyxin B sulphate (500,000 units) or sodium colistimethate (150 milligrams) were tested only after the initial cooling.

Cephalothin pellets were tested immediately after preparation, at twenty-four hours, and at 112 days of dry storage by adding one, two, or three pellets to broth tubes containing a standard inoculum, as against control pellets without antibiotic in the broth inoculum. The tubes were incubated at 37 degrees centigrade for twenty-four hours and then examined for inhibition of growth. The broth of those showing inhibition was transferred to blood agar plates to assess the bactericidal effect. The cultures were saved to watch for delayed inhibition of bacterial growth. A similar broth study was carried out with gentamicin sulphate at dose levels of ten, fifty, 200, and 500 milligrams per batch. These pellets were tested immediately and after twenty-four hours of dry storage.

The leaching out of antibiotics from cement was studied employing gentamicin sulphate and kanamycin sulphate, one gram per batch. Each pellet was placed in a tube containing ten milliliters of normal saline and was incubated at 37 degrees centigrade. The saline was drained off daily and an aliquot of the saline was analyzed for antibiotic activity [1]. Three tablets of each type were similarly assayed each day for antibiotic activity [1] until the supply of pellets was exhausted or no inhibition of bacterial growth was found.

For comparison, pellets from the same batch stored under dry conditions were assayed. A similar study was carried out on clindamycin at one gram and two grams per batch. Analysis of the saline aliquot was carried out by the *Bacillus globigii* method.

The release of gentamicin sulphate and clindamycin sulphate from cement, as implanted in rabbits, was done on female New Zealand white rabbits weighing approximately four kilograms each. All surgery was performed under standard operating room conditions and with sterile technique. General anesthesia with ketamine hydrochloride, methoxyfluorine, and oxygen was employed. One rabbit was killed and its serum was used to set up antibiotic standards. The medullary canal of one femur in each of four rabbits was filled with cement through a window in the cortex. In two animals one gram of gentamicin sulphate was added to each sixty-gram batch of cement while in the other two cement without antibiotic was implanted. Blood specimens were drawn and muscle adjacent to and remote from the cement was removed prior to insertion of the cement, immediately after insertion, after the cement had solidified (approximately ten minutes after instillation), and at four hours and twenty-four hours after solidification. These specimens were assayed for gentamicin activity using the *Bacillus globigii* method. We homogenized the tissue samples in 0.5 milliliter of sterile normal saline and ran the control blood and tissue samples with and without penicillinase added to the plates. Some of the serum and tissue samples were run after we had stored them for several days at -20 degrees centigrade. Gentamicin sulphate has been shown to be stable under these storage conditions. The cement in each of the two experimental rabbits contained 0.080 gram of gentamicin sulphate.

Fresh pellets prepared as in the initial experiments were inserted into the quadriceps muscle of four additional rabbits. Serum and tissue samples were taken at ten minutes and at twenty-four hours after pellet placement. Each pellet in this experimental group contained 0.024 gram of gentamicin sulphate. To ascertain the activity level of gentamicin sulphate in the pellets, a series of pellets was incubated in normal saline at 37 degrees centigrade and assayed in parallel with the serum and tissue samples from the rabbits.

At eight and at thirty-seven days after placing the pellets in the rabbits, two pellets from each rabbit in the series were surgically removed and incubated in ten milliliters of normal saline at 37 degrees centigrade for twenty-four hours. The supernatant fluid was then assayed for gentamicin sulphate. Pellets kept in normal saline, which was changed daily according to the protocol previously described, were also analyzed.

Our *in vitro* studies of gentamicin sulphate to be described showed it to have a relatively short half-life in cement. An *in vivo* study was therefore done with clindamycin sulphate. The protocol followed was the same as for the study of the two rabbits with gentamicin sulphate, except that only at twenty days after the operation were the four pellets in the two rabbits removed and assayed for clindamycin.

To be certain that no factor was operative in the rabbit that would inactivate the antibiotics and result in no detection by the *Bacillus globigii* microassay, in two rabbits one gram of clindamycin powder was placed directly into the soft tissues of the thigh. Serum samples alone were taken from these rabbits immediately prior to placing the powder in the thigh and then at intervals of one, four, and twenty-four hours after placement. These were submitted to the *Bacillus globigii* microassay.

