

MINISTRY OF HEALTH OF THE REPUBLIC OF BELARUS

Belorussian Engineering Academy

Gomel State Medical Institute

**CLINICAL AND EXPERIMENTAL ASPECTS
OF THE EFFECT OF INCORPORATED
RADIONUCLIDES UPON THE ORGANISM**

Edited by Professor Yu.I. Bandazhevsky and
Professor V.V. Lelevich

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The results of clinical and experimental studies of the effect of incorporated radionuclides upon the conditions of vital systems are presented. The relationship between a number of pathological conditions and the dose of accumulated radioactive elements is described.

For specialists in medical biology investigating the problems of radiation medicine.

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FOREWORD

To eliminate the aftermath of the Chernobyl disaster is one of the major tasks of health care in the Republic. Research aimed at elucidating the pathogenesis and fundamental clinical manifestations of disorders and conditions produced by radioactive elements is a challenging task both practically and scientifically. The monograph "Clinical and Experimental Aspects of the Effect of Incorporated Radionuclides upon the Organism" edited by Professor Yu.I. Bandazhevsky and Professor V.V. Lelevich describes the condition of vital systems affected by accumulated radionuclides in the organism. The results of several years of investigation accomplished by the research team based at the Gomel Medical Institute are presented to the medical community.

By combining clinical and experimental methods in their research the authors have obtained the results which have a valuable scientific and practical significance. I hope that this effort to develop methods of diagnosis, treatment and prevention of the effects of radionuclides upon the organism will find its continuation.

I.M. Drobyshevskaya
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The Chernobyl disaster is one of the largest ecological disasters of the present century on the globe. Its consequences will remain in the focus of radiobiologists, experts of radiation medicine, clinical doctors, ecologists and other specialists for a long time.

It is a strong warning to the mankind on its evolution from biosphere to noosphere considered by V.I. Vernadsky as an environment restructured by the human intellect and power to satisfy needs of the society.

A huge number of publications have appeared in the recent years on the Chernobyl disaster written by journalists, scientists and politicians.

Regretfully, the problems of the disaster have been and still remain the subject of political commercialism and career promotion. Even the belated manuscript by the Academician L.A. Ilyin "The Realities and Myths of Chernobyl" resembles an essay created in a cozy and quiet Moscow library room and written for self-exemption and self-redemption rather than to achieve a profound scientific result.

Therefore, the present manuscript is the first kernel of the objective scientific information on the radionuclides effect upon the organism. It evaluates the risk to the whole world such disasters may incur and the extent of justified optimism of those who lived and continues to live on the native victimized soil.

A.S. Shaginyan,

**Academician, Vice-President of
the International and Belorussian
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INTRODUCTION

The Chernobyl disaster in 1986 has been a severe grievance for the people of Belarus. Alongside with socioeconomic cataclysms the radiation factor has affected the people living in contaminated areas. At least 180 million Curies of radioactive substances were released into the environment. They have spurred a number of diseases, specifically neoplasm of the thyroid, endocrine and cardio-vascular pathology. Considering the entire variety of the factors it is a problem to discriminate those which are decisive in the evolution of pathological processes. While initially short-living isotopes (^{137}I , ^{89}Sr , inert gases krypton, xenon and others) were the leading contributors into the dose rate, subsequently ^{137}Cs , ^{134}Cs and ^{90}Sr became dominating. These elements both govern the external radiation background and penetrate into organisms with food, water and air. They accumulate in tissues and organs and destroy their normal functions. Though 9 years have passed after the disaster the lives of the people in these areas remain under threat. Only the nature of this effect undergoes changes. While a lot of information has been obtained about the effect of external radiation, quite little is known how incorporated isotopes affect the human organism.

The present manuscript deals exactly with this problem. It contains the results of studies accomplished at the Gomel and Grodno Medical Institutes. The authors do not claim that their conclusions are beyond argument, they believe it is their duty to make the results of this 5-year long study initiated by the Gomel Medical Institute known to the international medical community. It is hoped that this information will be a useful step stone for further investigation of the effect of radioactive substances upon the human organism with a view to develop methods of protection and treatment.

**Professor Yu.I. Bandazhevsky
Professor V.V. Lelevich**

Chapter 1

PREGNANCY EVOLUTION, FETUS AND NEWBORN DEVELOPMENT DURING ENDOGENOUS RADIATION INCORPORATION

The problem of ionizing radiation effect upon the human organism has been an ambitious task since the Chernobyl disaster. It is a current specific feature that ionizing radiation chronically affects the organisms of humans and animals through its incorporation with food [11]. During recent years the disaster effects already touch greater contingents of people exposed to the ionizing radiation.

These conditions render the population group of pregnant women and infants critical [1, 15, 17]. Extensive research has been accomplished to get insight into the problem how radioactive exposure affects pregnancy, fetus and newborn development [3—5, 9, 13, 14]. It has been revealed that the embryo is specifically vulnerable during "critical periods" when this or that systems undergoes differentiation [13]. Chronic exposure is particularly risky affecting various types of embryonic cells damaging a great number of germinal organs during such critical periods of their development, very small doses of ionizing radiation causing the maximum injury. Among other products of nuclear fission released by Chernobyl ^{137}Cs and ^{90}Sr having a long half life period are most detrimental for the biological chain. Hence small doses of these radionuclides are continuously incorporated by living organisms with food.

So far there is insufficient insight into the effects of small doses of chronic endogenous incorporation of ^{137}Cs and ^{90}Sr upon the processes of prenatal and postnatal development. The objective of the present study is a comprehensive morphofunctional investigation of the features of prenatal and postnatal development of common white rats when these radionuclides are incorporated with the components of their diet.

The major objectives of the study are the following:

1. To investigate the features of prenatal development of common white rats during endogenous incorporation of radionuclides (^{137}Cs and ^{90}Sr) with food.

2. To reveal the features of postnatal development of common white rats during endogenous incorporation of radionuclides (^{137}Cs and ^{90}Sr) with food.

3. To investigate the pattern of accumulation of ^{137}Cs during its endogenous incorporation with grain by the mother and the offspring.

4. To reveal possible metabolic changes in the organism of female white rats in pregnancy during endogenous incorporation of radionuclides (^{137}Cs and ^{90}Sr) with food.

The study covered 137 pregnant female common white rats, 745 embryos and 344 infant rats. The first day the sperm would be found in vaginal smears was assumed the pregnancy start. The animals were kept in the vivarium. The fetuses were extracted on the 20th day of intrauterine development because by this time the orthogenesis and placenta evolution would be completed [6].

In order to investigate the features of prenatal and postnatal development the animals were kept on a diet providing a daily incorporation of radionuclides (beef and oats). The concentration in the food (meat and grain) for the diet of the control and test animals is shown in Table 1.1.

The daily incorporation of radionuclides by pregnant rats was estimated based on the food consumption (Table 1.2).

Therefore, the food radioactivity was primarily rated based on ^{137}Cs .

The test and control animal groups to investigate prenatal and postnatal development were determined based on the qualitative composition of the diet (beef meat or oats) and the concentration of radionuclides (Table 1.3)

The following techniques were applied to achieve the objectives:

1. The method of Wilson modified by A.P. Dyban (A.P. Dyban et al., 1970) to investigate the condition of internal organs of fetuses.

In order to evaluate the condition of internal organs and the brain the embryos fixed in the Buen mixture were placed on a paraffin table and a series of parallel sections were made with a razor.

2. The Dowson method modified by A.P. Dyban [6] to investigate the bony skeleton of rat fetuses. The size of skeletons (the pariet-to-coccygeal size) was measured with compasses. The ossification analogue size was measured with the help of an ocular micrometric microscope (MPC-10, ocular 8, magnification 2).

Table 1.1

Concentration of radionuclides in rat's diet

Radionuclides specific activity, Bq/kg	Groups			
	meat		grain	
	test	control	test	control
¹³⁷ Cs	5587	49.0	445.7	44.2
⁹⁰ Sr	23.7	1.7	15.5	1.7

Table 1.2

Daily incorporation of radionuclides by pregnant rats with food
(Bq/kg)

Groups	¹³⁷ Cs	⁹⁰ Sr
meat-test	84	36×10^{-2}
meat-control	74×10^{-2}	3×10^{-2}
grain-test	16	54×10^{-2}
grain-control	155×10^{-2}	6×10^{-2}

Table 1.3

Numbers of rats, embryos and offspring in experiments

Groups	Prenatal development		Postnatal development	
meat-test	20	115	4	69
meat-control	23	100	10	37
grain-test	24	292	15	127
grain-control	24	238	17	111
TOTAL	91	745	46	344

3. A common technique for hemoglobin, erythrocyte, leucocyte count and the leucocytary formula.

4. Measurement of ¹³⁷Cs accumulation in the mother and the offspring. The ¹³⁷Cs concentration in female common white rats during pregnancy and lactation and in the offspring during lactation was checked in vivo based on γ -emission using a gamma -radiometer L - 92.

5. A "Synchron" analyzer produced by Beckman (USA) served to evaluate biochemical indicators of the blood serum of pregnant rats.

6. Evaluation of ¹³⁷Cs and ⁹⁰Sr concentration in food was performed using spectrometric and radiochemical technique of the Gomel Regional Sanitation and Hygiene Center. The variational statistics technique was used for data processing.

The study has revealed that in case female white rats since the 1st day of pregnancy are kept on a diet of meat with the ¹³⁷Cs concentration 5587 Bq/kg and ⁹⁰Sr 23.7 Bq/kg there is a valid increase of preimplantation death — 2.27 ± 0.52 ($p < 0.05$). Consumption of grain with ¹³⁷Cs concentration 445.7 Bq/kg, ⁹⁰Sr — 15.5 Bq/kg by pregnant rats would not produce any essential preimplantation death rate compared with the control group. The postimplantation death rate in the two test groups corresponded to the control.

The data about higher preimplantation death rate during radioactive exposure correlate with other reported data about the death rate of fetuses.

during this period [16, 18]. The analysis of publications indicates that radiation during the preimplantation period induces more frequent mutations of two types or mutations of genes and chromosomes. The genetic mutations kill the zygote, non-lethal mutations may be the cause of monsters [12].

The study has revealed that mothers after dieting during pregnancies on meat and grain with a higher ^{137}Cs and ^{90}Sr concentration in the test group would have an offspring with a valid reduction of the average size of bony skeletons (the pariet-to-coccygeal size) compared with the relevant control (Table 1.4).

The mothers after dieting on meat with a higher ^{137}Cs and ^{90}Sr concentration would have an offspring with the valid absence of the anlagen of ossification of metacarpal and metatarsal bones compared with the control. No significant difference between the test and control offspring if mothers would diet during pregnancy on the grain (Table 1.4).

Investigation of the condition of bony skeletons of the test fetuses after feeding mothers with the meat containing ^{137}Cs has revealed a valid symmetrical hypoplasia of the anlagen of ossification of all the bones manifested to different extent, the bilateral absence of anlagen of pubic bones, absence of points of ossification of the 2nd and 4th metacarpal bones, 2nd, 3rd and 4th metatarsal bones (Fig. 1.1).

Similar studies of bony skeletons of test fetuses born by mothers after dieting with a higher ^{137}Cs and ^{90}Sr concentration in the grain has revealed a valid bilateral reduction of the size of anlagen of ossification of all the bones, excepting the 4th metacarpal and 2nd and 3rd metatarsal bones (the difference with the control group is insignificant). Also a bilateral absence of anlagen of pubic bones, the 2nd metacarpal, the 4th metatarsal bones (Fig. 1.2) were found.

Evaluation of the results and comparison with the reported data allow to assume that the major causes of misformation of the bony system among white rat fetuses can be a disordered inductive interaction between the somato-pleural mesoderm and the coating endoderm or the death of mesenchymal cells caused by the incorporated ^{137}Cs . Another possible cause may be the abnormalities occurring during separate stages of the bone tissue morphogenesis [8].

Table 1.4

Development of bony system of 20-day old white rat fetuses

Indicators	meat		grain	
	test	control	test	control
Skeleton size of fetuses, mm	21.39±0.48	24.70±0.41	23.84±0.39	25.55±0.35
Fetuses without anlagen of ossification of metacarpal and metatarsal bones, %	31.4	13.0	33.8	25.9

The 45-day old offspring of the mothers after the meat diet with a higher radiation concentration manifested valid reduction of leucocytes $6.27 \pm 0.75 \times 10^9 / l$ ($p < 0.05$), lymphocytes $72.38 \pm 3.35\%$ ($p < 0.02$) in the peripheral blood against the increased eosinophil count $2.63 \pm 0.65\%$ ($p < 0.05$) compared with the control group.

Daily ^{137}Cs incorporation by female rats since the 1st day of pregnancy and throughout the lactation period with oats containing 445.7 Bq/kg would result in its significant accumulation in the mother's organism. The offspring would not manifest any pronounced accumulation of ^{137}Cs (Fig. 1.3).

Similar dynamics was manifested by the control group also when oats contained 44.2 Bq/kg of ^{137}Cs , but it was less pronounced. The ^{137}Cs concentration among the test female rats by the end of pregnancy was 132.77 ± 10.77 Bq/kg and the control rats had 9.22 ± 2.90 Bq/kg. The test offspring had the ^{137}Cs concentration 6.47 ± 2.18 Bq/kg, the control group had 1.61 ± 0.87 Bq/kg (Fig. 1.4).

Persistent differences in the ^{137}Cs accumulation between the test and control female rats at the end of pregnancy and during lactation can probably be explained by the fact that the ^{137}Cs absorption is stimulated resulting in its growing accumulation in the mother's organism.

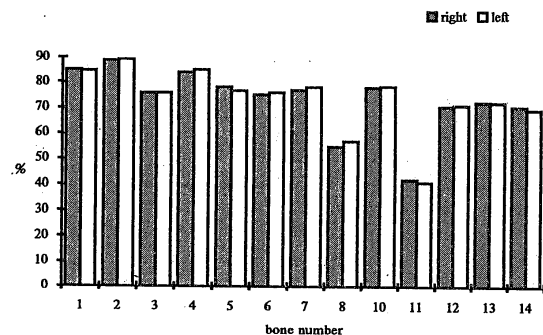


Fig. 1.1. Size of anlagen of ossification of skeleton in 20-day fetuses of test group (experiment with meat)

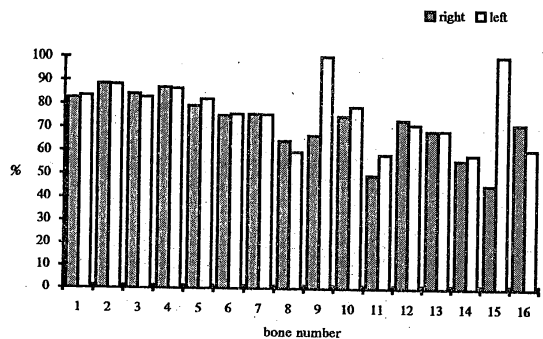


Fig. 1.2. Size of anlagen of ossification of skeleton in 20-day fetuses of test group (experiment with grain)
 1 — parietal bone; 2 — mandible; 3 — scapula; 4 — clavicle; 5 — humerus; 6 — elbow bone; 7 — radius; 8 — metacarpal III; 9 — metacarpal IV; 10 — iliac bone; 11 — ischial bone; 12 — femur; 13 — tibia; 14 — fibula; 15 — metatarsal II; 16 — metatarsal III

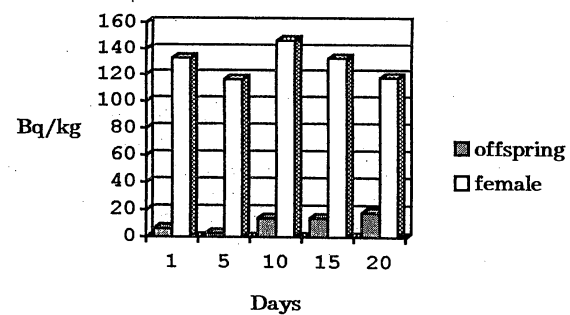


Fig. 1.3. Dynamics of ^{137}Cs accumulation by mothers and offspring in test group during lactation

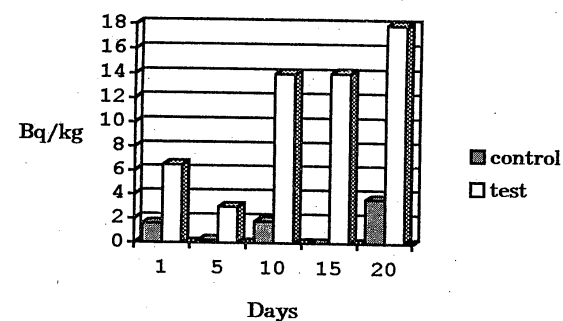


Fig. 1.4. Dynamics of ^{137}Cs accumulation by offspring during lactation

Persistent differences in the accumulation of this radionuclide between the mother and the offspring and its essential absence in the organism of newly born rats is explained by the barrier function of the mother's organism.

