A QUANTITATIVE DETERMINATION IN THE FROG OF CERTAIN MICROELEMENTS IN THE REGENERATED TISSUE DURING HEALING OF A FRACTURED FEMUR

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New physicochemical methods (microchemistry, spectral analysis, electron microscopy, etc.) have enabled us to extend our knowledge of the body's content of micro- and ultramicroelements. They are present in extremely small amounts from 10^{-3} to 10^{-12} g, and their accumulation is known to depend not only on their physicochemical nature, but on the metabolic processes. These elements include Cu, Fe, Mn, Al, B, Be, Co, Mo, etc.

Although a great deal of experimental work [1-5] has been done on the content of microelements of human and animal tissues and on their physiological function, at present little is known about the way these elements accumulate in regenerating bone.

There have been no reports of changes in the chemical composition of the minerals containing the microelements in the bone which develops after a fracture.

In studying the biological significance of the microelements for the physiological control of the organism, as well as for the regeneration of bone, we have set out to determine on the one hand the content of copper, iron, aluminum, and manganese at different times during the formation of bone in the damaged region, and on the other the extent to which the above-mentioned microelements take part in the metabolic processes of the skeleton after fracture. For the determination we have used the method of emission spectral analysis.

METHODS

The work was carried out on adult frogs maintained during June to August in aquaria under uniform living and feeding conditions. In 18 of the frogs the right femur was broken in the middle third of its length. In 14 of the frogs, in order to determine the microelements under normal conditions, a study was made of the cortical layer of the bone.

The animals were killed 3, 5, 12, 15, 20, 25, 30 and 40 days after the fracture (three frogs on each day). Spectrographic studies of the callus were made after 12, 15, 20, 25, 30 and 40 days, and the microelements were determined in the undamaged left femur after 3, 5, 12, 15, 20, 25, 30 and 40 days. Studies of the ends of the broken fragments were confined to the 3rd, 5th, 12th, 15th, and 20th days.

The preparations were carefully freed from bone marrow, dried in a desiccator at 100° until the weight was constant, after which for one day the samples were weighed three times daily; if they remained constant, the sample was accepted for analysis.

For determining the microelements, we used aqueous solutions of the samples, and standards which had been prepared synthetically.

The analysis was made on a quartz spectrograph of moderate dispersion, mark ISP-28; the slit width was 0.015 mm, and a 3-lens system was used for illumination. The source of excitation was an alternating current arc generator, giving a current of 8 amp at a voltage of 125 v. A three-minute exposure was given, the substance was not calcined, and the interelectrode distance was 2 mm. Finally, the spectrum of the iron electrodes was measured by means of a 9-step attenuator on spectral plates type I, 0.7 units, GOST (State Export-Import Office). The analytic lines used were: copper—3247.54 A, aluminum—3082.16 A, iron—3020.64 A, manganese—2798.27 A. Photometric

measurements of the analytical lines were made on an MF-2 microphotometer, and the lines were compared with the background. The background was placed at a distance of 0.05 mm from the analytical line in the short-wave end of the spectrum. A calibrated curve was constructed having coordinates log I/I and log C, from which the concentration of the samples was determined.

From spectrographic studies of the diaphysis of the femur in the 14 control animals, it was found that the average contents of elements in the bone were as follows: copper – 0.00026%, aluminum – 0.0014%, iron – 0.0025%, and manganese – 0.0019%.

In the regenerating bone, during the early stages (12th day) the copper had risen to 0.0034%, which was 13 times the value in the control. Fifteen days after the fracture, the copper in the regenerating bone was somewhat less than on the 12th day, and had fallen to 0.0011%, which was nevertheless 4.2 times greater than the amount in the control (Fig. 1).