Results

Effect of Cement on Bacterial Growth

Cool pellets had no effect on the growth of Enterobacter, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens,* alpha Streptococcus not group D, and Streptococcus group D (Enterococcus), and none on beta Streptococcus group A with the exception of the test run at forty days, when a 0.5-millimeter ring of inhibition of growth about the pellets was noted. Slight inhibition of growth of penicillin-resistant *Staphylococcus aureus* was noted up to forty days. The inhibition zone averaged 1.5 millimeters beyond the circumference of the pellet. Penicillin-sensitive *Staphylococcus aureus* showed spotty inhibition of the same magnitude for up to forty days. Penicillin-resistant *Staphylococcus epidermidis* showed slight inhibition of growth (one-millimeter zone beyond the pellet) immediately after preparation and at the forty, fifty, and sixty-day intervals. Penicillin-sensitive *Staphylococcus epidermidis* showed a similar slight inhibition for up to seventy days except at the thirty-minute and twenty-four-hour intervals, after which there was no effect. The factor in the cement responsible for the slight inhibition of growth of the several organisms was not identified.

Effect of the Polymerization of Cement on Bacterial Viability

To measure this effect the kill rate was determined: that is, the average number of viable organisms in the polymethylmethacrylate cups, subtracted from the number of viable organisms in the control glass tubes, divided by the number of viable organisms in the glass tubes. At three hours the kill rate was 57 per cent of *Escherichia coli,* 60 per cent of *Staphylococcus aureus,* and 48 per cent of *Staphylococcus epidermidis.* The inhibition was most effective against *Staphylococcus aureus.* Over the next three hours, the organisms were noted to proliferate in both the polymethylmethacrylate cups and the glass control tubes; however, there was continued inhibition of growth in the polymethylmethacrylate cups.

In the similar study carried out on two-hour-old polymethylmethacrylate cups, there was a kill rate after thirty minutes of 26 per cent of *Escherichia coli,* 17 per cent of *Staphylococcus aureus,* and 25 per cent of *Staphylococcus epidermidis.* At three hours the kill rates were 12 per cent, 18 per cent, and 12 per cent, respectively, with *Staphylococcus aureus* remaining most susceptible. In the two-day-old cups, at thirty minutes only a 7 per cent kill rate was encountered with *Escherichia coli*; there was no initial kill of *Staphylococcus aureus* and *Staphylococcus epidermidis.* At three hours there were kill rates of 8 per cent, 11 per cent, and 20 per cent, respectively.

Effect of Antibiotic-Cement Mixtures on Bacterial Growth

Cephalothin-polymethylmethacrylate pellets showed strong inhibition of the growth of all the test organisms except *Serratia marcescens,* which was completely resistant. The largest zones of inhibition were seen in the penicillin-sensitive strains of *Staphylococcus aureus* (thirty-seven to forty-six millimeters) and *Staphylococcus epidermidis* (forty-one to fifty millimeters), and in alpha Streptococcus (thirty-eight to fifty-nine millimeters). Beta Streptococcus group A was also strongly inhibited (thirty-five to forty-two millimeters). All the other organisms showed approximately 50 per cent of the inhibition of the groups previously mentioned. Under dry sterile storage at 37 degrees centigrade no diminution in the inhibitory effect of the pellets was noted with time. Even at 112 days after manufacture, 100 per cent inhibition of broth cultures of *Escherichia coli,* penicillin-resistant *Staphylococcus aureus,* and penicillin-resistant *Staphylococcus epidermidis* was noted.

The other antibiotics studied were tested only against *Escherichia coli,* penicillin-resistant *Staphylococcus aureus,* and penicillin-resistant *Staphylococcus epidermidis.* Immediately after solidification all of the antibiotic-containing pellets showed substantial antibacterial activity against the organisms within their usual spectra. The weakest activity was in colistimethate, polymyxin B, nafcillin, and lincomycin. The strongest activity was seen in penicillin G and methicillin, which were both significantly active against all three organisms. Erythromycin showed an intermediate range of effectiveness. At twenty days after preparation penicillin, methicillin, and erythromycin were unchanged in potency. Lincomycin and nafcillin showed complete loss of their antibacterial activity. Polymyxin B and colistimethate were not tested at twenty days.

Broth culture studies of pellets containing cephalothin stored dry up to 112 days after preparation showed 100 per cent inhibition of bacterial growth in all three test organisms. Control pellets showed no inhibition of growth.

Similar broth studies carried out on gentamicin sulphate pellets up to twenty-four hours after preparation showed 100 per cent inhibition of bacterial growth in all three test organisms. The associated controls showed no inhibition. In those tubes observed for delayed bacterial growth at the ten-milligram level employing one disc in the broth, some growth of *Staphylococcus aureus* was encountered.

