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INVESTIGATION OF THE HUMAN SPLEEN BY X-RAY MICROANALYSIS

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Qualitative and quantitative topographic analysis using X-ray fluorescence (XRF), X-ray powder diffraction (XRD) and scanning electron microscopy was performed in tissue samples of rat and human spleens. The presence of silico-aluminium and silico-calcareous particles of various sizes could be seen. The presence of the inorganic substances mentioned in the human red pulp cords is assumed to be a consequence of the purifying function of the spleen.

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1 Introduction

Elementary analysis of animal organs and tissues with respect to the determination of the location of the individual chemical elements remains an intricate problem. In studying ortho- and pathomorphology of animal organs, a range of methods can be used to prove oligo- and polysaccharides, proteins, fats, etc., to provide specific evidence for the organic tissue constituents. A permanent problem concerns identification of low molecular weight substances that are either insufficiently autosomal or lack specific chemical characteristics accessible to microscopic studies. Due to a variety of industrial activities, such substances are gradually increasingly getting into the environment and, in turn, into the internal environment of animals and plants. They may, at other occasions, accumulate in various organs due to some disturbances of systems involved in their elimination.

Until recently, the tools of physical investigation available to identify the elementary composition of organs, tissues and cells in special cases only included polarisation microscope to make visible anisotropic substances.

Analytical methods based on X-ray radiation and electron beams [1,2] utilize various products. The resulting product of the interaction between primary electrons and a sample are transmitted electrons, backscattered electrons (interaction depth from 100 nm to 1 μm), secondary

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electrons (from 5 to 50 nm), Auger electrons, electrons absorbed in the sample, typical X-ray radiation (from 5 μm), light photons. Emission of electrons from the surface area of the sample depends on number of factors including accelerating voltage V, surface morphology and the angle of incidence of the primary beam, sample quality (proton number, density) and surface charge of the sample. The depth R to which electrons can penetrate until their energy falls below the excitation energy of the given element is characterized by the relationship.

$$\rho R = k(E^{\mathrm{n}} - \bar{E}^{\mathrm{n}}), \tag{1}$$

where k and n are constants for given proton number and atomic mass of the material, E is the energy of the primary beam, $\bar{E}$ is the ionisation energy of the given element, and ρ is the material density [3].

Since electrons are unable to penetrate deep into biological samples ($k = 0.033$ A/Z, $n = 1.5$, $R \approx 3\mu$m) and the volume analyzed is small, generally a thin superficial layer only several micrometers thick can be investigated.

Of the products mentioned, morphological analysis utilizes secondary electrons mainly. Secondary, backscattered and absorbed electrons may appropriately be used to determine the composition of the sample studied [4].

X-ray photons are also used for the determination of the elementary composition of the excited volume of a sample. Since this is a local method, we also may, to a certain extent, study the area distribution of the elements in the material studied.

Since atom energy levels are typical of the individual types of atoms, it is evident that the X-ray photon energy is specific for each element and that it provides the information on the chemical composition of the sample.

Silicon and oxygen are the two most common elements in the Earth's crust, so perhaps their diverse modes of organization are not so unexpected. The natural abundance, geological and geophyzical importance, and technological properties of silica makes it one of the most throughly studied compounds.

Its polymorphism is very complex. There is not less than nine different ways of organizing SiO_2, referred to as silicon dioxide or silica. An alternate name for the Quartz Group is the Silica Group [5]. But in reality it is simply a matter of the temperature and pressure, especially at the time of crystallization, that determines which form silicon dioxide will organize into.

