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EVENTS OF RUSSIAN HISTORY OF THE LATE MIDDLE AGES IN THE LIGHT OF NEUTRON ACTIVATION ANALYSIS DATA

1. Introduction

The study of the elemental composition of human remains makes it possible to learn a lot about the circumstances of life and death of a person and return from oblivion his fate, which is extremely important not only for experts, but also of interest to history buffs. In world practice the human remains composition study concerned well-known historical figures, for example, the Renaissance astronomer Tycho Brahe (d. 1601), Francesco Caracciolo (d. 1608), as well as ordinary people of the XVth-XVIth centuries, monks and laity (Rasmussen *et al.* 2008, 2013; Kučera *et al.* 2017).

Russian and Soviet researchers also carried out similar investigations. In the 1960s the repair and restoration work in the necropolis of Archangel Cathedral in Moscow Kremlin gave the opportunity to open for the first time the burials of Tsar Ivan the Terrible, his sons Ivan and Fyodor, as well as Prince Skopin-Shuisky (State Research Institute of Forensic Medicine 1964). It was for the first time in Russia, when the studies on microelement composition of the medieval human tissues were carried out. In the 1990s and at the beginning of the XXIst century, in the process of studying the tombs of Russian grand duchesses and tsarinas from the necropolis of the Ascension Cathedral of Moscow Kremlin, the database of the microelemental composition of human remains of the XVth-XVIIth centuries was significantly expanded due to application of modern research methods. So far as the study encounters difficulties in accessing to human remains of the XVth-XVIth centuries, experiments of this kind are carried out quite rarely, and, therefore, new data on the elemental composition of the samples are of significant scientific interest. In 2018 the study of Russian historical figures remains from the necropolis of the Archangel Cathedral, and the Ascension monastery of Moscow Kremlin was carried out at the Frank Laboratory of Neutron Physics (FLNP), Joint Institute for Nuclear Research (JINR) in Dubna, Russia.

2. Samples

The following unique samples were obtained:

– part of the rib of Maria Borisovna, the first wife of Grand Prince Ivan III, d. 1467 (Fig. 1a);
– part of the brain and organic matter from the skull of Grand Princess Elena Glinskaya, the mother of Tsar Ivan the Terrible, d. 1538 (Fig. 1c-d);

Fig. 1 – a) The fragment of the rib of Grand Princess Maria Borisovna; b) the small bone of Tsarevna Feodosia; c) the part of brain of Elena Glinskaya; d) organic matter from skull of Elena Glinskaya; e) the rib bone of M.V. Skopin-Shuisky; f) the strand of hair of Tsarina Anastasia Romanovna; g) the rib bone of Tsarevich Ivan Ivanovich; h) fragments of the rib of Tsaritsa Maria Nagaya. All the samples are presented before cleaning.

– hair of Anastasia Romanovna, the first wife of Ivan the Terrible, the first Russian Tsarina, d. 1560 (Fig. 1f);
– the rib of Tsarevich Ivan Ivanovich, the son of Ivan the Terrible, d. 1581 (Fig. 1g);
– the bone of Tsarevna Feodosia, granddaughter of Ivan the Terrible, d. 1594 (Fig. 1b);
– three parts of the rib of Tsarina Maria Nagaya, the last, sixth wife of Ivan the Terrible, d. 1611 (Fig. 1h);
– the rib of Prince Mikhail Skopin-Shuisky, d. 1610 (Fig. 1e).

To determine the elemental composition of the remains, the method of neutron activation analysis was used. The experiment with the remains of Anastasia, Tsarevich Ivan and Prince Mikhail is described in detail in Panova *et al.* (2018). The work with the last five samples is shown below.

3. Samples analysis

3.1 *Samples preparation*

Preparation of samples for irradiation was carried out in the same way as described in Panova *et al.* 2018, 128-129. The bone samples were cleaned with cotton swabs, first moistened with distilled water, then with ethyl alcohol. Finally, the samples were washed under a stream of distilled water. Samples of the brain and organics were cleaned from visible large dirt, mold, sand, and fibrous inclusions with tweezers.

The samples were dried at temperature of 40°C to a constant mass for about one day. A planetary mill was used for grinding equipped with an agate grinding bowl and six agate balls. Grinding time was chosen from 3 to 5 minutes, speed – 600 rps. Brain and organics samples were grinded by hand in an agate mortar. Three subsamples with mass of 0.10 ± 0.01 g were prepared from each sample. The following standards with similar mass were used: 1633C, 1635A, 2431, 2586, 2684C, 2710A, 2782, 50C, 87A. In addition, flux monitors were prepared – zirconium samples with mass ranged from 0.12 to 0.15 g, which were used to calculate fluxes of thermal and resonance neutrons.