The growing ^{137}Cs accumulation in the test offspring while they grow and develop is assumed to be due to a gradual reduction of lactation and partial transition to independent nutrition [20].

The test animals on the grain diet with a higher ^{137}Cs and ^{90}Sr concentration manifest the albumin concentration 13.83 ± 1.01 ($p < 0.05$) and calcium 1.87 ± 0.16 ($p < 0.05$) on the 20th day of pregnancy. It can possibly be explained by the processes of compensation and adaptation in the pregnant animals' organism during endogenous incorporation of radionuclides [10].

Summarizing the obtained facts it can be concluded that daily incorporation of relatively small amounts of radionuclides (^{137}Cs — 16.84 Bq/kg, ^{90}Sr — 36—54 Bq/kg) by test animals in pregnancy can cause noticeable disorders of the offspring prenatal and postnatal development.

CONCLUSIONS

1. Incorporation by of radionuclides (^{137}Cs and ^{90}Sr) with food (meat and grain) affects the offspring prenatal and postnatal development manifested by embryotoxic effects and abnormal bony system development.

2. When female rats since the 1st day of pregnancy consume meat containing 5587 Bq/kg of ^{137}Cs and 23.7 Bq/kg of ^{90}Sr embryotoxic effects are manifested by a higher preimplantation death rate of fetuses.

3. When female white rats since the 1st day of pregnancy consume beef meat containing 5587 Bq/kg of ^{137}Cs and 23.7 Bq/kg of ^{90}Sr and oats containing 445.7 Bq/kg of ^{137}Cs and 15.5 Bq/kg of ^{90}Sr it results in abnormal formation of the offspring bony system manifested by the reduced skeleton size of fetuses, various degrees of hypoplasias of anlagen of ossification of skeleton bones, absence of points of ossification of metacarpal and metatarsal bones.

4. The postnatal pathology after incorporation of radionuclides (^{137}Cs and ^{90}Sr) by female white rats with food (meat) in pregnancy and lactation manifests reduction of leucocytes, lymphocytes and a higher

eosinophil count in the peripheral blood when the offspring reaches the 45th day.

5. Endogenous incorporation of ^{137}Cs with food by female common white rats in pregnancy results in its significant accumulation in their organisms and its significantly less accumulation by the offspring.

6. Daily incorporation of radionuclides (^{137}Cs and ^{90}Sr) by pregnant rats with food (meat and grain) does not cause any substantial disorders of metabolic process compared with the control. The increased albumin and calcium concentration in the blood serum after consumption of grain with a higher concentration of radionuclides is assumed to be due to the process of compensation and adaptation induced by the radionuclides in the pregnant animal's organism.

7. The obtained experimental data can be useful for development of diagnostic actions, prevention and treatment of pathological conditions of the mother and the fetus exposed to radiation.

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Chapter 2

EFFECT OF RADIONUCLIDES UPON FEMALE REPRODUCTIVE SYSTEM

The female reproductive system is highly vulnerable to ionizing radiation [2, 4]. Repeated experiments and clinical data evidence that modifications in ovaries caused by incorporated substances are similar to the effect of external gamma- and X-exposure. In the majority of cases they are manifested by dystrophy and death of the follicular apparatus [3].

The pathological modifications are primarily detected in the epice-
lium and oocytes of mature follicles. As doses of radiation increase (200 R and up) dystrophic changes appear in mature follicles, oocytes being the first to perish. Very high doses (600—800 R) induce destructive processes in primary follicles. Similar effects are produced by incorporated radioactive isotopes [2].

So, when rats received uranium fission products perorally in the amount 10 mCuries per gram maturing or about to be mature follicles would be completely destroyed.

Similar changes have been observed after intraperitoneal and peroral introduction of ^{90}Sr , ^{60}Co , the degree of damage would be the function of the dose an introduced radionuclide [3].

An adequate reflection of pathological changes in ovaries is the condition of the estrous cycle. In response to the dose of entirely introduced radioactive substances experimental animals demonstrated changes of various nature. Introduction of uranium fission products in the dose 7 mCuries per gram would cause a longer diestrous and metoestrous phase and a corresponding reduction of the proestrous and estrous phase. The dose 2.5 mCuries per gram would just increase the diestrous phase [3].

The experimental results have been confirmed clinically. Variations of the menstrual cycle have been registered among female patients living in the contaminated areas [1]. At the same time the pattern of the female reproductive system variations in response to the level of endogenous in-

corporation of radionuclides penetrating into the organism through natural chains remains almost obscure.

In this connection a clinical and experimental study has been performed to investigate the effect of endogenous accumulation of radionuclides in the female organism upon the reproductive system.

The objectives have been the following:

1. To evaluate the status of the reproductive systems among pubescent females in response to the level of gamma—irradiation.
2. To test the female animals in order to clarify the relationship between the dose of accumulated radionuclides incorporated through the food diet and the morphofunctional condition of the reproductive system.

I. CLINICAL PART

Gynecological examinations of 650 female patients living under continuous exposure to small doses have been performed. Among them 170 patients aged between 18 and 40 years. This age cohort was split into groups based on the dose of accumulation of radionuclides. The first group comprised females with the accumulated dose of ^{137}Cs up to 20 Bq/kg, the second with the dose between 20 and 30 Bq/kg, the third with the dose between 30 and 40 Bq/kg, the fourth group with the dose above 40 Bq/kg. Blood was sampled among all the patients based on the menstrual cycle (on the 7th and the 21st days) in order to estimate biochemical indicators: total bilirubin, LDG, phosphorus, alkaline phosphates, ALT, creatinine, total protein, albumin's, AST, cholesterol and also estradiol and progesterone. The results were split among the groups. Comparative analysis of the results has revealed valid differences in the concentration of progesterone and estradiol during the 1st and 2nd phases of the menstrual cycle between the 1st and the 4th groups (Fig. 2.1—2.4). Accumulation of radionuclides in the organisms in excess of 40Bq/kg is accompanied by a pronounced disbalance in the production of sexual hormones, specifically by the reduction of progesterone and simultaneous increase of estradiol during the 2nd phase of the menstrual cycle, by the increase of progesterone and reduction of estradiol during the 1st phase. Without doubt, it affects the health condition of women, the functions of the reproductive system.

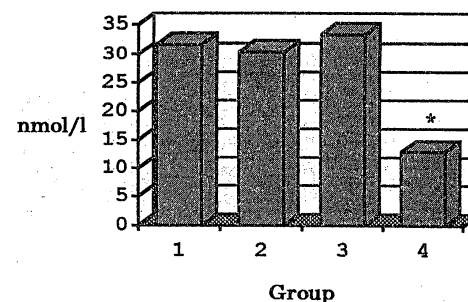


Fig. 2.1. Level of progesterone in II phase of menstrual cycle; * — in respect to the 1st group ($p < 0.05$)

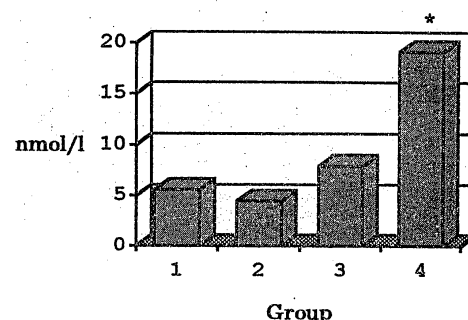


Fig. 2.2. Level of progesterone in I phase of menstrual cycle; * — in respect to the 1st group ($p < 0.05$)

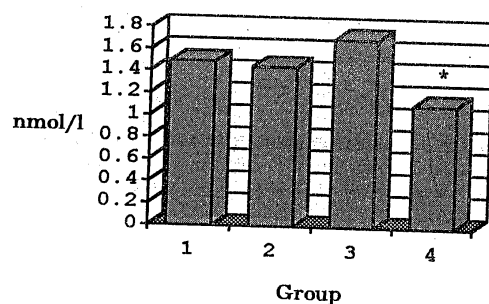


Fig. 2.3. Level of estradiol in I phase of menstrual cycle; * — in respect to the 1st group ($p < 0.05$)

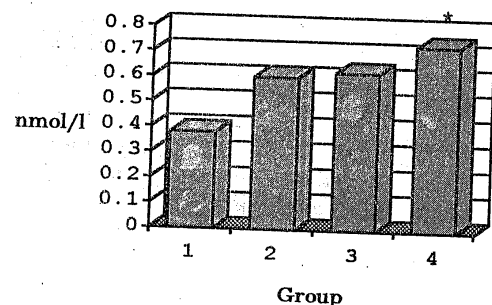


Fig. 2.4. Level of estradiol in II phase of menstrual cycle; * — in respect to the 1st group ($p < 0.05$)

The patients were interviewed and examined gynecologically in order to evaluate the health status of pubescent females from the gynecological point of view. A tendency has been detected that the incidence of the following diseases in response to accumulation of radionuclides is on the rise: inflammatory disorders of adnexa, disorders of the menstrual cycle, uterine myomas and infertility, to a lesser extent (Fig. 2.5—2.8).

The major pathological conditions have been confirmed by ultrasonic body screening.

II. EXPERIMENTAL PART

The results of experimentation are based on the observation of 100 common female white rats with the body weight 200—250 grams. The animals were kept on a standard diet in the vivarium. 50 female rats were included into the experimental group which were kept during two weeks on a diet to achieve incorporation of ^{137}Cs in a daily dose 84 Bq by feeding them with radioactive grain. The control group included 50 animals on the same grain diet containing 1.5 Bq of ^{137}Cs .

After this term vaginal smears were examined in the microscope to trace the condition of the estral cycle. In order to assess possible changes in the uterus and ovaries rats of the two groups were decapitated based on the estral cycle phases. 50 animals were examined in the estrous stage, 50 in the diestrous stage. On the day of decapitation the animals were weighed and the accumulation of ^{137}Cs was checked.

After decapitation blood was sampled to determine hematological indicators, the level of hormones (estradiol — progesterone). To estimate changes in the reproductive system rat ovaries and uteruses were fixed in 10% formalin and then in paraffin to make histological preparations 5—8 mm thick colored with toxilin and eosin.

The histological preparations were examined under the microscope and described. Secondary follicles were counted over the entire organ section in nuclei. All the uterine layers were measured to check thickness. The results were processed statistically using the Student criterion.

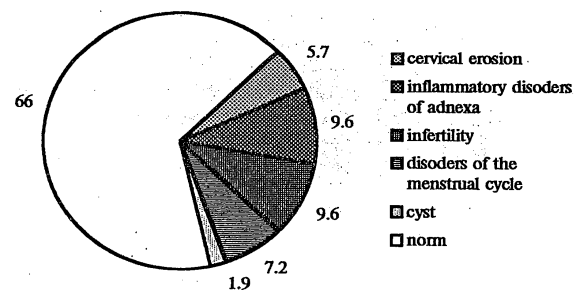


Fig. 2.5. Percent distribution of frequency of pathological conditions of female reproductive system in 1st group

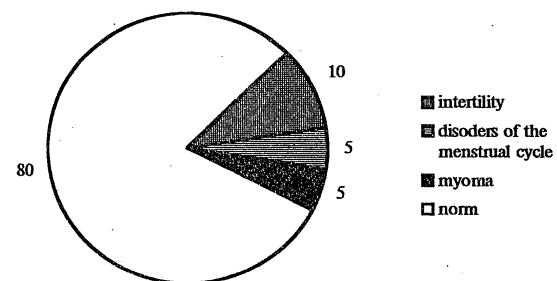


Fig. 2.7. Percent distribution of frequency of pathological conditions of female reproductive system in 3rd group

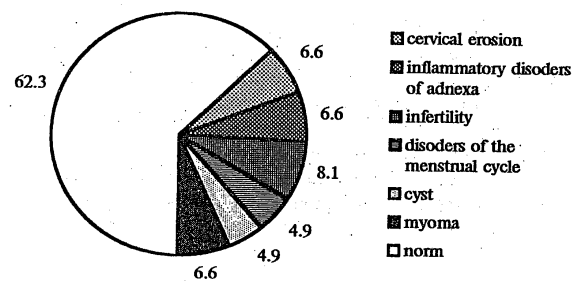


Fig. 2.6. Percent distribution of frequency of pathological conditions of female reproductive system in 2nd group

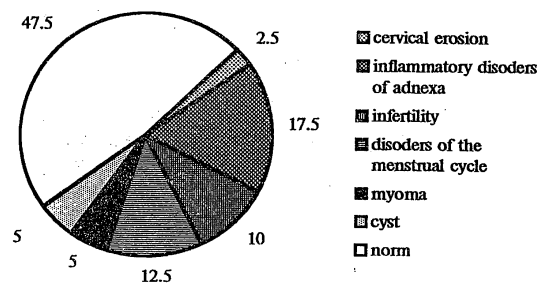


Fig. 2.8. Percent distribution of frequency of pathological conditions of female reproductive system in 4th group

The results have revealed a valid increase of ^{137}Cs concentration in the organisms of experimental animals ($54.30 \pm 6.28 \text{ Bq/kg}$) compared with the control (14.05 ± 3.31 — $p < 0.001$). It was accompanied by the reduced concentration of progesterone in the estrous stage in the experiment to $23.96 \pm 6.94 \text{ nmol/l}$ (the control was $61.01 \pm 15.66 \text{ nmol/l}$) and reduced thickness of the uterus respectively (the experiment was 3.91 ± 0.99 at $p < 0.05$ compared with 6.57 ± 0.52 conventional units in the control).

Thus, accumulation of radionuclides in the organisms of experimental animals alters metabolism relating to the production of progesterone during separate phases of the estrous cycle resulting in morphofunctional changes in the uterine structure. It should be noted that the numbers of secondary follicles in ovaries did not manifest any valid difference.

At the same time no valid reduction of the number of follicles during different stages of development of experimental animals compared with the control has been detected.

Taking into account the above result pronounced functional modifications of the endocrine system of the animals can be assumed affecting the pituitary body, ovaries and the uterus. Changes in the other endocrine organs, specifically in adrenal and thyroid glands, should not be missed.

