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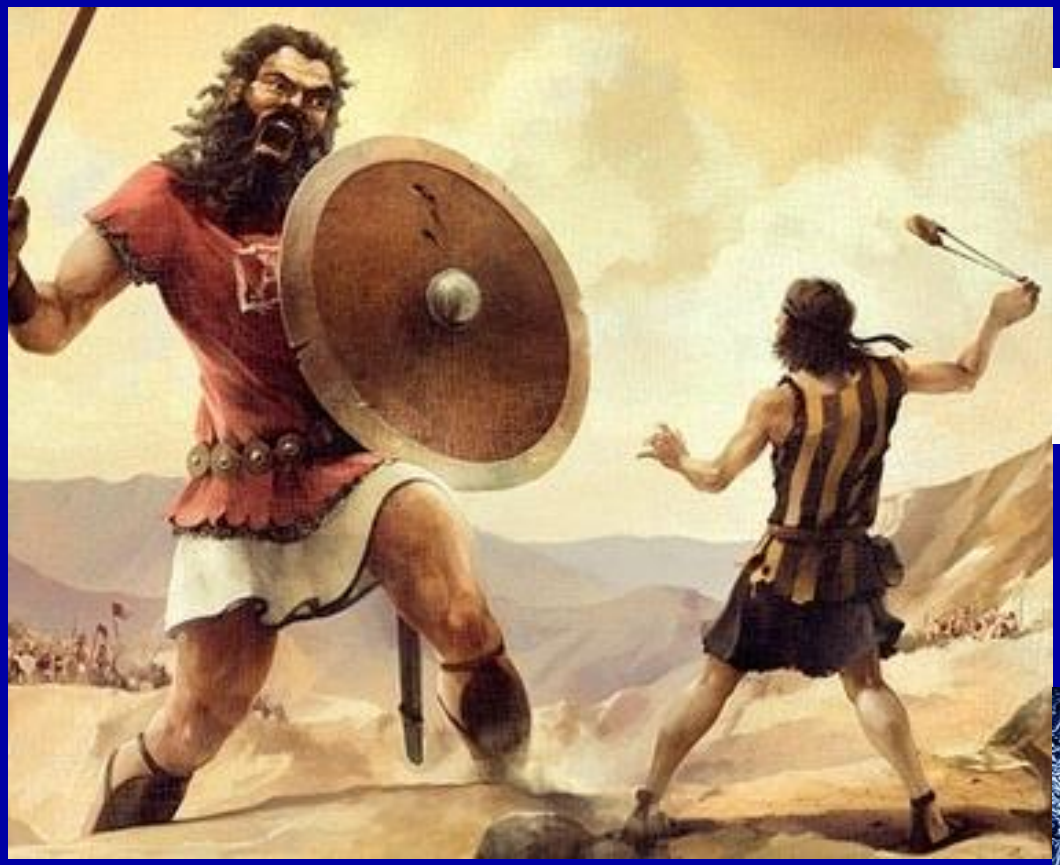
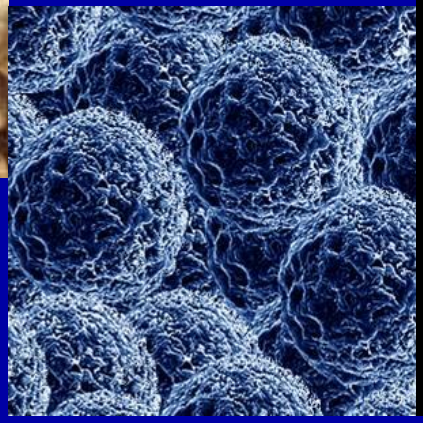
Tracing and Quantitative Measurements of Inorganic Nanoparticle Amounts in Biological Tissues by Nuclear-Physical Methods

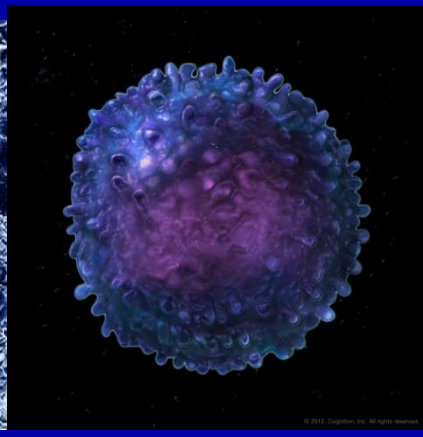
A.A. Antsiferova, Yu.P. Buzulukov, V.A. Demin, V.F. Demin, P.K. Kashkarov

