EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Labeled Citrate Metabolism in Bone Fractures and Impaired Innervation

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Changes in ^{14}C incorporation into regenerate after bone fracture and impairment of mandibular innervation, and injection of [3-^{14}C]cytrate corresponded to the stages of reparative osteogenesis: after 1 week ^{14}C incorporation in the cellular-fibrous callus surpassed its release, after 2 weeks the rates of ^{14}C incorporation and release in the chondroid callus become similar, and after 4 weeks the release of the label predominated in the primary bone callus. Denervation reduced ^{14}C incorporation into regenerate, which impaired bone remodeling. Citrate in the bones is characterized by high metabolic activity.

Key Words: *bones; fracture; denervation; metabolism; [3-^{14}C]citrate*

Bone tissues differ from other tissues by chemical composition and metabolism [14]. About 1% bone weight belongs to citrate, its content being tens of times higher than in other organs, even in the liver with its intense citrate cycle. Bones can be regarded as a citrate depot.

Citrate metabolism in bones includes mineralization, demineralization (resorption), and remineralization (remodeling) [7], *i.e.* with metabolism of Ca and phosphates presented by hydroxyapatite crystals constituting more than half of total bone weight. In addition, bones contain carbonate, fluoro- and strontium-containing apatites, tricalcium phosphate, and other compounds. Phosphate and Ca metabolism in bone fractures is described in many reports, but citrate metabolism received little attention. There are no data about the effects of fractures with partial or complete denervation on metabolism of labeled citrate, though radioisotope analysis is the most sensitive method showing the direction of transport of the compounds and atoms. On the other hand, bone fracture is associated with impairment of innervation or at least affects the nervous system.

We investigated the effect of bone fracture on metabolism of labeled citrate in bones and the effect of denervation on these processes. The results can be useful for determining the regularities of reparative regeneration, in the treatment of injuries and other bone diseases.

MATERIALS AND METHODS

Experiments were carried out on 1-2-month-old albino rats kept on standard vivarium rations. The main object of the study was the mandibular bone. Fractures of this bone are almost inevitably associated with damage to the trigeminal and sympathetic nerves, which impedes bone regeneration.

[3-^{14}C]Citrate was injected intraperitoneally in a dose of 0.05-0.1 μCi/g. The ratio of radioactivity per 1 g bone (1 g bone regenerate or 1 ml serum, respectively) to radioactivity injected per gram body weight

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was calculated. The technique of measuring mild β-radiation on a gas-discharge and scintillation counters was described previously [3,4]. Reparative bone regeneration was studied after infliction (under Nembutal narcosis) of a mandibular fracture as described previously [2]. Specimens of the mandibular bone, diaphysis and metaepiphyseal part of the femoral bone, and serum were weighed before drying. Radioactivity in cpm was determined in 10 mg dry residue. The results were calculated for wet weight.

Three experimental series were carried out. In the first experimental series the dynamics of label content in intact rat bones after injection of labeled citrate was studied. Ten minutes after injection of the radioisotope 4 rats were sacrificed, 5 animals were sacrificed after 20 min, 5 after 60 min, and 3 after 180 min.

In the second experimental series fracture of the right half of the mandibular bone was modeled. In this series and series III the radioisotope was injected for 20 min (time of maximum label incorporation). One week after fracture 12 rats were sacrificed, 11 animals were sacrificed after 2 weeks and 7 after 4 weeks, these periods corresponding to stages of bone regeneration: a soft callus (cellular fibrous) forms after 1 week, chondroid callus after 2 weeks, and primary bone callus after 4 weeks [2].

The third experimental series was carried out on 8 rats without fractures 2 weeks after crossing the right inferior alveolar nerve [2].

The results were statistically processed using Student's *t* test and were expressed as $M \pm m$.

RESULTS

In the first experimental series the label maximally incorporated in the mandible and femoral bone diaphysis 20 min after injection (Fig. 1). This term was used in the second and third series. In the metaepiphyseal part of the femoral bone incorporation was almost the same after 10, 20, and 60 min. In two other bone specimens the maximum 20 min postinjection was significantly higher than 10 and 60 min postinjection ($p<0.05$ and $p<0.01$). Differences in the label incorporation 10 and 60 min postinjection were minimum in all 3 bone specimens. After 180 min the incorporation was 2-3-fold lower than the maximum level in all bones. Hence, citrate in bones and bone regenerate is metabolically active and not inert.