CONCLUSION

The clinical and experimental study has revealed an adverse effect of endogenous incorporation of radionuclides upon the status of the female reproductive system. Specifically, accumulation of radioactive elements, primarily ^{137}Cs , is accompanied by profound functional modifications of the endocrine system manifesting themselves in disorders of progesterone and estrogen production. Experimental animals have demonstrated both clinically and experimentally the relationship between the dose of accumulated radionuclides and production of the above hormones. The consequences of such hormonal disbalance in the form of morphological changes of the uterus present interest and practical significance.

Such modifications can be caused both by the direct effect of radionuclides upon the oocytes and the inflammatory disorders in the uterus due to the reduction of specific and non-specific resistance.

As a final result the functional disorders of the system of ovaries and the uterus in response to the incorporated dose are manifested by relevant pathological conditions, such as disorders of the menstrual cycle, infertility, tumoral processes.

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Chapter 3

IMMUNITY CONDITION OF CHILDREN FROM AREAS CONTAMINATED WITH RADIONUCLIDES

The immunity system is one of the most vulnerable systems to radiation.

Numerous studies after the Chernobyl disaster among the population in the contaminated areas have failed to yield any explicit answer what modifications evolve in the immune system and what is their result. Publications report reduction of NK-cells, T-suppressors, T-killers, natural killers and the phagocyte activity of neutrophils [9, 10, 11]. The humoral immunity condition is appealing. The level of all immunoglobulins is reported to go down [8], only JgA is reported to reduce [7]. The increase is reported for JgM [1, 6] and JgG [7].

The blood system of children is particularly vulnerable to radiation. A valid reduction of T- and B-lymphocytes, major classes of immunoglobulins [3, 4], suppressed proliferation of T-cells under stimulation by mitogens [2] have been observed. A typical feature is inhibition of the suppresser link accompanied by the growing concentration of JgM and JdG [5]. Yet, the majority of the data relate to the population in the areas where the ^{137}Cs contamination exceeds 5 Curies per km^2 completely ignoring the areas with the ^{137}Cs contamination between 1 and 5 Curies per km^2 where the population is much greater.

Hence, the objective of the present study is to evaluate the condition of the immune system using a number of lab indicators widely practiced clinically among children exposed to small radiation doses. Totally 1313 children have been examined in four groups: the first group (235 children) residing in Gomel (the ^{137}Cs contamination level is 1 to 5 Curies per km^2); the 2nd group (137 children) residing in Svetlogorsk after relocation from the sites with the ^{137}Cs soil contamination above 40 Curies per km^2 ; the 3rd group is the 1st control group (827 children) residing in Zhlobin (the ^{137}Cs contamination level is under 1 Curie per km^2); the 4th group is the 2nd control group (114 children) residing in Grodno (the ^{137}Cs contamination level is under 1 Curie per km^2). The children were

divided into two age subgroups: (a) 3 — 6 years; (b) 6 — 8 years. The sex was also considered.

In addition to the general clinical examination lab tests were performed to evaluate the following indicators of the peripheral blood: the count of erythrocytes, hemoglobin, leucocytes, trombocytes, cellular elements in the blood smear, ESR, the phagocyte activity of neutrophil leucocytes, the concentration of JgA, G, M, M-ROK and E-ROK. The mean values were determined within the groups and compared.

The results were processed statistically using the Student criterion. No significant deviation of the mean values of the hematological parameters within the groups from the established physiological parameters has been detected. At the same time there were valid differences between the groups and subgroups. Specifically, the ESR in Gomel was much higher than among children from Grodno and Svetlogorsk in the age group 6 — 8 years. This indicator among the children in Svetlogorsk within 6 — 8 years corresponds to that among the Gomel children, it is much lower among the children within 3 — 6 years of age both boys and girls than in the other groups (Tables 3.1, 3.2)

Pronounced differences have been traced in the JgA concentration. It was reduced among the children relocated to Svetlogorsk. At the same time the JgM concentration among the children within 3 — 6 years of age in Gomel was validly higher than among the children of the same age in Grodno and Zhlobin. The JgI concentration was the highest among the children in Zhlobin and it was distinctly different from the concentration among the children in Gomel and Grodno (Tables 3.1, 3.2). So, the studies have revealed that the children in the contaminated areas have not suffered from any significant deviations in the blood pattern compared with the children in the "clean" areas. At the same time the suppressed phagocyte activity of neutrophil leucocytes, reduced JgA concentration and increased JgM concentration have been registered. These changes prove disordered functions of a number of immunity system links, specifically the phagocytosis, generation of A and M immunoglobulins. It should be emphasized that the children relocated from the "strictly" controlled areas have the hematological and immunological indicators corresponding to the control level, excepting the ESR and JgA.

Table 3.1

Immunological indicators among children within 3 — 6 years in studied areas

Indicators	Gomel group N 1	Svetlogorsk group N 2	Zhlobin group N 3	Grodno group N 4
ESR	10.25±0.57**	3.56±0.41**	8.71±0.25**	6.62±0.42
Phagocyte activity of neutrophil leucocytes	36.69±0.88**	51.74±2.01	45.08±0.56	47.83±1.37
JgA (g/l)	1.04±0.05*	0.94±0.09**	1.46±0.05	1.38±0.08
JgM (g/l)	1.28±0.06*	1.23±0.13	0.93±0.03	1.01±0.06
JgG (g/l)	11.60±0.28	10.53±0.86	13.46±0.27*	12.32±0.49

* — $p < 0.05$ compared with group N 4

** — $p < 0.01$ compared with group N 4

It evidences that the blood forming and the immune systems are capable to restore their structures when the effect of radionuclides is eliminated.

The obtained results correlate with those after examination of other groups of children which suffered from the Chernobyl disaster. So, the children from the areas exposed to radiation would have more frequent respiratory disorders and their immunity status would be reduced due to the inhibited functions of the cellular and humoral links, specifically by the generation of JgA [2, 3, 4, 5]. Taking into account the above stated it is a challenging scientific and practical task to perform laboratory studies of experimental animals to evaluate the relationship between the accumulated dose of radionuclides, the composition of immunocompetent cells in the peripheral blood.

Table 3.2

Immunological indicators among children within 6 — 8 years in studied areas

Indicators	Gomel group N 1	Svetlogorsk group N 2	Zhlobin group N 3	Grodno group N 4
ESR	8.71±0.83*	8.80±0.87*	6.63±0.30*	4.06±0.43
Phagocyte activity of neutrophil leucocytes	33.10±1.69*	39.70±1.21	44.30±0.73	42.24±3.17
JgA (g/l)	1.32±0.10*	-----	1.54±0.08	1.89±0.27
JgM (g/l)	1.35±0.13	-----	1.25±0.06*	0.88±0.15
JgG (g/l)	12.54±0.43	-----	15.90±0.36*	11.87±0.97

* — $p < 0.05$ compared with group N 4

To accomplish it the immunity status of laboratory animals was evaluated during endogenous incorporation of radionuclides with the assessment of the extent of expression of differentiated antigens LCD-5, LCD-8, LCD-4 together with the antigens of monomorphous determinants of the MNS class using the monoclonal antibodies (MCA) in an indirect test for immunofluorescence (IF).

Fourteen (14) male "Wistar" rats were kept on a standard food diet. Daily they would receive 35 grams of grain (oats). The concentration of radionuclides in the grain of the test group (7 animals) was ^{137}Cs — 445.7 Bq/kg, ^{90}Sr — 15.5 Bq/kg, in the control group (17 rats) it was ^{137}Cs — 44.2 Bq/kg, ^{90}Sr — 1.7 Bq/kg. The animals of the test and control groups weighed 250 — 320 grams.

Considering their food consumption the male rats of the test group would daily receive with grain (oats) 16 Bq of ^{137}Cs , $54 \cdot 10^{-2}$ Bq of ^{90}Sr ,

the male rats of the control group would receive $155 \cdot 10$ Bq of ^{137}Cs , $6 \cdot 10^{-2}$ Bq of ^{90}Sr . Thus, the grain radioactivity was governed primarily by ^{137}Cs .

At the moment the animals were killed the average radioactivity of the test animals was 62.76 ± 3.84 Bq/kg and control animals 9.76 ± 1.77 Bq/kg. Peripheral blood lymphocytes of the rats were separated from the heparinized blood sampled in the heart cavity (10 units of heparin "Richter" per 1 ml of blood, on the ficoll-pack density gradient ("Pharmacy") using Verografin ("Spofa"), the density 1.083 was produced by centrifuging 800 g at 4°C during 15 min. Cells were rinsed with the Hanks fluid containing antibiotics with the final concentration of penicillin 50 units per ml, streptomycin 50 mkg per ml and they were resuspended to achieve concentration 10^6 ml of the cultural medium containing the Hanks fluid and 5% of the calf embryonal serum (from the factory producing bacterial preparations in Minsk). Survivability of the separated cells was 96 — 98% based on the exception of the tripane blue. The monoclonal antibodies MRC OX-19, MRC OX-6, W 3/25, MRC OX-8 were used in the study (V.L. Yurin, the Research Institute of Medical Genetics of the Ministry of Health of the Russian Federation), Table 3.2.

The MCA were diluted 1:50 in 0.2 M of PBS with pH 7.2 — 7.4 with the addition of 0.4% of BCA and 0.02% of sodium aside. After introduction of the MCA incubation was performed during 1 hour at $t=4^\circ\text{C}$, than the preparation was rinsed three times with the rinsing buffer containing 0.4% of BCA. During the next stage FITC traced F (ab 2) — fragments of rabbit antibodies ("DAKOPATS", Danemark) diluted to the optimum concentration were added. Incubation and rinsing were repeated. The rat spleen cells served for positive control and lymphocytes of the rat peripheral blood served for negative control.

The results were rated per 100 cells using the "phase contrast" technique with the help of a luminescent microscope. The Student criterion was used for statistical processing. The study has revealed that the available modern technique of estimating the number of immunocompetent cells fails to validate differences in their concentrations between the test and control animals (Table 3.3, 3.4).

Table 3.3

Antigen spectrum of monoclonal anticells used in the study

Mca	Cell colonies	Differentiation cluster
MRC OX-19	pan T-cell T- cells and thymocytes	LCD5
MRC OX-6	Primarily with B cells, macrophages	Monomorphous determinants of MNC class II
W 3/25	Helper colonies, some macrophages	LCD4
MRC OX-8	Suppressor colonies, some thymocytes	LCD8

Table 3.4

Relative concentrations of immunocompetent cells in test and control animals

Mca	Test n=7 m±m	Control n=7 m±m	t
MRC OX-19	16.43±4.03	25.86±4.96	1.48
MRC OX-6	19.71±3.79	20.86±2.11	0.26
W 3/25	14.0±4.53	18.00±4.61	0.62
MRC OX-8	24.57±5.49	21.86±3.18	0.43

So, accumulation of ^{137}Cs in the indicated doses by bodies of "Wistar" line rats during its enteral incorporation with grain (oats) does not reveal any valid changing in the relative shares of immunocompetent cells compared with the control.

The results of studies of the immunity peripheral organs, such as palatine tonsils specifically, can also be an evidence of the effect of radionuclides upon the immunity system. These immunocompetent organs fulfill a number of very essential functions protecting the organism against environmental effects. For this purpose laboratory animals were studied in order to be able under experimental conditions to evaluate the effect of radioactive substances upon a certain organ. Rabbits of the "White giant" breed with pronounced lymphoid formations in the pharynx primary portions were studied experimentally. The lab animals were kept on a diet of oats containing ^{137}Cs and ^{90}Sr in the respective amounts 445.7 Bq per kg and 15.5 Bq per kg during two weeks with a simultaneous control diet (oats) containing 44.2 Bq per kg of ^{137}Cs and 1.7 Bq per kg of ^{90}Sr .

Investigation of the morphological structure of palatine tonsils after 2 weeks of experimentation has revealed a valid increase in the number of immunocompetent cells per unit area of lymphoid follicles to 107.67 ± 3.50 compared with 67.31 ± 1.06 in the control ($p < 0.01$). Specifically the concentration of β -globulins in the globulin serum and a higher relative concentration of albumin (Table 3.5).

After four weeks of experimentation the number of cells per unit area of lymphoid follicles reached 110.95 ± 3.50 on the average compared with 86.97 ± 3.58 ($p < 0.001$) in the control. The blood serum of these animals showed a reduced concentration of α_2 -globulins compared with the control (Table 3.5).

These data prove that radionuclides significantly affect the condition of the immune system peripheral organs of experimental animals. The growing number of immunocompetent cells in lymphoid follicles may signify intricate compensating and adaptation processes taking place in the organism under the effect of radionuclides. It should be emphasized that the synthesis of globulin protein is inhibited to some extent, specifically β and α_2 -globulins.

Table 3.5

Protein spectrum of blood of test and control animals

Description of indicators	After 2 weeks		After 4 weeks	
	test	control	test	control
total protein	73.92 ± 1.81	80.50 ± 2.74	61.74 ± 5.54	60.90 ± 3.51
albumin (%)	$65.28 \pm 1.0^{**}$	58.75 ± 0.83	54.98 ± 2.71	56.50 ± 1.13
globulin (%)	$34.72 \pm 1.00^*$	41.25 ± 0.83	45.02 ± 2.71	43.50 ± 1.13
α_1 -globulin (%)	4.56 ± 0.69	5.38 ± 0.50	8.14 ± 2.18	11.46 ± 1.80
α_2 -globulin (%)	9.14 ± 0.79	8.17 ± 0.20	$8.46 \pm 0.62^*$	11.30 ± 1.03
β -globulin (%)	$7.42 \pm 0.76^{**}$	12.47 ± 1.28	10.92 ± 1.44	7.68 ± 1.70
γ -globulin (%)	13.54 ± 1.58	15.23 ± 1.72	17.40 ± 2.24	13.06 ± 1.59

* — $p < 0.05$

** — $p < 0.01$

The experimental data have been validated clinically. A greater number of immunocompetent cells per unit area of lymphoid follicles of palatine tonsils has been detected among the children from areas contaminated with radionuclides and operated due to the hypertrophied palatine tonsils (Table 3.6).

So, the study has revealed that children living in the areas affected by the Chernobyl disaster with the level of contamination $1 - 5 \text{ Ci/km}^2$ of ^{137}Cs have definite modifications in their immunity system compared with the control, which are reflected by the reduced phagocyte activity of neutrophil leucocytes, concentration of JgA, some loss of balance in the production of JgM and JgG. Still, these effects would remain, as a rule, within the physiological parameters. No relationship has been revealed between their extent and the dose of internal incorporation of radioactive

Table 3.6

**NUMBER OF CELLS IN LYMPHOID FOLLICLES OF
PALATINE TONSILS AMONG CHILDREN FROM AREAS
CONTAMINATED WITH RADIONUCLIDES**

Soil contamination with ¹³⁷ Cs (ci/km ²)	Number of cells per unit area of lymphoid fol- licles of palatine tonsils of children
5 — 15	176.11±4.89**
1 — 5	118.51±2.14*
< 1 — control	81.69±7.03

* — compared with control $p < 0.01$

** — compared with control $p < 0.001$

elements. At the same time, processes evolve in the immunity peripheral organs, such as palatine tonsils, producing structural changes of lymphoid formations reflected by a greater number of cellular elements as a demonstration of the morphofunctional loss of balance. It has been revealed both clinically and during experiments with animals.

These clinical and experimental studies prove intricate modifications of the immune system under the effect of radioactive elements.

Further studies, both clinical and experimental, are required to evaluate structural and functional modifications of the immunity system with the consideration of doses of internal and external incorporation of radioactive elements.

