NEUTRON ACTIVATION ANALYSIS of Br, Ca, K, Mg, Mn, and Na CONTENTS in BENIGN PROSTATIC HYPERTROPHIC TISSUE

Professor **Vladimir Zaichick**

Ph.D. (nuclear physics), D.Sc. (radiobiology)

Fellow of the Royal Society of Chemistry,

Medical Radiological Research Center, Obninsk, Russia

E-mail: vezai@obninsk.com

Postulate 2 of Medical Elementology:

The levels and metabolic fluxes of chemical elements are controlled by homeostatic regulation (Differential homeostasis)

In all organisms differential homeostasis of chemical elements is carried out, i.e. at all levels of their organization (the internal environs, organs, tissues, cells, etc.) the content of chemical elements is maintained at certain levels. These levels can change with age and under the influence of various exogenous and endogenous factors, within, however, the certain ranges and limits. Differential homeostasis is a cause of irregularity in distribution and of difference in exchange velocity of chemical elements in organs, tissues, fluids and other structural formations of the organism.

Zaichick V., Agadjanyan N.A. Vesti Vosstan. Med., 2004, vol. 3, №9, 19-24.
Zaichick V. J. Radioanal. Nucl. Chem., 2006, Vol. 269, No. 2, 303-309.

Introduction

Benign prostatic hyperplasia (BPH) assigns most of men after the age of fifty and represents the most common urologic disease among elderly males. BPH is histologically defined as an overgrowth of the epithelial and stromal cells from the transition zone and peri-urethral area of prostate. The excessive cell proliferation associated with BPH causes benign prostatic enlargement, bladder outlet obstruction, and lower urinary tract symptoms, which afflict the patients.

The prevalence of benign enlargement of the prostate rises sharply with age. Using the criteria of a prostate volume > 30 mL and the results of autopsy studies, it may be estimated that the prevalence of BPH increases from approximately 8% in men aged 31 to 40 yr to roughly 50-60% of men at 50 yr of age. Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old. To date, we still have no precise knowledge of the biochemical, cellular and molecular processes underlying the pathogenesis of BPH. Although the influence of androgens and estrogens has been demonstrated, hormonal factors alone may not fully explain BPH development.

In our previous study a significant positive correlation between age and Ca mass fraction in the prostate was observed. High intraprostatic Ca concentrations are probably one of the main factors acting in prostate cell proliferation. Moreover, a significant positive correlation was seen between the prostatic Ca and Na contents. Hence it is possible that besides Ca, some other elements also play a role in the pathophysiology of the prostate.

Aims

This work had four aims. The first aim was to assess the Br, Ca, K, Mg, Mn, and Na mass fractions in intact prostate of healthy men aged over 40 years using instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). The second aim was to evaluate the quality of obtained results. The third aim was to compare the levels of chemical elements in the prostate gland of age-matched patients, who had BPH. The final aim was to estimate the intercorrelations of chemical element mass fractions in normal and BPH glands.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk

Material

All patients suffered from BPH (n=32, mean age M±SD was 67±6 years, range 56-78) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Intact prostates were removed at necropsy from 37 men (mean age 55±11 years, range 41-79) who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized. The pounded sample weighing about 100 mg was used for chemical element measurement by INAA-SLR.

Method

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [Zaichick, 1995]. In addition to SSB, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material (CRM) IAEA H-4 (animal muscle) sub-samples weighing about 100 mg were treated and analyzed in the same way as prostate samples to estimate the precision and accuracy of results.

Details of the relevant facility for INAA-SLR and the results of quality control were presented in our earlier publications [Zaichick, 2011].