3.2 *Samples irradiation*

The irradiation was carried out for 2 days on the 3rd channel of the IBR-2 reactor. The IBR-2 reactor operated at average power of 1.75 MW. The flux of thermal neutrons was equal to $7.2 \cdot 10^{11}$ n/(cm^2·s), resonance – $1.2 \cdot 10^{11}$ n/(cm^2·s·eV).

3.3 *Data collection and spectra processing*

Samples and standards were repackaged into clean plastic containers five days after the end of irradiation. The first measurements of the spectra

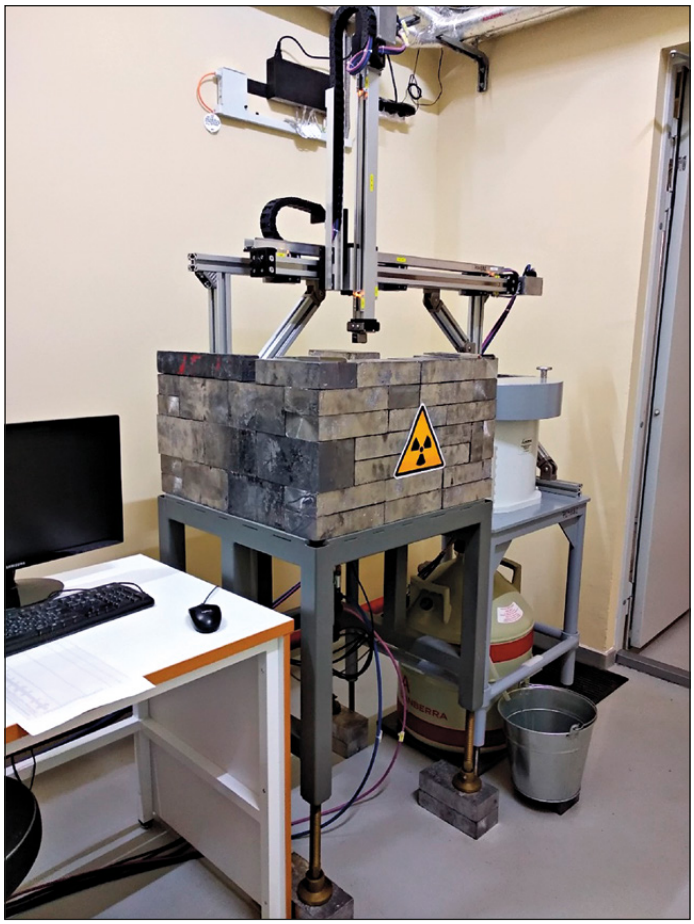

Fig. 2 – Automation system for spectra measurement.

of the induced activity for 1.5 hours were carried out immediately after the repackaging. The second measurements for 4 hours started 22 days after the end of irradiation. Automation system for spectra measurement (Pavlov *et al.* 2014; Frontasyeva *et al.* 2016) (Fig. 2) developed at FLNP JINR was used for data collection. Spectra were measured using the HPGe Canberra GC4018 detector with resolution 2.1 keV for gamma-line of ^{60}Co with energy 1332.5 keV.

The Genie-2000 program was used for spectra processing. The "Concentration" program (Dmitriev, Borzakov 2019) created at FLNP JINR was used to calculate mass fractions of the elements.

