JAROSLAV JAMBOR

CHANGES IN BONES
OF PREHISTORIC POPULATIONS
CAUSED BY ENVIRONMENTAL
INFLUENCE

ABSTRACT — A model of the interaction of bones and environment has been worked out and with the help of the model the content of a number of elements in connection with the degree of contamination was tested. From the dependence thus found it follows that the ageing of bones takes place very unevenly. From the proportional presence of some elements, however, we can determine the degree of contamination of each sample, either with soluble or with insoluble components of the environment, thus facilitating the interpretation of the results. One of the important conclusions is the importance of the reproducible selection of all samples.

KEY WORDS: Ancient diet — Bone chemistry — Postmortem alteration.

The knowledge of the exact composition of bones is a basic condition for deriving various demographic data, especially the eating habits of the historical populations. Researchers mostly analyse only the strontium and lead content, less the content of Zn, Na, Cu, Mg, V and others (Lambert et al. 1984). The interpretation of the results is greatly complicated by the natural process of diagenesis, changing composition of all bone components as compared with the premortal situation. The conditions influencing the diagenesis and the mechanism of its effects are known only aproximately and the individual physico-chemical processes have been studied regardless of mutual relations and under conditions very distant from the real ones. It has been documented experimentally (Klepinger et al. 1986), that the process of degradation of the bones does not form a linear function of time. This, however, does not mean that the degree of the contamination of the bones by the environment and an possible extrapolation of results are impossible. It requires more detailed knowledge of the changes of contents of the individual elements in dependence on the degree of contamination, with regards to the content of the same elements in non-burried bones, to the chemical composition of the soil in general, and in the positions of direct contact with the bones in particular, to the chemical and physico-chemical properties of the given elements, and all this with respect to the natural dispersion of these elements in the bones and also in the environment surrounding them.

To answer some of these questions one can make use of the collection of skeletal finds from the regions along the northern and southern limes of the Roman Empire dated to the period spanning the 1st to 4th centuries A.D. For analytical evaluation the following elements were chosen: Ca, Sr, Na, K, Li, Co, Ti, Ag, Zn, Cu, Ni, V, Cr, Pb, Mn.

For determining these basic and some trace elements various methods were used, first of all atomic absorption spectrometry (Szpunar et al. 1979), (Schoeninger 1979), ICP spectroscopy (Klepinger et al. 1986), neutron activation analysis (Brätter et al. 1977); however emission spectroscopy with flame extinction for alkaline metals and earth has also given creditable results (Otruba et al. 1983), and the same

applies with arc excitation for heavy metals (Otruba et al. 1982, Le Roy et al. 1974). The presence of alkaline metals and alkaline earths is determined from the solution of bone sample aspirated into acetylene-nitrous oxide flame for the determination of Ca and Sr, and into the acetylene-air flame for the analysis of Na, K and Li. In order to reach a satisfactory detection limit and to suppress spectral interference, it is advisable to use a monochromator with a high degree of dispersion and low level of stray light. Spectral analysis with the excitation in an arc of alternating current permits the determination of the remaining elements with sufficient sensitivity and acceptable accuracy.

METHODOLOGY

Selection of samples

The samples came from the localities Abrahám, Sládkovičovo, Gerulata I, II and III and Wadi Quitna (Smrčka et al., Anthropologie XXVI/1). The samples from localities Abrahám, Sládkovičovo and Gerulata II included parts from the greater trochanter proximal parts of the femur and apex of the petrous bone, from the localities Gerulata I and III of the proximal parts of the femur opposite the lesser trochanter and from the greater trochanter. The samples from Wadi Quitna include the proximal, medial and distal parts of the femur and of the same parts of the tibia.

Preparation of the samples

No uniform methodology has been accepted as regards either the methodology of samples and of their processing. In some cases the samples are just dried and the results are expressed in weight units per one gramme of the dry sample. The main advantage of this procedure is simple operation and speed, the disadvantage includes possible neglection of the various degrees of destruction of the organic components of the sample, resulting in unequal and hardly comparable concentration levels of the elements being analysed. This shortcoming can be eliminated by expressing the concentrations not only in absolute values, but also in the ratio of concentrations of the elements to be determined to the calcium concentration in the bones; the latter is a relatively constant value. In the other case the samples are ashed at temperatures of 400—600 °C, so as to burn the entire organic component. In this way we can do away with the problem of unequal degree of destruction of organic matter, but it appears that losses of some other elements also occur. During the decomposition of collagen some metal elements are reduced, and at higher temperatures they can volatilize. Substantial losses occur among other elements also in Hg, Sn, As and Pb. Not entirely negligible losses were also observed in alkaline metals.

