METHODS

A Method for Evaluating the Fluctuations in the Intensity of Substance Transport between Blood and Bones in the Predominant Direction

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The dynamics of fluctuations in the intensity of the blood-bone flow of substances in the predominant and opposite directions was evaluated using the percent radioactivity difference coefficient and its graphic presentation. The technology of calculating this coefficient is described.

Key Words: *percent radioactivity difference coefficient; transport fluctuations; blood; bone; rats*

The intensity of metabolism in the bone and transport of a substance to the bone with blood is evaluated by changes in the content of this substance in the bone. However, the content of substance in the bone is a final result of its synthesis, degradation, and blood-bone exchange. For this reason it is impossible to evaluate precisely the excess in the intensity of the substance transport from the blood into the bone in the predominant direction over that in the opposite direction for a certain period.

The method for determining the difference between substance transport from the blood into the bone in comparison with that in the opposite direction can be useful for biochemists, physiologists, pathophysiologists, traumatologists, orthopedists, dentists, oncologists, radiologists, specialists in aerospace medicine and combined radiation and traumatic injuries.

Our aim was to develop a method for estimating the excess of the substance transport intensity in the predominant blood-bone direction over that in the opposite direction.

MATERIALS AND METHODS

Bone content of carbonate, citrate, and selenate is 3-4%, about 1%, and hundredth fractures of percent, respectively. Carbonate is essential for solubility and solidity of the main bone crystal hydroxyapatite $Ca_n(PO_4)_6(OH)_2$ [5,8,9,11]. Citrate transports Ca between blood and bone [4,6,9,11,13,14]. Selenium (antioxidant) maintains the normal bone status [1,6,7,10,12,15].

Rats of different age were intraperitoneally injected with [^{14}C]carbonate, [3-^{14}C]citrate, and [^{75}Se]selenate in a dose of 0.5 μCi/g body weight. Blood and bone specimens were collected repeatedly after injections for studies of radioisotope dynamics. The bones were cleansed from soft tissues and blood, dried, and crushed into powder. Serum and bone radioactivity was evaluated as described previously [2] with our modification. The data were statistically processed using Student's *t* test.

The dynamics of coefficient of difference (CD) in percent radioactivity (PRA), repeatedly evaluated

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from the two nearest PRA, was evaluated by our patented method [3]. The first step of this procedure is estimation of the radioisotope incorporation percentage in the mandibular bone and serum by the percent ratio of cpm/g bone (Fig. 1, *c*) or 1 ml blood (Fig. 1, *d*) to the cpm/g body weight injected.

The results of PRA estimation are presented in Fig. 1, *b*. PRA_1, PRA_2, PRA_3, *etc.* (PRA_0 before injection of the isotope) were calculated for rats under certain experimental conditions after certain terms following the injection of radioisotope by the formula:

$$PRA=\frac{\%\text{ of radionuclide incorporation into bone}}{\%\text{ of radionuclide incorporation into blood}}$$

PRA more than 1 indicated a higher content of the label in the bone, PRA less than 1 indicated that the label content was higher in the blood, and PRA=1 indicated equal content of the label in the bone and blood.

Predominant transport of the substance in bidirectional transport was estimated using the coefficient of PRA difference (CD_{PRA}): $CD_{PRA_0}=PRA_1-PRA_0$, $CD_{PRA_1}=PRA_2-PRA_1$, $CD_{PRA_2}=PRA_3-PRA_2$, *etc.* The value of each CD_{PRA} precisely reflects changes of transport in the predominant direction for the time of estimation of the two successive PRA after injection of the radionuclide. Higher values of CD_{PRA} indicate higher intensity of radionuclide transport.

A total of 4-12 rats were examined per step of the study (after injection of radionuclide).

RESULTS

Labeled carbonate was assayed 5, 10, 20, 45, 90 min, 3, 6, 12, 24, 48, and 192 h after injection of the radioisotope (Fig. 1).

Bone level of the radioisotope depends on its blood level (Fig. 1, *d*, *c*): similar peaks by min 20, followed by reduction until the end of the experiment with slight changes in the rate of reduction. A minor second wave was observed for the serum (from min 90 until the 3rd hour).