Table 1 INAA-SLR data of chemical element contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work
	Mean	95% confidence interval	Type	Mean±SD
Br	4.1	3.5 – 4.7	C	5.0±0.9
Ca	188	163 – 213	C	238±59
K	15800	15300 – 16400	C	16200±3800
Mg	1050	990 – 1110	C	1100±190
Mn	0.52	0.48 – 0.55	N	0.55±0.11
Na	2060	1930 – 2180	C	2190±140

Results

Table 1 depicts our data for six chemical elements in ten sub- samples of CRM IAEA H-4 (animal muscle) and the certified values of this material. Good agreement with the certified data of certified reference materials indicate an acceptable accuracy of the results obtained in the study. Good agreement of the Br, Ca, K, Mg, Mn, and Na mass fractions analyzed by INAA-SLR with the certified data of CRM IAEA H-4 indicates an acceptable accuracy of the results obtained in the study of chemical elements of the prostate presented in Tables 2.

Table 2. Comparison of mean values (M±SEM) of Br, Ca, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal and BPH prostate tissue

Element	Prostatic tissue		Ratio	
	Normal	BPH	BPH/Normal	Student's *t*-test
Br	32.9±3.6	30.4±3.6	0.92	NS
Ca	2280±178	2030±165	0.89	NS
K	11211±414	14470±740	1.29	$p \leq 0.01$
Mg	1118±76	1200±80	1.07	NS
Mn	1.24±0.07	1.19±0.09	0.96	NS
Na	11100±408	11610±870	1.05	NS

Discussion

The K mass fraction was significantly higher in BPH tissues than in normal tissues. It is well known that K is mainly intracellular electrolyte. Thus, the abnormal high level of K in prostate tissue could be a consequence of BPH transformation.

It was found by us that Ca levels in the human prostate are almost 3-7 times higher than in other soft tissues and whole blood (Zaichick et al. 2013 a,b). The high content of Ca in the prostate suggests that Ca may play a role in prostate function and health.

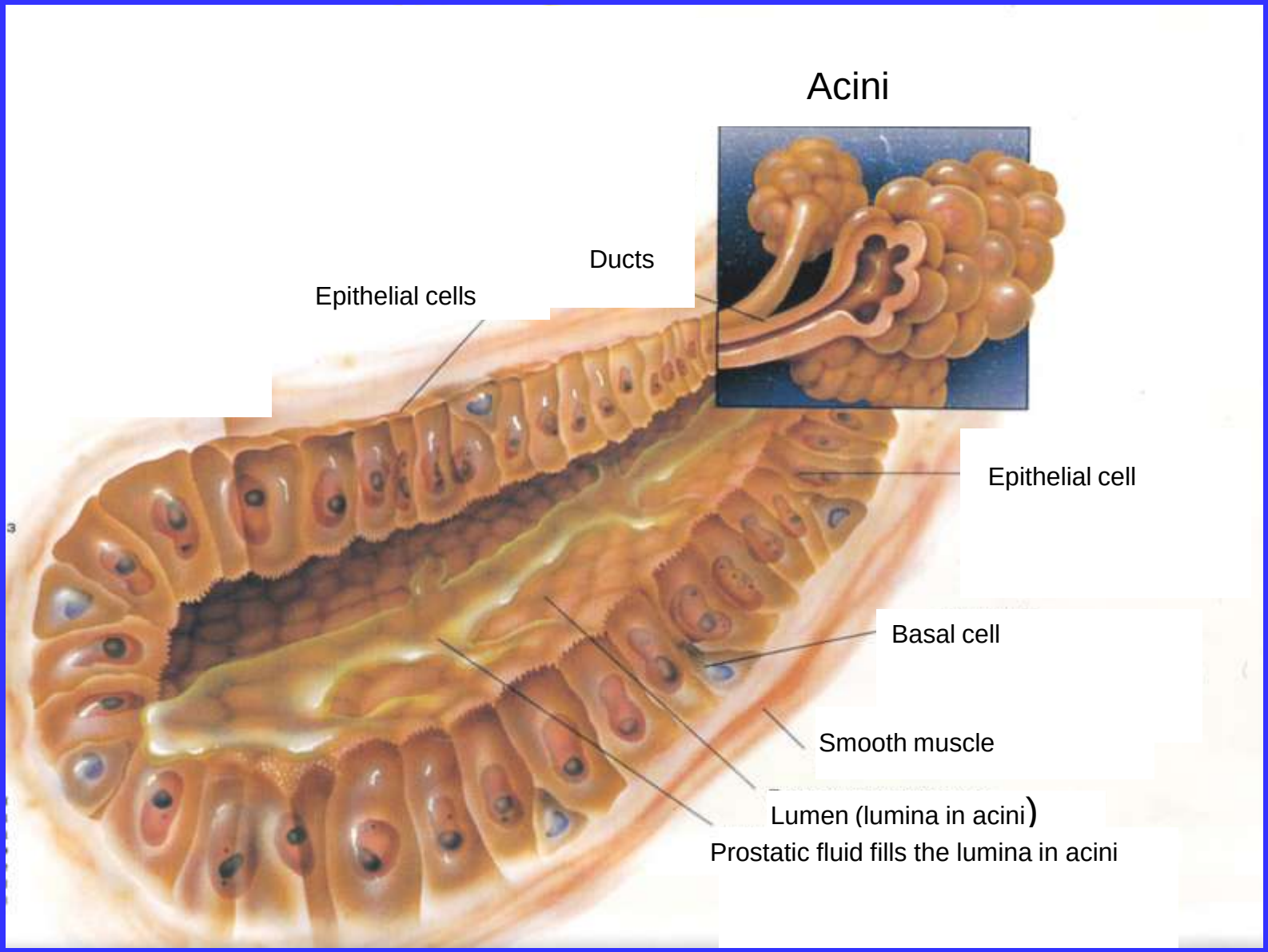
In our previous studies it was shown that during puberty and postpuberty, when there is a significant increase in circulating androgens, Ca mass fraction is almost 2 time greater than in prostate of before puberty (Zaichick et al. 2013 a,b; Zaichick et al. 2014). These results confirm that the Ca mass fraction in prostate tissue is an androgen-dependent parameter. However, it is well known that after age 30-40 years the levels of androgens in blood, including testosterone, decrease, but our findings show that Ca mass fraction in prostate tissue continues to increase. For example, in prostate of 50 years old men the mean Ca was 2.3 times greater than in prostate of 16 years old persons (Zaichick et al. 2011). Why?!