In the second experimental series ^{14}C incorporation into mandibular regenerate and femoral bone sharply increased 1 week after the fracture ($p<0.001$) and decreased to the control level after 2 weeks (Fig. 2). After 4 weeks label incorporation into regenerate was even lower than in the control and in the femoral bone almost at the same level as after week 2. Hence, changes in citrate metabolism in the skeleton were generalized, but more pronounced after injury. Two weeks after denervation without fracture label incorporation decreased in comparison with the control ($p<0.05$).

In the third experimental series the label incorporation from the blood decreased 1 and 2 weeks after the fracture, this decrease being more pronounced for the bone regenerate than for the femoral bone (Fig. 3). The isotope transfer was less pronounced after denervation. After 4 weeks the penetration of ^{14}C into the regenerate was lower than in the control. The levels in the femoral bone did not differ from the control.

In vitro experiments showed that by minute 20 labeled citrate from aqueous solution maximally bound to synthetic hydroxyapatite crystal, but the label was slower released from the crystal than *in vivo* [11], presumably, because of the absence of fluid circulation.

Changes in citrate content during reparative osteogenesis clearly corresponded to the consolidation phases [2,12]. First at the stage of cellular-fibrous soft callus the level of radioactive carbon in the regenerate sharply increased as the entry predominated over release. At the stage of chondroid callus the rates of label incorporation and release approximated. At the stage of primary osseous callus the release predominated over incorporation.

During the initial period of consolidation activity of citrate synthase and succinate dehydrogenase in the callus increased, by week 4 activity of NAD-dependent isocitrate dehydrogenase and malate dehydro-

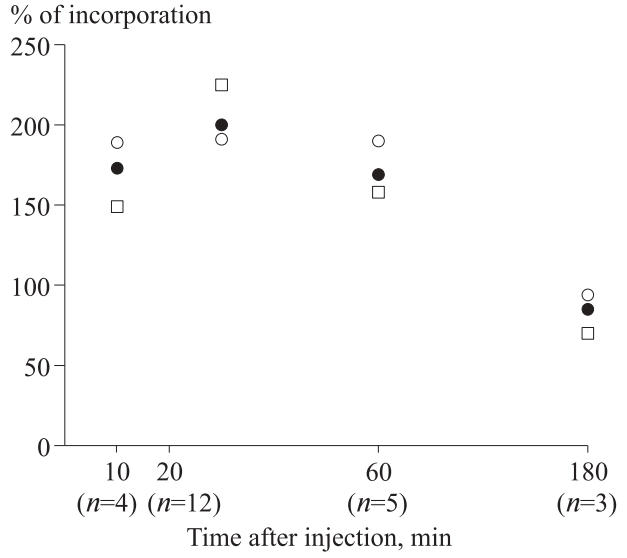


Fig. 1. Radioactive carbon incorporation in the metaepiphyseal part of the femoral bone (light circles), mandibular bone (dark circles), and femoral bone diaphysis (light squares) of young rats after intraperitoneal injection of [3-^{14}C]citrate. *n*: number of rats in experiment.