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Chapter 4

EFFECT OF RADIOACTIVE ELEMENTS RELEASED INTO ENVIRONMENT AFTER CHERNOBYL DISASTER UPON MYOCARDIUM CONDITION

Among other systems the cardiovascular system changes its functions when exposed to radiation, the pathological effects being detected primarily when the organism is exposed to external doses. In fact, exposure of lab animals to a dose of 15 Grays produces degenerative or necrotic changes in the myocardium [1] inhibiting the contractile activity of myofibrils, reducing the volume rate of coronary blood circulation [9]. Presumably these changes are caused by the damage of blood vessels, arterioles specifically [4] or capillaries [12], modification of the metabolism of catecholamins and electrolytes in vascular walls [7].

Experimental studies of lab animals are confirmed by reported results of examinations of the population after Chernobyl exposure.

Specifically 95.5% children and adolescents have manifested functional disorders of blood circulation in the form of the impaired cardiac rhythm, conductivity of pulses, redox processes in the myocardium, oppressed tolerance of physical exertion, higher arterial pressure. Such functional disorders are most pronounced among children aged 6—10 years considered healthful [8]. Dr. I.S. Tsubluskaya with colleagues observed pronounced electrocardiographic modifications, such as severe rhythm disorders, alteration of the teeth of the ventricular complex, disorders of conductivity and processes of repolarization, hypoxia, even among 74.4% of infants during their first year of life living in the areas contaminated by ^{137}Cs from 5 to 20 Ci/km^2 . It is noted that such modifications are caused by disorders of the neurohumoral regulation of the cardiac tone and contractility of the myocardium [6].

Also, it is a challenging scientific and practical task to investigate the responses of the cardiovascular system to doses of incorporated radionuclides remembering their extensive migration in the environment. This aspects still remains obscure.

Hence, the goal of the present study is to evaluate clinically and experimentally the morphofunctional modifications in the cardiac muscle of the maturing organism in response to incorporated doses.

The objectives were the following:

1. To investigate electrocardiographic changes among children living on contaminated and "clean" territories.
2. To evaluate relationships between the incidence of such changes and the incorporated dose together with the changes of major metabolism indicators.
3. To investigate morphofunctional changes in the myocardium during endogenous incorporation of radiation with food among experimental animals.

So, the study was divided into the clinical and experimental parts. The first clinical part was devoted to investigating electrocardiographic and metabolic changes among the children on contaminated territories. The study covered 227 children aged between 3 and 7 years living in the area with soil ^{137}Cs contamination 1 to 5 Ci/km^2 (Gomel).

Control groups included 118 children in the Gomel region (Zhlobin) and 104 children in the Grodno region of the same age living in places where soil ^{137}Cs contamination was under 1 Ci/km^2 . At the moment of examination the children were assumed healthy and attended nurseries and schools. In addition to general examinations their cardiovascular systems were rated electrocardiographically in 12 spots according to the standard routine [3]. In order to investigate metabolism in response to the cardiovascular condition triiodine thyronine (T_3), thyroxine (T_4), cortisol were counted using radioimmune techniques and standard instruments of the Institute of Biochemistry of the Belorussian Academy of Science, total bilirubin, urea, creatinine, uric acid, total protein, albumin, cholesterolin, glucose, phosphates, calcium triglycerides were counted, the alkaline phosphates (AP), creatine phosphokinase (CPK), γ -glutamate transpeptidase (GGTP), alanine amine transferase (ALT), aspartate amino transferase (AST), lacto dehydrogenase (LDH), choline esterase activity was registered using a biochemical analyzer "Synchron-4" of Beckman Co. Doses of internal accumulation of radionuclides were registered among children from Gomel and Grodno using the medical radiological monitoring net.

The experimental group included 39 "Wistar" line male rats, the control group included 29 rats, all weighing 210—290 grams. During experiments the animals were kept in standard laboratory cells. The test group was kept during 10 days on the diet of oats (with the daily rations being 35 grams per animal and similar in the two groups) the ^{137}Cs concentration 445.7 Bq/kg and ^{90}Sr concentration 15.5 Bq/kg.

During this period the control group was kept on a diet of oats with ^{137}Cs concentration 44.2 Bq/kg and ^{90}Sr concentration 1.7 Bq/kg. The concentration of radiation was measured on the 11th day using a radiation counter based on the emission of gamma-sources. Similar measurements of the heart, liver, kidney, the bony skeleton and the skeleton musculature were performed after decapitation. After that pieces of myocardium were preserved in a 10% formalin solution to be used later for making paraffin wax sections dyed with hematoxylin and eosin. Polarization microscopy served to assess metabolic disorders in myocardium muscular cells by registering the size of A-discs with the help of a morpho-optical system "Vidas-Video" manufactured by "Opton" (Germany).

Also, blood was sampled among 8 animals in each group, the serum was separated in order to assess the concentration of phosphates, calcium, creatinine, total bilirubin, albumin, cholesterol, urea, total protein, activities of alkaline phosphatase (AP), aspartate amino transferase (AST), alanine amino transferase (ALT).

A Synchron analyzer manufactured by Beckman was used to assess the activity of enzymes. The results were processed statistically using variational statistical techniques to determine mean arithmetic magnitudes, the mean quadratic error, the mean quadratic deviation and the Student - Fischer criterion.

The study has revealed that incorporation of radiation by the maturing organism, primarily ^{137}Cs , produces significant structural and functional changes in the myocardium. This effect has been traced both clinically and experimentally among lab animals.

Clinical electrocardiography of children aged between 3 and 7 years, attending nurseries and complaining about cardiovascular troubles, has allowed to assess electrophysiological processes in the cardiac muscle. In combination with a number of biochemical techniques for assessing the metabolic processes in the organism cardiography allows to rate the car-

diovascular condition of the human organisms in various environments during exogenous and endogenous incorporation of radiation.

The Monitoring network served to assess incorporation of radiation from gamma-sources, primarily ^{137}Cs . Radiological soil measurements were used to specify the areas where the children lived. It allowed to assess the myocardium condition in the areas with stronger contamination (1—5 Ci/km²) with milder contamination under 1 Ci/km² of ^{137}Cs in the control zone and to include endogenous incorporation.

Cardiography has revealed that cardiac disorders were most frequent in the main group (Gomel) — 72.25% and somewhat less frequent in the two control groups (Zhlobin and Grodno) — 55.93% and 66.35%. These variations are due primarily to the cardiac pulse disorders with the incidence among all these groups 49.78%, 40.68% and 39.42%, respectively.

Examination of the children from contaminated areas (Gomel) has revealed a significant link between the dose of endogenous incorporation and cardiographic disorders. Though the average incorporated doses did not differ in any valid way between the two groups and amounted to 30.32 ± 0.66 Bq/kg in the test and 29.74 ± 0.67 Bq/kg in the control ($p > 0.1$) the children in Gomel manifested a more pronounced and statistically valid inverse relationship between the incorporated dose and cardiographic disorders with valid differences between the subgroups 11—25.9 Bq/kg and 37.0—74.0 Bq/kg. (Fig. 4.1)

The children with such incorporated doses from Grodno did not manifest any valid difference in the incidence of cardiac disorders (Fig. 4.2).

Such relationship in the main group is primarily produced by disorders of intraventricular conductivity due to an incomplete blockade of the His right fascicle cruse (Fig. 4.3, 4.4).

Thus, studies of electrophysiological processes in the cardiac muscle among children from areas with stronger contamination have revealed a number of electrocardiographic changes. In our view, such relationship can be explained by the fact that children in Gomel are exposed to the external gamma-radiation primarily from the soil containing ^{137}Cs in amounts 1 — 5 Ci/km² in addition to endogenous radiation incorporation.

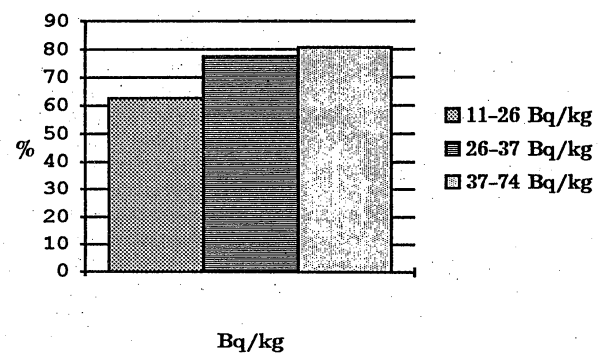


Fig. 4.1. Frequency of ECG modifications among children in Gomel in response to the incorporated dose of radionuclides

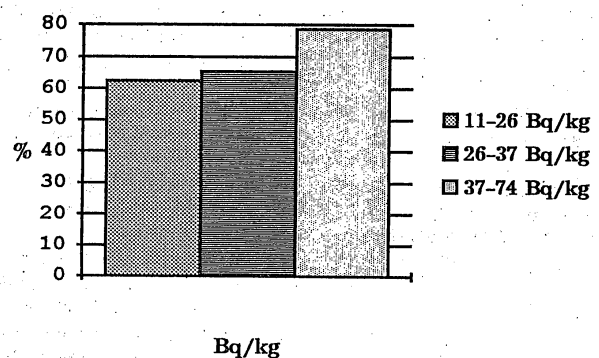


Fig. 4.2. Distribution of cardiac pathology based on the incorporated dose among children in Grodno

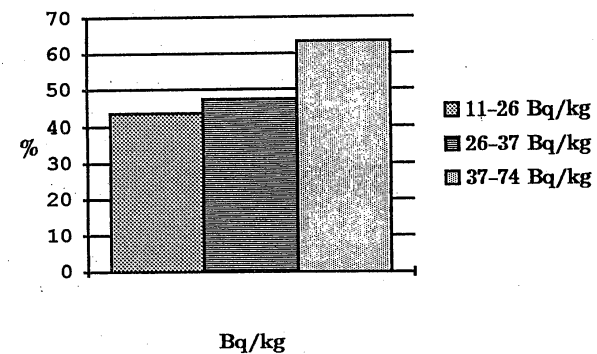


Fig. 4.3. Distribution of cardiac conductivity disorders among children in Gomel

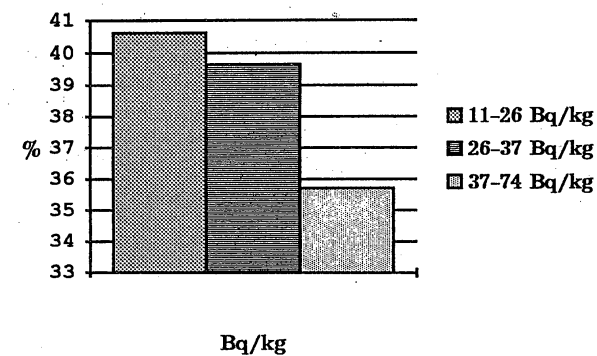


Fig. 4.4. Distribution of conductivity disorders based on the incorporated dose among children in Grodno

It is supported by the results of simultaneous evaluation of the metabolic status of Gomel and Grodno children. It has been traced that there are valid differences between the two children groups in the count of creatinine, total protein, cholesterol, albumin, ALT, AST, LDH, calcium, cortisol, triiodine thyronine, thyroxin (Fig. 4.5—4.15). These differences remain in comparison with other indicators within the limits corresponding to the relevant detected doses. Yet, there is no correlation between the changes of these parameters and internal incorporated doses (Fig. 4.16—4.21).

Considering a stronger ALT, AST activity and reduced LDH activity and concentration of total protein, albumin, creatinine and cholesterol in the blood serum of the main group compared with the control one, metabolic disorders in the liver and the heart should be noted.

Such changes in their majority remain within the standard physiological parameters. They may be caused by the processes in the myocardium as much as they relate to AST [5] or damage the myocardium. Specifically a higher thyroxin concentration compared with the control can be a factor impairing metabolic process in the cardiac muscle.

Thus, external exposure to radionuclides in the environment is acting as gamma-sources affecting metabolism and cardiac disorders in addition to the endogenous incorporation.

Apart from external exposure causing cardiac disorders there is a larger share of children in the main group with the incorporated dose 11.0—25.9 Bq/kg and 37.0—74.0 Bq/kg than in the control group with the dose 26.0—36.9 Bq/kg (Fig. 4.22), irrespective of similar average incorporated doses.

Experimental studies ("Wistar" line rats) prove the effect of endogenous incorporation upon the myocardium.

The lab animals were kept on a diet of oats during 10 days with the concentration of radiation 10 times higher than for the control (^{137}Cs concentration in the grain was 445.7 Bq/kg and ^{90}Sr was 15.5 Bq/kg).

As a result the test animals accumulated 63.35 ± 3.58 Bq/kg compared with 5.43 ± 0.87 in the control group ($p < 0.001$).

Thus, the above animals are capable to accumulate within a relatively short period radionuclides, particularly ^{137}Cs .

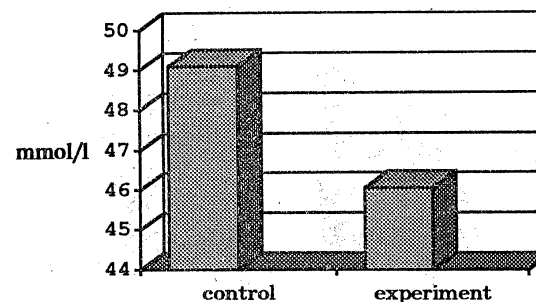


Fig. 4.5. Comparative concentrations of creatinin in the blood serum of children in the main and control groups; $p < 0.01$

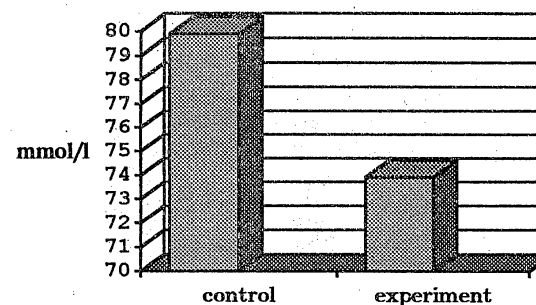


Fig. 4.6. Comparative concentrations of total protein in the blood serum of children in the main and control groups; $p < 0.001$

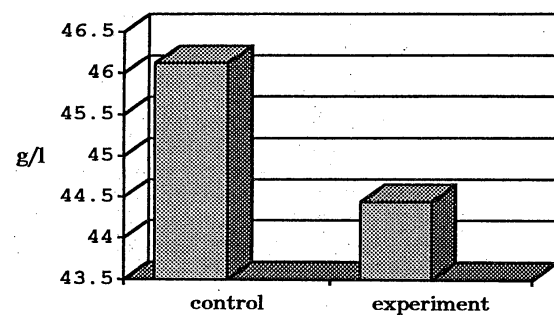


Fig. 4.7. Comparative concentrations of albumins in the blood serum of children in the main and control groups; $p < 0.01$

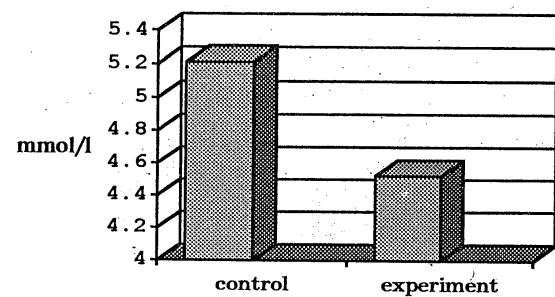


Fig. 4.8. Comparative concentrations of cholesterol in the blood serum of children in the main and control groups; $p < 0.001$