Taking into account all the above factors, a combined method has been chosen for the treatment of the elements to be analysed. For the determination of alkaline metal and earth content a solution is used.

The sample is only dried till it reaches a constant weight and the results are expressed in absolute terms and also with regards to calcium. The spectral analysis of other elements requires solid samples, which, however, must be homogenized. In some cases this causes problems and is not practicable with contemporary samples. Thus we had to work out a compromise method of carbonizing the sample, i.e. its ashing at 250—270 °C. At these temperatures the collagen is decomposed and it occured carbon in the amount of roughly 8 per cent of the original collagen content. Thus the contemporary samples can easily be ground; the reduction of the above elements takes place in a very limited extent (with the exception of Hg) and the tension of vapour eventually reduced metals is up to one order lower than with the usual ashing temperature. The effect of the varying degree of destruction of the organic components also decreases, which is further eliminated through the necessary evaluation of the concentrations as the ratio of the intensities of spectral emissions of the elements to be determined to the basic constant element in the sample, which, fortunately, is again calcium.

Analytical procedure

The determination of the alkaline metal and alkaline earth is achieved by weighing 50—100 mg of dried sample, dissolving by heating in 5 ml concentrated HNO_3, then cooling it down, pouring into a 50 ml graduated flask, and filling up with water. Then one places 20 ml of the solution in a 25 ml graduated bottle, adds 1 ml of NaCl solution at concentration of $50 mg . ml^{-1}$ Na, and fills it up. In this solution one then determines the Ca and Sr content in the flame of acetylene-nitrous oxide. From the original solution take 2 ml in a 25 ml graduated bottle, dilute it up to the mark, and measure the Na content in acetylene-air flame, in the same way as K and Li content in the original solution.

The heavy metal content can be determined in the following way: heat up approximately 100 mg of sample up to 250—270 °C, if possible during the night, cool it down, ground it and fill the homogenized sample into graphite electrodes. Excite the sample with alternating current arc in a suitable spectrograph in the usual way. Evaluate the proportion of transformed values of the density of spectral lines to the value of the density of the selected Ca line. For the construction of calibration curves use the prepared secondary standards.

Standardization

One of the principal problems of the standardization of the methods is the lack of suitable reference materials and the synthetic preparation of the standard solutions meets with difficulties in modelling the complex composition of bones. The phosphate matrix of the bones often causes a considerable systematic error in the determined values. The most correct, but very laborious method is the preparation of several standard samples through the analysis of suitable samples in a sufficient amount, following

TABLE 1. *Diagram of the mutual influence of bones and the environment.*

$\Downarrow H_2O$ + air + soil

deposited particles		fast material	colloid	ions			
reactions		filtration	sedimentation	adsorption	difusion	ion-exchange	destruction of organic
reaction	disintegration	leaching		desorption	elution	ion-exchange	leaching
leached particles	parts of bone	fast material + colloids + ions		ions			fast mater. + colloids + CO_2 + N_2

perfect homogenization and standardization of determined elements by the proper analytical procedure in solution with the method of standard additions. In elements determined through flame spectroscopy one can make also direct use of some of the samples of every analysed series for testing the setting of the instruments through the method of standard additions.

RESULTS AND DISCUSSION

Relation of the bones to the environment

To make easier the review of possible processes of the reaction between the buried bone and its environment (not to mention the microbiological effects), we have presented them systematically in *Table 1*. Rain water, and sometimes also ground water causes the soaking of the earth into the bones in the form of various particles, colloidal solutions and ions. Bigger particles and a great deal of colloidal solutions are effectively filtered by the surface layers of the compact bone and do not reach the deeper layers. The fact that in the localities mentioned both the cavities and the cancellous parts of the finds are mostly or often entirely filled with earth, should be ascribed to infiltration from inside, through the natural openings of the bones. This fact is well documented by the mineralogical composition of the infiltrated earth, corresponding to the slight share of the surrounding earth; it is also documented by the dimension of the particles of materials found in the cavities of completely intact bones. They were up to 0.3 mm in size. The solutions of the dissolved salts also penetrate the bones through the natural holes, but also through compact walls and the individual ions according to their character are absorbed and are caught through the ion exchange again already on the surface layers, and only some get deeper to the compact bone. This is accompanied by an opposite process

of washing out parts of the bones and also of the sedimented particles of earth, desorption and elution of ions.