Amorphous silicon is currently not considered as pathogenic material. Information concerning its occurrence are occasional, above all because of the lack of methods for its localisation in tissues, organs and cells. SiO_2 particles in the form of crystals that rotate the level of polarized light, from 1 to 5 μm in size, are considered pathogenic, i.e. disease inducing [6,7]. Having got into the lungs, they are engulphed by macrophages. The lysozome apparatus of the macrophages is unable to process them, and the macrophages sooner or later dye. SiO_2 particles therefore get outside of the cells, again. The body attempts to separate such particles from the environment by producing collagen fibres at least. This process however gradually reduces the area available for gas exchange in the lungs, causing subsequent complications. SiO_xH_y particles (crystals?) that induce the process mentioned in animals have been made visible in several works [8,9,10] using light microscope utilizing polarized light. Only few data on the presence of silicon in animal organs, its origin, localisation and impacts have been published so far. The database system PUB-MED [11] contains only four items of information about the occurrence of silicon in hu-

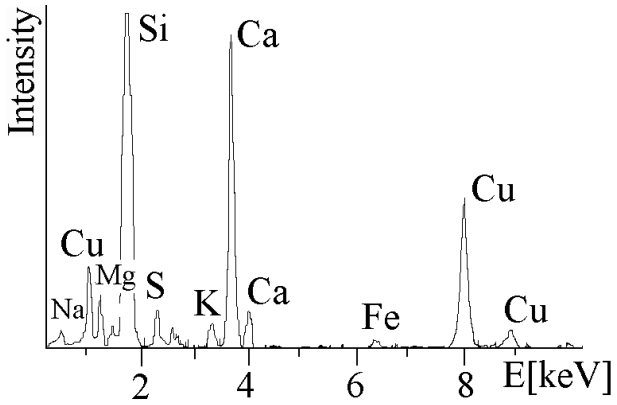

Fig. 1. X-ray microanalysis reveal the presence of silicon, calcium, sulphur, iron, and magnesium. Spectral line of copper is caused by copper layer on the surface. Range 0 - 10 keV. Real time 200 s, live time 140 s, dead time 30%, 1 900 counts/s.

man and laboratory rat spleen, published between 1962 and 2000. We therefore should like to describe our own findings concerning Si and X-ray fluorescence.

2 Sample preparation and experiment

A total of five samples of human spleen tissue and three samples taken from the spleens of Wistar strain rats-males weighing 230 g (Breeding Station of the Slovak Academy of Sciences, Dobrá Voda, Slovakia) were analyzed. The first three samples were from patients suffering from autoimmune thrombocytopenia (AITP). Two control samples were taken from the spleens of stillborn fetuses. All the samples were fixated in 3% glutaraldehyde solution (Serva, Heildelberg, FRG) [12], and were dehydrated in increasing acetone series. The samples were dried in a CPD 030 apparatus (Bal-Tec) at the CO_2 critical temperature (31.2 $^\circ$C, 7.38 MPa). The samples were attached to aluminium or carbon supports and metalised by a 20 - 25 nm thick copper layer by ion sputtering in an SCD 050 device (Balzers, Vaduz, Lichtenstein) [13] to prevent the generation of disturbing charge on the surface of the sample studied.

Investigations were performed in a scanning electron microscope BS 340 (Tesla, Brno, Czech Republic) at the accelerating voltage of 20 kV.

The elementary analysis was performed in a Quantum energy-dispersion spectrometer (Kevex, Valencia, CA) at the accelerating voltage of 20 kV. The spectrum collection time took 200 s within the range of energies between 0.160 and 10.230 keV.

Histological sections from spleens were stained with hematoxylin and eosin. Thus prepared sections were examined by light microscope in polarized light.