The dynamics of PRA (Fig. 1, *b*) differed significantly from the dynamics of the incorporation percentage: the maximum values were recorded during the 5th and 45th minutes and during the 192th hour, the decrease being observed during the 10th minutes and 12th hour, which is explained by incomplete synchronization of deceleration of incorporation in the blood and bone. A significant elevation of PRA from the 12th through 192th hours is due to a greater reduction in the percentage of incorporation in the serum in comparison with bone.

Repeated estimation of CD_{PRA} gives the actual picture of the dynamics of fluctuations in the intensity of the blood-bone transport in the predominant direction.

The dynamics of CD_{PRA} values and direction (Fig. 1, *a*) often do not coincide with PRA shifts. Only CD_{PRA_0} is equal to PRA_1 by value and direction, as PRA was equal to zero before injection of the radionuclide. CD_{PRA_1} is much lower than CD_{PRA0}. The value then increases to CD_{PRA_3}, after which it decreases to CD_{PRA_5}, increases to CD_{PRA_8}, and decreases to $CD_{PRA_{11}}$.

The level of labeled citrate in the mandibular bone after intraperitoneal injection depended on animal age (Fig. 2). The radioisotope incorporation in rats aged 1 month was several-fold higher in comparison with animals aged more than 1 year during the entire experiment (Table 1). Significant differences in the fluctuations of CD_{PRA} were observed within age groups. In 1-month-old rats, the minimum CD (CD_{PRA_1}) was 0.19, the highest (CD_{PRA_6}) 31.54 (more than 160 times higher). In rats aged

TABLE 1. Dynamics of Radionuclide Incorporation in Mandibular Bone and Serum of Rats of Different Age after Intraperitoneal Injection of [3-^{14}C]Citrate (%; $M\pm m$)

Object of study; age of animals	Term of observation					
	10 min	20 min	1 h	3 h	24 h	96 h
Mandibular bone						
1 month	173.6±4.8	206.1±10.5	169.4±4.1	87.7±1.6	36.2±1.2	16.7±0.3
over 1 year	—	58.1±3.4**	52.3±2.9**	20.2±1.4**	7.3±0.9**	4.0±0.2
			Serum			
1 month	97.7±0.7	104.8±1.6	12.1±0.8	8.3±0.1	1.5±0.1	0.30±0.05
over 1 year	—	98.9±15.6	24.7±1.3**	8.1±0.4	2.9±0.4*	1.50±0.05**

Note. Here and in Table 2: *p<0.05, **p<0.001 compared to the other age group.