Of great importance for the stability and resistence of the bone against contamination is the collagen matrix. As long as the collagen is decomposed to a small degree only, the above processes are very slow and are mostly limited to the surface layers only. With the progressive degradation of the collagen an increasing amount of microcrystalline hydroxyapatite is deprived of its protective layer, the influence of the above processes speeds up in these places, and the corrosion of crystals accelerates. The denuded crystals more easily dissolve and get washed out; secondary cavities arise, and the effects of the above processes intensify. The protective layer of collagen also protects the bones against sorption and against the exchange of ions of the solution overlapping on the surface of the crystals.
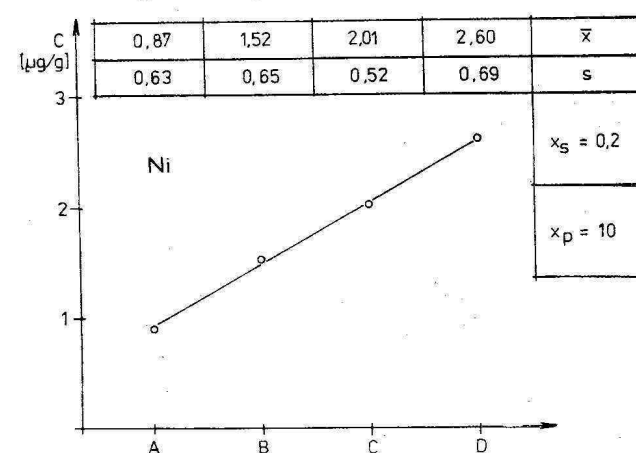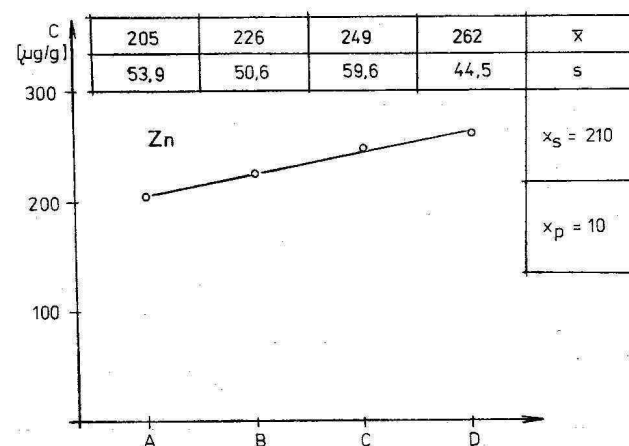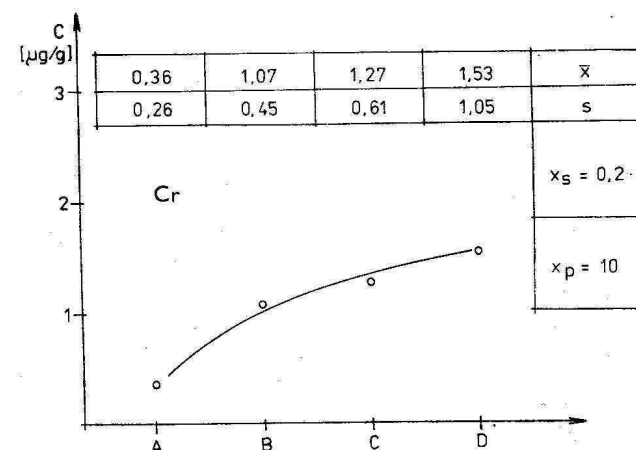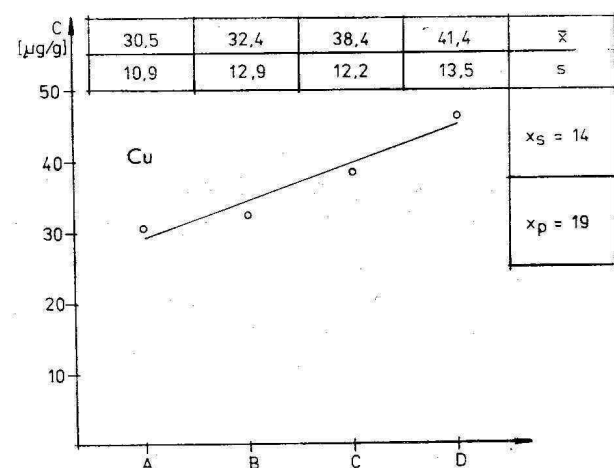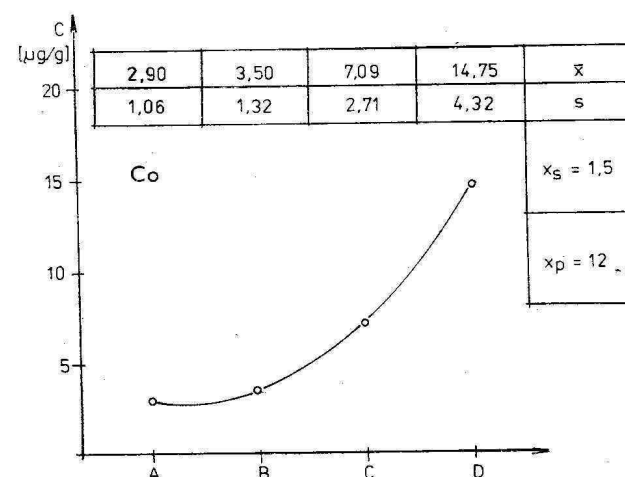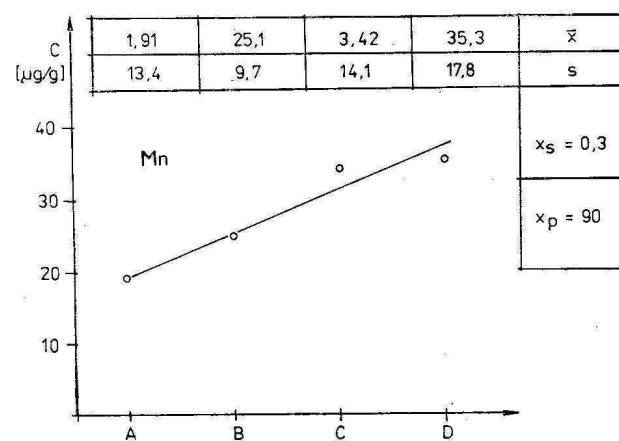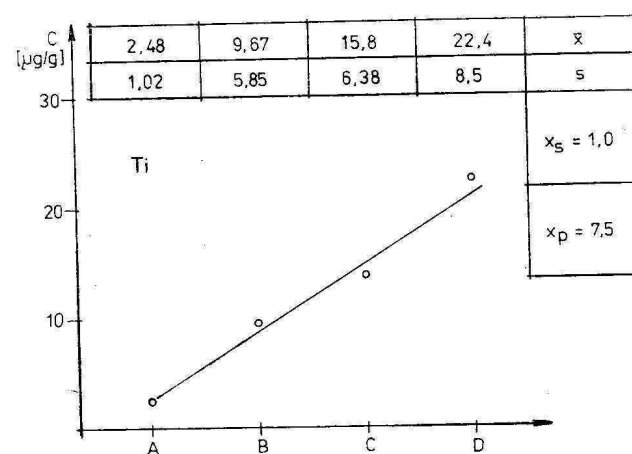
All these influences result in the fact that the process of degradation of buried bones is not a linear function of the time; it takes place rather unevenly and in dependance on the complex effects of all environmental components. In order to evaluate the share of the invidual elements in the course of contamination and the authentication of the distribution of these elements in various parts of the bones we studied the content of various elements in part of the bones of the assemblage found in the Gerulata locality.

