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FUNCTIONAL ELIGIBILITY OF IODCASEIN FOR IODINE DEFICIENCY PROPHYLAXIS

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Not less than a milliard and a half people worldwide and more then 100 thousands of Russian live in iodine deficient territory. Join programs of UNISEF, ICCIDD and WHO take steps for the elimination of iodine deficiency [1]. At the moment, the most commonly used program is salt iodization. Along with the positive results of iodine deficiency prophylaxis with iodized salt, there are some negative effects as iodine induced disorders (IID), caused by increased iodine inflow into a thyroid [7,11]. In the proceedings of Meeting on salt iodization program quality assurance (October, 1996) it was stated that due to the imperfection of existing technology it is difficult to achieve steady mixing of potassium iodide (iodate) in common salt. The content of potassium iodide (iodate) in salt varied from 0 to 600 mg per 1 kg, and average level – from 24 to 148 mg per 1 kg [3]. The increase of hyperthyroidism cases as well as revealing of other medical complications coinciding with iodine prophylaxis in population of iodine deficient territory of various countries, stipulated the necessity of the international symposium in March, 1996 in Brucline, USA [6, 10].
Probability of iodine deficiency prevention with organic iodine compounds was known since the end of XIX century. The similarity of physiological action of iodized proteins and thyroid hormones was demonstrated by Wormser in 1897 during experimental therapy with iodized casein in dogs with ablated thyroid and in myxedema patients. In period from 30^{th} to 60^{th} of XX century, fundamental study of iodine organic compound, including iodtyrosine and tyroxine, have been performed. In 1943 Reineke and Turner published the improved technology of the Iodcasein synthesis and the results of it high thyroid activity and influence on reproduction processes in farm animal [10]. In 1936 Abelin noted that at alkaline hydrolysis of artificial iodized casein, product imitating thyroid hormnes have been formed [7]. In 1956 American physiologist L.Van Middlesworth reported that feeding of rats with casein containing small amount of iodine (20-50 mcg/kg) prevent goiter formation [12]. Further, casein ability to prevent goiter disease in iodine deficient rats and mice was reported by Leblond and Axelrad [12]. These and many other studies of artificial iodized protein metabolism made our choice of iodcasein as the mean for iodine deficiency prevention.

Materials and methods

Iodcasein (milk protein, casein, iodized on amino acid residues) is a yellow water soluble powder with iodine contents of 7-9%. Functional eligibility of Iodcasein (made by Medbiopharm company) was studied in vivo and in vitro by radiometric methods.
Tests were performed in 170 white outbreed rats of both sexes, (weigh 160-200 g). Animals were divided into two groups; the first group had iodine deficient diet and the second one had solution of iodcasein, containing up to 5 mcg of iodine applied every day during a week. This corresponds to normal animal physiological iodine diet.

For the purpose of biological tests, chromatography pure ^{131}I-labeled Iodcasein with activity of 370 kBq was performed. Radioactive label into casein was entered by the following diagram. The solution of radioactive sodium iodide volume of 0,2 ml with general activity of 110 Mbq (3 mCu) was placed into glass tube and evaporated until dry. Then into the same tube the solution of 0,1 ml of 1 N iodine monochloride in 6 N hydrochloric acid obtained by Weygand-Hilgetag was added [4]. The tube content was mixed thoroughly and incubated for 20 minutes. Then, 1 ml of 10% casein solution was added to the carbonate buffer (pH 10,2). Iodcasein precipitate was transferred into the soluble form by increasing the pH of reaction mixture to 8,0, then the mixture was separated by chromatograph column 1,6x30 cm filled with sephadex G-25. Carbonate buffer was used as eluent. Detection was performed with a fluent ultraviolet detector and a gamma-counter. ^{131}I-casein with radiochemical purity of 99,9% (Fig.1) was obtained. Outcome of ^{131}I use in radiolabeling was 85%.