Age-related histological and Ca content changes in cellular component of nonhyperplastic prostate glands

The prostate gland is a vital part of the male reproductive system. It produces and excretes much of the liquid portion of semen (about 30-35% of the semen ejaculate). The prostate mixes its fluids with those from the seminal vesicles to transport the sperm made in the testes.

The prostatic tissue contains three main components: glandular tissue, prostatic fluid, and fibromuscular tissue or stroma. Glandular tissue includes acini and ducts. Epithelial cells (E) surround the periphery of the acini and luminal surfaces (L) in acini (glandular lumen). Prostatic fluid fills the lumina in the acini (glandular lumen). Stromal tissue (S) is composed of smooth muscle, connective tissue, fibroblasts, nerves, lymphatic and blood vessels. Thus, the volume of the prostate gland may be presented as a sum of volumes (E + L + S) and the volume of the prostatic cells as a sum of volumes (E + S). It is possible to quantitate morphological data using a stereological approach.

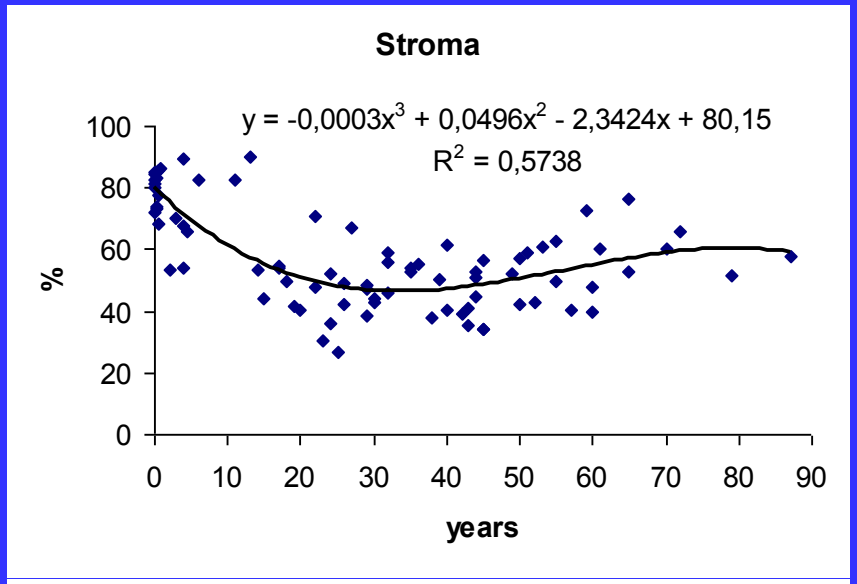
Morphology of prostate gland

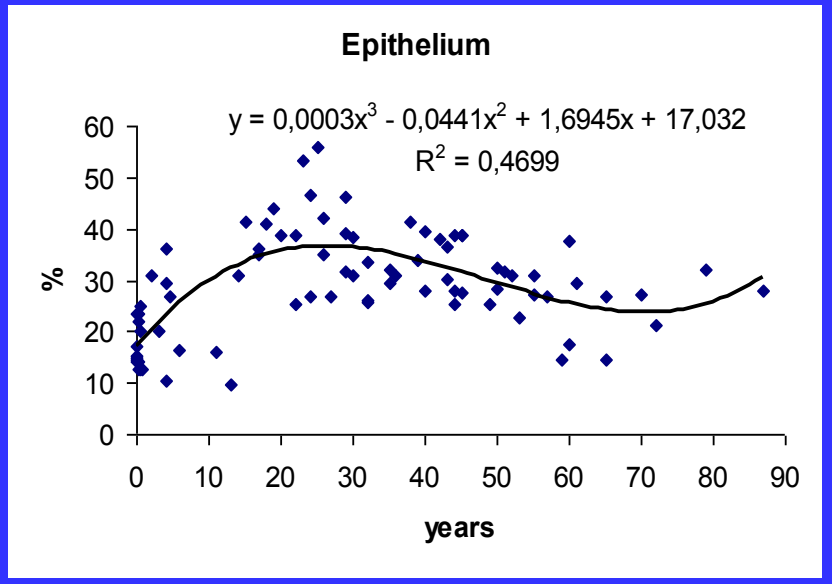


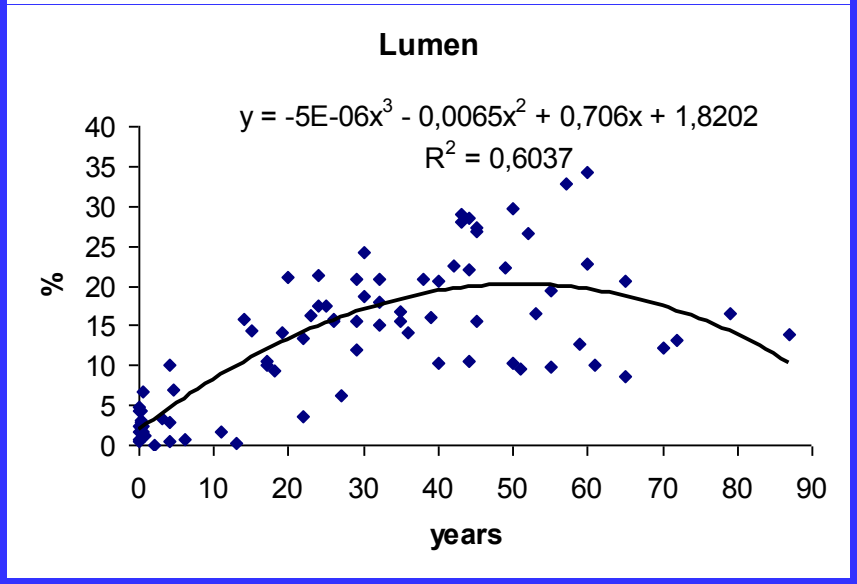
In order to clarify the age-related histological and Ca content changes in nonhyperplastic prostate glands, a quantitative morphometric and Ca content studies were performed, respectively. The prostates were obtained at autopsy from 101 subjects (European-Caucasian, aged <1–87 years) who died mainly from trauma. None of the subjects presented any clinical symptoms of prostatic disease and all prostates were classified as histologically normal. Each prostate was divided into two portions. One tissue portion was reviewed by an anatomical pathologist while another was used for the Ca mass fraction measurement. The mean percent volume of the stroma (S), glandular epithelium (E), and glandular lumen (L) were determinedfor for each prostate specimen. It was found that normal prostate tissue undergoes substantial changes during this period of life.