The *in vitro* leaching study of pellets containing gentamicin or kanamycin showed that the pellets continued to have significant levels of inhibition of bacterial growth. Over the eight-day testing period the gentamicin pellets had a twenty-four to twenty-five-millimeter zone of inhibition against *Escherichia coli,* from twenty-four millimeters decreasing to twenty-two millimeters against *Staphylococcus aureus,* and twenty-seven to twenty-nine millimeters against *Staphylococcus epidermidis.* The kanamycin pellets were tested up to three days and had zones of twenty-one to twenty-two millimeters against

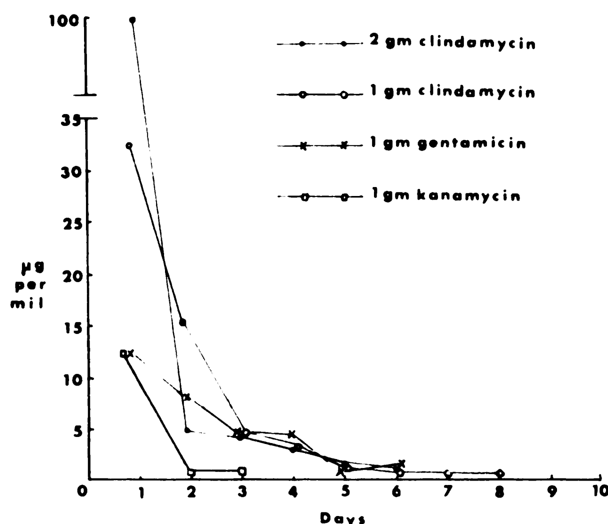

FIG. 1

Leaching of clindamycin, gentamicin, and kanamycin from polymethylmethacrylate pellets incubated in normal saline at 37 degrees centigrade. Each pellet of cement, containing one or two grams of clindamycin, one gram of gentamicin, or one gram of kanamycin per sixty-gram batch of cement, was incubated in ten milliliters of normal saline at 37 degrees centigrade. Every twenty-four hours the saline was exchanged for fresh saline and the concentration of antibiotic was determined by *Bacillus globigii* assay. Each point on the graph represents the average of three pellets. Control pellets of plain cement showed no activity. Note the rapid leaching rate within the first forty-eight hours.

Escherichia coli, eighteen to twenty-one millimeters against *Staphylococcus aureus*, and thirteen to fourteen millimeters against *Staphylococcus epidermidis*. At the same time a rather rapid diminution in the antibiotic level in the supernatant saline occurred (Fig. 1). During the first twenty-four hours 12.5 micrograms per milliliter of each antibiotic leached out. There was a 46 per cent decrease in the activity of the gentamicin in the saline by the second day and a 100 per cent loss of inhibitory activity in the saline of kanamycin. Thereafter the level of gentamicin in the saline decreased slowly until no inhibitory activity was present by seven days.

A similar study was carried out on clindamycin at dose levels of one and two grams per sixty-gram batch of cement. In this portion of the study, the antibiotic level in the saline alone was analyzed. This study was terminated at eight days after manufacture of the pellets, since there was rapid leaching of antibiotic into the saline during the first forty-eight hours and the level of antibiotic in the saline thereafter rapidly diminished such that the limits of measurability by the micromethod employed in this study were reached (Fig. 1). By calculation, nearly 100 per cent of the clindamycin in the pellets was leached out within the eight-day period.

Release of Gentamicin Sulphate and Clindamycin Sulphate from Cement Implanted in Rabbits

In the two rabbits with cement containing one gram of gentamicin sulphate per batch placed in the intramedullary canal of the femur, no gentamicin was detectable in any of the tissue or serum samples.

In the two animals in which pellets of cement containing two grams of gentamicin sulphate per batch were inserted in the soft tissues, no antibiotics could be detected in either the tissue or serum samples. A parallel series of pellets incubated in saline showed zones of inhibition an average of fifteen millimeters in diameter. This is equivalent to approximately twenty micrograms per milliliter of gentamicin and represents the twenty-four-hour accumulation of gentamicin in a ten-milliliter aliquot of saline. Residual gentamicin sulphate remained in the pellets taken from the rabbits at eight days, with 50 per cent more activity in the pellets incubated in the rabbits than in those incubated in saline. At thirty-seven days no detectable antibiotic remained in the pellets in either group. In a second run of this *in vivo* study using two grams of clindamycin sulphate per batch of cement, serum and tissue specimens again showed no antibiotic activity. Assay of the pellets from the rabbits twenty days after implantation showed the average amount of clindamycin in the aliquot of saline to be 8.9 micrograms per milliliter.