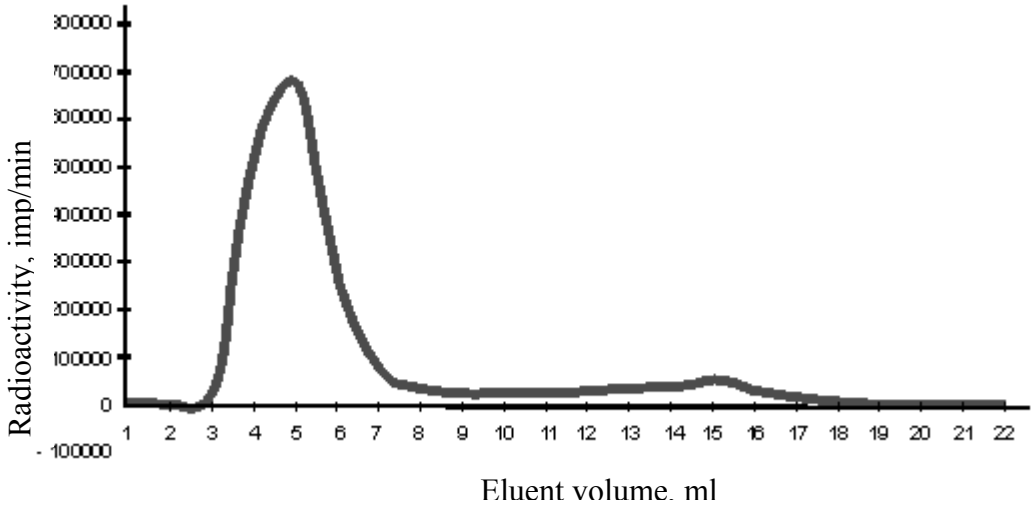

Fig. 1. **^{131}I-casein chromatogram**

As the source of iodine in the experiment, Na^{131}I, 370 kBq activity per head, similar in iodine action to potassium iodide, was used.

To study pharmacokinetics of labeled preparation, automatic gamma-counter GAMMA-550 (BECMAN USA) and scintillation gamma-camera MB-9200 (GAMMA Hungary) were used. Radioactive solution in 0,5 ml of physiological solution were injected by tube to lower third of an rat esophagus.
Scanning was performed in 30 minutes, 2,4,10 hours, 1,2,3,4,6 and 8 days to determine accumulation and elimination velocity of ^{131}I- casein и Na^{131}I from an animal organism. Scanning conditions were standardized during all observation periods. As a phantom, polyethylene 100 ml stoppered vial filled with water and ^{131}I activity of 370 kBq. At the same terms, animals were unitized; organs were removed, weighed and sampled. Sample portions were up to 1 g. Separately, daily urine and faeces were collected. Radiometry of tissue samples and defecations was performed under standard conditions in automatic gamma-counter. Statistical processing was made by Student's test criteria.

Results and discussion

First group of experiments have been performed on animals with insufficient iodine diet. The results of organ radiometry of the first group are presented in Table 1 and 2 correspondently. According to these data, thyroid iodine accumulation graph peak (77,2%) on the fourth hour after the treatment with ^{131}I-casein injection and then decreased to 40% of injected amount to 24 hours. When Na^{131}I injected, maximum accumulation of ^{131}I in thyroid occurs in 2 hours (64,5%), and then decrease almost twice to 4th hour. Slower accumulation of Iodine in thyroid from iodcasein is explained be the fact that before entering a thyroid, there is hydrolysis of a protein to amino acids in a gastrointestinal tract with following release of iodine under liver deiodinases. This process conditions gradual entering of iodine into blood in comparison with iodine from inorganic salt. Apparently, this is connected with higher accumulation of iodine from iodcasein in thyroid. In most organs, except thyroid

and liver, iodine content in 24 hour after treatment, had not exceed 1% of injected amount. Dynamics of accumulation-elimination of ^{131}I-casein and Na^{131}I in thyroid is presented in Fig.2. The analysis of thyroid iodine accumulation demonstrates that integral content of iodine in thyroid during three days after Iodcasein injection is about 38% of injected amount and about 24% when sodium iodide is injected, i.e. half as much (P <0,01).

