Institute of Forensic Medicine[1] and Second Department of Surgery[2], Medical University, Debrecen, Hungary, Center for Functional Imaging[3], Lawrence Berkley National Laboratory, University of California, Berkely, Institute of Inorganic Chemistry[4], L. Kossuth University, Debrecen, Hungary

Distribution of inorganic elements in human autopsy tissue

G. Somogyi[1], E. Jenei[1], J. Szokol[1], L. Buris[1], L. Buris jr.[2], D. Nagy[3] and L. Pap[4]

In the practice of forensic medicine, we find many of cases of death where the actual cause is not determinable with autopsy, histological or toxicological examinations. In these cases of death, we can consider cardiac dysfunction of unknown origin, in the background of which such a physiologic cardiac insufficiency occurs that cannot be detected with the previously mentioned methods. The dysfunction is possibly associated with a significant change in certain inorganic elements, primarily in the conduction system of the heart. In the absence of published data, our goal was to determine the concentration of inorganic elements in the specialized rhythm determining muscle cell groups in the heart: sinus node (SN), atrioventricular node (AV), septum (SE), left ventricle anterior wall (LVAW). With microwave technology we destroyed the muscle tissue and measured the concentration of ions (Na, Mg, K, Ca, Mn, Fe, Cu, Zn, P, S) using Inductive Completed Plasma Atom Emission Spectrometry (ICP-AES) equipment. Of the 24 cases examined, the average ion concentrations in μg/g were the following; Sinus: Na 2602 ± 493, Mg 120 ± 24, K 1787 ± 347, Ca 244 ± 41, Mn 0.129 ± 0.011, Fe 58 ± 12, Cu 2.171 ± 0.46, Zn 10.4 ± 2.027, P 1147 ± 227, S 2301 ± 245; Septum: Na 1452 ± 315, Mg 243 ± 56, K 3269 ± 689, Ca 105 ± 26, Mn 0.17 ± 0.05, Fe 74 ± 16, Cu 3.557 ± 0.952, Zn 25.75 ± 8.4, P 2764 ± 494, S 3001 ± 523; Av: Na 2614 ± 517, Mg 242 ± 40.2, K 2010 ± 395, Ca 271 ± 27.3, Mn 0.13 ± 0.029, Fe 54 ± 12, Cu 2.369 ± 0.297, Zn 15 ± 3.2, P 1625 ± 291, S 2535 ± 346; Lvaw: Na 1340 ± 201, Mg 250 ± 37, K 3659 ± 532, Ca 88 ± 22, Mn 0.175 ± 0.05, Fe 76 ± 19, Cu 3.62 ± 0.58, Zn 27.13 ± 3.1, P 3025 ± 441, S 3140 ± 440.

1. Introduction

We recall homeostasis to be the total of biochemical and biophysical occurrences which guarantee the system, and within this the normal working of the heart muscle. Homeostasis also covers the comparative constancy of the ion concentrations.

Any significant change in this can easily lead to disturbances in the system, or in some cases to death. For this reason the precise information about different organs inorganic elemental composition is important in medical practice. There has been a demand for the determination of ions qualitatively and quantitatively since it was discovered that the elements which are present serve important biological functions – often in very small concentrations. The first articles related to this subject appeared in the 1930's. For quite a while the examinations were limited by the instrumentation as well as the inadequacy of the available methods. A strange paradox was that in contradiction to the almost boundless development of technical possibilities, the majority of data in the growing number of publications were incomparable and almost useless. The main reason for this: the use of differing methods, imprecise sampling, differing sample preparation, and often the use of uninterpretable concentration units (eg: in the case of muscle tissue μg/ml).

Whether the ratio of potassium to sodium is suitable for the early diagnosis of myocardial infarction has already been discussed in 1977 [1]. For similar reasons Speich [2, 3] examined the concentrations of Mg, K, Na, Ca, Zn, Pb and Cd in the myocardium, while Lockvard and Bloom [4] examined the mineral content of the same muscle. More relationships were found between the functional disturbance in the myocardium and the ion concentration.

Iyengar et al. [5] published a book in 1978 containing a large amount of data. However, possible reasons for the large deviations were not examined. Versieck [6] calls our attention to the need for examining possibilities for error, while Stacchini [7] emphasizes that reliable data are key in clinical, -toxicological practice, or as the case may be, in the preparation of environmental studies.

