4. References

[1] Carson, B. L., Ellis, H. V., and McCann, J. L., Toxicology and Biological Monitoring of Metals in Humans, Lewis Publishers, Inc., Chelsea, MI (1986).

[2] Joselow, M. M., Tobias, E., Koehler, R., Coleman, S., Bogden, J., and Gause, D., Amer. J. Publ. Health 68, 557 (1978).

[3] TerHaar, G. L., Griffing, M. E., Brandt, M., Oberding, D. G., and Kapron, M., J. Air Poll. Control Assoc. 25, 858 (1975).

[4] Tsalev, D. L., and Zaprianov, Z. K., eds., AAS in Occupational and Environmental Health Practice, Volume I, CRC Press, Boca Raton, FL (1983).

[5] Gianutsos, G., Seltzer, M., Saymeh, R., Wu, M., and Michel, R., Arch. Tox. 57, 272 (1985).

[6] Paul Saltman, University of California at San Diego, personal communication.

[7] NIOSH/OSHA “Health Guide for Manganese,” in “Occupational Health Guidelines for Chemical Hazards,” DHHS (NIOSH) Publication 81-123 January, 1981.

[8] Versteeg, J., and Cornellius, R., Anal. Chem. A16, 217 (1980).

[9] Buchet, J. P., Roels, H., Lauwerys, R., Bruxa, P., Clays-Cloetoo, L., Lafontaine, A., and Verduyn, G., Env. Research 22, 95 (1980).

[10] Subramanian, K., and Meranger, J., Anal. Chem. 57, 2478 (1985).

[11] Veillon, C., Anal. Chem. 58, 851A (1986).

[12] Laboratory Procedures Used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (HANES II) 1976–1980, Elaine W. Gunter, Centers for Disease Control Laboratory Manual, Atlanta, GA 30333.

[13] Jarvisalo, J., Olinuorin, M., Tossavainen, A., Virtamo, M., Ristola, P., and Aitio, A., in Chemical Toxicology and Clinical Chemistry of Metals, Brown, S., and Savory, J., eds., Academic Press, London (1983).

[14] Wald, A., Sequential Analysis, Wiley, New York (1947).

[15] Welz, B., Atomic Absorption Spectroscopy, Verlag Chemie, Weinheim/New York (1976).

[16] Slavin, W., Carrick, G., Mannig, D., and Pruszkowska, E., At. Spectrosc. 4, 69 (1983).

[17] Slavin, W., and Carrick, G., At. Spectrosc. 6, 157 (1985).

Appropriate Reference Parameters for the Evaluation of Elemental Analysis Data from Biomedical Specimens

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Studies on the elemental composition of biological systems can be divided into four stages: experimental design, collection of valid samples, chemical analysis and data evaluation (and interpretation). Each of these steps is important for the overall success of an investigation. In our opinion, one aspect of data evaluation and interpretation of trace element studies, namely relating the elemental analysis data to a meaningful base, has not received adequate attention. In many cases this has resulted in wrong conclusions being reached, even if the elemental analysis has been carried out correctly. This frequently happens when the biological material consists of several components with different elemental content, and the ratio of these components differs from sample to sample [1].

Elemental analysis in bone samples is an example of this phenomenon, as several elements are unevenly distributed between the different bone compartments. The fluorine content of the trabecular bone, for instance, is greater than that in the compact substance by a factor of 3 [1]. As the ratio of spongy to compact substance varies along the bone, different fluorine contents are estimated for the whole sample, according to where the sampling has been carried out, although no changes in the fluorine levels in the different bone tissues have occurred. Forbes et al. [2] have reported a similar phenomenon for lead within a rib; expressed on the
basis of ash, Pb content decreases as the distance from the costochondral junction increases. This has been attributed to the increased ash content of the samples as the fraction of marrow changes. Therefore, it is necessary to specify the location of the bone section analyzed and its marrow content.

Similarly, the results of elemental analysis of whole blood may depend on the percentage of red blood cells in the sample [1]. This should always be considered when the elemental content is much higher in erythrocytes than in the blood plasma, as for instance, in the case of iron and lead. If not, changes in the hematocrit, which can occur as a function of several factors (such as pregnancy, age or disease) may lead to false conclusions being drawn about the quantities of these elements in the body.

Other examples of this source of error are the changes in the age distribution of erythrocytes after blood loss [3], which results in a higher zinc content of the red blood cells, changes in the water content of serum samples according to the body position during sampling (recumbent or upright) [4], or changes in the amount of fat or residual blood in a tissue, all of which can lead to alterations in the content of several elements.

Errors can also occur by redistribution of elements or water in certain organs due to the interruption of metabolic processes. For instance, significant changes in the weight of the whole organ can occur under post-mortem conditions [5]. This can lead to great differences in elemental concentrations, depending on whether they are related to the wet or the dry weight of the material. In such cases, it is necessary to know the total weight of the organ, as well as its water content, to be able to account for changes in elemental concentrations. It is obvious that meaningful data interpretation is not possible without this knowledge.

Many of the errors alluded to above can be avoided if one does not express the analytical value only in the usual way of amount per volume or weight of the sample, but also relates the data to other parameters such as the number of certain cells or the content of a protein. This enables changes in the tissue composition to be taken into account. For instance, in whole blood analysis errors due to changes in the hematocrit can be excluded, if the amount of hemoglobin, which reflects the number of red blood cells, is used as a base for expressing elemental concentrations.