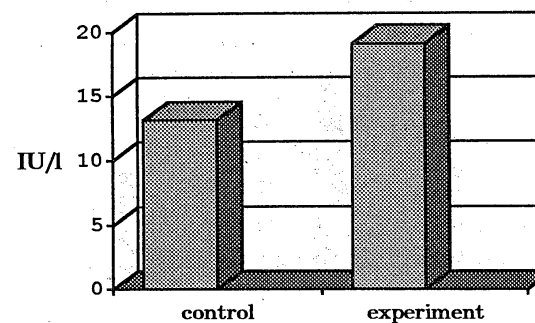


Fig. 4.9. Comparative ALT activities in the blood serum of children in the main and control groups; $p < 0.01$

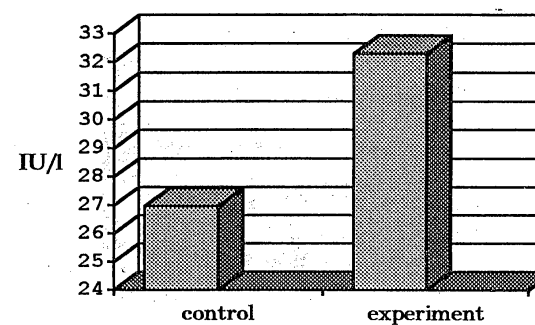


Fig. 4.10. Comparative AST activities in the blood serum of children in the main and control groups; $p < 0.01$

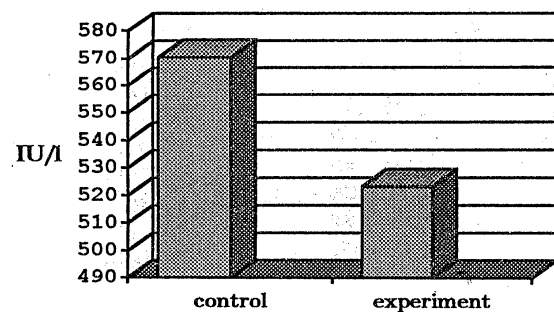


Fig. 4.11. Comparative LDG activities in the blood serum of children in the main and control groups; $p < 0.001$

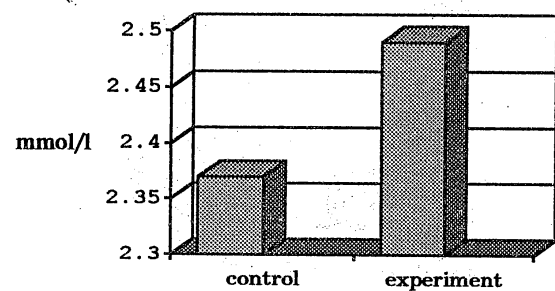


Fig. 4.12. Comparative calcium concentration in the blood serum of children in the main and control groups; $p < 0.001$

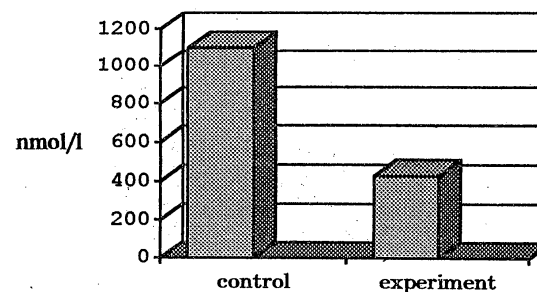


Fig. 4.13. Comparative concentrations of cortisol in the blood serum of children in the main and control groups; $p < 0.001$

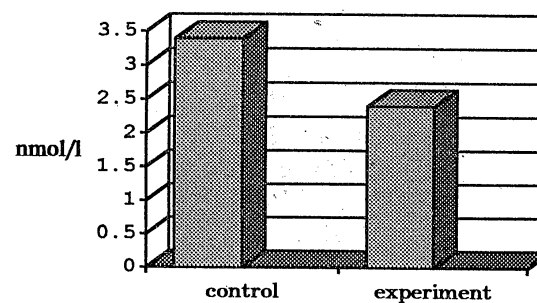


Fig. 4.14. Comparative concentrations of triiodine thyronine (T_3) in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg

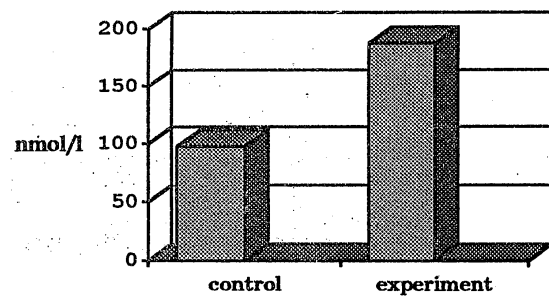


Fig. 4.15. Comparative concentrations of thyroxin (T_4) in the blood serum of children in the main and control groups; $p < 0.001$

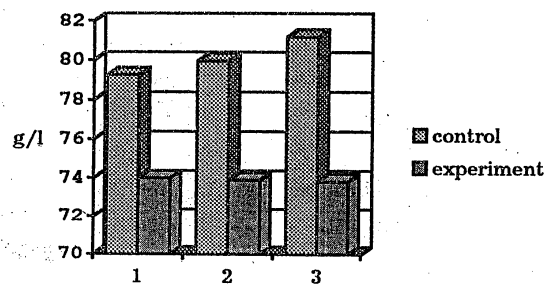


Fig. 4.16. Relationship between dose and total protein concentration in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg

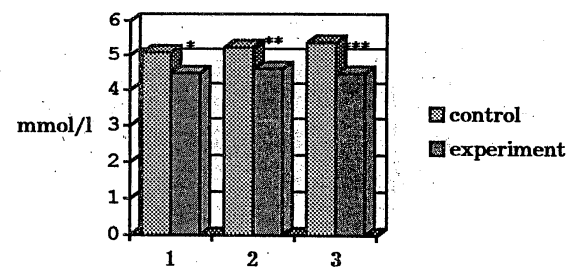


Fig. 4.17. Relationship between dose and concentration of cholesterol in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg; * — $p < 0.05$; ** — $p < 0.02$; *** — $p < 0.01$

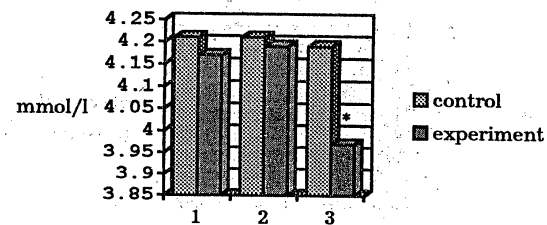


Fig. 4.18. Relationship between dose and concentration of glucose in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg; * — $p < 0.05$

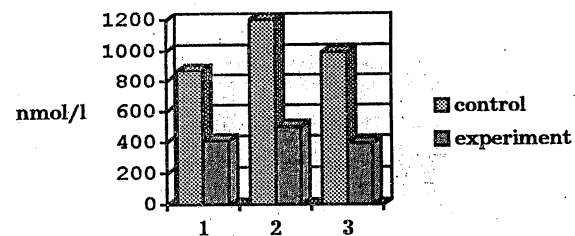


Fig. 4.19. Relationship between dose and concentration of cortisol in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg; $p < 0.001$

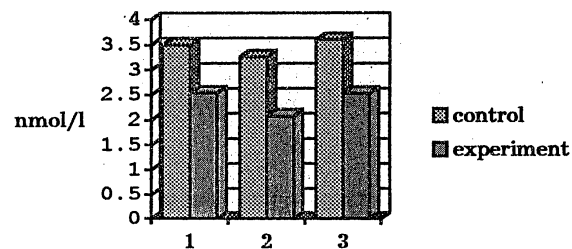


Fig. 4.20. Relationship between dose and concentration of triiodine thyronin (T₃) in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg; $p < 0.001$

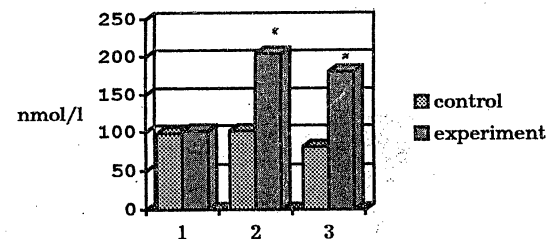


Fig. 4.21. Relationship between dose and concentration of thyroxin (T₄) in the blood serum of children in the main and control groups; 1 — 11—25.9 Bq/kg; 2 — 26—36.9 Bq/kg; 3 — 37—74 Bq/kg; * — $p < 0.001$

The fact that there is a directly proportional relationship between the accumulated ¹³⁷Cs dose in the animal organisms and the concentration of the element in the grain. Evaluation of the experimental results has revealed that the myocardium tissue accumulates this radioactive element most (Fig. 4.23). Its accumulation in the animal myocardium was accompanied by pronounced morphofunctional disorders, such as modifications of polarization behavior of cardiomyocytes due to the growing number of A-discs, inhibition of alkaline phosphates and creatine phosphokinase (Fig. 4.24, 4.25). The blood serum manifests greater AST activity and creatinine concentration (Fig. 4.26) also proving the myocardium damage. Considering that creatine phosphokinase is a key enzyme of the cardiac muscle energy metabolism promoting links between macroergic phosphate and creatinine [11] its inhibited activity significantly deducts from the functions of cardiomyocytes. Thus, the experiment has demonstrated that incorporation of radionuclides by experimental animals (primarily ¹³⁷Cs) with food damages myocardial cells resulting in relevant structural and functional modifications.

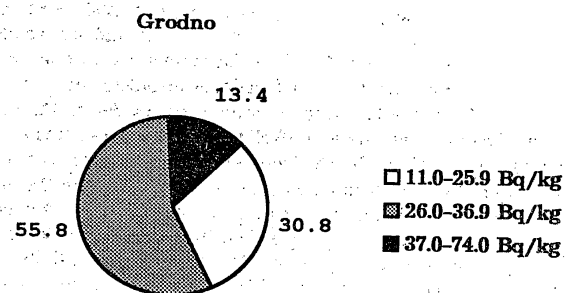
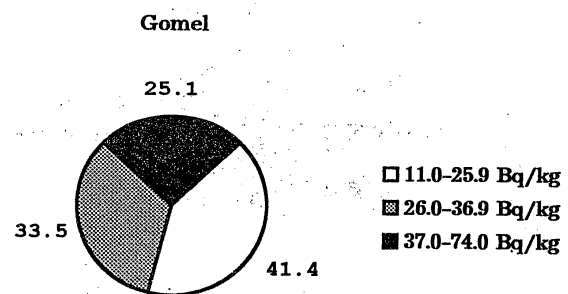


Fig. 22. Percent frequency of distribution of children in Gomel and Grodno in accordance with dose

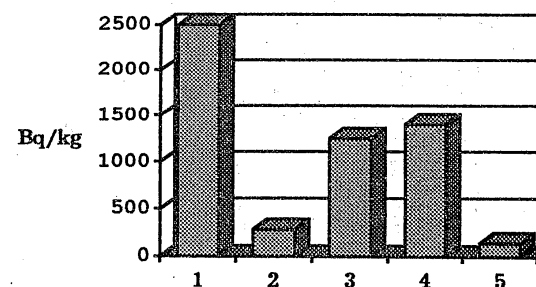


Fig. 4.23. ^{137}Cs contamination in organs and whole bodies of the control animal groups: 1 — heart, 2 — liver, 3 — spleen, 4 — kidneys, 5 — whole body

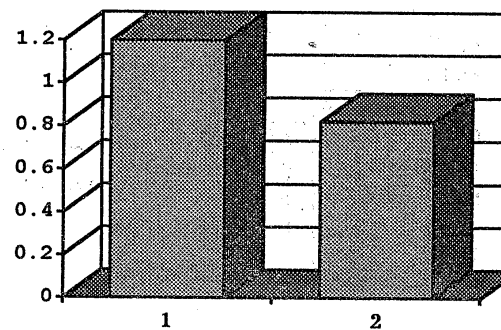


Fig. 4.24. Anisotropic disks of myocardium of the tested animal groups: 1 — test, 2 — control, $p < 0.05$

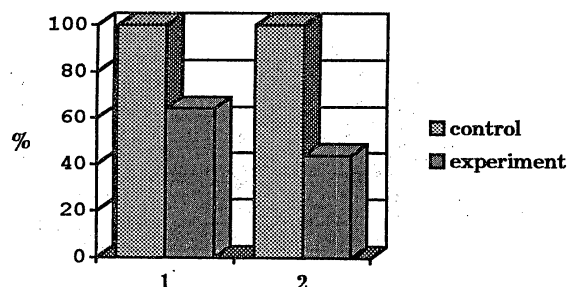


Fig. 4.25. Modification of ferment activity in myocardial tissue of the tested animal groups (in % to the control); 1 — alkaline phosphatase, 2 — creatine phosphokinase, $p < 0.05$

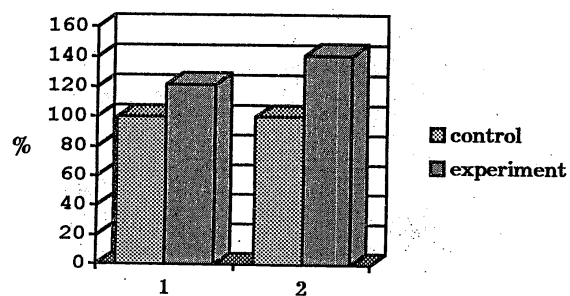


Fig. 4.26. Basic metabolic indicators modification in the blood serum of tested animal groups (in % to the control); 1 — creatinine contamination, 2 — AST activity, $p < 0.05$

Both clinical and experimental studies have allowed to observe a significant response of myocardial cells of the maturing organism to incorporated doses. A whole combination of changes evolve evidencing both direct damage to the cardiac muscle and the damage of organs and systems controlling its functions.

Therefore, it is a challenging task to reduce doses of internal accumulation of radionuclides, including application of sorbents [2], development of techniques improving the metabolic processes in the myocardium. Comprehensive clinical and experimental investigation is needed using morphofunctional techniques.

CONCLUSIONS

1. Endogenous incorporation of gamma-emitting radionuclides by the growing child organism, primarily ^{137}Cs , causes significant morphofunctional modifications in the cardiac muscle.

2. Children between 3 and 7 years living on the contaminated territory, primarily with ^{137}Cs between 1 and 5 Ci/km^2 manifest pronounced electrocardiographic changes and their frequency is directly proportional to the concentration of ^{137}Cs in the organism.

3. Among such changes disorders of intracardiac conductivity in the form of incomplete His right fascicle crus dominate followed by significantly less frequent disorders of redox processes and vegetative vascular distonias.

4. The organisms of children living on the territory contaminated with ^{137}Cs between 1 and 5 Ci/km^2 manifest significant metabolic changes evidenced by stronger activities of ALT, AST and concentration of thyroxin, inhibited LDH activity and lesser concentration of total protein, albumins, creatinine, cholesterolin and triiodine thyronine in the blood serum.

5. Incorporation of radionuclides, specifically ^{137}Cs , by the organisms of laboratory animals with oats ("Wistar" line rats) results in their endogenous accumulation, specifically in the heart, producing pronounced morphofunctional changes of the myocardium in the form of degradation of muscular tissues proved by stronger anisotropy, inhibited activity of alkaline phosphatase and creatine phosphokinase in the myo-

cardial tissue, stronger activity of aspartate amino transferase and greater creatine concentration in the blood compared with the control.

6. These results emphasize the need for diagnostics and prevention of premature cardiac disorders among children in contaminated areas to reduce accumulation of radiation in their organisms.

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Chapter 5

EFFECT OF INCORPORATED RADIONUCLIDES UPON THE NEUROMEDIATORS IN THE BRAIN OF RATS

Neurochemical effects of ionizing radiation are intricate. It is not easy to achieve a clear idea about variations of the metabolism of neuromediators and relative compounds and to produce any satisfactory explanation of behavioral and vegetative changes caused by radiation, including dose-induced effects.