3 Results

In addition to current histological [14] examinations including that using polarisation microscope, also X-ray fluorescence and X-ray powder diffraction was performed. No pin-like forma-

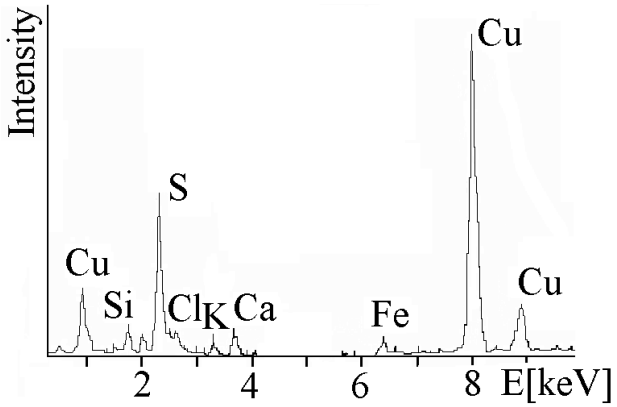

Fig. 2. X-ray microanalysis of particles the multi-elemental composition reveal. Spectral line of sulphur is dominant. Range 0 - 10 keV. Real time 200 s, live time 140 s, dead time 30%, 1 900 counts/s.

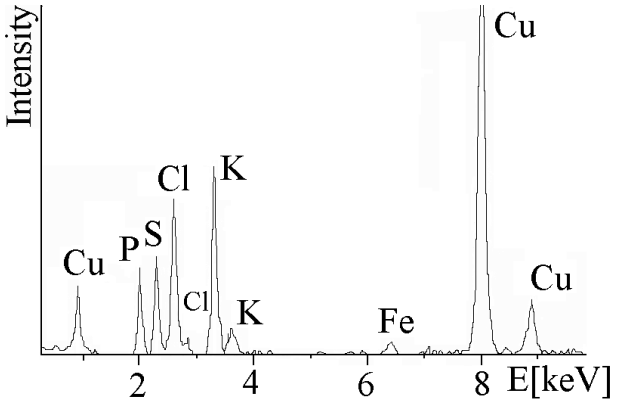

Fig. 3. X-ray microanalysis of stillborn human fetuses. Spectral line of silicon is not present. Range 0 - 10 keV. Real time 200 s, live time 140 s, dead time 30%, 1 900 counts/s.

tions rotating the polarized light level could be seen in polarized light. Multi-elementary composition of particles present in spleen samples could be demonstrated using XRF (Figs. 1 and 2). XRF also enabled to detect silico-aluminium and silico-calcareous particles in the cytoplasm of macrophages in the red pulp cords in spleens of patients with AITP (4, 17 and 25 years old) as well as in the spleens of Wistar rats. They were not found in the spleens of stillborn human fetuses (Fig. 3). The formations were mostly of irregularly shaped various sizes (10 to 30 μm) (Figs. 4 and 5). In rare cases, they were polygonal in shape. Some structures were sometimes also located outside of the macrophage cytoplasm.

Moreover, the presence of magnesium, sulphur, potassium and iron was found in the samples. Unlike our observations, Roperto et al. [15] could also detect zinc. The elements that these authors observed in polarisation microscope appeared as light-blue pin-shaped crystals. Also, they could observe polyhedric and spherical crystals.

The spectral bands (K_α 8.040 keV, K_β 8.904 keV, L_α 0.930 keV) are due to the presence of

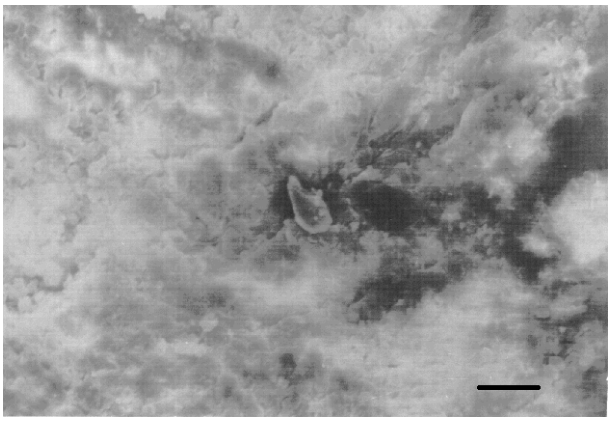

Fig. 4. The particle contain silicon, calcium, sulphur, iron, and magnesium. SEM micrograph, line size is 10 μm.