Zaichick et al., Andrology, 2013, Vol. 1, pp. 139-146.
Zaichick et al., Age, 2014, Vol. 36, pp. 167-181.
Zaichick et al., Biol. Trace Elem. Res., 2014, Vol. 157, pp. 195-204.
Zaichick et al., Biometals, 2014, Vol. 27, pp. 333-348.

Individual data sets for the percent volume (stroma, epithelium, lumen, and glandular component) and Ca mass fraction in the nonhyperplastic prostate gland of males between ages <1–87 years and trend lines







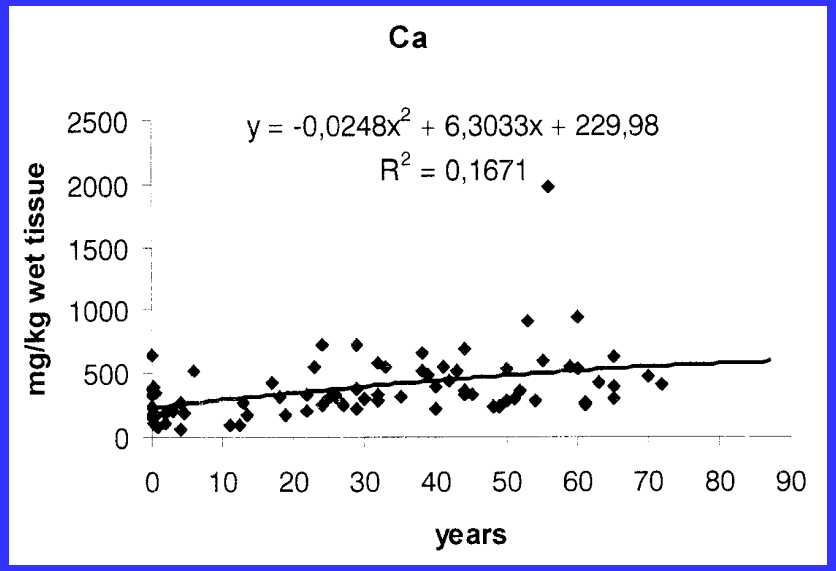
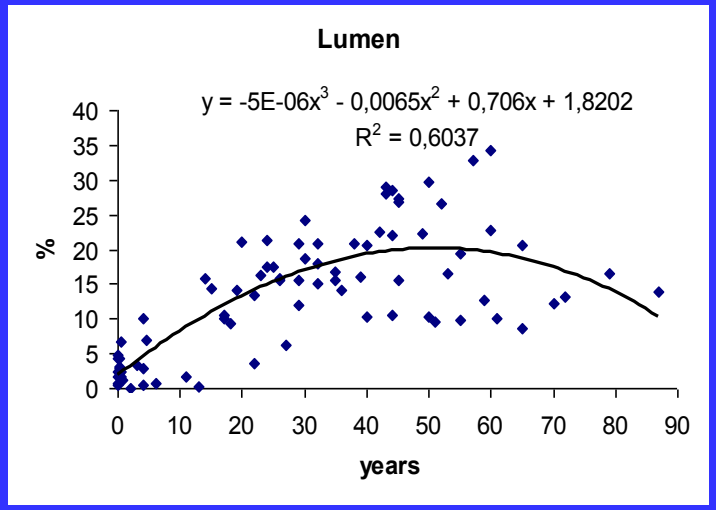