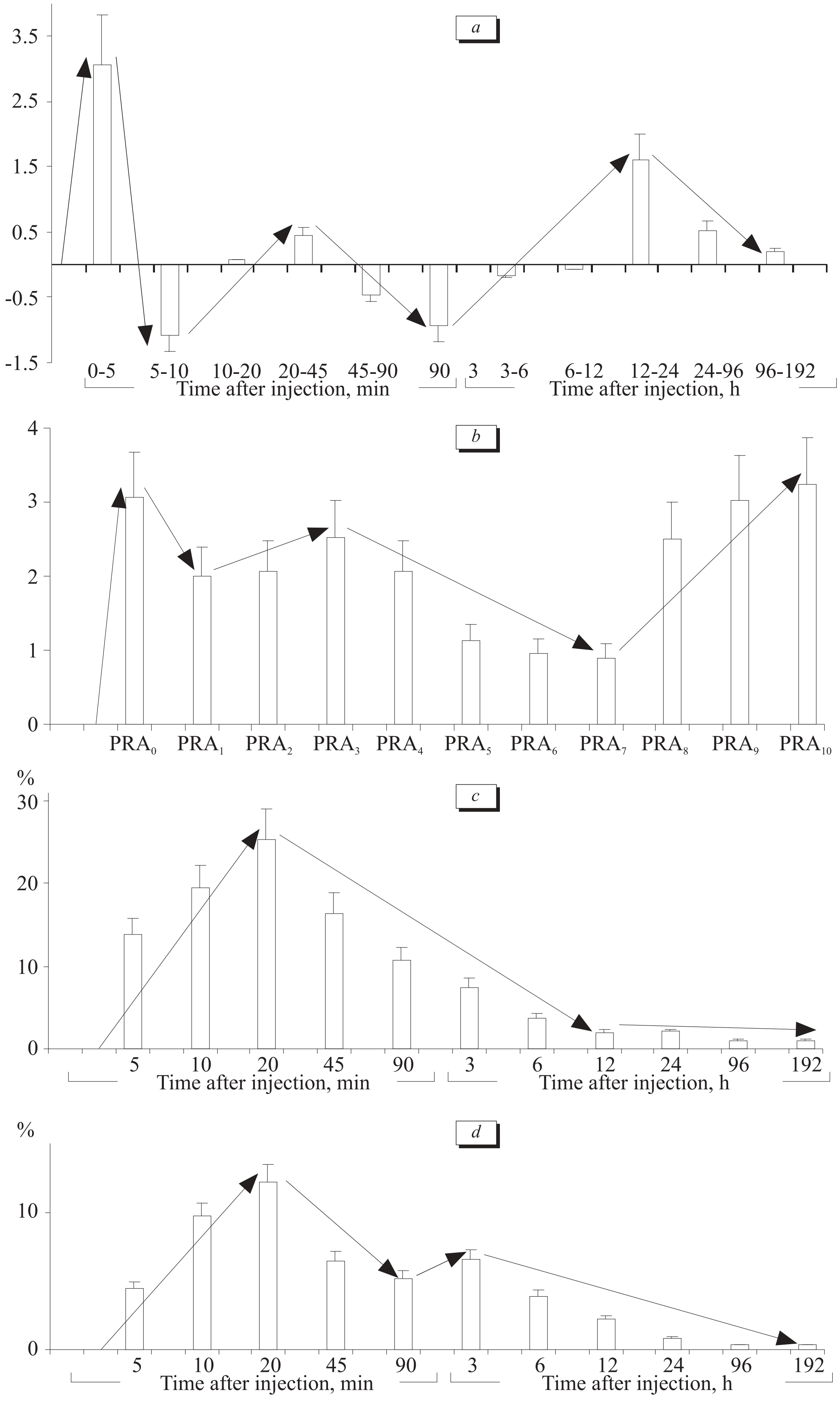

Fig. 1. Dynamics of radioisotope incorporation (coefficient cpm/ml serum or cpm/g bone to cpm/g body weight) in the serum (*d*) and mandibular bone (*c*), PRA (*b*) and CD coefficients of two nearest PRA (*a*) in rats.