Topicality: Action of Nanoparticles



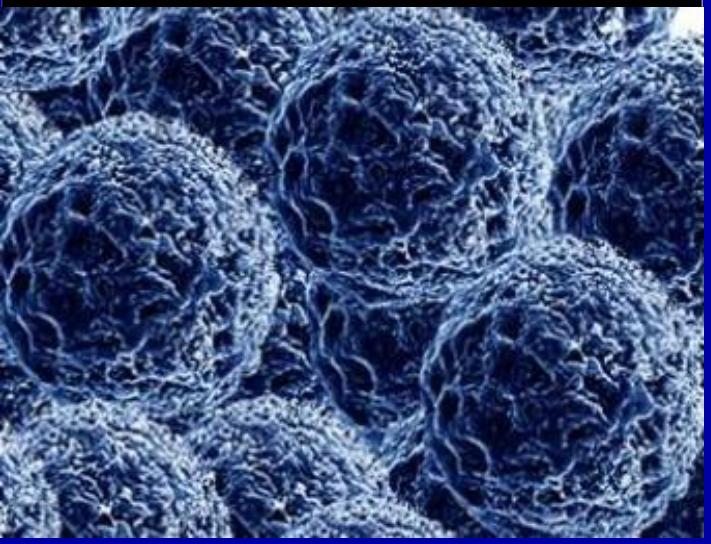
Size difference – 1000 times





Despite relatively small size of nanoparticles they significantly affect cells due to their high penetrability and high surface area to volume ratio.

Application of Silver Nanoparticles



Properties of silver

- Antibacterial
- Antiviral
- Fungicidal

- **Silver nanoparticles demostrate toxic effect onto bacterial cells as well as on mammal organisms taking them up as medicine.**
- **Standards of silver nanoparticles administration is not regulated documentary.**

Application of TiO_2 Nanoparticles and Following Problems

- White dye (90 % from the whole use of white dye)
- Cosmetics
- Sunscreens (due to the property to absorb UV-radiation)
- Air cleansing
- Toxicity
- Phototoxoicity
- Penetration through nantural barriers of the organism
- Study of TiO_2 biokinetics

Subject and Purpose of Work

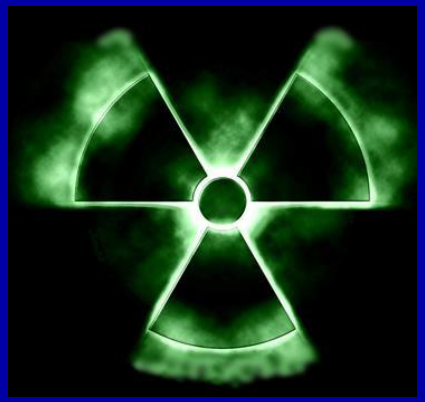
Development and application of a precious technique for quantitative amount control of widely-used inorganic nanoparticles contained in drugs, food suppliments as well as in complex biological matrices of laboratory animal tissues being tested in studies of nanoparticle biokinetics for certification and risk assessment.

Nanoparticles Concentration Measurements in Biological Tissues and Fluids

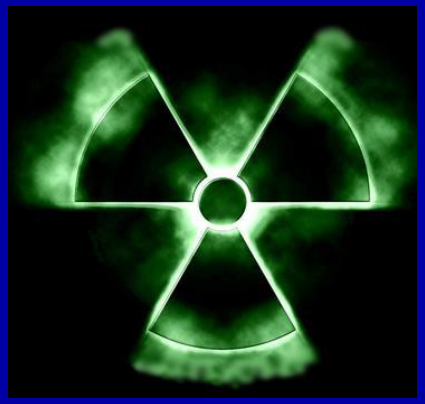
Method	Advantages	Disadvantages
Electron Microscopy (SEM, TEM)	Visualization of measurement results	Low representativeness (microsections and microsamples), complexity of sample preparation, impossibility of biophilic nanoparticle analysis
Optical Spectroscopy and Mass-Spectrometry	Relatively high accessibility	Relatively low representativeness (microcuts and microsamples), difficulties in converting of solid samples into liquids, destructive techniques, low accuracy, impossibiliy of biophilic nanoparticle analysis
Neutron and Proton Activation Analysis (in conjunction with Gamma Spectrometry detection)	Possibility of nondestructive macrosample analysis (whole tissues or their parts), possibility of biophilic nanoparticle analysis (Zn, Fe, Se etc.), high sensitivity (up to 10^{-11} g) and metrological accuracy (~1-10%)	Relatively low accessibility