	Fe	Ni	Zn	As	Se	Sr	Sb	Hg
Ivan Ivanovich, rib bone	1200 ± 97	-	620 ± 20	0.19 ± 0.1	-	-	0.21 ± 0.05	0.36 ± 0.07
Mikhail Skopin-Shuisky, rib bone	1400 ± 100	-	460 ± 20	0.23 ± 0.1	-	-	< 0.1	0.2 ± 0.06
Anastasia Romanovna, hair	< 1170	-	< 100	1.2 ± 0.2	-	-	2.7 ± 0.4	47 ± 1.2
Maria Borisovna, rib bone	690 ± 30	5.4 ± 1.1	1800 ± 50	1.6 ± 0.1	0.6 ± 0.05	210 ± 10	0.15 ± 0.01	< 0,42
Elena Glinskaya, brain	2900 ± 200	4.4 ± 0.3	70 ± 3	0.3 ± 0.04	36 ± 2	19 ± 1	0.17 ± 0.01	14 ± 1
Elena Glinskaya, organic	3000 ± 280	6.2 ± 0.6	66 ± 5	0.4 ± 0.1	34 ± 1	17 ± 2	0.19 ± 0.01	15 ± 1
Feodosia, bone	150 ± 40	-	250 ± 10	0.5 ± 0.05	-	120 ± 5	1 ± 0.03	1.2 ± 0.1
Maria Nagaya, rib bone	2000 ± 90	-	665 ± 20	0.4 ± 0.06	0.5 ± 0.05	110 ± 5	0.07 ± 0.004	0.9 ± 0.1
ribs of modern Russian people *	135 ± 10	-	89.7 ± 1.6	< 0.1	≤ 0.03	286 ± 19	< 0.01	≤ 0.008
hair of modern Russian people *	95.5 ± 7.5	-	165 ± 3	≤ 0.05	0.248 ± 0.008	43 ± 4	0.0616 ± 0.0053	0.145 ± 0.009
* from Zaichick and Zaichick 2011								

Tab. 1 – Mass fractions of elements, mg/kg.

4. Results

The obtained results are presented in Table 1 and some conclusions can already be drawn. Our study showed the increased arsenic content in the rib of Maria Borisovna, the first wife of the Grand Prince Ivan III of Moscow. Maria was the daughter of Prince Boris of Tver. By mutual decision of Prince Vasily II of Moscow and Prince Boris, the children were betrothed at a very early age in order to strengthen ties between Moscow and Tver. At 26 Maria suddenly died at night.

One of the Russian chronicle composed in Novgorod the Great contains a story about the poisoning of Maria by "mortal potion". Novgorod the Great has been in opposition to Moscow for a long time, defending its Republican rules. The boyars from Novgorod finally obeyed Moscow exactly during the reign of Ivan III of Russia in 1478, and thus in the 1460-70s the relations between the Novgorod boyars and the Moscow princes were especially embittered. It is no coincidence that the version of the poisoning of Maria appeared in the Novgorod chronicle.

Elemental analysis of bone remains from the entombment of Maria in the Ascension monastery of Moscow Kremlin made in 1998 showed the increased content of lead, arsenic and mercury. Lead excess is not surprising for the Middle Ages, it was the result of using cosmetics and therapeutic ointments. Arsenic and mercury are known to have been part of some medieval paints. In 1998 researchers using XRF found out in the bone remains of Maria increased arsenic content (1-6 mg/kg in different samples) and concluded that this amount can be explained by deliberate poisoning (Alexandrovskaja 2015, 233). On the other hand, in the same 1998 emission spectral analysis did not reveal arsenic, but showed increased values of mercury (0.28 mg/kg) (Makarenko 2015, 230).

Our study found out approximately the same mercury content – less than 0.42 mg/kg, it is 18 times higher than the amount of mercury in the bones of modern people (a fragment of a rib was studied in both experiments, in 1998

and 2018). The same quantity of mercury is found out by Rasmussen's group during studying of remains from a medieval monastic cemetery – 0.12-0.48 mg/kg (Rasmussen 2008, 2305). Rasmussen concludes that this amount of mercury can be justified using by monks mercury-containing paints (cinnabar) in the scriptorium, or, more likely, the participation of the friary members in the treatment of patients with the help of mercury ointments. It is therefore possible that mercury entered the body of the Grand Princess for quite a long time. The remarkable thing is that anthropologists have documented fractures along her several ribs, thus it is entirely possible to assume the long-term application of therapeutic ointments, which included mercury and lead (the norm of the latter is also exceeded). Keeping in mind the suddenness of Maria's death, it can be assumed that such a quantity of mercury is not a marker of acute poisoning. However, it was in Maria's bone we found the highest content of arsenic compared to all other samples – 1.6 mg/kg, which is 16 times higher than the norm of this element in modern humans. Perhaps, the quick-acting poison (arsenic) was chosen for Maria. These results most likely confirm the data of the Novgorod Chronicle about the poisoning of the young princess owing to rivalry of Novgorod or Tver with Moscow.

The major mass fraction of mercury in the brain and organics from the skull of Elena Glinskaya, also attracted our attention. In 1533 Elena became regent of young Ivan, in fact, the ruler of Russia, but in 1538 she died unexpectedly. The only source indicating the cause of Elena's death is the "Notes on Muscovite Affairs" written in Latin in 1549 by the diplomat Herberstein: "according to rumors the widow was put to death by poison" (Panova 2017, 88). And if arsenic, according to our study, is 3-4 times higher than the norm, then the quantity of mercury showed maximum values of 15 mg/kg (!).