Extensive incidence of neuropsychic disorders among residents exposed to radiation after the Chernobyl accident pushed through experimental studies of chronic exposure effects upon the status indicators of neurotransmitter systems.

It has been reported that the neurochemical mechanism of muscular tone disorders is produced by the external irradiation destroying reciprocal relationships between the dopamine, serotonin and cholinergic systems [1, 7]. Variations of the density of β -receptors in the cerebellum of rats after X-ray exposure have been reported [14]; variations of the mono amino oxides (MAO) have been described; reduction [13] or increase [4] of tryptophane, specifically after introduction of the serotonin precursor, has revealed the phase pattern of changes in the MAO activity. It has been positively correlated with the serotonin concentration [8].

There is quite a few data on the affinity between dopamine and serotonin receptors and specific legends [18]; it has been demonstrated that the bonding between the γ -aminobutyric acid (γ -AOA) and muscimole by receptors with high mutual affinity declines at a dose 9.5 Grays.

It has been demonstrated that the dopamine activity goes up and it is accompanied by a stronger mediator synthesis on the first day after rats are exposed to 20 Grays; the dopamine system under the phenamine effect is activated more pronouncedly among exposed rats [10]. Nevertheless, lesser exposure doses would reduce the dopamine concentration in the striatum [7].

Concentration of the mediator in the brain after exposure to 4—9 Grays has been reported to tend to reduce in the serotonin system leading to a greater concentration of the serotonin final metabolite or the 5-oxiindoleacetic

acid [17]. Increased penetrability of the hematoencephalic barrier by serotonin has been noted after exposure to average lethal doses [2].

The γ -AOA concentration in cerebral segments would increase after exposure [11, 12]. The mechanism of such increase is attributed to the growing concentration of glutamate as a γ -AOA precursor [11].

Hence, rather extensive facts have been accumulated so far relating to the effect of ionizing radiation upon neurotransmitters in the CNS. Still, caution should be exercised in applying the obtained results to explanations of pathogenetic sequences in neurological disorders among humans affected by radionuclides in contaminated areas. All these studies have related to external even or local, more frequently acute, exposure. Lack of correlation among the results may be explained by various physical characteristics of radiation and their intricate effect upon the neurotransmitter systems. The components of this effect (upon the transport systems, biosynthesis and distribution of mediators, characteristics of the bonding of receptors, degradation enzyme systems) may have various behavior in time. Biochemical effects of external and internal radiation are also known to differ to various extent. Therefore, it is specifically challenging to investigate the effect of incorporated radionuclides of ^{137}Cs and ^{90}Sr upon the metabolism of neuromediators and also to perform it dynamically when they are incorporated chronically.

The effect of incorporated radionuclides upon the number of neuroactive compounds in the brain segments has been studied among 36 white male rats kept on a standard vivarium diet. Experimental animals received 445.7 Bq/kg radioactive oats and control animals 44.2 Bq/kg oats during 10 and 28 days in two independent experiments.

Rats were decapitated and brain segments were extracted during 3 minutes in refrigerated conditions: cerebral hemispheres, the cerebellum, the thalamus region (the thalamus and the hypothalamus) and the striatum. The tissues were instantly frozen in liquid nitrogen.

Concentration of the studied compounds was evaluated in chloric acid extracts of tissues from brain segments. Weighed portion of the tissues were homogenized in the ratio 1:10 in 0.2N HClO_4 containing 50 mg/l of sodium meta bisulphate and 20 mg/l of EDTA together with the internal standards: the dopamine homologue 3,4-dioxi benzyl amine (400 μM) and the γ -AOA homologue β -amine valeric acid (25 μM) [5]. The homogenates were

centrifuged for 15 min. at 15000 g, after that the extracts were passed through 0.45 mcm membrane filters Millex (Millipore, USA).

The concentration of biogen amines (noradrenaline, dopamine, serotonin, serotonin precursor (tryptophan) and acidic dopamine and serotonin metabolites was determined with the help of ion-couple high power liquid chromatography (EFA) with a bicomponental ion-coupled reagent and by electrochemical detection using the technique [6]. The concentration of transmitters of amine acids (aspartate, glutamate, glycine, taurine and γ -AOA) and related compounds were determined with the help of the phase-reverted EFA with specimens derived in advance with ophtalic aldehyde and 2-mercaptoethanol and by detecting the fluorescence of isoindolic derivatives [5, 15]. The chromatographic system of Waters Assoc., MA, USA was calibrated using the internal standard.

Compounds were identified and quantitatively processed with the help of the Multichrome hardware (Russia) using the software 3d BMDP Classic (BMDP Statistical Software, CAUSE).

The results have indicated that incorporation of radionuclides with food during 10 days does not produce any statistically significant variations in the concentration of the above compounds in the cerebellum, striatum and in the thalamic region (Tables 5.1, 5.2), while the concentration of γ -AOA increases in the cerebral hemispheres and the concentration of dopamine tends to increase (Table 5.1). Yet, the experiments have not yielded any valid data proving the activation of the dopamine system, though a more protracted accumulation of radionuclides in the striatum increases the concentration of the acidic dopamine metabolite — the 3,4-dioxiphenyl acetic acid (Table 5.4). It evidences a stronger oxidizing deamination of dopamine as demonstrated by an invariable concentration of its final metabolite — the homovanilic acid. It results from the O-methylating effect of catechol-O-methyl transferase localized in the synaptic slot. Its activity has not demonstrated any significant radiobiological effects at sublethal exposure doses [149]. The tendency of the γ -AOA concentration to increase in cerebral hemispheres remains when the experiments last 28 days (Table 5.3), it correlates with the data obtained during external irradiation [3]. Moreover, the cortex of the hemispheres is the only brain structure which does not reveal any premature reduction of the γ -AOA shunt activity[11].

Table 5.1

Concentration of biogenic amines, their precursors, metabolites and neuroactive amine acids in cerebral hemispheres and in cerebellum of rats after 10 days of incorporation of radionuclides with food (nmol/g)

Indicator	Cerebral hemispheres		Cerebellum	
	control	test	control	test
Noradrenaline	1,56±0,33	1,19±0,02	—	—
Dopamine	0,64±0,07	0,99±0,17**	—	—
3,4-dioxiphenyl acetic acid	0,17±0,04	0,14±0,02	—	—
Tryptophane	19,2±1,84	22,3±2,41	24,2±1,69	23,3±3,26
Serotonin	1,59±0,12	1,96±0,29	0,18±0,03	0,17±0,03
5-oxiindolacetic acid	1,16±0,15	1,76±0,33	0,36±0,06	0,35±0,09
Aspartate	1854±149	1719±876	1080±96	1142±145
Glutamate	7496±496	7053±463	5414±403	5728±510
Glutamine	4048±272	4288±113	3775±191	4001±341
γ -AOA	1432±44	1620±84*	1801±119	2086±146
Serin	527±58	708±42	927±62	948±61
Glycine	616±109	766±10	901±35	974±70
Threonine	230±61	212±25	367±68	286±52
Phosphoethanol amine	480±24	468±20	2131±111	2186±124
Alanine	276±18	304±16	464±32	470±25
Taurine	2249±102	2302±85	4070±194	3984±334

* - $p < 0.005$ versus control

** - $p < 0.1$ versus control

Table 5.2.

Concentration of biogenic amines, their precursors, metabolites and neuroactive amine acids in the thalamus region and the striatum of rats after 10 days of incorporation of radionuclides with food (nmol/g)

Indicator	Thalamus		Striatum	
	control	test	control	test
Noradrenaline	7,80±0,48	7,20±1,29	—	—
Dopamine	2,06±0,12	1,76±0,23	88,6±10,3	70,6±7,19
3,4-dioxiphenyl acetic acid	0,33±0,04	0,38±0,04	5,75±0,69	4,80±0,64
Gomovaniline acid	0,05±0,006	0,07±0,01	2,55±0,35	2,36±0,39
Tryptophane	21,9±1,52	20,3±1,72	22,8±2,27	25,1±1,77
Serotonin	3,95±0,24	3,40±0,44	1,88±0,03	2,01±0,15
5-oxiindolacetic acid	4,04±0,41	3,29±0,42	3,37±0,32	3,83±0,58
Aspartate	1423±80	1341±92	1227±100	1203±73
Glutamate	5381±3062	5258±413	6820±459	7188±410
Glutamine	5055±131	4983±455	3698±599	4613±496
γ-AOA	5757±275	5129±452	1801±119	2086±146
Serin	619±69	583±52	927±62	948±61
Glycine	1956±147	1682±139	901±35	974±70
Threonine	399±124	247±55	367±68	286±52
Phosphoethanol amine	1910±79	1757±130	2131±110	2187±124
Alanine	276±48	279±30	421±64	462±66
Taurine	1970±136	1746±179	4070±194	3984±334

Table 5.3.

Concentration of biogenic amines, their precursors, metabolites and neuroactive amine acids in cerebral hemispheres and in cerebellum of rats after 28 days of incorporation of radionuclides with food (nmol/g of tissue)

Indicator	Cerebral hemispheres		Cerebellum	
	control	test	control	test
Noradrenaline	5,56±0,61	1,34±0,32*	0,90±0,13	0,80±0,15
Dopamine	0,21±0,04	0,34±0,12	—	—
3,4-dioxiphenyl acetic acid	0,14±0,03	0,14±0,02	—	—
Tryptophane	16,3±2,61	11,3±1,53	17,3±2,87	18,9±2,24
Serotonin	4,51±0,63	1,56±0,35*	0,30±0,06	0,29±0,06
5-oxiindolacetic acid	3,95±0,86	0,85±0,17*	0,45±0,10	0,28±0,06
Aspartate	2432±707	2197±246	2305±470	2023±304
Glutamate	4554±982	8012±507*	8310±1612	6950±681
Glutamine	2486±319	3597±191*	5114±600	4042±482
γ-AOA	1369±75	1580±72**	1539±131	1281±57
Serin	323±51	648±59*	575±78	523±94
Glycine	4950±217	1420±127*	1374±178	1307±193
Threonine	645±133	463±82	731±86	557±131
Phosphoethanol amine	577±54	1267±184*	504±20	429±22*
Alanine	273±53	463±37*	439±72	383±45
Taurine	904±98	2486±94*	2543±161	2149±84*

* - p < 0.005 versus control

** - p < 0.1 versus control

Table 5.4.

Concentration of biogenic amines, their precursors, metabolites and neuroactive amine acids in the thalamus region and the striatum of rats after 28 days of incorporation of radionuclides with food (nmol/g of tissue)

Indicator	Thalamus		Striatum	
	control	test	control	test
Noradrenaline	6,17±0,32	6,33±0,80	1,95±0,73	5,35±1,76
Dopamine	1,37±0,37	0,97±0,24	8,18±3,21	12,7±4,11
3,4-dioxiphenyl acetic acid	0,21±0,04	0,25±0,05	0,32±0,08	0,62±0,09*
Gomovaniline acid	0,11±0,03	0,09±0,01	0,47±0,27	0,25±0,02
Tryptophane	19,1±3,53	11,6±1,95**	19,6±3,49	11,9±1,12**
Serotonin	6,26±0,61	7,40±0,38	4,13±1,60	6,89±0,99
5-oxiindolacetic acid	5,90±1,11	3,43±0,35*	2,71±0,65	2,29±0,35
Aspartate	2488±668	2251±255	2351±1023	2785±305
Glutamate	5814±1232	5092±604	7543±2104	7610±960
Glutamine	3212±333	2529±263	4185±1189	3399±292
γ-AOA	2957±371	2090±255**	2273±918	2735±678
Serin	371±64	326±37	653±83	590±76
Glycine	3286±469	2839±424	2067±784	2109±562
Threonine	609±112	416±62	589±140	503±109
Phosphoethanol amine	933±94	829±73	1254±138	1316±166
Alanine	276±48	279±30	421±64	462±66
Taurine	1092±98	1072±205	2176±36	2531±264

* - $p < 0.005$ versus control

** - $p < 0.1$ versus control

Thus, the CNS incorporated radiation leads to the activation of the γ AOA-ergic system in cerebral hemispheres which is apparently not accompanied by any stronger synthesis of the mediator taking into account that 10-day irradiation produces no changes and a greater concentration of glutamate is found in cerebral hemispheres after 28 days.

28-day irradiation reveals a lower concentration of noradrenaline in cerebral hemispheres with the unchanged dopamine concentration (Table 5.3). Suppression of the activity of dopamine-β-hydroxylase can be assumed as the most probable mechanism, though a stronger mediator degradation can be admitted. The latter fact correlates with a higher concentration of 3,4 dioxiphenylacetic acid concentration in the striatum, as indicated above considering that the oxidizing deamination of both dopamine and noradrenaline is catalyzed primarily by the isoenzyme MAO-B. At the same time the hemispheres demonstrate reduced concentrations of serotonin and 5-oxiindolacetic acid as its final metabolite. It is more probably due to a reduced concentration of its precursor tryptophan which tended to reduce in the striatum and in the thalamic region of the experimental animals (Table 5.4). Since the tryptophan concentration in the brain is a comparatively monotonous magnitude and, as a rule, it is almost the same in various sections [21], it can be assumed that the effect of lesser serotonin concentration is moderated by the precursor concentration. In its turn, a lesser tryptophan concentration can be due both to changes in the functioning of the transport systems and to the activation of the alternative paths of the tryptophan metabolism through animizing and pyrrolase cracking. The results of the study do not allow to make any final conclusion in this respect still the depressive effect of small radiation doses upon the serotonin system is apparent.

A pronounced increase of the concentration of glutamate and glutamine in the cerebral hemispheres should be highlighted among other effects of irradiation of the system of amino acids-transmitters during 28 days (Table 5.3). A significant glycine reduction in the same brain section and the tendency of γ-AOA concentration to increase allow to assume a pronounced loss of balance between concentrations of amine acids which act as excitants (aspartate, glutamate and suppressing (γ-AOA, glycine) transmitters. The taurine concentration in the cerebral hemispheres exceeded that in the control group 2.5 times. Since taurine produces an exciting action in the CNS [9], it

greater concentration may be a factor equalizing the ratio between the suppressing and stimulating transmitters and inhibiting the convulsive syndrome possible when this ratio shifts significantly towards exciting amino acids. Nevertheless, the taurine concentration in the cerebellum tends to reduce, still this brain portion does not evidences any pronounced shifts of concentrations of other neuroactive amino acids either.

Opposite shifts of taurine concentration in the cerebral hemispheres and in the cerebellum are accompanied by similar shifts of the phosphoethanol amine as a structural taurine analogue. Such greater phosphoethanol amine concentration in the cerebral hemispheres may be caused by a greater serin concentration in the same brain portion, because it has a pronounced activity of ethanol amine kinase [19], thus phosphoethanol amine is a product of serin transformations.

Variations of concentrations of the above compounds in the thalamus region and in the striatum both after 28 days and 10 days of irradiation are much less pronounced than in the cerebral hemispheres. In addition to the tendency of tryptopane to reduce the concentration of 5-oxiindolacetic acid reduces in the thalamus region and γ -AOA concentration tends to reduce (Table 5.4). Assuming that the reduction of metabolite concentration at a constant serotonin concentration is due to the reduction of activation of its oxidizing deamination, it is apparent then that the effect of irradiation upon the MAO activity on the cerebral hemispheres and in the thalamic region is oppositely directed. Yet, it should be remembered that the thalamus region includes, both functionally and metabolically, quite different structures, such as the thalamus and the hypothalamus characterized by different concentrations of serotonin and its derivatives alongside with the differing MAO activity [16]. Hence, it is more probable that the structures included into the thalamus region experience reduction of the synaptic release or a stronger serotonin attraction under a protracted irradiation effect, it results in the weakening of the oxidizing deamination process not related to the direct effect upon the MAO activity.