Radioactive Labeling Technique and Neutron Activation Analysis

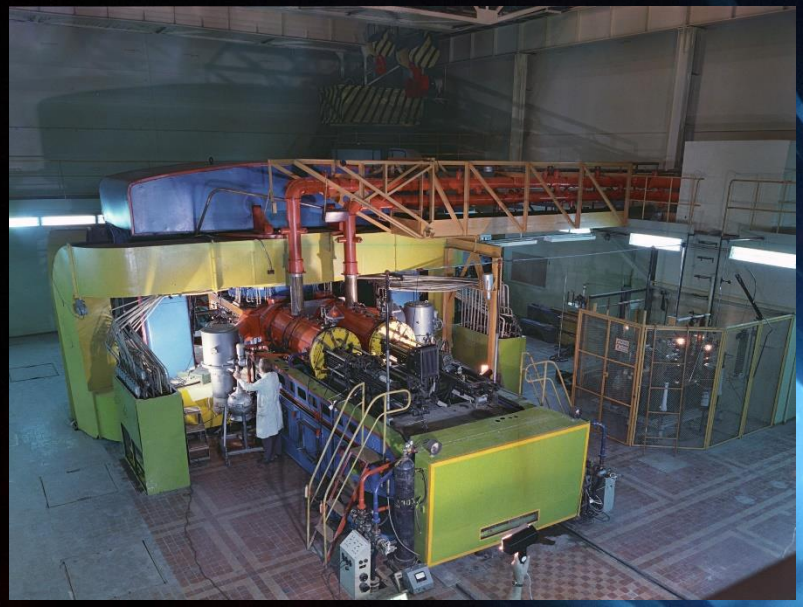
1.)



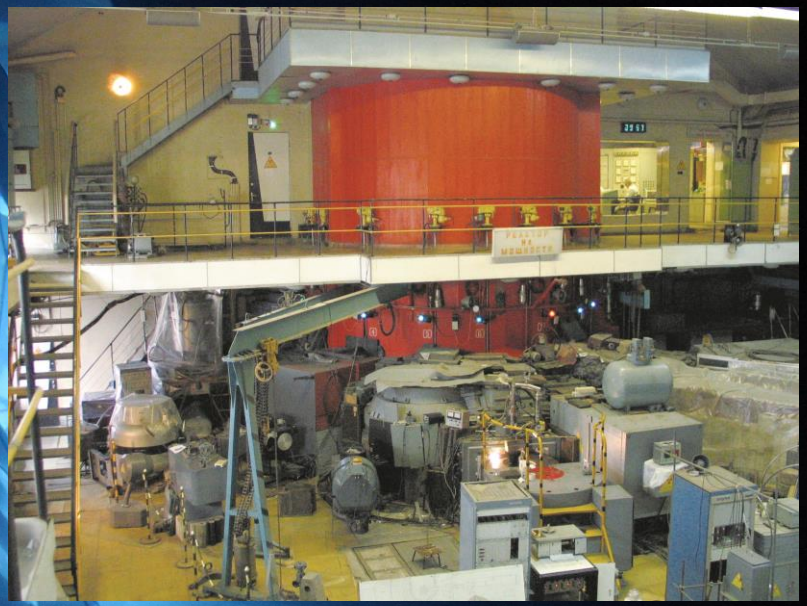
2.)