However, it is necessary to take into account the data of the mercury mass fraction in the soft tissues that is usually higher than in the bones. We also note that, according to toxicologists, it is the brain tissue that is particularly affected by the accumulation of mercury. Rasmussen, whose group carried out an analysis of its content in the remains of people in Danish cemeteries of the XVIth century, confirmed the increased content of mercury in soft tissues compared to bones. Spanish researcher Garcia found out the mass fraction of mercury in the bones of modern man – 0.05 mg/kg, while in the kidneys and liver its content was 0.25 and 0.14 mg/kg (Rasmussen *et al.* 2008, 2295). But even in comparison with these values, the amount of mercury in organic matter from the skull of Elena Glinskaya – 14-15 mg/kg (!) – looks significant. Comparing this amount with the increased mercury content in the bones of medieval Danish monks who used substances comprising mercury – 0.12-0.48 mg/kg (Rasmussen *et al.* 2008, 2305) – we can see that the quantities of mercury in the bones of people who used substances based on it are much lower. It is difficult to acquire gradually the quantity of this toxic mineral

discovered in Elena's body, so we can assume a rapid and deliberate poisoning of the ruler of Russia with a large dose of mercury salts.

Our study also revealed a significant amount of mercury in the hair of Anastasia Romanovna. The young Tsarina died after a short illness in 1560, when she was about 26. Tsar Ivan the Terrible wrote later about the poisoning of his first wife: "Tsarina Anastasia was harried by the enemy's slander, and by malevolent people's witchcraft, and by poisons" (Panova 2017, 96). However, historians have not reached a consensus regarding the death of Anastasia. For a long time, the inanition of the body was considered as the cause of the Tsarina's death, including due to numerous births (by the age of 26 the Tsarina gave birth to six children).

Studies on the Tsarina's entombment in 1998 revealed the elevated mercury levels of 48 mg/kg in hair, 3 mg/kg in decay, and 5 mg/kg in her clothes (Panova 2017, 99). According to the researchers, human bones concentrate mercury much less efficiently than hair, usually the transfer of mercury to bones is 10-100 less than the corresponding concentration in hair (Rasmussen *et al.* 2013, 1193). The results of our study of Tsarina's hair confirmed an increased amount of mercury in it – 47 mg/kg, but also showed an increased value of arsenic – 1.2 mg/kg. Since all other samples from the burial place of Anastasia are represented by fragments of bone tissue or organics from the skull, we present for comparison data on the quantity of mercury in hair of the famous Renaissance astronomer Tycho Brahe. The researchers explain the high quantity of mercury in Brahe's hair – 16.4 mg/kg by using of mercury-containing medicines.

Basing on the norms of mercury and arsenic in the hair of modern people, according to the analysis of Zaichick (Zaichick, Zaichick 2011, 120), it can be calculated that the amount of mercury in Anastasia's hair is 300 times higher the normal level, and arsenic 24 times. At the same time, the amount of arsenic exceeds the values of almost all other samples, which are higher the normal level from 2 to 5 times (the exception is the samples of Maria Borisovna – 16 times higher than the norm). Since arsenic is a quick-acting poison, we can suppose a gradual poisoning with a mixture of arsenic and mercury. This explanation corresponds to the historical facts about the time-consuming Anastasia's illness. However, the data of analysis needs further interpretation, since the amount and time of accumulation of elements in the hair depends on their length. In any case, our research confirms that the young Tsarina fell victim to the intrigues of the court nobility, who tried to remove representatives of the Zakharyins-Koshkins (Romanov ancestors) from the Royal entourage (Voronova, Panova 1998).

Exceeding of mercury was noted in the bone of granddaughter of Ivan the Terrible Tsarevna Feodosia –150 times higher than normal level. Feodosia died at the age of one and a half year in 1594. Studies of her skeleton in 1998 showed excesses of many elements (Panova 2017, 138). Mercury and arsenic exceeded the norm by 5 and 6 times, respectively. Our study in 2018 confirmed

the data on arsenic – 5 times higher than normal level, but gave significantly higher mercury indicator – 1.2 mg/kg, which 150 times exceeds the norm. The infant age of the Tsarevna does not allow to suppose such an accumulation of mercury during her life. This makes us to suggest poisoning, which put an end to the Ivan I Kalita dynasty and plunged Russia into the Time of Troubles.