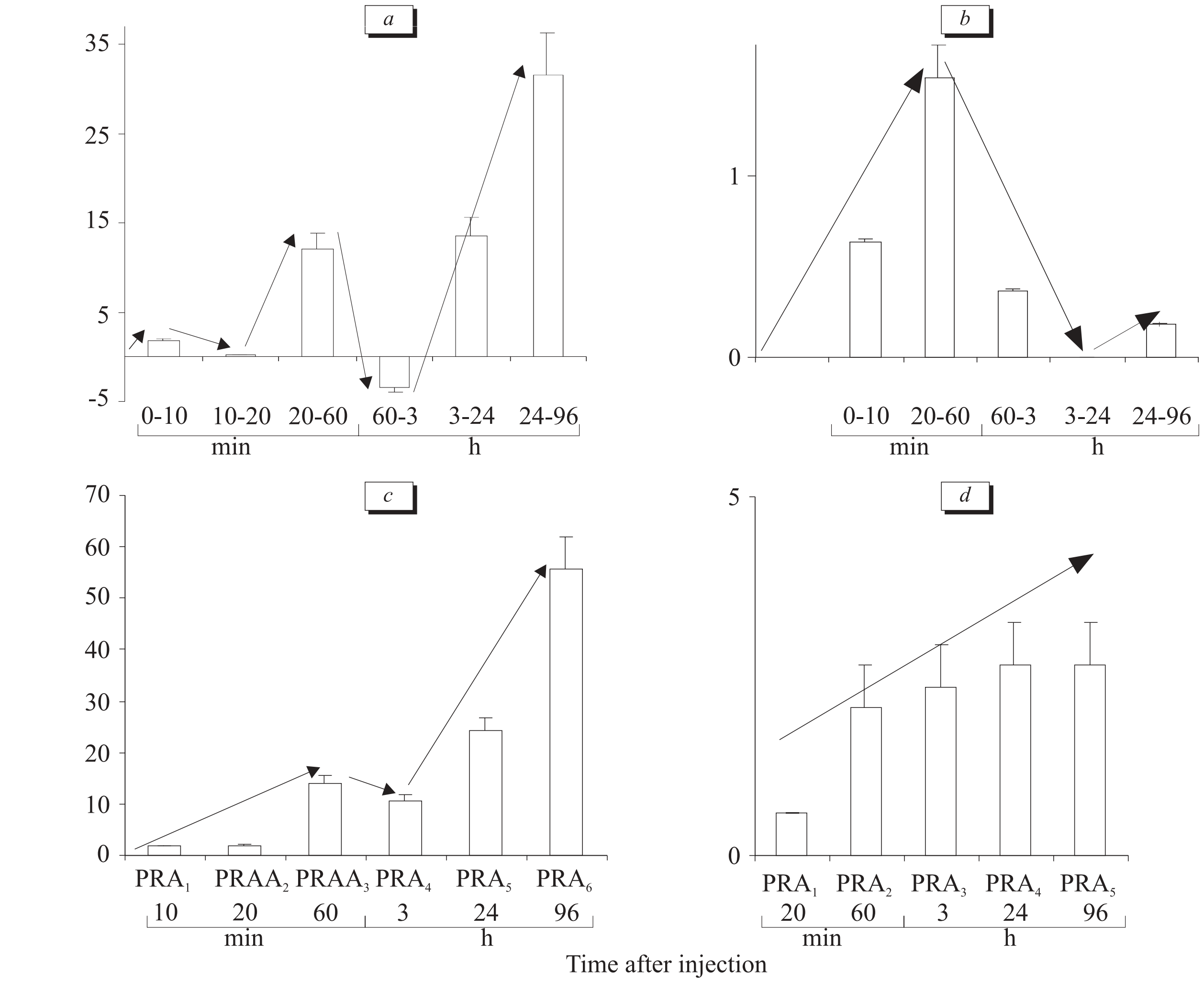

Fig. 2. PRA (*c*, *d*) and CD_{PRA} (*a*, *b*) in rats aged 1 month (*a*, *c*) and above 1 year (*b*, *d*) after injection of [3-^{14}C]citrate.

over 1 year, the minimum value of this parameter was 0.03 (CD_{PRA_3}), maximum 1.53 (CD_{PRA_1}; 50-fold difference).

The rhythm of fluctuations was also different (particularly for the last three CD_{PRA}). Elevation from CD_{PRA3} to CD_{PRA6} with more than 10-fold difference (from -3.43 to 31.54) was observed in 1-month-old rats, while in animals aged over 1 year the fluctuations were slight, approaching the zero line (CD_{PRA_2}=0.37, CD_{PRA_4}=0.15).

The dynamics of labeled selenate was also traced for two age groups (Table 2). The level of iso-

TABLE 2. Dynamics of Radionuclide Incorporation in Mandibular Bone and Serum of Rats of Different Age after Intraperitoneal Injection of [^{75}Se]Selenate (%; *M*±*m*)

Object of study; age of animals	Term of observation, h						
	1	3	6	12	24	48	192
Mandibular bone							
1 month	45.8±5.6	51.6±4.8	59.7±5.2	41.0±2.9	20.6±2.0	30.8±4.4	10.4±0.9
3 months	—	—	18.5±2.9**	26.6±2.6**	30.6±3.2*	31.1±4.4	14.7±1.2*
Blood							
1 month	77.7±16.4	76.6±10.6	59.4±3.6	61.8±7.4	41.0±4.8	29.6±3.9	14.6±2.3
3 months	—	—	83.0±15.6	52.9±1.3	66.8±0.4**	68.4±0.4**	66.2±0.05**