Thus, the data allow to conclude that under the effect of small doses of ionizing radiation the portions of the brain of rats with different functional and metabolic orientation experience a strong loss of balance between concentrations of compounds the main neurotransmitting systems: neuroactive amino acids and biogenic monoamines. Radionuclides accumulated just during 10 days in the cerebral hemispheres spur the γ -AOA

concentration and dopamine concentration tends to increase, i.e. the loss of balance manifests itself in the generation of funds of neuroactive compounds. A significantly more pronounced shifts of the studied indicators are observed after 28 days which become most manifest in the cerebral hemispheres. The main neurochemical effects of radionuclides after 28 days are the following: first, γ -AOA-ergic system activation not related to a stronger decarboxylation of glutamate; second, deranged relationship between exciting and suppressing transmitters as the concentration of glutamate acting as an exciting mediator in the CNS [20] and γ -AOA, goes up, while the concentration of glycine as a suppressing transmitter reduces; third, the serotonin system loses its activity, it is presumably due to a lesser accessibility of the precursor. The cerebral hemispheres manifest a higher concentration of taurine which is a neuromodulator with a predominantly suppressing action behaving also as a radioprotector [9]. It is possible that such increase serves also to compensate and level the processes of excitation and suppression in the CNS. It should be noted that phosphoethanol amine concentration changes similarly to taurine, the former is a major substrate of the lipid metabolism in the CNS, it may possibly be moderated by the changes in the concentration of serin, its precursor.

These data evidence that incorporated radionuclides produce an intricate pattern of changes in concentrations of biogenic amines and neuroactive amino acids compared with the changes produced by external irradiation, still, a number of effects of medium lethal and superlethal doses (suppression of the serotonin system, premature activation of the γ -AOA system) are repeated. The effects follow the same direction during relatively short (10 days) and longer (28 days) exposure. The loss of balance of biogenic amines, their precursors, metabolites and neuroactive amino acids in the brain tissue under the effect of incorporated radionuclides may be one of the factors fostering neuropsychic disorders among the communities affected by radioactive contamination.

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Chapter 6

METABOLIC EFFECTS OF INCORPORATION OF RADIONUCLIDES

Radiation biology and medicine has accumulated a considerable clinical and experimental data on studies of biochemical and molecular fundamentals of close and remote effects of radiation upon the organism. It has been noted that ionizing radiation causes numerous biochemical deviations in the metabolism of nucleic acids, hydrocarbons, macroergic compounds, proteins and membrane lipids [3, 9, 11]. Such disorders may to a certain extent be conditioned by changes in the hormonal status and other regulatory systems [8]. The biological effect of small doses of ionizing radiation is one of the most intricate and least studied in the radiation medicine. The data accumulated so far fail to explain fully the pathological pattern of radiation injury. The biochemical processes in organs and tissues during incorporation of radionuclides are the least studied.

The knowledge of radiation and biochemical deviations is needed to expand ideas about their effects and to develop the optimum diagnostic techniques and the correction of emerging disorders.

In this connection an experimental study has been made (the experiment is described in Chapter 5) of the intestine, the blood plasma, the liver, the heart to check the indicators of hydrocarbon (glucose, glucose-6-phosphate dehydrogenase, transketolase, lactate dehydrogenase, creatine phosphokinase), protein (AST, ALT, GGTP, total protein), lipid (general lipids, cholesterol, phospholipids) and hormonal (insulin, corticosteroids, T_3 , T_4 , TG) metabolism. Alongside with isolated intestine cells, the activity of enzymes of membrane digestion, alkaline and acidic phosphatases, catalase in the blood serum and antioxidizing activity were studied. The valid significant shifts are shown in the appended tables.

Several circumstances attract the study of biochemical processes in the small intestine. First, the gastrointestinal tract being the major entrance for food continuously experiences the main portion of radioactive substances [2]. Second, quite high concentrations of radioactive isotopes

can accumulate and persist in the epithelial cells of the small intestine as a result of hepatointestinal recirculation of nutrients including the phase of repeated absorption, deposition of radionuclides in crypts and subsequent intestinal excretion [6, 10]. In its turn, it results both in the longer exposure and greater doses in the mucus of the gastrointestinal tract. Third, finally the fine intestine epithelium has an extremely high proliferate activity and a tendency to restoration making it a suitable object for investigation of processes of compensation, adaptation and restoration evolving under the effect of the organism irradiation [14].

To this end the activity of maltase, saccharase, alkaline phosphatase and gamma glutamyl transpeptidase (GGTP) was studied in epithelial cells of different types isolated in the villus crypt direction [5]. The results of the investigation in Table 6.1 prove that rats after a standard vivarium diet have a pronounced cryptal apical gradient of membrane enzymes in the fine intestinal enterocytes. Highly specific cells of the apical portion of villuses have a great activity of the above enzymes compared with poorly differentiated cells of crypts, it well correlates with reported results [15]. Rats after dieting on a ration with radionuclides manifest a different distribution of enzymes. The activity of disaccharidase and GGTP in the villus types of cells is significantly inhibited compared with control animals. The alkaline phosphatase concentration under such conditions did not differ essentially from control indicators. Also, the homogenates of poorly differentiated cells of crypts did not manifest any significant modifications of the activity of the above enzymes. These data definitely indicate significant deviations in the biogenesis of membrane enzymes of the intestine epithelial cells. Since this intricate process comprises several stages [12] an effort has been made to verify the most probable mechanism of the described changes. In order to evaluate the activity of enzymes in the cytosol and membrane-bonded fractions of the cellular homogenate a technique of differential centrifuging of cells was applied. Fig. 6.1 — 6.2 demonstrate the relative activity of disaccharidase, alkaline phosphatase, GGTP in the membrane and cytosol fractions of apical cells. Rats during enteral incorporation of radionuclides manifest reduction of the percent concentration of all the above membranes in the membrane portion of enterocytes of villuses.

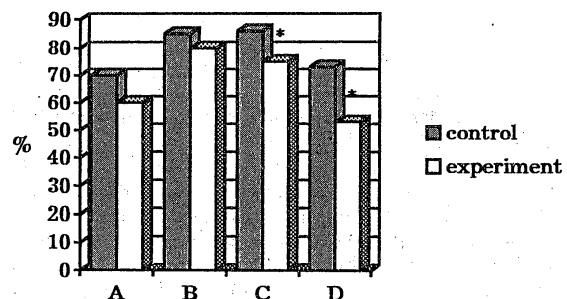


Fig. 6.1. Relative activity of alkaline phosphatase (A), maltase (B), saccharase (C), GGTP (D) in membrane fractions of apical enterocytes of control rats (1) and rats after 10 days of incorporation of radionuclides (2). * statistically significant variations between control and experimental groups

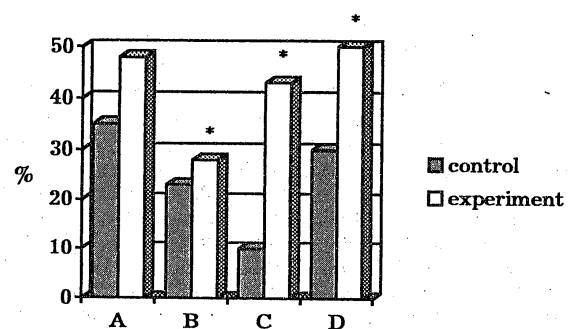


Fig. 6.2. Relative activity of alkaline phosphatase (A), maltase (B), saccharase (C), GGTP (D) in the cytosol fraction of apical enterocytes of control rats (1) and rats after 10 days of incorporation of radionuclides (2). * statistically significant variations between control and experimental groups

Table 6.1

Activity of enzymes in isolated enterocytes in intestines of rats during 10-days incorporation of radionuclides

Enzymes (mcmol/min mg of protein)	Group of rats	Apical cells	Cells of the median portion of villuses	Crypt cells
Maltase	control	0,193±0,017	0,107±0,018	0,076±0,011
	test	0,076±0,008*	0,110±0,014	0,080±0,004
Saccharase	control	0,015±0,001	0,020±0,003	0,007±0,001
	test	0,010±0,002*	0,017±0,002	0,007±0,001
Alkaline phosphatase	control	1,53±0,26	1,06±0,15	0,72±0,05
	test	1,46±0,15	0,97±0,17	0,69±0,09
GGTP	control	0,089±0,013	0,057±0,006	0,046±0,006
	test	0,047±0,006*	0,038±0,004	0,052±0,007

* — $p < 0.05$

Table 6.2

Biochemical indicators of blood plasma of rats during 10-days incorporation of radionuclides

Indicators	Control	Test	P
Glucose	2,82±0,11	3,42±0,14	< 0,01
Alkaline phosphatase, nmol/l min	180,0±13,6	292,0±14,6	< 0,01
T ₃ , nmol/l	0,70±0,05	1,15±0,12	< 0,01
T ₄ , nmol/l	28,30±2,66	70,70±10,54	< 0,001
TG, nmol/l	4,78±1,20	0,50±0,10	< 0,01
Corticosterone, nmol/l	165,0±45,5	228,0±53,0	> 0,1

The most profound changes have been registered for saccharidase and GGTP, 24 and 36%, respectively. On the other hand, the relative concentration of maltase, saccharase and GGTP in the soluble cytosol fraction of these cells increased significantly (Fig. 6.2). Hence, internal irradiation of the small intestine by enterally incorporated radionuclides causes damage to the final stage of the biogenesis of intestinal enzymes, their incorporation into the enterocyte plasmatic membrane, it can probably be due to the physical and chemical condition of these structures of cells [7, 13].

Incorporation of radionuclides during 10 days with the diet increases the glucose concentration in the blood (Table 6.2) by 21% compared with the control. It is apparently caused by hormonal transformations among animals even after a brief incorporation of radionuclides. It is manifested by the statistically significant increase of thyroxin (T_4) and triiodine thyronin (T_3) and the tendency of corticosterone (Table 6.2) to increase in the blood plasma of the experimental animals. The results indicate the evolution of a stress-like response after incorporation of radionuclides with food during 10 days. It is confirmed by a greater mass of adrenal glands (mg, 40.1 ± 0.61 compared with 47.4 ± 1.10 in the experiment, $p < 0.001$) and a greater concentration of corticosterone (by 28 %, $p < 0.05$) in this tissue. Variations of the thyroid functional activity also indicated a significant reduction of thyreoglobulin (Table 6.2).

A significantly stronger activity of alkaline phosphatase in the blood serum (by 62 %, Table 6.2) in the experimental group may be attributed to the effect of incorporated radionuclides upon the skeletal tissue [4].

The experimental animal group manifests a significant reduction of the creatin phosphokinase activity in the liver (up to 35 %) and in the heart (up to 44 %) as against the control level. It reflects a disordered metabolism of macroergical compounds under the effect of radionuclides, it is confirmed by oppositely directed variations of the phosphatase activity in the liver and a greater acid phosphatase activity in the skeletal muscle (Table 6.3).

Incorporation of radionuclides during 10 days does not cause any significant changes in the functioning of the centose phosphate tract in the studied tissues. A greater transketolase is remarked only in the muscular tissue (Table 6.3).

Table 6.3

Biochemical indicators in tissues of rats during 10-days incorporation of radionuclides

Indicators	Control	Test	P
Liver			
Creatine phosphokinase, $\mu\text{mol}/(\text{mg of protein min})$	$2,07 \pm 0,12$	$0,74 \pm 0,04$	$< 0,05$
Alkaline phosphatase, $\mu\text{mol}/(\text{mg of protein min})$	$9,69 \pm 0,25$	$8,23 \pm 0,46$	$< 0,05$
Acid phosphatase, $\mu\text{mol}/(\text{mg of protein min})$	$19,10 \pm 0,25$	$21,30 \pm 0,55$	$< 0,05$
Heart			
Creatine phosphokinase, $\mu\text{mol}/(\text{mg of protein min})$	$1,98 \pm 0,12$	$0,87 \pm 0,04$	$< 0,05$
Muscles			
Transketolase $\mu\text{mol sedoheptulose-7-P}/(\text{mg of protein min})$	$0,51 \pm 0,06$	$0,73 \pm 0,1$	$< 0,1 > 0,05$
Acid phosphatase, $\mu\text{mol}/(\text{mg of protein min})$	$1,81 \pm 0,12$	$3,76 \pm 0,35$	$< 0,05$
Lactate dehydrogenase $\mu\text{mol}/(\text{mg of protein min})$	$192,0 \pm 10,7$	$374,0 \pm 45,8$	$< 0,05$

A stronger activity of lactate dehydrogenase in the skeleton muscle of the experimental animals indicates the activation of the anaerobic glycolysis in the tissue. Thus, brief incorporation of radionuclides causes a stress-resembling condition with the change in the hormonal status.

Also, a certain reduction of the intensity of energy metabolism occurs, while the metabolism of hydrocarbons, lipids and proteins does not change significantly. The pattern and the extent of metabolic damage by irradiation is governed by the dose and duration of the effect of radionuclides [1].

Table 6.4

**Biochemical indicators of blood plasma of rats during 28-days
incorporation of radionuclides**

Indicators	Control	Test	P
Glucose, mmol/l	3,72±0,27	6,05±0,48	< 0,05
Insulin, mcUnit/ml	7,90±1,02	5,32±0,03	<0,1>0,05
Corticosterone, nmol/l	249,0±51,0	391,0±95,0	<0,1>0,05
Alkaline phosphatase, nmol/(min · l)	329,0±30,1	393,0±11,4	<0,1>0,05
GGTP, mcml/ml	2,43±0,43	6,11±0,93	< 0,01
Counteroxidizing activity, %	56,30±5,20	34,10±9,50	<0,1>0,05
Catalase, mcml/min · ml	3,04±0,56	4,21±0,81	<0,1>0,05

During 28 days of incorporation of radionuclides with food a hyperglycemia develops (Table 6.4).

It is probably caused by the reduction of insulin concentration in the blood plasma alongside with a growing concentration of corticosterone. The latter modification may be assessed as a manifestation of the stress response. It should be emphasized that the hormonal lack of balance observed after 10 days of incorporation of radionuclides persists until the end of the first month of the experiment. It is confirmed by changes in the indicators of peroxide oxidation of lipids in the blood plasma. A reduced antioxidation activity and a stronger catalase activity are observed (Table 6.4).

A persistent stronger activity of the alkaline phosphatase in the blood plasma on the 28th day after incorporation of radionuclides can be an evidence of its accumulation in the skeletal tissue.

At the same time reduced activity of the acid phosphatase in the blood serum (by 10 %) in the experimental animal group should be noted (Table 6.4).

The activity of gamma glutamyl transpeptidase in the blood serum increases parallel to the alkaline phosphates activity (Table 6.4), yet this enzyme demonstrates a pronouncedly stronger activity compared with the alkaline phosphatase.

After 28 days of incorporation of radionuclides the lactate dehydrogenase activity in the liver reduces. A significant inhibition of the transketolase activity is observed in the skeletal tissue (34.3 %). The activity of the enzymes tends to reduce by 20 and 15.4 %, respectively, in the liver and the myocardium (Table 6.5). These effects may be due to a greater vulnerability of adaptive enzymes to radiation, which also include liver enzymes [8].