It is interesting to note that similar data showed the studies of the bones of Maria Nagaya, who died at the age of 43-45: arsenic is 4 times higher the normal level, and mercury is more than 110 times. Maria Nagaya survived her husband Ivan the Terrible. Data on the quantity of mercury in her remains does not indicate the intentional poisoning

The cause of death of Ivan the Terrible's son Ivan Ivanovich, who died, like his mother, at the age of 27, is still a cause of controversy for historians. As it turned out during the study of his remains in the 1960s, the Tsarevich caught a venereal disease quite early, in the age of adolescence (Buzhilova 2009). It is known that diseases with skin manifestations, including leprosy and syphilis, were treated in the Middle Ages with mercury-containing ointments. According to our research, the amount of mercury in the rib of Ivan Ivanovich is 0.36 mg/kg. It is important to compare this value with data of Danish medieval cemeteries studies, where many cases of syphilis have been confirmed.

The average mercury level in individuals with marked signs of syphilis on their bone material is 0.053 mg/kg (Rasmussen 2008, 2303). However, since the object of the study was the medieval cemetery of the laity in Odense, not all victims of syphilis could receive treatment for obvious reasons. Therefore, a certain proportion of human remains with syphilis symptoms show only background levels of mercury. Six samples have a mercury level high enough to suggest treatment with mercury-containing products – 0.14 to 0.42 mg/kg. The maximum value in 2 samples is up to 3.12 mg/kg (Rasmussen 2008, 2297). Thus, it turned out that the amount of mercury determined by our study in Ivan's bones corresponds to the data characteristic of medieval people who have signs of the disease confirmed by anthropologists and used mercury-containing medicines. Moreover, the amount of mercury in the Ivan's rib exceeds the current mercury norm by 45 times. In comparison with other samples (300 times higher the norm in remains of his mother Anastasia) this fact can be interpreted as chronic mercury poisoning.

Prince Skopin-Shuisky was the statesman and military leader of the Time of Troubles and the nephew of Tsar Vasili Shuisky. Prince Mikhail showed interest in military affairs early enough and proved to be a talented commander. He became a popular man and stood out against the background of his mediocre relatives – Tsar Vasily and especially his brother – the boyar Dmitry Shuisky. People enthusiastically praised Prince Mikhail and they were ready to set him to the tsar throne. This situation predetermined the fate of the Prince. In 1610, at one of the feasts, the wife of the boyar Dmitry Shuisky brought to young relative a cup

of honey. The death symptoms of the 23-year-old healthy man appeared immediately at the feast, and testified to acute poisoning in the gastrointestinal form. The total amount of mercury (up to 2.66 mg/kg) and arsenic (0 to 1.3 mg/kg) revealed in 1964 in decay, bones, hair and nails, was considered sufficient to talk about the poisoning of this young man; his death was clearly violent.

But at the beginning of the XXIst century ideas about the background content of toxic elements in a human body changed. Our study showed the presence of 0.23 mg/kg of arsenic and 0.2 mg/kg of mercury in Skopin-Shuisky's bone, which exceeds the norm, respectively, by 2.3 and 25 times. Considering the suddenness of death and comparison with other samples (for example, the remains of Anastasia, who had been ill for some time, and had arsenic 20 times higher and mercury 300 times higher the norm), it is possible to assume that Skopin-Shuisky was poisoned with plant toxin, which traces it is almost impossible to reveal after four centuries. Skopin-Shuisky died due to the conspiracy of envious relatives who eliminated his potential rival in the struggle for power.

Thus, thanks to the use of the modern NAA method, physicists can help historians to clarify the circumstances of the life and death of historical figures. This research allows to introduce into scientific circulation the exact values of mercury, arsenic and other elements in samples from the graves of Russian historical figures of the XVth-XVIth centuries.

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ABSTRACT

The qualitative and quantitative elemental analysis of the unique remains of the Russian nobility of the Middle Ages was carried out in the Frank Laboratory of Neutron Physics at the Joint Institute for Nuclear Research (Dubna, Russia). Neutron activation method was used for experiment. In the course of the research, bones, organics from the skull and hair of seven Russian historical figures who died in the 15th-early 17th centuries were studied. The mass fractions of several elements, including arsenic and mercury (part of the most common medieval poisons) were found in these samples. The comparison of the obtained results with data from similar Russian and European studies made it possible to make assumptions about the probability of the deliberate poisoning of some representatives of the higher Russian nobility. It also gave an opportunity to replenish the elemental composition database of the human remains of that period.