There are very few articles dealing with the examination of post mortem samples. Webb [8] examined the element composition of cattle and pig heart and came to the conclusion that there was a unclear connection between the elemental composition and the physiological function.

Brandt [9] measured the quantity of Na, K, Mg and Ca taken from the left and right sides of human myocardium. He discovered a striking difference between the element concentrations of the left and right ventricles. At the same time, he did not find an acceptable connection between the element concentration and the deaths.

Elwood [10] studied the myocardial tissue of individuals who died of ischemic heart disease. He found that there was a significant difference in the content of Mg and Ca when comparing samples from individuals who died of other causes. These results were supported by the hypomagnetic model examinations of Beasly [11] and Fiset [12].

Smythe [13] examined the myocardial tissue of individuals who died of chronic uremia. A significant increase was found in the content of Ca, Cd, Sr and Mo while the K and Rb content was significantly decreased. Dialysis did not significantly influence the inorganic ion concentration. In similar examinations, Kenneth [14] found increased concentrations of Fe (increased hemachromatosis) and P, and stressed Co as a pathologically influencing ion.

Aalbers [15] tested the relationship between arteriosclerosis and the concentration of inorganic elements. He found that autolysis is insignificant for 2 days after death, thus ion concentrations are nearly stable for 2 days.

Martin [16] studied the connection between age and the concentration of 9 elements in post mortem tissues. He observed that with the advancement of age, the concentrations of K, Mg and Cu are substantially lower than the average, while Mn is significantly higher. The accumulation of Cr and Al was prominent.

Durak [17] investigated the relationship between the Ca deposits in heart valves and the ion concentrations of Fe, Cu, Zn, K, Ca, Na, carbonate and phosphate. He found that the ratio of ions compared to each other was important, especially the change in the ratio of Mg and Ca.

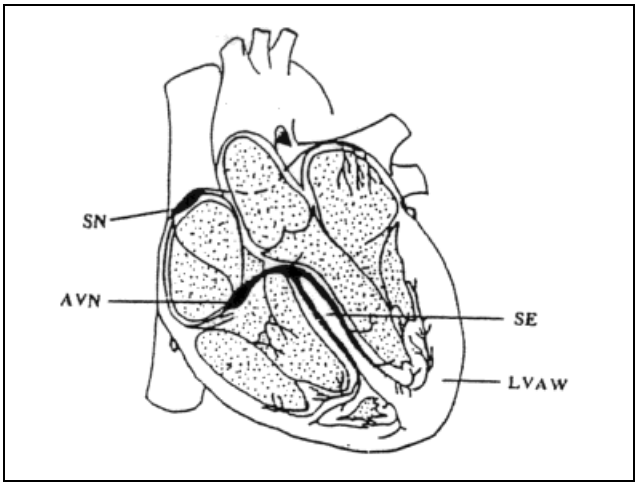

Fig. 1: Conduction system of the heart from which areas samples were taken. SN: sinus node, AVN: atrioventricular node, SE: septum, LVAW: left ventricle anterior wall

Oster [18] et al. tested the connection between coronary disease and the concentrations of Se, Cu, Zn, Fe, Mg, K and P. They found a relationship between the psychological functional parameters of the left ventricle and the concentration of ions. Especially significant was the content of Cu and Se, while the content of K and Mg had no important effect. The increase in the concentration of Fe and Zn increased the effectiveness of the myocardium, and decrease in P content resulted in decreased contraction due to the appearance of fibrotic tissues.

Meyers [19] and Corti [20] investigated the role of iron in diseases of the heart and the vascular system. They were unable to unambiguously prove their presumption that iron catalyzes the formation of free radicals.

In the practice of forensic medicine we see several cases of death, where a concrete cause cannot determined by autopsy, histological or toxicological examinations. In these cases, it is possible to state that the cause of death is a heart failure of unknown origin. The functional disturbance is probably related to a significant change in the concentrations of certain elements, particularly in the heart's stimulus producing and conducting system.

Our aim was to determine the average concentration values of microelements that are present in the rhythm determining sites of the heart sinus node (SN), atrioventricular node (AV), septum (SE), left ventricle anterior wall (LVAW).