The evaluation of the samples according to the degree of their soiling was done visually and microscopically and the samples were divided into four groups.
Group A — fully compact, clean and colourless samples,
B — coloured, slightly non-homogeneous samples, with only vestiges of soiling,
C — non-homogenous samples, with easily perceptible vestiges of soiling,
D — spongy samples, with their cavities filled with earth.

2,48	9,67	15,8	22,4	x̄
1,02	5,85	6,38	8,5	s

Ti — $x_s = 1,0$ — $x_p = 7,5$

1,91	25,1	3,42	35,3	x̄
13,4	9,7	14,1	17,8	s

Mn — $x_s = 0,3$ — $x_p = 90$

2,90	3,50	7,09	14,75	x̄
1,06	1,32	2,71	4,32	s

Co — $x_s = 1,5$ — $x_p = 12$

30,5	32,4	38,4	41,4	x̄
10,9	12,9	12,2	13,5	s

Cu — $x_s = 14$ — $x_p = 19$

0,36	1,07	1,27	1,53	x̄
0,26	0,45	0,61	1,05	s

Cr — $x_s = 0,2$ — $x_p = 10$

205	226	249	262	x̄
53,9	50,6	59,6	44,5	s

Zn — $x_s = 210$ — $x_p = 10$

0,87	1,52	2,01	2,60	x̄
0,63	0,65	0,52	0,69	s

Ni — $x_s = 0,2$ — $x_p = 10$

FIGURES 1—7. *The dependence of the content of the given element in the bone according to the degree of contamination of the sample. x̄ expresses the mean value of 14 samples, s respective standard deviation, X_s is the mean content of the given element in recent bones, X_p is the content of the element in the soil extract of the Gerulata locality. All values are indicated in µg/g. In Graph for Mn instead of x̄ 3.42 read 34.2*