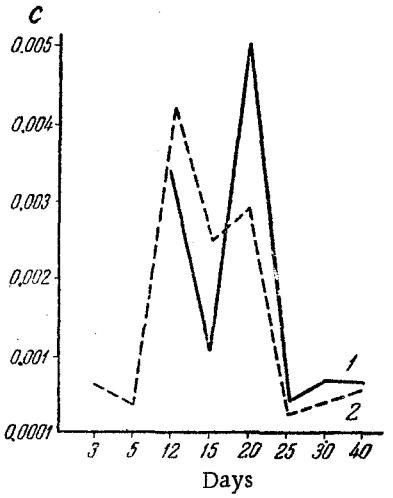

Fig. 1. Amount of copper in the developing callus and in the undamaged femur, at various times after fracture. ____ callus; ---- undamaged femur.

In the regenerating bone, we found the copper had risen on the 20th day to a maximum value of 0.005%, almost 20 times the normal amount. A reduction in the percentage of copper took place after the 25th day. Between the 30th and 40th days the amount was 0.00058-0.00068%, i.e., it varied over approximately the range of control values.

It should be noted that the curve of variation of the amount of copper in the undamaged limb to some extent followed the fluctuations of the amount in the regenerating bone. Between the 5th and 12th days, there was an increase in the percentage of copper in the left femur, where the amount was 0.004-0.0042, a value almost 19 times the control figure (see Fig. 1). Fifteen to twenty days after the operation, the amount of copper was somewhat lower (0.0025-0.0028%) than it had been previously, although, as before, it was higher than in the control (10 times higher). Later, on the 30th-40th days, the amount of copper in the intact bone varied between 0.00031 and 0.00058%, values which were rather greater than in the control group.

In the early stages, a similar relationship in the variation of the copper content was established by spectrographic studies of the ends of the damaged bone. On the third day after the fracture, the percentage of copper was 2.5-2.7 times higher than it was in the control, and on the 5th day there was a marked rise in the proximal fragment to 0.0037%, and in the distal end to 0.0047%. The maximum copper content of the ends was reached on the 12th day. It exceeded the control value 20-22 times, and was 0.005% in the proximal and 0.0055% in the distal fragment. Subsequently, between the 15th and 20th days, there was a reduction to between 0.0022 and 0.0046%.

Quantitative changes in the amount of aluminum in the regenerating bone resembled those of copper. Thus, 12 days after the fracture, the regenerating bone contained 0.006% of aluminum, i.e., 4.5 times as much as in the controls, and by the 15th day the value had risen to 0.0042% (Fig. 2). The maximum amount of aluminum in the regenerating bone was found to occur, as for copper, on the 20th day when it exceeded the control value 7 times, reaching a value of 0.009%.

Subsequently, the amount of aluminum fell, and by the 30th-40th day after fracture it varied over the range of control values, between 0.001 and 0.0012%.

In the intact femur, we found the following changes in aluminum content. On the third day, it varied within a range of 0.00062%, i.e., it was within the control range, though later the amount increased to a value 2-3 times greater than that of the control (see Fig. 2). The maximum amount of this substance in the left bone occurred on the 15th-20th day, when it was 6-7 times greater than the control, and varied between 0.0072 and 0.009%. Subsequently, when studying the diaphysis of the intact femur, we showed that the percentage of aluminum varied within the control range (0.00083-0.00098%).

In the damaged ends of the bone, the aluminum content was generally 3-4 times greater than in the controls, although on the 12th day, in the proximal portion, it was 0.0085%, and in the distal 0.011%, i.e., 6-7.2 times greater than normal.

On the 12th day, in the regenerating bone there was 0.012% of iron, i.e., 5.2 times more than in the controls. However, on the 15th day, the iron fell to 0.0059%, only two times more than in the control, though by the 20th day there was 7.6 times more than in the control (0.19%). The percentage of iron was reduced from the 25th day onwards, and by the 40th day it had fallen to 0.0024%, i.e., within normal limits (Fig. 3).