In the two rabbits in which one gram of clindamycin powder was placed directly into the soft tissues of the thigh, at one hour an average of twenty-five micrograms of clindamycin per milliliter of serum was found. Four hours after implantation 19.5 micrograms per milliliter was present. At twenty-four hours, this level had diminished to 4.4 micrograms per milliliter.

Discussion

Our pellet study indicates that polymethylmethacrylate itself has a slight long-term inhibitory effect on the growth of staphylococci. The polymerization of the cement in an *in vitro* situation is capable of killing up to 66 per cent of the organisms in an inoculum in contact with its surface. This is probably due to the heat of polymerization, but the elaboration of the monomer may also play a significant role. It is reasonable to assume that the long-term bacteriostatic effect of the pellets against staphylococci is due to leaching of monomer.

In clinical use many of the effects of polymethylmethacrylate itself on micro-organisms on its surface are attributable to circumstances not under consideration in this paper. Thus, it has been shown by Charnley and others that after implantation of cement there is a small zone of necrosis adjacent to the cement, probably due either to the heat of polymerization of the cement or to the toxic effect of monomer. This non-viable tissue may predispose to the proliferation of micro-organisms that might be deposited in or about this zone. A second circumstance is the production of a dead space on the irregular surface of the cement. In the present study we have shown that the great majority of micro-organisms deposited on the surface of cement at the time of polymerization are killed. Additionally, a bacteriostatic effect was shown to persist for at least seventy days in the case of such commonly encountered organisms as staphylococci, and this may pertain to micro-organisms deposited hematogenously at the cement-tissue interface.

This study has shown that all twelve of the antibiotics studied maintained their bactericidal activity for long periods after incorporation into cement prior to polymerization. The design of this study did not permit the assessment of the rate of loss of potency of the antibiotics after incorporation into cement. It appeared, however, that with the possible exception of colistimethate and polymyxin B, a clinically effective level of potency was maintained for several weeks.

When incubated in saline, rapid leaching of the antibiotics from the cement occurs such that up to 80 per cent may be removed in the first forty-eight hours, and by eight days no antibiotics could be detected in saline by the methods employed. Some residual antibacterial activity, however, remained in the pellets exposed to daily change of the saline leaching solution, and also *in vivo* after eight days, probably due to retained antibiotics. These antibiotics are probably loosely trapped by the polymethylmethacrylate and may continue to leach out slowly in minuscule amounts (they are not detectable in the saline nor in the tissues adjacent to the implanted cement). The leaching phenomenon *in vivo*, under the conditions of this study, suggests that antibiotic may be released at levels not detectable by the methods we employed. Alternatively, perhaps extremely rapid release of the antibiotic occurred and the timing we used for testing did not permit its detection. The detection of residual antibiotic activity in pellets which had been in rabbits for eight days would suggest that the first explanation is the more likely one. By thirty-seven days, however, all the antibiotics had disappeared both *in vitro* and *in vivo*.

These findings are consistent with those of Buchholz and Engelbrecht; they could not detect any antibiotic in the serum of patients after incorporating one gram of gentamicin into each batch of cement used in total hip arthroplasty. The assay method that we employed could detect as little as one microgram per milliliter of aminoglycosides. Therefore, it is likely that the serum level in the rabbit at any given time is less than one microgram at dose levels below two grams of aminoglycoside per sixty-gram batch of cement.

Our study showed that many antibiotics can be incorporated into cement without losing their antibacterial activity, but that leaching out of the antibiotics occurs rather rapidly. It should be emphasized that our results may support the thesis that antibiotics incorporated into the cement may be antibacterial, but only over a very short period of time, at most two to four weeks.

Incorporation of antibiotics into the cement during total joint replacement has a number of additional theoretical advantages and disadvantages. Although we found no prolonged systemic release of antibiotics *in vivo*, very low levels of release could have occurred. Systemic infection with non-sensitive organisms could then be a problem. If an allergic reaction to the antibiotic were to occur in the postoperative period, the consequences could be disastrous. The necessary immediate removal of *all* of the offending agents may not be possible, and medical treatment of the allergic reaction might be difficult indeed. Moreover, if a long-term low-level systemic release of antibiotics induced delayed sensitivity, this might also be disastrous.

At the present time the inclusion of antibiotics into cement as a prophylaxis against or treatment for infection is still an experimental procedure because its efficacy remains to be proved. The incorporation of antibiotics into the cement may entail such risks as heightened chances of superinfection or allergic reactions, or mechanical failure of the cement.

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