Sources of Ionizing Radiation in NRC Kurchatov Institute



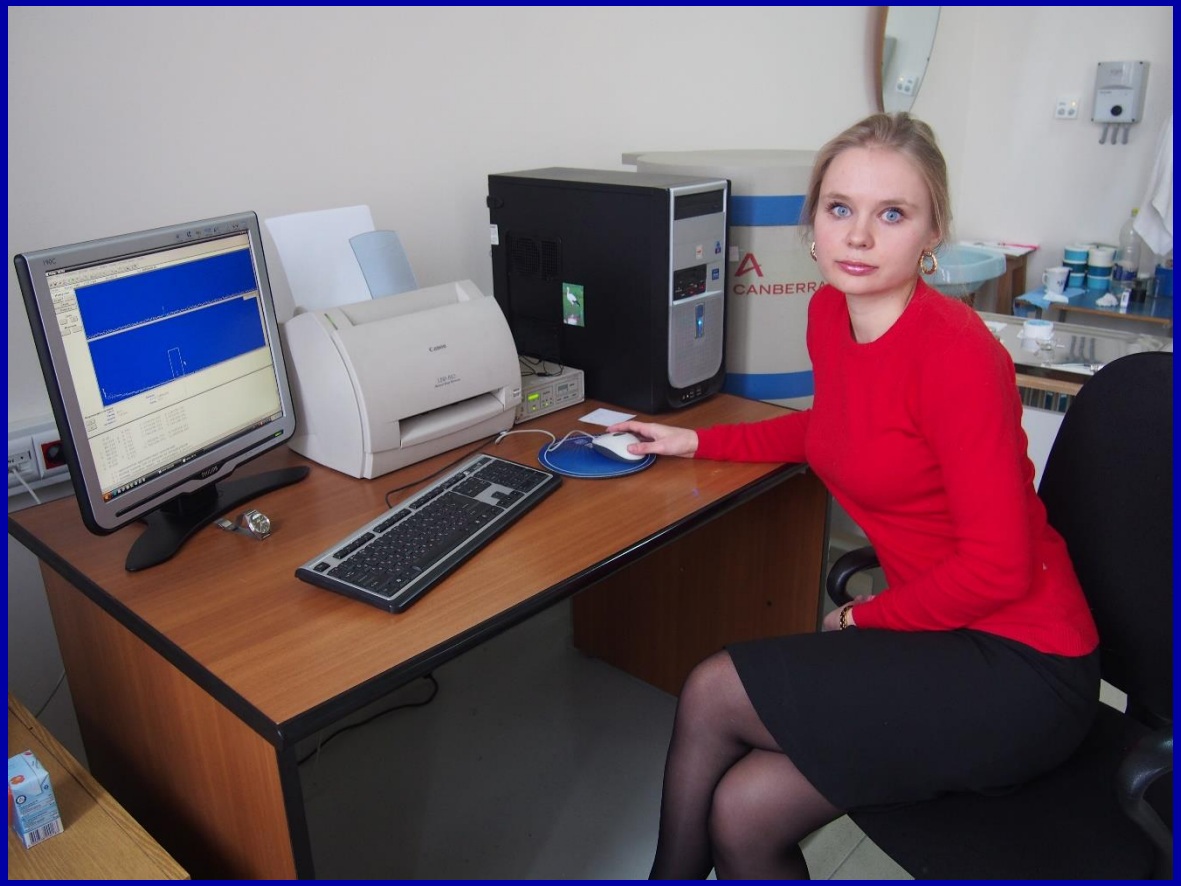
Cyclotron of NRC Kurchatov Institute – source of fast protons



Source of thermal neutrons is a nuclear experimental reactor IR-8 in NRC KI with neutron flux of no less than 10^{12} $cm^{-2}c^{-1}$;

Auxiliary Equipment

High precision Gamma ray Spectrometer CANBERRA with germanium crystalline detector, certified by Federal Metrology Agency, Russia