Additionally we hoped to discover a relationship, for instance between age, sex, previous illness, cause of death, or, as the case may be harm from the environment and the measured ion concentrations. We were further interested in the question whether occasional or regular use of drugs influences these values. We wish to place special emphasis on the Retrometabolism Based Drug Design (RBDD) method of determining substances [21, 22].

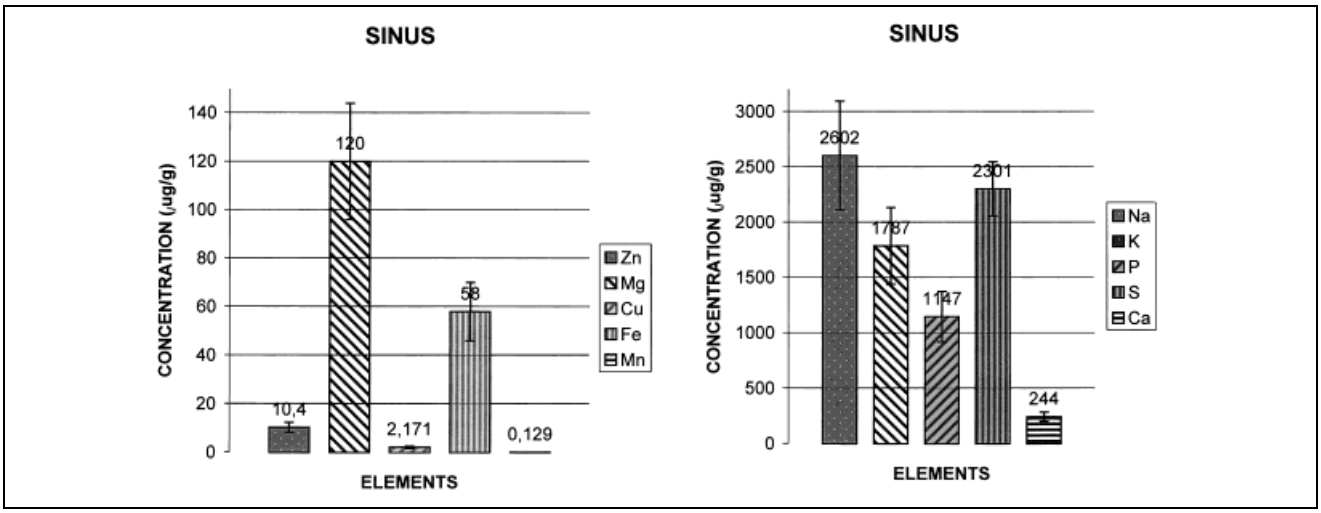

Fig. 2: Distribution of trace elements in the sinus node

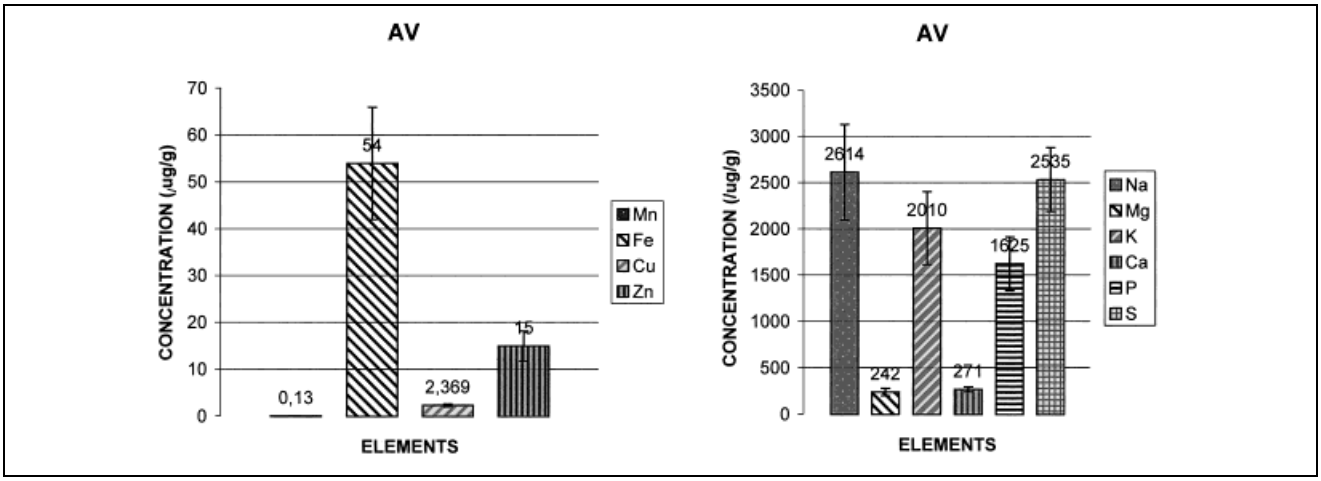

Fig. 3: Distribution of trace elements in the av node

Until now, our work has shown well-reproducible methods (destruction: microwave technique [23] concentration determination: ICP-AES method [24]) for sampling and the determination of the concentration of ten elements (Na, Mg, K, Ca, Mn, Fe, Cu, Zn, P, S). In our present article, we demonstrate the elements composition in the myocardium of 24 corpses. We wish to use data as a reference domain.

2. Investigations and results

2.1. Sampling

We examined the myocardium of 14 male and 10 female corpses. (The corpses ages were between 16 and 32 years). Some were accident victims that had no prior history of illness. Drugs and alcohol were not detected during the post mortem toxicological screening. In order to decrease contamination, the samples of myocardium were cut out on a teflon plate. Sharpened quartz tubes were used for the cutting.

2.2. Preparation of samples

The extracted samples were cooled in liquid nitrogen to minus 190 °C and crushed to powder immediately afterwards in a teflon mortar. Immediately following this we transferred 1–2 g to a quartz pot. We added a mixture of HNO_3 and H_2O_2 and let it stand for 24 h.

2.3. Microwave destruction

We destroyed the earlier prepared samples by means of microwave energy and avoided contamination.

2.4. Determination of concentration

The destroyed samples were put into divided test tubes which were sterilized using gamma radiation, and the element concentrations are measured by the ICP-AES method. For Zn and Cu element the concentrations were also measured by atom absorption spectrometry.

2.5. Results

Fig. 1. shows the exact places from which the samples were taken. Figs. 2–5 demonstrate the average determined element concentration (μg/g) and their standard errors.
Tables 1 and 2 show the average ion concentration and the deviation from average in percentage, respectively. The average value is considered to be 100 %.

3. Discussion

According to the data in Table 1, nearly identical concentrations were measured in the SN and AV node samples, with the exception of Mg. Very similar concentrations, but differing from those mentioned previously, were measured in the SE and LVAW samples. The latter valves were sig-