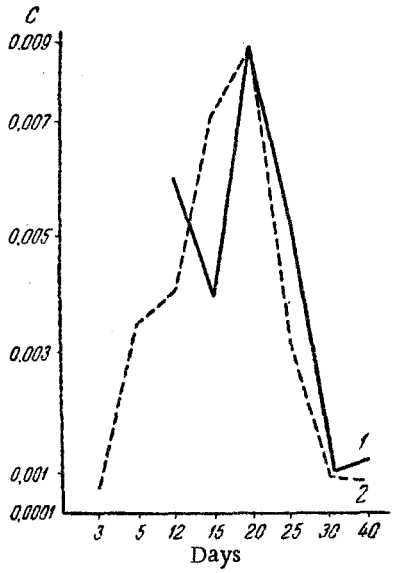

Fig. 2. Amount of aluminum in the developing callus, and in the undamaged femur, at various times after the fracture. Indications as in Fig. 1.

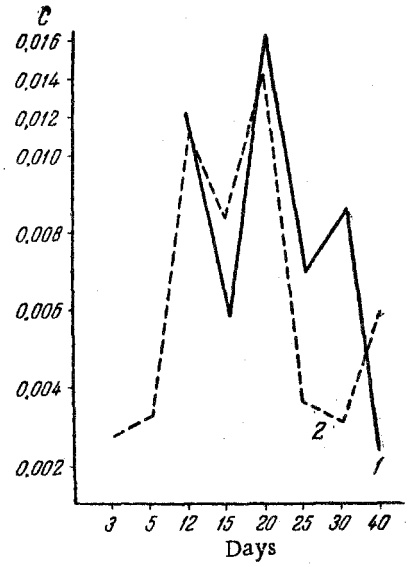

Fig. 3. Amount of iron in the developing bony callus of the undamaged femur, at various times after fracture. Indications as in Fig. 1.

The curve for the amount of iron in the intact femur was similar to that for iron in the regenerating bone. In the diaphysis of the femur, on the 3-5th day after fracture, we found that the percentage of iron varied between the normal limits of 0.0028 and 0.0033%, though by the 12th day it was seven times greater, at 0.011%. The maximum iron content in the undamaged femur occurred on the 20th day, when it was 0.014%, and later between the 30th and 40th days, it had returned almost to normal limits (0.0031-0.0059%) (see Fig. 3).

There was no appreciable rise in the amount of iron in the end portions of the fragments. Only in one case, on the 12th day after the fracture did the iron in this region exceed the control figure by 4-4.5 times (0.015% in the proximal, and 0.01% in the distal fragment).

Manganese accumulated in regenerating bone rather differently from copper, aluminum and iron. The maximum amount in the regenerating bone occurred on the 12th-15th day, when it was 0.0021-0.0023%, or 1.1 times the control value. Later, during the development of the callus, the amount varied within normal limits (0.0012-0.0018%), i.e., the variations in the percentage of manganese at this period in the regenerating bone were insignificant, whereas the change in the percentage of manganese in the end portions of the damaged bone, and also in the undamaged left femur showed that it was quite actively involved in mineral metabolism.

Our studies of the accumulation of microelements in regenerating bone, and our measurements of the metabolic processes both in the bone fragments and in the opposite undamaged femur have shown that copper, aluminum, iron, and manganese play an active part in the metabolism of regenerating bone, both in the various parts of the damaged bone, and in the early repair process. The most marked changes in metabolism of the microelements occurred during the early stages of new bone formation.

SUMMARY

Emission spectrum analysis was used in a study of the copper, aluminum, iron and manganese contents of the femoral diaphysis of healthy frogs, and in the regenerating bone of a femur fractured in the middle third of its length; measurements were also made in the splinters of the femoral bone, and in the opposite intact femur. In the diaphysis of the femoral bone of healthy animals there was 0.00026% of copper, 0.0014% of aluminum, 0.0025% of iron and 0.0019% of manganese. At the early stages of callus formation there was an accumulation of microele-

ments, and their percentage was very much higher than in the control animals. The metabolic processes of the end portions of the fractured bone and of the uninjured femur were markedly affected by the trauma. During regeneration, the percentage of the microelements listed was greatly increased.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