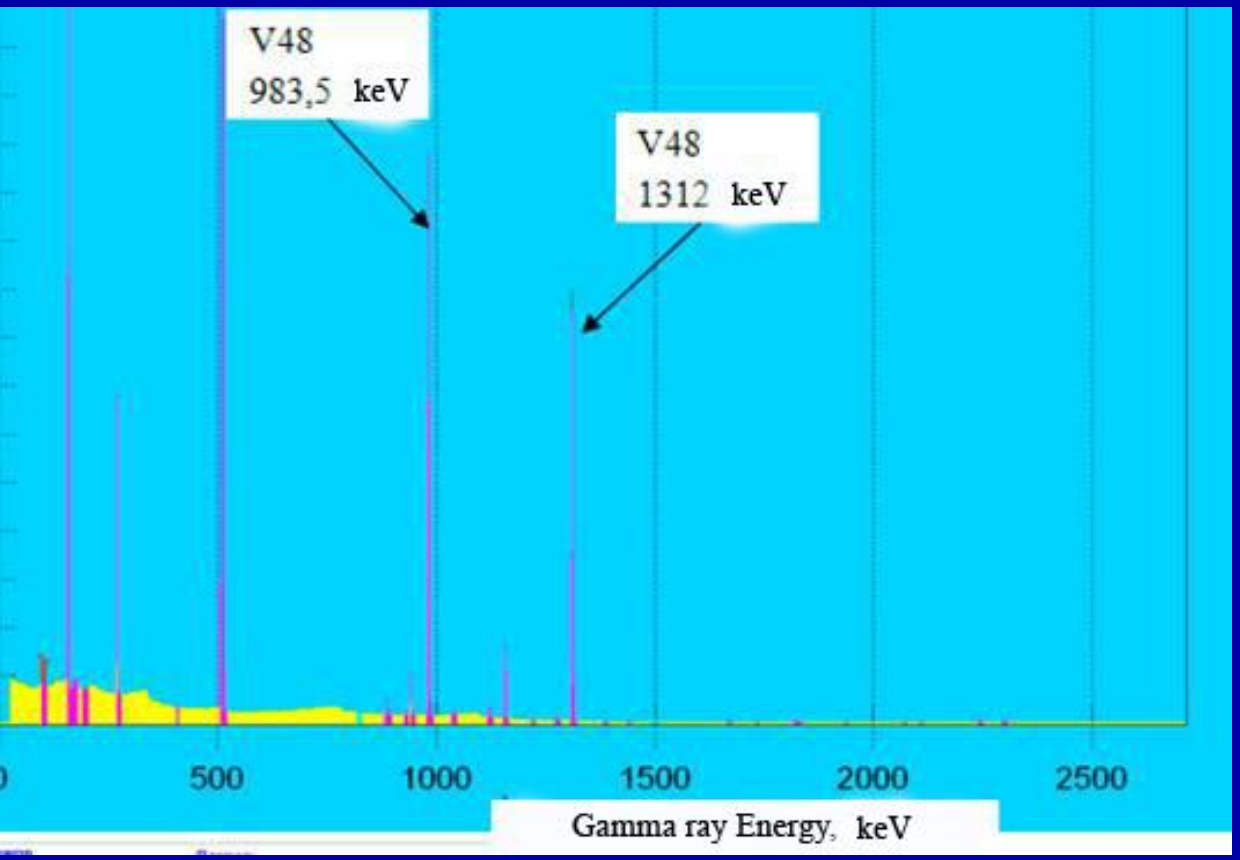
Concentration measurements of nanoparticles containing active isotopes of investigated elements are held with the gamma-ray spectrometry of activated samples.

Detection limit (metrologically approved) is 10^{-9} g.

Characteristics of Different Activation Techniques

Technique	Advantages	Disadvantages
Radioactive Labeling	⑩ Possibility of measuring quantitative amounts of biophilic elements	1. Specialized laboratories are required to conduct the experiment
Neutron Activation Analysis	1. No need to conduct experiments in specialized laboratories	⑩ Impossibility to measure quantitative amounts of biophilic elements