Table 1. Accumulation of ^{131}I in organs of iodine deficient animals after ^{131}I-casein injection.

Organs and tissues	*Term after injections, hours*							
	0,5	**2**	**4**	**10**	**24**	**48**	**72**	**144**
Blood	26,2±2,0	7,9±3,1	2,6±0,5	1,3±0,9	0,9±0,5	0,9±0,5	0,6±0,2	0,4±0.1
Lung	2,7±0,5	0,6±0,3	0,3±0,1	0,3±0,1	0,1±0,1	0,2±0,1	0,1±0,1	0,1
Heart	0,8±0,3	0,1±0,1	0,1	>0,1	>0,1	>0,1	>0,1	>0,1
Thyroid	27,2±3,1	72,6±4,1	77,2±2,8	47,5±1,3	40,0±1,0	25,4±3,4	16,0±1,0	4,5±0,5
Liver	17,4±2,0	6,3±1,8	3,3±0,7	2,4±0,3	1,8±0,2	2,7±0,2	2,6±0,2	4,5±0,9
Kidney	5,7±1,1	1,4±0,6	1,3±0,4	0,5±0,1	0,3±0,1	0,3±0,1	0,1±0,1	0,2±0,1
Spleen	1,1±0,2	0,2±0,1	0,1	>0,1	>0,1	>0,1	>0,1	>0,1
Duodenum	2,4±1,1	1,3±0,2	0,1	>0,1	>0,1	>0,1	>0,1	>0,1

Notes: Here and in the Tables 2 - 4 values of $M \pm m$ are the percentage of injected activity.

Table 2. . Accumulation of ^{131}I in organs of iodine deficient animals after Na^{131}I injection.

Organs and tissues	*Term after injections, hours*							
	0,5	**2**	**4**	**10**	**24**	**48**	**72**	**144**
Blood	29,7±4,1	12,2±1,1	6,3±0,7	0,8±0,3	1,2±0,5	0,9±0,3	0,6±0,1	0,3±0.1
Lung	4,4±1,1	1,8±0,3	0,3±0,1	0,2±0,1	0,1±0,1	0,2±0,1	0,1±0,1	0,1
Heart	1,6±0,3	0,4±0,1	0,2	>0,1	>0,1	>0,1	>0,1	>0,1
Thyroid	22,5±6,8	64,5±3,4	34,3±2,4	30,7±0,3	27,1±0,5	22,3±1,2	13,1±0,7	3,5±0,5
Liver	22,4±0,3	7,3±1,6	1,9±1,1	0,5±0,2	1,6±0,2	2,4±0,3	2,6±0,2	1,5±0,9
Kidney	4,7±1,1	0,9±0,4	0,3±0,1	0,1±0,1	0,1±0,1	0,2±0,1	0,1±0,1	0,1±0,1
Spleen	1,8±0,7	0,8±0,1	0,1	>0,1	>0,1	>0,1	>0,1	>0,1
Duodenum	1,2±0,4	0,2±0,1	0,1	>0,1	>0,1	>0,1	>0,1	>0,1