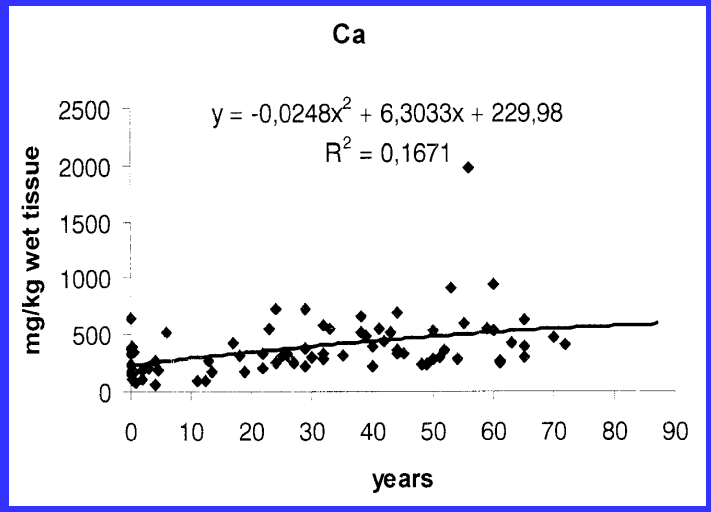
Table 4 Age-dependence of mean values of Ca mass fraction (mg/kg, wet mass basis) in prostatic cells

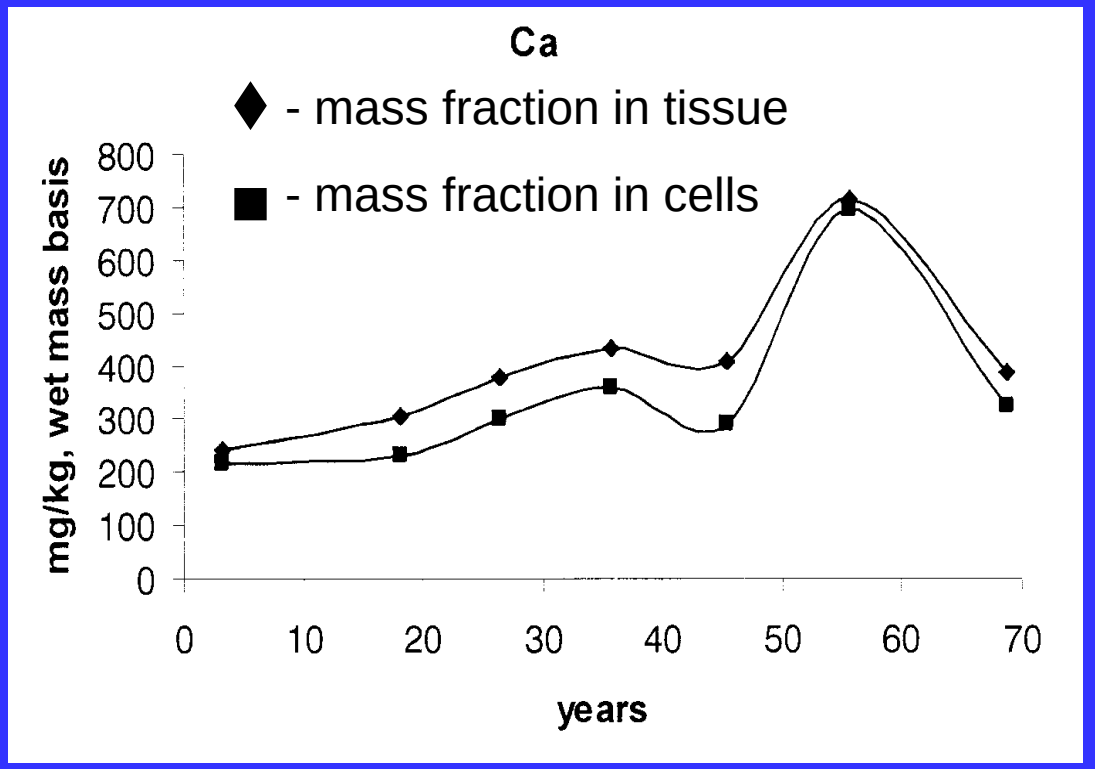
Group No	Age Mean	Ca(T)	L	C=S+E	Ca (L)	Ca (C)	Ca mass fraction in cells (Ca=Ca(C) /C)
Group 1	3.3	241	0.045	0.955	36	205	215
Group 2	18.2	305	0.13	0.870	104	201	231
Group 3	26.4	378	0.156	0.844	125	253	300
Group 4	35.8	435	0.168	0.832	135	300	361
Group 5	45.4	408	0.227	0.773	182	226	292
Group 6	55.6	717	0.205	0.795	164	553	695
Group 7	68.8	392	0.136	0.864	109	283	327