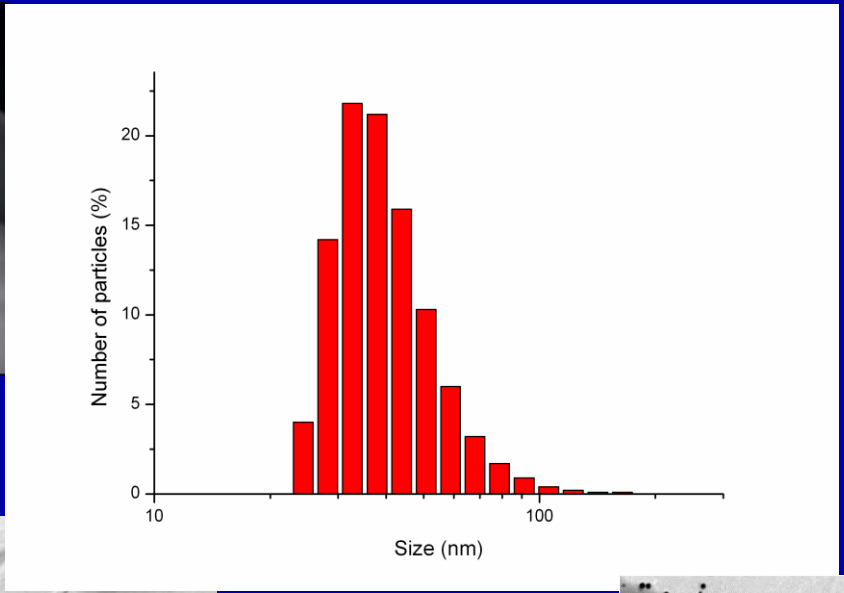
Application of Fast Protons for Radioactive Labeling



- Irradiation of rutile nanoparticles (TiO_2) with fast protons generated by cyclotrone
- The problem is in the high recoil energy in the reaction with fast protons which could lead to the high activated isotope (^{48}V) output from the sample
- Nevertheless, the study has shown that the output is negligible

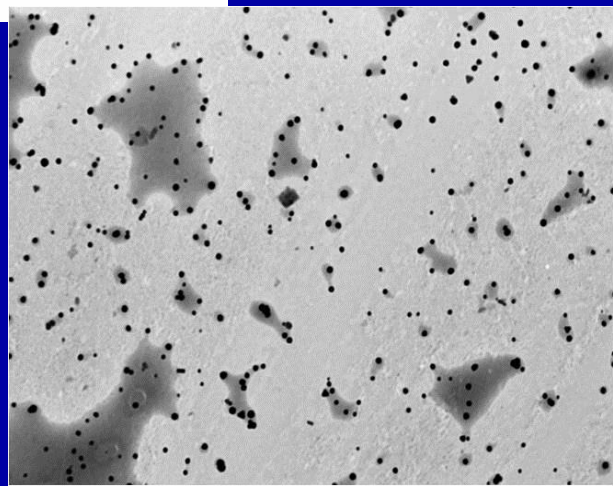
First Experiments: Materials

- Silver nanoparticles (Argovit™) stabilized with polyvynilpyrrolidone. An average size was 34±2 nm
- Unstabilized gold nanoparticles with average size of 8±5 nm

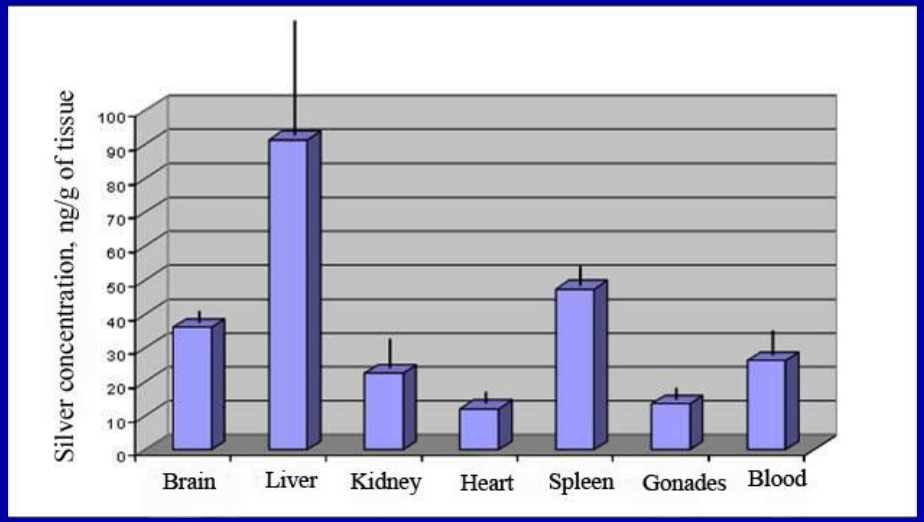


Ag NP Argovit

Rats (Wistar™)