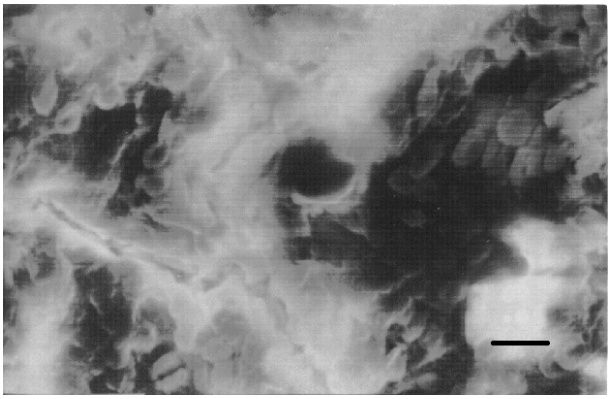

Fig. 5. Particles of inorganic material are visible. Seize and shape of particles are various. SEM micrograph, line size is 10 μm.

copper on the surface of the sample studied. Gold dusted samples give more contrast. On the other hand, the spectral band for gold (M_α2.121 keV) may hide the presence of phosphorus (K_α 2.013 keV) and sulphur (K_α 2.307 keV) [16,17].

The sample 631017 examined by XRD method is a little more crystalline than the other which are wholly cryptocrystalline. Some samples have several diffractions of small ones intensities, but not all concering the silicon. The source of these diffractions is unknown.

4 Discussion

The pathogenic effects of Si angular masses in the pulmonary tissue have been sufficiently studied from a number of aspects [18 - 23]. In amorphous or crystalline state, silicon and/or its compounds have not yet been subject to broader conceived studies. Reports dealing with the presence of silicon in the human spleen or in spleens of experimental animals are rare [24 - 27].

The major reason why the topographic localisation of silicon has not attracted closer attention yet has been the fact that light microscopy does not offer satisfactory methodological approaches. Unfortunately, both XRF and XRD have so far been only rarely used by biological morphological laboratories.

The clarification of the problem in question also requires methodological solution to the following question: Is the presence of silicon (silica) in the spleen due to the biological function of the organ or is it an artificial finding due to the sample processing (contamination from vessel walls)? Silicon has not been identified in samples of human spleens taken from stillborn fetuses, and it is therefore assumed that silicon enters human spleens and to the spleens of Wistar rats in natural ways rather than artificially.

On the other hand the question arises why silicon does not get to stillborn human fetuses from maternal blood circulation via the placenta. Being a substance with a low molecular weight, it is expected to cross the feto-placental barrier without any problems. Two possibilities can be offered to explain the situation:

1. Maternal spleen performs as a blood filter so well that no silicon or its compounds SiO_xH_y can get into the fetal circulation;

2. The amounts of silicon that enter splenic circulation of the fetus remain under the threshold values detectable by XRF. Neither can the fact be ruled out that spleens of human fetuses remain excluded from fetal systemic circulation during the embryonal development [28].

From the X-ray diffraction pattern the presence of some (certain) amorphous, cryptocrystalline or glassy-like phase (compound) is seen. After a light combustion or burning the brown coloured matrix began to be black and carbonise and remained only a trace of black residues as powder or agglomerates of very fine structure.

5 Conclusion

Being a non-destructive method for the investigation of the elementary composition of biological materials, XRF may help to extend our knowledge on some diseases and to help precise their diagnosis.

Our measurements identifying the presence of silicon particles in the red pulp of the human and rat spleen were not artifacts due to tissue processing for morphological studies.