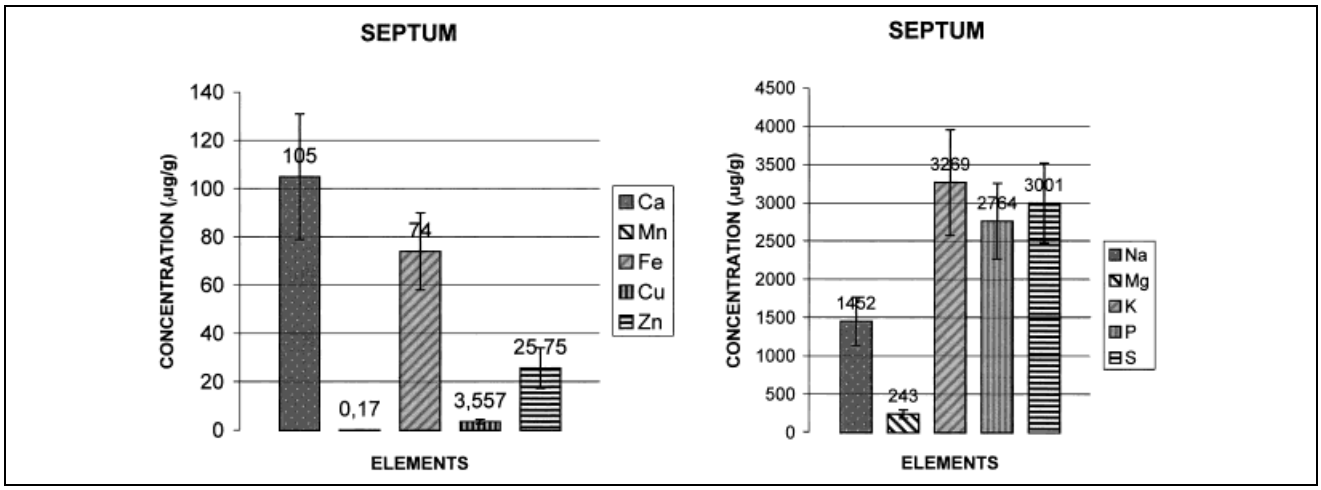

Fig. 4: Distribution of trace elements in the septum

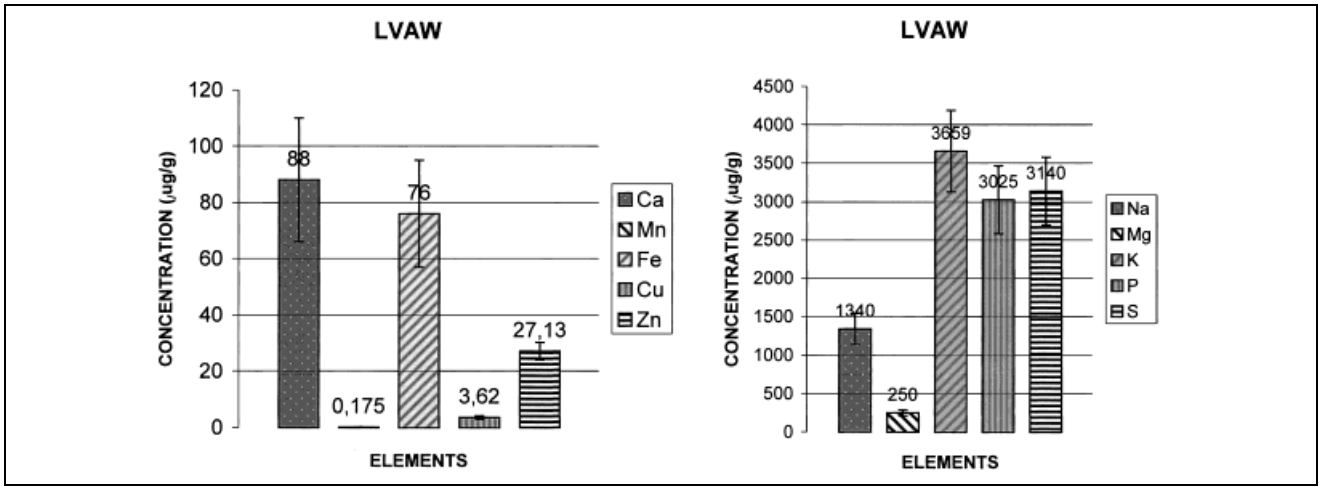

Fig. 5: Distribution of trace elements in the left ventricle anterior wall

Table 1: Average ion concentration in μg/g

	SN	SE	AVN	LVAW
Na	2602	1452	2614	1340
Mg	120	243	242	250
K	1787	3269	2010	3659
Ca	244	105	271	88
Mn	0.129	0.17	0.13	0.175
Fe	58	74	54	76
Cu	2.171	3.557	2.369	3.62
Zn	10.4	25	15	27.13
P	1147	2764	1625	3025
S	2301	3001	2535	3140

Table 2: Deviation from average concentrations in %

	SN	SE	AVN	LVAW
Na	18.9	21.7	19.7	15.0
Mg	20.0	20.0	16.6	14.8
K	19.4	21.0	19.6	14.5
Ca	16.8	24.7	10.0	25.0
Mn	8.52	29.4	22.3	28.5
Fe	20.6	21.6	22.2	25.0
Cu	21.1	26.7	12.5	16.0
Zn	19.4	32.6	21.3	11.4
P	19.8	17.8	17.9	14.5
S	10.6	17.4	13.6	12.7

nificantly higher, with the exception of Na and Ca where the measured concentration was lower in the SE and LVAW. The Mg concentration in the AVN was also nearly identical to the concentrations found in the SE and LVAW samples. Only in the SN sample a significantly lower concentration was found (approximately half). Larger deviations occurred in those elements where the concentrations were very low: Mn, Cu, Zn. The lowest deviation was found for S.

As there is no published data, we can consider these ion concentrations as reference values. The standard error values are quite good (±8.52–32.6%) especially for a biological matrix.