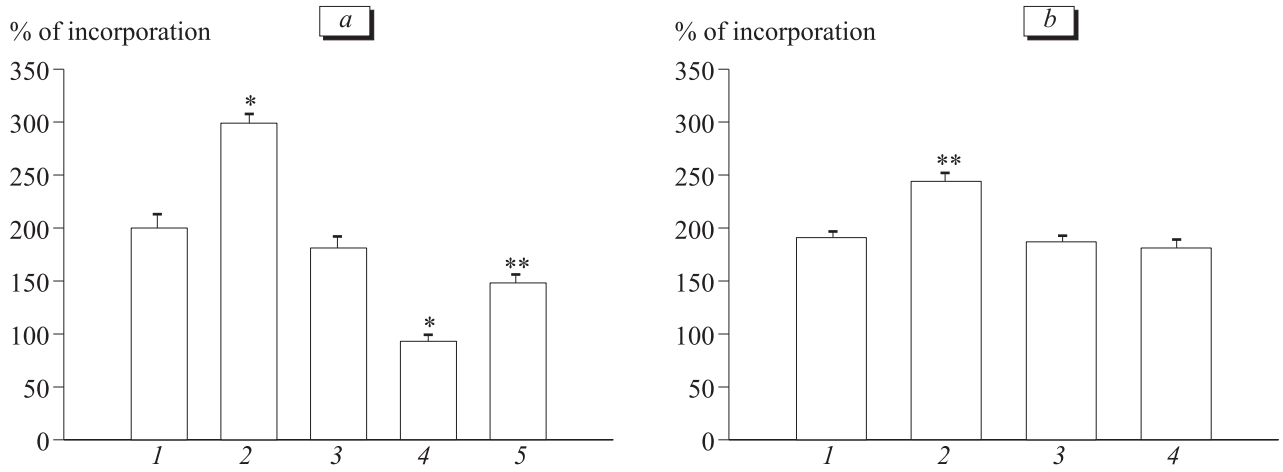


Fig. 2. Incorporation of radioactive carbon in mandibular bone (*a*) and metaepiphyseal part of the femoral bone (*b*) 20 min after intraperitoneal injection of [3-^{14}C]citrate to young rats. Here and in Fig. 3: *1*) control; *2*) 1 week after mandibular fracture on the right side; *3*) after 2 weeks; *4*) after 4 weeks; *5*) 2 weeks after crossing of the right inferior alveolar nerve. *p<0.001, **p<0.05 compared to the control.

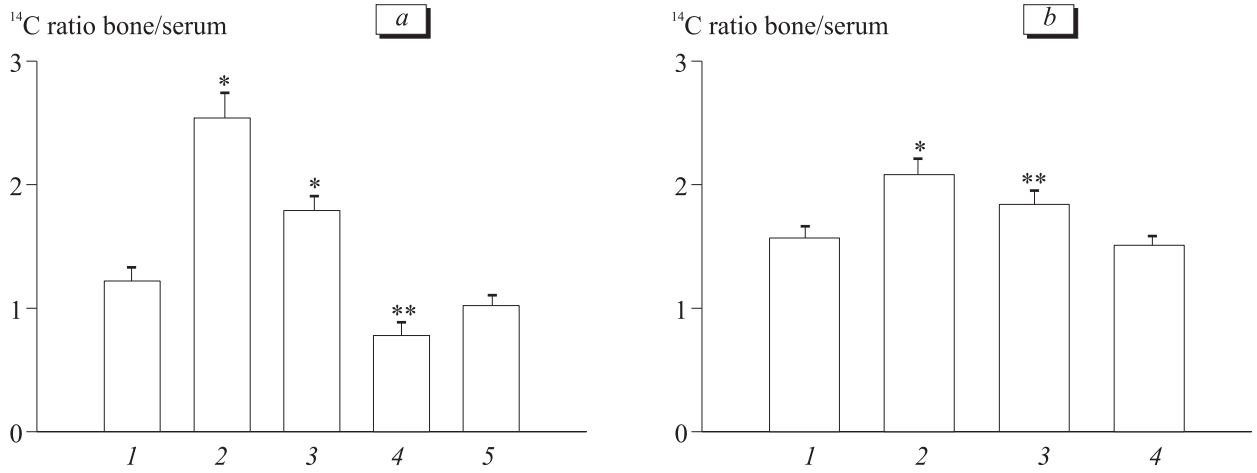
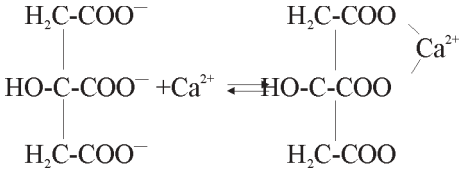


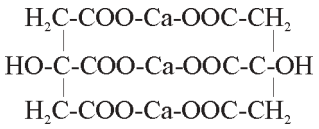
Fig. 3. Ratio between the content of radioactive carbon in the mandibular bone (*a*) and metaepiphyseal portion of femoral bone (*b*) and in the serum after intraperitoneal injection of [3-^{14}C]citrate to young rats.

genase increased, which seemed to be due to the need in the production of ATP for synthesis [1].

It is known that citrate transfers [10] Ca from the blood into the bone or into the regenerate and vice versa in the form of Ca monocitrate chelate [13], thus optimizing conditions for the formation or resorption of apatite structures:



Previously we detected 2 phases of citrate binding to the bone: fast and slow [4]. This was confirmed by other authors, who showed that biphosphonate inhibited fast binding of citrate to the surface of synthetic hydroxyapatite crystal but did not modulate slow binding [11]. It was found that precipitation of tricalcium bis-citrate was also possible [13]:



Apart from Ca monocitrate and tricalcium bis-citrate, phosphocitrate and calcium citrate peptide complexes, modulating the formation of protein matrix of the bone, were detected in bones and regenerate [15].

The detected decrease in labeled citrate incorporation in bones of young rats after inferior alveolar nerve crossing confirms that denervation can to a certain measure prevent remodeling. That is why the role of innervation disorders should not be neglected in case of changed citrate metabolism after bone injury [6, 8,9]. This deserves attention because clear-cut changes in the anatomical shape of the mandible appear in

1-month-old rats [5] 1-2 months after crossing of the inferior alveolar nerve.

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