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References

[1] J. Garaj: *Physical and physichemical analytical methods*, Alfa n.p. Bratislava, Bratislava 1977, pp. 239-261

[2] M. A. Hayat.: *Principles and techniques of scanning electron microscopy. Biological Applications*, Van Nostrand Reinhold Company, New York 1974, vol. 1, pp. 9-18

[3] J. C. Russ.: *Fundamantals of Energy Dispersive X-ray Analysis*, Butterworths 984, pp. 42-45

[4] N. Mahy, A. Prats, A. Riveros, N. Andrés, F. Bernal: *Acta Neuropathology* **98** (1998) 217

[5] HTTP://GALLERIES.COM/MINERALS/SILICATE/QUARTZ.HTM#KEATITE

[6] E. Rubin, J. L. Farber: *Pathology*, J. B. Lippincott Company, Philadelphia 1994, pp. 593-596

[7] J. B. Walter, M. S. Israel: *General Pathology*, Churchill Livingstone, Six. Ed., Edinburg 1987, pp. 151-153

[8] C. Brambilla, J. Abraham, E. Brambilla, K. Benirschke, C. Bloor: *Am. J. Pathol.* **96** (1979) 149

[9] P. J. Canfield, T. L. W. Rothwell, J. M. Papadimitriou, J. D. Moore: *J. Comp. Pathol.* **100** (1989) 199

[10] H. J. Hansen, F. M. Jama, C. Nillson, L. Norrgren, O. S. H. Abdurahman: *J. Veter. Med.* **A36** (1989) 189

[11] HTTP://WWW.NCBI.NLM.NIH.GOV/ENTREZ/QUERY.FCGI?DB=PUBMED

[12] P. Mráz, J. Polónyi.: *Electron microscopical methods of animal tissues*, Veda, Bratislava 1988, pp. 33-37

[13] Š. Polák: *Czech. Patol.* **32** (1996) 12

[14] Š. Galbavý, M. Ružičková, E. Surmíková, L'. Danihel, J. Porubský, J. Papinčák, S. Holeša, J. Trnka: *Czech. Patol.* **32** (1996) 19

[15] F. Roperto, A. Troncone, A. Tranquillo, A. Galati: *J. Comp. Path.* **112** (1995) 97

[16] Edax Peak Identification Chart. Edax Inc, 91 McKee Drive, Mahwah, NJ 07430

[17] HTTP://WWW.CSRRI.IIT.EDU/PERIODIC-TABLE.HTML

[18] D. J. Cook: *Cellular Pathology*, Butterworth-Heinemann 1998, pp. 154-155

[19] D. L. Gardner: *Pathological Basis of the Connective Tissue Diseases*, 1. Title. Edward Arnold, London 1992, pp. 498, 320, 964

[20] W. M. Haschek, C. G. Rousseaux: *Handbook of Toxicologic Pathology*, Academic Press Inc., San Diego 1991, pp. 806-808

[21] J. M. Kissane: *Anderson's Pathology*, C. V. Mosby Company, St. Louis 1990, vol. 1, pp. 35-36, 233-235, 996-999

[22] J. D. Harley, J. Margolis: *Nature* **189** (1961) 1010

[23] K. Stadler, W. Stober: *Nature* **207** (1965) 874

[24] S. Barlogova, L. Ulrich: *J. Hyg. Epidemiol. Microbiol. Immunol.* **21** (1977) 247

[25] L. I. Privalova, B. A. Katsnelson, A. B. Osipenko, B. N. Yushkov, L. G. Babushkina: *Environ. Health perspect.* **35** (1980) 205

[26] L. I. Privalova, A. V. Osipenko, V. N. Frash: *Biull. Eksp. Biol. Med.* **82** (1976) 1480

[27] R. Mariani-Costantini, F. S. Jannotta, F. B. Johnson: *Am. J. Clin. Pathol.* **78** (1982) 785

[28] F. Tischendorf: *Blutgefäss – und lymphgefässapparat innerseketorische Drûsen. Sechste Teil Feil die Milz.* In: Handbuch der Mikroskopischen Anatomie des Menschen. (Eds. W. Mõllendorf, W. Barfmann), Springer Verlag, Berlin 1969, pp. 419