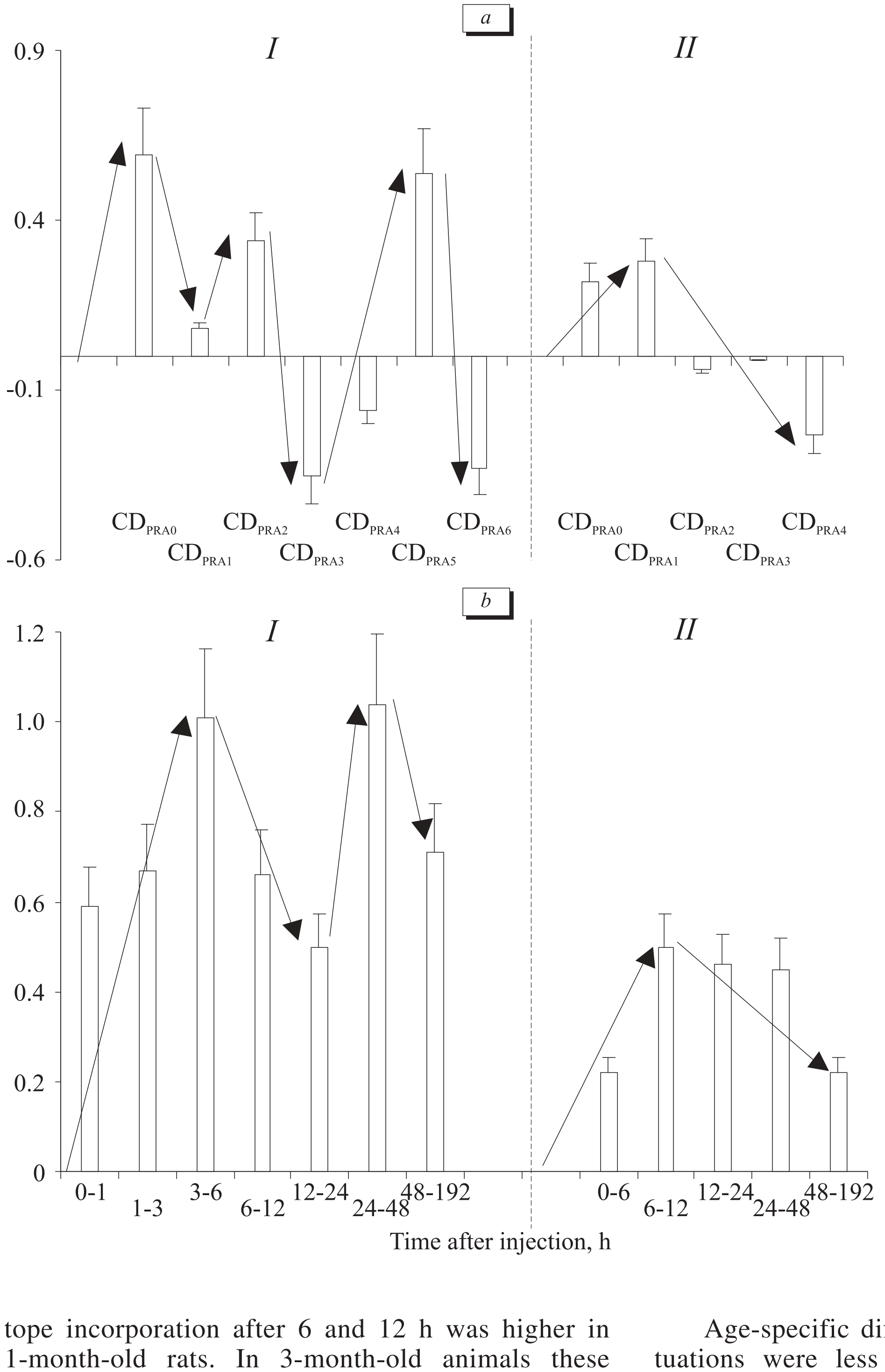

Fig. 3. CD_{PRA} (*a*) and PRA (*b*) in mandibular bone of rats aged 1 month (*I*) and 3 months (*II*) after injection of [[75]Se]selenate.