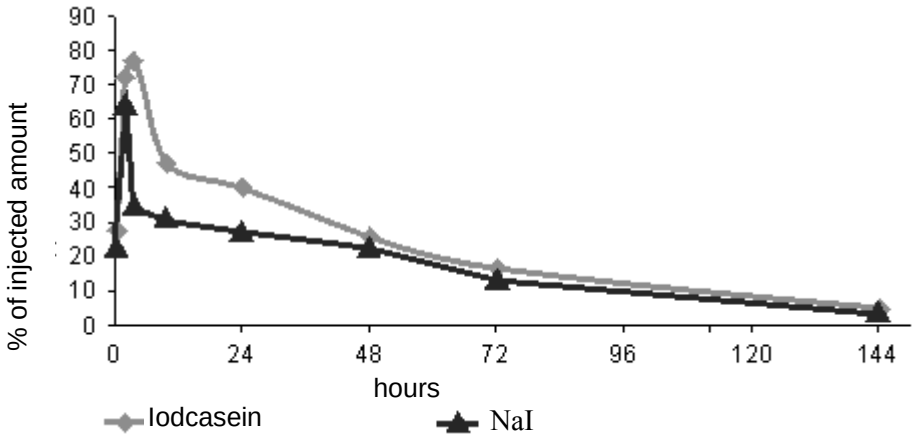

Fig 2. Dynamics of accumulation-elimination of **^{131}I** solution in iodine deficient animal thyroid under injection of ^{131}I-casein and Na^{131}I

The second group of experiments was performed in animals which iodine sufficient diet. In Tables 3 and 4 accumulation-elimination of iodine preparation in organs of such animals are presented.

Table 3. Accumulation of ^{131}I in organs of iodine sufficient animals after ^{131}I-casein injection.

Organs and tissues	*Term after injections, hours*								
	0,5	**2**	**4**	**10**	**24**	**48**	**72**	**96**	**192**
Blood	29,4±2,0	19,2±2,2	5,1±0,4	1,2±0,3	2,6±0,5	1,5±0,3	0,9±0,2	0,3±0,1	0,4±0.1
Thyroid	23,6±3,1	48,8±2,1	66,9±2,7	40,1±1,3	26,7±1,2	19,8±1,1	16,6±1,0	12,6±0,8	3,7±0,5
Liver	23,9±2,0	14,5±1,4	6,7±0,8	1,1±0,4	1,4±0,2	1,4±0,1	1,1±0,2	0,3±0,1	0,3±0,1
Kidney	7,2±3,1	3,4±0,6	2,0±0,4	0,1±0,1	0,2±0,1	0,2±0,1	>0,1	>0,1	>0,1
Duodenum	5,3±1,3	2,7±0,4	0,6±0,2	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1

Table 4. Accumulation of ^{131}I in organs of iodine sufficient animals after Na^{131}I injection

Organs and tissues	*Term after injections, hours*								
	0,5	**2**	**4**	**10**	**24**	**48**	**72**	**96**	**192**
Blood	27,2±4,3	16,3±3,1	2,5±0,3	2,5±0,2	2,6±0,4	1,1±0,2	0,3±0,2	0,2±0,1	0,4±0,1
Thyroid	26,7±3,4	63,8±1,2	58,3±2,5	50,6±1,4	47.6±1,8	26,6±2,1	23,1±1,3	11,9±0,6	3,8±0,3
Liver	20,4±2,3	8,8±0,6	5,5±0,3	1,2±0,3	1,2±0,2	0,8±0,1	0,9±0,2	0,6±0,1	0,6±0,1
Kidney	1,8±0,4	0,9±0,3	0,3±0,2	0,1±0,1	0,2±0,1	0,1±0,1	>0,1	>0,1	>0,1
Duodenum	3,3±0,6	0,8±0,3	0,4±0,2	>0,1	>0,1	>0,1	>0,1	>0,1	>0,1

The results of the second group experiments show that the peak of maximum thyroid accumulation of iodine from ^{131}I-casein was observed in fourth hour after injection as was in the first group, but its was approximately 10% less. Iodine elimination from thyroid was also smooth. Maximum thyroid iodine accumulation under Na^{131}I occurs in 2 hours after injection, similarly as in the first group, but iodine elimination from thyroid was slowed down. Accumulation of ^{131}I in other organs shows no significant differences within two groups. Iodine accumulation-elimination dynamics from thyroid in animals in the second group is presented in Fig.3. In the first animal group after Na ^{131}I injection, fast iodine elimination from thyroid was noted, contrary, in the second group it was slowed, especially from 10th to 24th hours. Due to this fact, iodine 'integral content' in thyroid during three days in iodine sufficient animals after ^{131}I- casein injection amounted about 31%, and after Na ^{131}I - 40%. I.e. in animals of the second group dynamics of accumulation-elimination differed with the first group.