First Experiments: Results

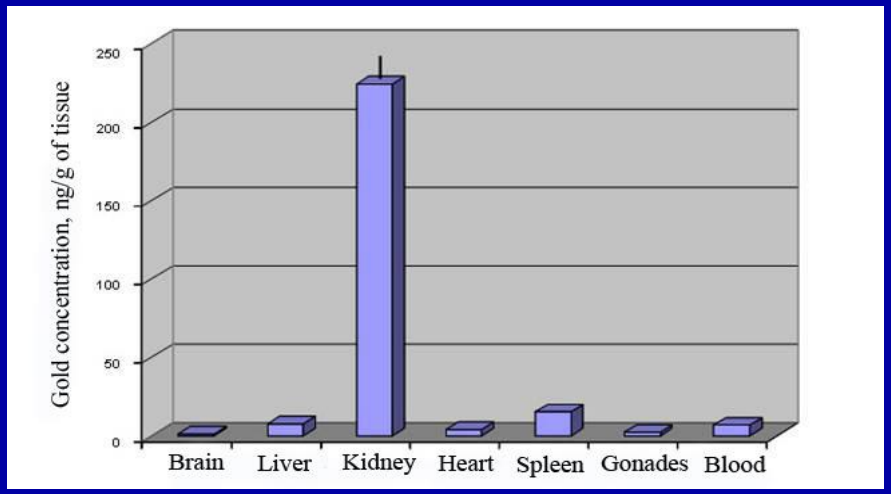


•Oral administration of silver NPs in the quantity of 100 mg per kilogram each day for 28 days (5 animals);
•Silver nanoparticles penetrate mostly into liver, spleen and **brain** as a result of long time injection

•Silver Nanoparticles penetrate through blood-brain barrier:

$$m_{bb} = \frac{A_{Fe}^{brain}}{a_{Fe}^{blood}};$$

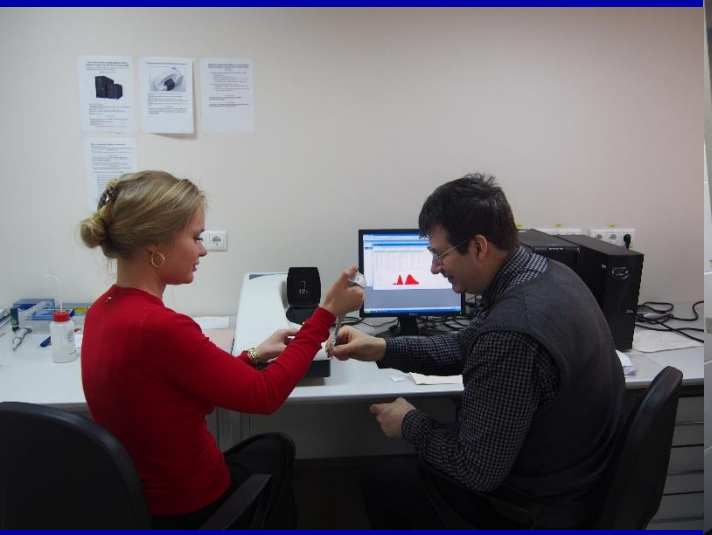
$$\tilde{A}_{Ag}^{bb} = a_{Ag}^{blood} m_{bb} << A_{Ag}^{brain}$$



•Oral administration of gold NP in the quantity of 100 mg per kilogram each day for 14 days (5 animals);
•Gold nanoparticles penetrate mostly into kidney and **not into brain**

•Gold nanoparticles hardly penetrate through blood-brain barrier

Studies on Accumulation and Excretion of Silver Nanoparticles: Experiment



White mice SHK

-1) control group, oral administration of pure water, period of maintaning 2 months (2 mice);

-2) control group, oral administration of pure water, period of maintaining 3 months (2 mice);