tope incorporation after 6 and 12 h was higher in 1-month-old rats. In 3-month-old animals these differences were absent 24-192 h after injection. Analysis of CD_{PRA} of rats aged 1 and 3 months showed slight age-specific differences (Fig. 3, *a*, *b*). The amplitude of fluctuations was approximately the same in both age groups. In 1-month-old rats the minimum value (CD_{PRA_4}) was -0.35, the maximum 0.59 (2.7 times higher), while in 3-month-old animals the minimum value was -0.23 (CD_{PRA_5}) and the maximum (CD_{PRA_1}) 0.28 (2.2 times higher).

Age-specific differences in the rhythm of fluctuations were less pronounced after injection of selenate than after injection of [3-[14]C]citrate. The time of elevation and reduction of the predominant direction of transport coincided for the last three CD_{PRA} of [3-[14]C]citrate and [[75]Se]selenate.

Changes in the content of the substance and/or incorporation value do not indicate the predominant direction of the substance transport between the blood and bones. Radioisotope nanotechnology used in our study represents the dynamics of intensity of the substance flow in the predominant direction in comparison with the opposite flow.

REFERENCES

1. M. V. Kozlova, V. N. Ivanov, I. S. Pinelis, and Yu. A. Petrovich, *Patol. Fiziol. Eksper. Ter.*, No. 2, 35-37 (1997).
2. R. D. Ozrina, Yu. A. Petrovich, E. P. Senchenkov, and M. B. Shvyrkov, *Ukr. Biokhim. Zh.*, **54**, No. 1, 69-72 (1982).
3. Yu. A. Petrovich, R. P. Podorozhnaya, and S. M. Kichenko, *Byull. Izobr. Otkrytii*, No. 34 (2004).
4. Yu. A. Petrovich, R. P. Podorozhnaya, S. M. Kichenko, and I. M. Dmitriev, *Byull. Eksp. Biol. Med.*, **136**, No. 7, 96-99 (2003).
5. Yu. A. Petrovich, R. P. Podorozhnaya, S. M. Kichenko, and I. M. Dmitriev, *Ibid.*, No. 8, 156-159.
6. Yu. A. Petrovich, R. P. Podorozhnaya, S. M. Kichenko, and M. V. Kozlova, *Ibid.*, **137**, No. 1, 85-88 (2004).
7. C. Delibasi, S. Demiralp, and B. Turan, *J. Oral Sci.*, **44**, No. 2, 85-90 (2002).
8. M. Hasegawa, Y. Doi, and A. Uchida, *J. Bone Joint Surg. Br.*, **85**, No. 1, 142-147 (2003).
9. H. J. Heller, J. R. Poindexter, and B. Adams-Huet, *J. Clin. Pharmacol.*, **42**, No. 1, 1251-1256 (2002).
10. F. Jakob, K. Decker, E. Paar, *et al.*, *Methods Enzymol.*, **347**, 168-179 (2002).
11. A. M. Kennj, K. M. Prestwood, B. Biskup, *et al.*, *Osteopor. Int.*, **15**, No. 4, 290-294 (2004).
12. R. Moreno-Reyes, D. Egrise, J. Neve, *et al.*, *J. Bone Miner. Res.*, **16**, No. 8, 1556-1563 (2001).
13. L. Patric, *Altern. Med. Rev.*, **4**, No. 2, 74-85 (1999).
14. S. Sasaki, H. Iwata, N. Ishiguro, *et al.*, *Nutrition*, **10**, No. 6, 538-543 (1994).
15. B. Turan, S. Bayari, C. Balcik, *et al.*, *Biometals*, **13**, No. 2, 113-121 (2000).