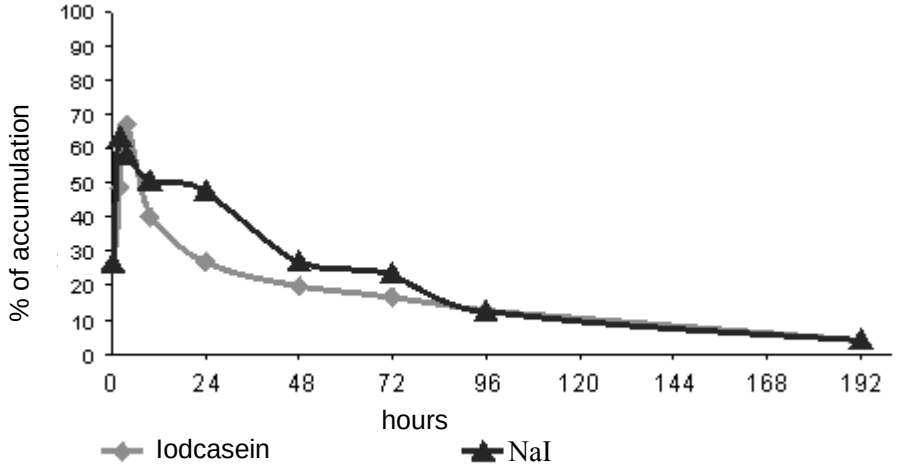

Fig 3. Dynamics of accumulation-elimination of **^{131}I** in iodine sufficient animal thyroid under injection of ^{131}I-casein and Na^{131}I

Radiometry of rats' feces under Na^{131}I injections shows that in the second group 90-95% of iodine was excreted with urine, corresponding to the almost the same amount in animals of the first group.

Curves of iodine in thyroid under 131I-casein injection in animals of the first ad the second groups were similar and differ only in iodine accumulation level (Fig. 2, 3)/ Integral content of iodine in thyroid on the third day of iodine sufficient animals was reliably less ($p<0,05$). We suppose, there is direct connection between low necessity of iodine in these animals regulating role of the liver when iodine is entered in organic form. Our results of feces radiometry demonstrated that 60% of iodine is excreted with urine and with the rest of the stool that proves the liver role in iodine metabolism.
Liver regulating role when iodine enters in the organic form was studied in 40-50th (8,9,10,11,12). Iodized protein in gastrointestinal tract first under protein-degrading enzyme decay into amino acids, including iodtyrosine. Then iodized amino acids enter to the liver, where iodine is released. Deiodization in a liver is performed by intracellular deiodinases, which fixed on endoplasmatic reticulum membranes, mitochondrions and microsomes. Limited Iodcasein deiodization can occur after it hydrolysis in gastrointestinal tract, on brush border and inside epithelial cells. But this process has no physiological significance [8]. Activity of the liver deiodinases depends on iodine insufficiency. Exceed iodised amino acids converted into glucuronids, and then return back to the intestine by biliary tracts [6]. It is well known that iodine is eliminated from an organism mainly by urine, At the same time, the elimination of organic forms of thyroxine and it metabolites takes place through biliary tracts. That's why Asimov and Zawadowsky named liver and kidney as 'final iodine filters'.

Obtained in the present study results aloow to make following conclusions:

1. When Iodcasein is entered under iodine deficiency state, thyroid consumes iodine in greater amount in comparison with inorganic salt.
2. The peak of iodine accumulation under Idcasein injection is observed in four hours; when iodized salt is injected – only in 2 hours. The elimination of iodine, entering into an organism in the form of Iodcasein under iodine deficiency, was slower.
3. Dynamics of thyroid iodine accumulation-elimination under 131I-casein injection was similar in iodine sufficient and iodine insufficient animals. Iodine accumulation was higher at iodine deficiency.
4. When iodine is entered in inorganic form, it retention in thyroid was significant greater in iodine sufficient animals that in iodine insufficient animals.
5. The results of study of iodine elimination when iodine is entered in organic form (Iodcasein) prove liver participation in iodine metabolism.