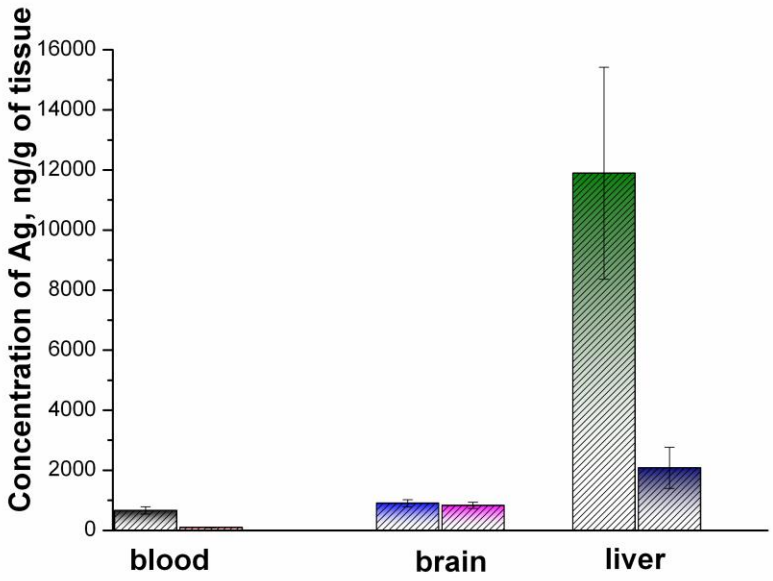
-3) experimental group: oral administration of 1 microgram per day of Ag nanoparticles for 2 months (6 mice);

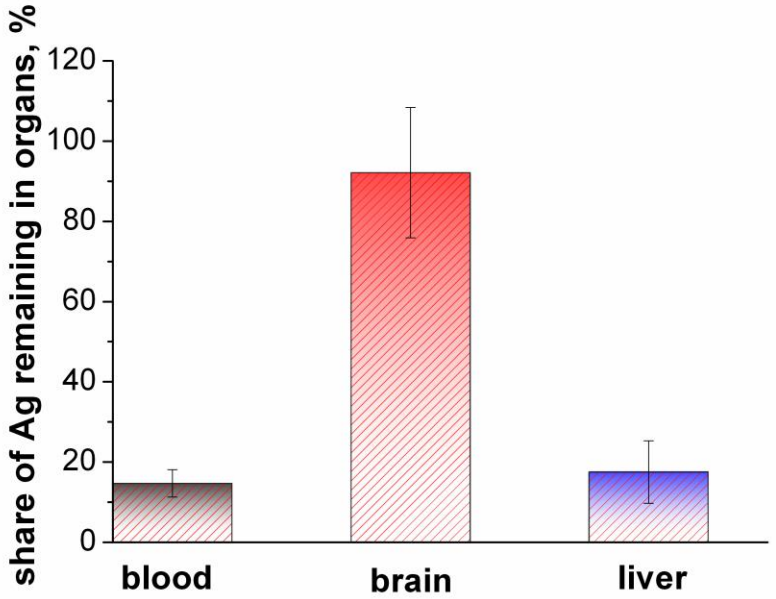
-4) experimental group: oral administration of 1 microgram per day of Ag nanoparticles for 2 months and administration of pure water during the following 1 month (6mice).

Ag nanoparticles performed by “Argovit” colloidal solution was studied in the experiment.

Average size of nanoparticles is 34 nm.

Studies on Accumulation and Excretion of Silver Nanoparticles: Results





- High rates of excretion of silver from blood and liver were shown (80% per month).
- Rather low level of silver nanoparticle excretion from brain equal 6% per month was demonstrated.
- This effect could be explained by properties of blood-brain barrier.

Conclusions

- Application of Nuclear-Physical methods for study of nanoparticle biokinetics were suggested.
- Nuclear-Physical methods for radiolabeling of TiO_2, Ag and Au nanoparticles were developed.
- It was shown that Au nanoparicles accumulate mostly in kidneys and Ag nanoparticles accumulate in liver, spleen and brain. Moreover Ag nanoparticles are able to penetrate through blood-brain barrier while Au nanoparticles are not.
- Level of excretion of silver nanoparticles from brain is rather low. However rates of excretion from liver and blood are relatively high.
- Effect of silver nanoparticles accumulation in brain is discovered.

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Thank You for Your Attention!