To ensure the continuity of the individual groups some burials were included in the evaluation only where the burial yielded samples belonging to at least three groups. The number of burials selected for the individual elements amounted to 14. The samples taken for analysis were cleaned mechanically, so that the changes mentioned in the graphs are soilings and contaminations difficult to remove mechanically.

The results for the individual elements are indicated in *graphs 1—7*. *Graph 8* is a summary of the previous graphs, including the integration of concentration scales through the calculation of the individual means for the contents of group A as 100 %. *Graphs 1—7* present in every group mean X and standard deviation s, while X_s is the content of the given element in recent bones and X_p the content of the element in the soil extract up to 1 M HNO_3 of the Gerulata locality.
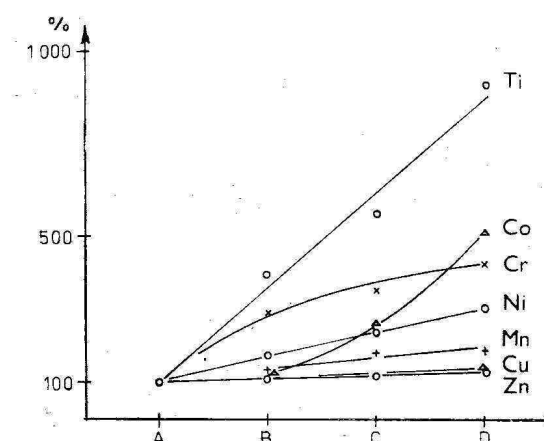

FIGURE 8. *Comparison of the dependences according to Figs. 1—7, by integrating the concentration scales.*

Although the degree of contamination was determined quite empirically, the relationship between the elements indicated is quite unequivocal. There are very conspicuous differences in the chemical character of the elements. The steepest dependence on the degree of contamination is exhibited by titanium, where the difference between the content in the soil (the value indicates the contents in soil extract, the overall Ti content in the given locality varies around 1 per cent) and between recent bones is very high. On the contrary the difference between Ti content in recent bones and bones in group A of the Gerulata locality (and also elsewhere) is small. The above contents and dependences show that titanium is a typical element causing surface contamination and can be suitably used as an indicator of surface contamination of a concrete sample.

The situation is similar with Co, Cr and Ni, only the respective curves are not so steep, in view of the rather low content of these elements in the soil.

Manganese exhibits the highest disproportions between the contents in recent bones and the contents in all groups. There is little probability that the skeletons of the populations studied exhibit increased content of the above elements. It follows from the dependence that manganese can form true solutions, it can penetrate into the bones and can be absorbed even by the compact parts. The relatively small dispersion of values also supports the conjecture that the Mn content is caused firstly by the sorption and by the ion exchange with the external environment. This dependence, on the other hand enables us to indicate through manganese content, the degree of the contamination of the bones with ions able to form true solutions in the conditions of the given soils.

Small changes were observed in potassium and lithium, corresponding to the changes of manganese content. In prehistoric bones there is a slightly increased content, not dependent very much on the place of sampling, documenting the permeability of the compact parts by the ions and the slight concentration of these elements through sorption, and above all through the exchange of ions. The increase in the content is relatively low and the applicability of this dependence for the indication of contamination can be successful in special cases only.

As regards copper content, there are small differences among recent bones, bones in the studied localities and in the soil, and contamination is of little importance.

Similarly there is also very low contamination with zinc, in view of the relatively low content of this element in the soil.

In other elements, such as Ca, Sr, Na, Ag, V and Pb the content, both in all groups of bones, and also in the soils, is very similar. If the dependences of the degree of contamination on the external conditions and on the chemical character of the elements are evaluable, as a rule they do not provide an easy survey, they are obviously covered by micro-non-homogeneities in the distribution of the elements.