4. Experimental

4.1. Sampling

The hearts extracted from the corpses were dried with filter paper and sliced according to Wichow. In order to avoid iron contamination a teflon knife was used. The extraction of the sample was done on a teflon plate using a 2 cm diameter sharpened quartz tube with a drill movement.

4.2. Sample preparation

The quartz tube extract samples were cooled to approximately −190 °C and were crushed to powder in a teflon mortar. Between 1–2 g was weighed correctly to five decimal places. Four ml of HNO_3 (p.a.) and 1 ml H_2O_2 (p.a.) was added. It was then left to stand for 24 h.

4.3. Microwave destruction

Milestone 1200 mega closed system microwave digestion was used (excited frequency: 2450 MHz, maximal performance: 600 w).

4.4. Analysis

Spectro spectroflame ICP-AES multielement method was used with argon gas (excited frequency: 27.1 MHz, nebulizer: concentric angle nebulizer, plasmagas velocity 1.5 l/min, cooling gas velocity 15 l/min, velocity of sample carrying gas nebulizer 1 l/min, velocity of sample entry 1 cm^3/min, integration time during the detection: 25–25 s)
Philips Ph 9200X monoelement method (Zn: wavelength 213.9 nm, slit width 0.5 nm, acetylene flow 1.2 l/min, Cu: 328.4 nm, 0.5 nm, 1.1 l/min).

This research paper was presented during the 2nd Conference on Retrometabolism based Drug Design and Targeting, May 11–14, 1999, Amelia Island, Florida, USA

References

1 Decastello, A. et al.: Acta. Morphol. Acad. Sci. Hung. **25**, 289 (1977)
2 Speich, M. et al.: Clin. Chem. **26**, 1662 (1980)
3 Speich, M.: J. Am. Cool. Nutr. **1**, 255 (1982)
4 Lockvard, V. G.; Bloom, S.: Am. J. Pathol. **139**, 565 (1991)
5 Iyengar, G. V.: Sci. Total Environ. **38**, 125, (1984).
6 Versieck, J.: Crit. Rev. Clin. Lab. Sci. **22**, 97 (1985)
7 Stacchini, A. et al.: Ann. Ist. Super Sanita. **25**, 379 (1989)
8 Weeb, J. et al.: Bioinorganic Chemistry **5**, 252 (1976)
9 Brandt, G. et al.: Virchows Arch. A Pathol. Anat. Histol. **369**, 335 (1976)
10 Elwood, P. C. et al.: Artery **9**, 200 (1981)
11 Elwood, P. C. et al.: Lancet 2 (8197), 720 (1980)
12 Fiset, C. et al.: J. Am. Coll. Cardiol. **27**, 1771 (1996)
13 Smythe, W. R. et al.: Ann. Intern. Med. **96**, 302 (1982)
14 Kenneth, S. et al.: Nephron. **34**, 93 (1983)
15 Aalbers, Th. G. et al.: Clin. Chem. **33**, 2057 (1987)
16 Martin, B. J. et al.: J. Trace Elem. Electrolytes Health Dis. **5**, 203 (1991)
17 Durak, I. et al. Am. J. Cardiology. **34**, 355 (1992)
18 Oster, O. et al.: Eur. Heart. J. **14**, 770 (1993)
19 Meyers, D. G.: Clin. Cardiol. **19**, 925 (1996)
20 Corti, M. C. et al.: Ann. Epidemiol. **7**, 62 (1997)
21 Bodor, N.; Buchwald, P.: Pharmacol. Ther. **76**, 1 (1997)
22 Bodor, N.; Buchwald, P.; Huang, M. J. in: Leszczynski, J. (ed.): Computational Molecular Biology. p. 569, Elsevier Amsterdam 1999.
23 Mizushima, R. et al.: Tohoku J. Exp. Med. **178**, 75 (1996)
24 Jenei, E.; Somogyi, G.; Pap, L.; Nagy, D.; Buris, L. jr.; Buris, L.: J. Anal. Toxicol. (submitted for publication).

Prof. Dr. Gábor Somogyi
Institute of Forensic Medicine
Univ. Medical School Debrecen
Nagyerdei Krt. 98
4012 Debrecen
Hungary
gsomogyi@jaguar.dote.hu