Introduction

The chemotherapy of bone infections is widely influenced by our information on the degree of penetration of an antibiotic into bone. But antibiotic levels in bone are often estimated without taking into account the amount of retained blood in the tissue under examination. The usually high variability of these results makes them questionable. If, in addition, the terms "chemotherapeutic concentration in bone" and "chemotherapeutic concentration in bone tissue" are used as synonyms, the interpretation of the measured antibiotic levels is not necessarily relevant.

Bone tissue is only the supporting substance (stroma) of the bone; the "antibiotic level in bone" means the amount of drug in the extravascular spaces of the skeletal system. According to these definitions we investigated the penetration of gentamicin into bone. Gentamicin was chosen for study first, because gram-negative bacteria also contribute in an increasing degree to bone infections.

Material and Method

We used bone specimens from patients operated on for arthroplasty of the hip. Prior to surgery the hemoglobin content in blood was determined. With initiation of anaesthesia a control sample of blood was taken and immediately thereafter 80 mg of gentamicin were administered intravenously. During surgery three samples of blood were taken simultaneously with three samples of bone:

1. the head of the femur,
2. cylindrical pieces of the acetabulum,
3. marrow from the femur shaft.

The control serum was tested for antibacterial activity by the agar-diffusion method on the same day. The three serum samples obtained during

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surgery were kept overnight at 4° C together with a gentamicin standard in a human serum pool. The bone specimens were freed of periosteum and soft tissue. Each sample was then separated into compact tissue, yellow marrow and red marrow. The marrow samples were easily homogenised in a two-fold volume (weight = volume) of phosphate buffer (pH 7.8) in a Bühler apparatus. By this procedure the gentamicin was extracted quantitatively.

All efforts to pulverise the compact tissue in grinders were unsuccessful, even after deep freezing of the bone fragments in liquid nitrogen. The best device to use was a simple mortar made of steel according to our specifications (Figure 1). By a few blows upon the steel pestle small pieces of compact bone tissue were pulverised without loosing or overheating the material. Afterwards the bone powder was suspended in an equal volume of phosphate buffer solution (pH 7.8) using a Whirlmix. To ensure the complete extraction of the gentamicin from the bone particles the homogenised samples were stored as a suspension overnight at 4° C. Similarly, a gentamicin standard in extraction fluid from hipbone and femur of untreated patients was kept overnight at 4° C. After centrifugation on the following day, the gentamicin activity of the extraction fluids and the serum samples was estimated in the agar-diffusion test according to Naumann (2).

By the homogenisation and extraction process of the bone fragments all retained blood in the bone vessels and sinuses of the marrow was released and the erythrocytes were lysed. The amount of blood in each sample was estimated by determining the hemoglobin concentration in the extraction fluid by the hemoglobin-cyanide method, using a spectrophotometer. For this procedure the extraction fluid had to be centrifuged again 8,000—12,000 x g. The extinctions were observed at wavelengths of 540 nm and 680 nm. The hemoglobin concentrations were calculated from the differences between these two values. All samples were tested twice.

**Results**

Figure 2 shows the antibiotic concentrations in the patients' sera during surgery. From 30 to 100 minutes after gentamicin injection the measurements within each ten-minute interval are recorded as one group. The average of each group is plotted as a circle in the middle of each time interval. The broken line connecting the first and the last of these averaged antibiotic levels demonstrates the approximate gentamicin concentration in the serum in relation to time. This curve is similar to that observed in healthy subjects (1, 3, 5), i.e. blood loss and infusions during the first 1.5 to 2 hours of surgery did not significantly influence the pharmacokinetics of gentamicin. Thus, during this time diffusion conditions from blood to bone tissue corresponded to the normal therapeutic situation.

In Table 1 all gentamicin concentrations in serum and bone specimens are summarized. The data from the head of the femur are used as representative values. They are shown in Figure 3 together with the gentamicin levels in the simultaneously removed serum samples. About 50 minutes after i.v. injection of 80 mg gentamicin the mean serum concentration was 4.25 mcg/ml but the compact bone tissue had practically no measurable activity. Similarly, in the compact tissue of the acetabulum only once did the gentamicin activity reach the minimal detectable quantity of 0.25 mcg/g of bone. In the yellow marrow of the femur head a mean activity of 0.36 mcg/g of tissue was measured. In contrast, the level in the red marrow was 0.82 mcg/g of tis-