Some examples of relevant parameters needed to evaluate elemental analysis data are shown in table 1. In table 2 additional features are listed, which should also be checked when elemental data are measured in biological materials.

The authors recognize that it is difficult to recommend a most suitable base, since this generally depends on the type of study and on the problems to be investigated. However, one can improve the present situation by paying adequate attention to documentation of supplemental information on relevant parameters and by including them in scientific publications. This would facilitate a meaningful intercomparison of results from different investigations.

In conclusion, it is important to present results from biological trace element investigations in an unambiguous way. A multidisciplinary approach is essential throughout the whole investigation. Only then will it be possible to eliminate the influence of presampling factors [6], to obtain biologically and analytically valid specimens for analysis [7,8] and to choose the most appropriate parameters for data evaluation and interpretation.

Table 1. Parameters for evaluation of elemental analysis data in biological specimens

| Specimens          | Parameters                                          |
|--------------------|-----------------------------------------------------|
| Body fluids        | Protein, water                                      |
| Serum, plasma      | Hematocrit                                          |
| Whole blood        | 24 h volume, creatinine                             |
| Urine              | Volume                                              |
| Sweat              | Meconium, cells                                     |
| Amniotic fluid     | Average daily output, fat content                   |
| Milk               | Sperm count                                         |
| Semen              | Volume                                              |
| Cells              | Cell number and Haemoglobin (for RBC)               |
| RBC, WBC, Platelets, Spermatozoa |                                 |
| Soft tissues       | Whole weight of the organ                           |
| Organ samples      | Age of the placenta                                 |
| Placenta           |                                                    |
| Hard tissues       | Percentage marrow, ratio spongy/compact, Ca/P ratio.|
| Bone               | Ratio pulp/enamel                                   |
| Teeth              | Distance from surface                               |
| Hair               |                                                    |
| Diet(s)            | 24 h collection weights, average body weights of subjects |
| Feces(s)           | 24 h collection weights, average body weights of subjects |

* Specific Gravity, dry/wet weight ratios, common for all fluids, moisture content common for both hard and soft tissues, diets and feces.
* Expressed as intake/day.
* Expressed as excretions/day.
Table 2. Checklist of essential features related to elemental analysis of biological systems

| Specimen        | Required information                                                                 |
|-----------------|---------------------------------------------------------------------------------------|
| Plasma          | Amount and type of anticoagulant and its chemical purity                               |
| Serum           | Body position during sampling, hemolysis status                                        |
| Milk            | Days past partum, specific fraction (hindmilk, foremilk) if entire volume not collected|
| Cells           | Viability, (cell age), amount. Type and purity of stabilizer used, trapped plasma      |
| Soft tissues    | Residual blood, decidual tissue while handling placenta, biopsy or autopsy              |
| Hard tissues    | Biopsy or autopsy, renal function status sampling location                              |
| bone            |                                                                                        |
| hair and nail   | Origin and washing procedures for hair and nail                                        |
| Diets           | Proximate composition, caloric energy                                                  |
| Feces           | Occult blood                                                                          |

References

[1] Behne, D., J. Clin. Chem. Clin. Biochem. 19, 115 (1981).
[2] Forbes, W. F., Finch, A., Esterby, S. R., and Cherry, W. H., Studies of trace metal Pb levels in human tissues—III. The investigation of Pb levels in rib and vertebra samples from Canadian residents, in Trace Substances in Environmental Health-X, Hemphill, D. D., (ed.), University of Missouri, Columbia, MO, 41 (1976).
[3] Gawlik, D., Behne, D., and Gessner, H., Trace Ele. Med. 2, 64 (1985).
[4] Juergensen, H., and Behne, D., J. Radioanal. Chem. 37, 375 (1977).
[5] Iyengar, G. V., J. Path. 134, 173 (1981).
[6] Iyengar, G. V., Anal. Chem. 54, 554A (1982).
[7] Iyengar, G. V., and Kollmer, W. E., Trace Ele. Med. 3, 25 (1986).
[8] Iyengar, G. V., J. Radioanal. Nucl. Chem. 112, 151 (1987).

Ultra-Trace Elemental and Isotopic Quantification for Neonatal Nutrition Studies

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Trace element accumulation in the human fetus occurs primarily in the third trimester of pregnancy, and premature birth interrupts this process. A study of zinc in low birth weight infants indicates that the fetus accrues 310 μg of Zn daily at the 30th week, increasing to 590 μg daily by the 36th week of gestation [1]. Similarly, the human fetus accumulates 80–90 μg of Cu/kg/day between 28 and 36 weeks, and by 40 weeks the fetus has accumulated almost 20 mg of Cu, one half of which is in the liver [2]. These and other essential trace elements play vital roles in the adult. Zinc is required for the synthesis of DNA, RNA, and protein, and as the zinc metallo-enzyme, regulates growth through DNA polymerase, RNA polymerase and thymidine kinase. References to these effects have been summarized [3]. Significant progress has been achieved recently using stable isotope tracers to assess the metabolic and nutritional roles of Ca [4], Zn [5], Se [6], and Mg [7] in adults and healthy infants. Techniques used for these studies include mass spectrometry, using electron impact ionization of metal chelates or thermal ionization of inorganic species. Trace elements at higher concentrations or in large samples have been determined with techniques such as atomic absorption (AA) or emission spectroscopy that do not address measurement needs for trace element and isotope tracer determinations in premature infants, healthy newborns, children, pregnant women, and other adults.