CONCLUSION

It has been proved that the bones buried in the soil are exposed to diagenetic changes in a greatly varying degree. Thus we can find parts differently contaminated in the same bone and the selection of the sample for the analysis is of special importance. The best results can be obtained from the edge opposite the lesser trochanter of the femurs (see *Fig. 9.*), where we find the largest compact area of the bone and the course of the diagenetic changes is the slowest. It is also important to ensure the possibility of taking samples which can be reproducible in the whole set of finds.

It is recommended to assess in all samples whether the given concrete sample is contaminated and the degree of contamination. It is very advantageous to make use of the determination of the content of titanium, possibly of cobalt, chrome or nickel as indicators of surface contamination. If the above elements appear in increased degree in the compact bone, we can conclude that the given area is degraded to a high degree, and the results of the analyses of all elements should be interpreted with great circumspection. An increased content of manganese, possibly of potassium and lithium, indicates the degree of ion
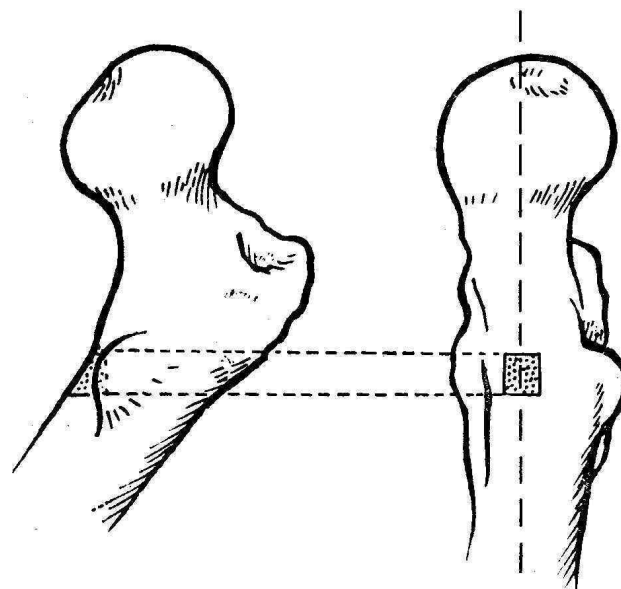
FIGURE 9. *Recommended place of sampliug.*

permeability of the compact bone. The determination of sodium, the most important element, as a rule is not influenced by changes in permeability—in view of its high content in the bones; on the contrary, we can expect a slight decrease through leaching.

REFERENCES

BRÄTTER P., GAWLIK D., LAUSCH J., RÖSICK U., 1977: On the Distribution of Trace Elements in Human Skeletons. *J. of Radioanal. Chem. 37:* 393—403.

KLEPINGER L. L., KUHN J. K., WILLIAMS W. S., 1986: An Elemental Analysis of Archeological Bone from Sicily as a Test of Predictability of Diagenetic Change. *Am. J. of Phys. Anthrop. 70:* 325—331.

LAMBERT J. B., SIMPSON S. O., SZPUNAR C. B., BUIK-STRA J. E., 1984: Ancient Human Diet from Inorganic Analysis of Bone. *Acc. Chem. Res. 17:* 298—305.

LeROY V. M., LINCOLN A. J., 1974: Spectrochemical Method for the Determination of 36 Elements in Industrial Effluent. *Anal. Chem. 46:* 369—373.

OTRUBA V., JAMBOR J., VRCHLABSKÝ M., SOMMER J., 1982: Multielemental Spectrochemical Analysis of Potable, Surface and Waste Waters. *Chem. listy 76:* 1199—1207.

OTRUBA V., JAMBOR J., SOMMER L., 1983: Determination of Small Amounts of Alkali Metals and Alkali Earths in Metallic Sodium. *Chem. listy 77:* 994—999.

SCHOENINGER M. J., 1979: Diet and Status at Chalcatzingo: Some Empirical and Technical Aspects of Strontium Analysis. *Am. J. Phys. Anthrop. 51:* 295—310.

SMRČKA V., JAMBOR J., SALAŠ M., 1988: Výživa v 1.—2. stol. n. l. na severní hranici římské říše. *Anthropologie, XXVI/1* (The Diet in the 1st—2nd Centuries A. D. along the Northern Limes of the Roman Empire).

SZPUNAR C. B., LAMBERT J. B., BUIKSTRA J. E., 1979: Analysis of Excavated Bone by Atomic Absorption. *Am. J. Phys. Anthrop. 48:* 192—202.

Dr. Jaroslav Jambor
Dept. of Analytical Chemistry
Faculty of the Natural Sciences
of the University of J. E. Purkyně
Kotlářská 2
611 37 Brno
Czechoslovakia