M – arithmetic mean, SEM – standard error of mean,
Ca(T) – calcium content (mg) in 1 kg wet tissue,
L – mass fraction of lumen (prostatic fluid), S – mass fraction of stroma, E – mass fraction of epithelium, C –mass fraction of prostatic cells (C=S+E),
Ca(L) – calcium of prostatic fluid (mg) in 1 kg wet tissue (it was accepted that Ca concentration in prostatic fluid does not depend from age and equal 802 mg/L [Kavanagh 1985]),
Ca(C) – calcium of prostatic cells (mg) in 1 kg wet tissue Ca(C)=Ca(T) - Ca(L) (it was accepted that density of prostatic tissue and fluid equal 1 kg/dm^3).

Age-dependence of Ca mass fraction in prostate tissue and cells







A cellular Ca mass fraction (about 200 mg/kg wet mass basis) remained at about the same level up to the 20-year age, the value of which almost did not exceed levels typical of cells of other organs and tissues (see Table).

Table. The mean and the range of Ca mass fraction in erythrocytes and some soft tissues of health human body (mg/kg wet mass basis)

No	Cells or tissue	Range of means
1.	Breast	210-340
2.	Heart	40-155
3.	Kidney	40-190
4.	Liver	54-133
5.	Lung	90-150
6.	Muscle (skeleton)	41-150
7.	Pancreas	140-150
8.	Skin	34-250
9.	Spleen	56-210
10.	Thyroid	286-550

There is moderate increase of intracellular Ca mass fraction between 20 to 40 and significant after the age of 40, and by 55 years old it is over 3-fold higher, on the average, than the level typical of men aged 20.

Naturally, BPH is a multietiological and multifactorial complex of diseases. BPH is caused by defects in the mechanisms underlying cell proliferation and cell death (apoptosis). It is well known that calcium ions are central to both phenomena, serving as major signalling agents with spatial localization, magnitude and temporal characteristics of calcium signals ultimately determining cell's fate.

These facts allow us to speculate that excessive intracellular content of Ca are probably one of the main endogenic factors acting at both proliferation and apoptosis. This speculation is confirmed by recent data obtained on the cultures of prostatic, breast, and lung cells [Roderick and Cook, 2008; Feng et al., 2010; Yang et al., 2010; Prevarskaya et al., 2013].

The assumed hypothesis on the role of excessive intracellular Ca is an explanation to some specific epidemiological features of BPH. Thus, the age-dependence of BPH prevalence agrees well with age dynamics of intracellular Ca accumulation. Dairy foods are the main source of Ca and high dairy products intake associates with higher risk of BPH [Koskimaki et al., 2000; Aslam and Neubauer, 2013].

The role of excessive intracellular Ca content at both cell proliferation and apoptosis of prostate cells does not exclude the influence of other factors, such as persistent and chronic inflammation, circulating hormonal level deregulation, and aberrant wound repair processes [Schauer and Rowley, 2011].

Conclusion

Nuclear analytical methods are the powerful analytical tools for the determination of chemical element content in prostate tissue. The methods can be successfully used both in experimental and clinical studies of benign prostatic hyperplasia diagnostics and etiology.

Thank you very much, indeed, for your attention !

Clinical sector of the Medical Radiological Research Center, Obninsk, Russia