The experimental animals manifest significant variations in the lipid metabolism. The concentration of phospholipids in the liver reduces by 43 % compared with the control evidencing the inhibition of their synthesis under the effect of radiation. Considering the functional significance of this class of lipids the obtained results indicates a profound disorder of the lipid metabolism in this tissue after 28 days of incorporation of radionuclides. It is proven by the reduced cholesterol concentration in the liver of the experimental group which is an indicator of the structural and functional liability of membranes of hepatocytes during internal irradiation.

A greater antioxidation activity is remarked by the end of the first month (Table 6.5), it is an aftermath of adaptation variations in the system of peroxidation of lipids. These variations occur after activation of the system of peroxide oxidation of lipids which is observed during early dates of incorporation of radionuclides.

Thus, the obtained results prove that incorporation of radionuclides with food causes numerous metabolic changes in various organs and systems.

One of the first organism responses to incorporation is the development of the stress reaction which appears already on the 10th day and persists in our version of the experiment during one month.

Table 6.5

**Biochemical indicators of blood plasma of rats during 28-days
incorporation of radionuclides**

Indicators	Control	Test	P
Liver			
Cholesterin, mg/g of tissue	3,79 ±0,24	2,92±0,36	<0,1>0,05
Phospholipids, mg/g of tissue	20,93±1,04	11,90±1,82	< 0,05
Counteroxidizing activity, %	39,60±4,97	47,70±3,40	<0,1>0,05
Lactate hydrogenase, nmol/mg of protein min	1,53±0,13	1,19±0,07	< 0,05
Heart			
Alkaline phosphatase, nmol/mg of protein min	41,1±2,47	24,0±1,81	< 0,05
Muscles			
Transchetolase, mcmol sedoheptu- lose-7-f/mg of protein min	0,35±0,03	0,23±0,03	< 0,05

Pronounced disorders of the hormone-producing function of the thyroid and adrenals may be a probable cause of the detected metabolism processes.

Also, the above metabolism variations may be partially caused by significant disorders of membrane digestive processes, in their turn the latter are just one indication of the total radiation-induced disorder of the gastrointestinal tract. The reported direct correlation links between morphostructural transformations and the activity of intestinal enzymes [5] corroborate this conclusion. It is also supported by the radiation-induced changes in the distribution of intestinal enzymes in the crypt-villus system proving disorders of the processes of biochemical

specialization and differentiation of enterocytes performing different functions.

On the other hand, a close integration between hydrolysis of absorption of nutrients in the intestine, existence of enzyme transport groups in epitheliocytes allow to conclude that internal irradiation of the small intestine by incorporated radionuclides damages both the membrane digestion and the adaptation of major nutritive components supporting the metabolic homeostasis. These changes in the metabolism during the initial period of incorporation of radioisotopes result in the inhibition of the energy metabolism intensity, in later days disorders of the hydrocarbon and lipid metabolism occur together with the peroxide oxidation of lipids. The above disorders in the metabolism of substances, hormonal regulation, the intestine functional activity may be assumed in the generalized version as a manifestation of disadaptation syndrome evolving even during a brief effect of incorporated radionuclides.

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Chapter 7

FREE AMINO ACIDS OF BLOOD PLASMA AND TISSUES OF ANIMALS AFTER INCORPORATION OF CESIUM AND STRONTIUM RADIONUCLIDES

Free amino acids of tissues are among the major indicators of homeostasis and they relate to various metabolic fluxes in a cell. Still, their metabolism during various types of radiation pathology remains unclear. Yet, the loss of balance of the amino acidic pool of the blood plasma manifests itself both after general and local exposure of the organism. An early response of the organism when it is exposed to ionizing radiation is taurinuria, which becomes especially strong in the case of a lethal outcome [1]. Changes in the spectrum of free amino acids, including a higher concentration of amino acids with the ramified carbon chain (leucine, isoleucine, valine), hypo- or hyperalaninemia, reduction of glutamine at a high level of urea and drop in the arginine/ornithine ratio are typical for an earlier period of acute exposure to different types of ionizing radiation [7]. Such higher urea concentrations result from the stimulation of its formation process in response to greater amounts of free amino acids released by intensified proteolysis [14]. Simultaneous drop of glutamine concentration which is synthesized normally together with urea, and disordered balance of other components of the urea formation cycle allow to assume a metabolic stress in the ammonia neutralizing system. These features of the blood plasma amino acidic pattern depend upon the radiation dose, its nature and reflect the profoundness of biochemical transformations [6]. There are also some general manifestations, such as hyperexcretion of taurine and a stronger consumption of glycogen amino acids [4].

Local exposure of separate skeleton muscles of rats to doses 10—15 Grays intensifies the processes of proteolysis and liberation of free amino acids by 60—70%. Alanine and glutamine account for about 45% of the amino acids liberated into the perfusate. However, since these amino acids in muscular proteins make up only 15% of the total amount of amino acids, their higher concentrations evidence that they are resynthesized

from pyruvate and glutamate. It is assumed that the non-protein contribution into the liberation of alanine accounts for 55—60% and that of glutamine 70% [15]. In addition to greater concentrations of alanine and glutamine during local exposure there is a higher concentration of proline, threonine, histidine, thyroxin, phenylalanine, glycine and lysin in the perfusate, proportionally to their original concentration in muscular proteins [15]. A significant leap in the concentration of these amino acids after exposure confirms the view that radiation causes protein disintegration in the skeleton muscle similarly what glucocorticoids do [16]. As these hormones penetrate into the organism they produce a generalized catabolic condition characterized by the reduction of the muscular mass and the loss of nitrogen with urine.

The origin of muscular proteins, which make amino acids biologically exposed after destruction by radiation, is unknown. Ultrastructural alterations prove local destruction of myofibrils and the swelling of the interfibrillar space. Yet, since no higher level of 3-methyl histidine is reported [15], the stronger liberation of amino acids during local exposure of the skeleton muscles occurs probably from sarcoplasmatic proteins, the enzymes of glycolysis and creatinkinase being their major portion.

The overwhelming majority of investigations have been accomplished for the conditions of general or local exposure, therefore the data are not suitable for treatment and prevention in the areas contaminated after the Chernobyl disaster. The population in these areas incorporates a significant proportion of radiation from the local food they produce.

We have experimented with rats which dieted on the grain contaminated with radionuclides (strontium-90, cesium-137) during one month.

An automatic amino acid analyzer AAA-339T (Czech) was used for determination of free amino acids in blood serum samples and animal tissues deproteinized with 6% chlorine acid.

Studies of free amino acids in liver tissues revealed absence of any valid variations of concentrations of individual acids excepting threonine, its concentration was 40% less. A similar reduction of the concentration of this amino acid was detected in the myocardial and skeletal muscles (Table 7.1, 7.2).

Table 7.1

Concentration of free amino acids in myocardial tissue of white rats during incorporation of radionuclides (nmol/g)

Amino acid	Control	Test	P
Cystic acid	92±4	89±3	> 0,05
Taurine	8383±113	8117±200	> 0,05
Aspartate	3243±467	2664±238	> 0,05
Threonine	793±88	445±23	< 0,01
Serin	433±64	365±29	> 0,05
Glutamate	5990±537	5124±264	> 0,05
Glutamine	3076±368	2223±37	< 0,05
Glycine	640±65	596±42	> 0,05
Alanine	1487±138	1331±70	> 0,05
Valine	196±18	148±10	< 0,05
Methionine	105±8	79±4	< 0,02
iso-Leucine	131±7	97±6	< 0,01
Leucine	309±29	219±18	< 0,05
Thyrosine	258±21	206±2	< 0,05
Phenylalanine	295±11	244±12	< 0,01
Ammonia	3386±444	2969±157	< 0,05
Ornithine	147±14	94±6	< 0,01
Lysin	124±14	119±15	> 0,05
Histidine	747±18	720±11	> 0,05
3-methyl Histidine	813±40	744±26	> 0,05

Table 7.2

Concentration of free amino acids in tissues of skeletal muscles of white rats after incorporation of radionuclides (nmol/g)

Amino acid	Control	Test	P
Cystic acid	44±4	36±4	> 0,05
Taurine	6354±190	6656±245	> 0,05
Aspartate	1170±63	983±76	> 0,05
Threonine	1326±208	648±62	< 0,01
Serin	929±126	562±38	< 0,05
Glutamate	2254±166	1816±96	> 0,05
Glutamine	2446±157	1472±105	< 0,001
Proline	434±89	405±140	> 0,05
Glycine	2943±237	2796±363	> 0,05
Alanine	2252±168	2164±210	> 0,05
Valine	209±10	226±40	> 0,05
Methionine	105±13	107±17	> 0,05
iso-Leucine	101±19	106±14	> 0,05
Leucine	255±12	244±22	> 0,05
Thyrosine	226±21	222±21	> 0,05
Phenylalanine	270±14	231±26	> 0,05
Ethanolamine	79±19	62±10	> 0,05
Ammonia	3508±754	3095±828	> 0,05
Ornithine	190±12	141±18	< 0,05
Lysin	734±59	618±38	> 0,05
Histidine	628±22	640±33	> 0,05
3-methyl Histidine	723±64	680±78	> 0,05

Threonine, together with lysin, is not involved in the reactions of repeated aminizin, in this case it can be viewed as an absolutely substitutable compound. Its synthesis de novo among mammals is impossible without suitable keto-analogs. The deficit of threonine in tissues in which its catabolism is the strongest (the liver) or which contain the major share of this amino acid (muscles), allows to assume both its lack in food and stronger disintegration.

The myocardial tissues among experimental animals had less glutamine and ornithine, the amino acids which are active agents of urine production. The free ammonia concentration would also reduce. The greatest changes in the myocardial tissue were observed in respect to non-substitutable amino acids. Recalculated for one gram of tissue the concentration of threonine would reduce 44%, valine and methionine 25%, isoleucine 26%, leucine 29%, phenylalanine 17% and the amount of thyroxin as a relatively substitutable amino acid would reduce 20%. The lack of amino acids with the ramified carbon chain in muscular tissues, aromatic amino acids and sulphur donors to produce other sulphur-containing amino acids (methionine) is a proof that the aminoacidic disbalance has been initiated (50% of the pool of free amino acids concentrates in the skeletal muscles) and it is apparently caused by their stronger catabolism [8].

Therefore, reduction of threonine concentration, thyronine, glutamine and ornithine for the myocardial and skeletal muscles, is common for all the studied tissues. Reduction of the glutamine, serin and simultaneous reduction of concentrations of amino acids with ramified carbon chains is a sign of inhibition of the glucose-alanine cycle (though the alanine concentration initially remains unchanged). No reduction of the intra/extracellular gradient of alanine and glutamine without their lesser intracellular concentration has been detected either after local irradiation of skeletal muscles with a dose 10 Grays of radioactive Co [15]. The observed muscular tissue changes after exposure may be caused by the protein metabolism, because these amino acids in the muscle result both from the protein degradation and from their synthesis de novo. It is accompanied also by reduced concentrations of other free amino acids (Table 7.2). These data prove a stronger disintegration of protein as one early significant metabolic disorder produced by radiation [9].

Table 7.3 shows the summarized analytical data of pools of free amino acids in the liver, myocardial and muscular tissue. It is apparent that incorporation of radionuclides reduces the concentration of free amino acids in the tissues and it is valid for the myocardial tissue.

The ratio between substitutable/non-substitutable amino acids in all the tissues is up, the concentration of amino acids with ramified carbon chains is down (it is valid for the myocardial tissue). Similar modifications in the concentration of aromatic amino acids in the tissues have been observed.

Table 7.4 presents changes in the structure of correlation links between individual amino acids. It is apparent that the tissues, according to the degree of disintegration of the old and appearance of the new highly valid correlation links ($r > 0.75$) can be rated as follows: the liver < the myocardium < the skeleton muscles. The correlation links between aspartate—glutamate and serin—iso-leucine in the myocardium tissue survive in the experimental group, but they change their sign to the opposite (Table 7.5).

The linear discriminatory and cluster analysis of the data has indicated that concentration of threonine, glycine, aspartate and taurine with the Fisher constants between 4.3 and 10.3 are most informative for the liver tissue.

The clusters of pools of free amino acids in the liver of the control and experimental groups stand quite apart (Table 7.3), irrespective of the lack of any valid difference between the studied indicators (excepting threonine).

Ornithine (the Fisher constant is 18.6) and glutamate (the Fisher constant is 8.1) are most informative for the myocardial tissue during the linear discriminatory analysis. Glutamine and leucine have the largest Fisher constants for skeleton muscles (39.5 and 4.6, respectively).

Thus, the studies of concentrations of free amino acids in the liver, myocardial tissues and skeleton muscles under the effect of incorporated radionuclides indicate that the strongest changes occur in the concentration of non-substitutable amino acids and it is most typical for the tissues of the myocardial and skeleton muscles. The deficit of such amino acids in muscular tissues may be a direct result of disorders in the synthesis of protein molecules and leads to disfunctions of muscles.

Table 7.3

Structure of total free amino acids in tissues of white rats after incorporation of radionuclides

Indicators	Tissue		
	Liver	Myocardial	Muscular
Total amino acids/ g			
control	55809±2831	30648±908	27213±1650
test	50506±2827	25862±236*	23842±2015
Substitutable/ non-substitutable (SU/NSU)			
control	6,40	14,69	8,07
test	7,16	18,14	9,94
Acids with ramified carbon chains (ARCC)			
control	3125±382	637±42	565±31
test	2761±187	464±34*	576±48
Aromatic amino acids (AA)			
control	1932±132	554±32	536±30
test	1692±131	450±13*	453±47
ARCC/ AA			
control	1,62	1,15	1,05
test	1,63	1,03	1,27

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.4

Relationships characterizing links between amino acids and their derivatives in the liver tissue during incorporation of radionuclides based on correlation factors (r)

Control		Test	
Clustered indicators	r	Clustered indicators	r
ASP-GLY	0,83	ASP-GLN	0,83
ASP-VAL	0,81	ASP-AAaA	0,95
ASP-MET	0,79	THRE-AOA	-0,81
ASP-ILE	0,93	THRE-VAL	0,90
ASP-LEU	0,80	THRE-MET	0,81
ASP-EA	0,80	THRE-LEU	0,80
GLU-ALA	0,79	THRE-THYR	0,97
GLU-LYS	0,87	THRE-PHE	0,95
ALA-ORN	0,81	THRE-NH ₃	0,96
VAL-HIS	0,83	THRE-ORN	0,96
ILE-HIS	0,81	SER-VAL	0,90
LEU-HIS	0,82	SER-MET	0,88
		SER-ILE	0,79
		SER-LEU	0,90
		SER-THYR	0,95
		SER-PHE	0,90
		SER-ORN	0,95
		GLN-MET	0,87
		GLN-ILE	0,87
		GLN-LEU	0,84
		AAaA-ALA	0,82
		ALA-THYR	0,77
		ALA-PHE	0,82
		ALA-NH ₃	0,77

Table 7.5

Relationships characterizing links between amino acids and their derivatives in myocardial tissue during incorporation of radionuclides based on correlation factors (r)

Control		Test	
Clustered indicators	r	Clustered indicators	r
TAU-CA	0,96	CA-SER	0,84
TAU-ASP	0,83	CA-GLY	0,75
THRE-SER	0,83	CA-ALA	0,80
ASP-GLU	-0,89	CA-VA	0,93
ASP-GLN	-0,91	CA-ILE	-0,92
ASP-ALA	-0,90	CA-LEU	-0,86
ASP-LEU	0,78	TAU-LYS	0,91
THRE-ILE	0,83	ASP-GLU	0,96
THRE-THYR	0,79	ASP-VAL	0,76
SER-GLY	0,95	THRE-GLY	0,93
SER-MET	0,83	SER-ALA	0,92
SER-ILE	0,85	SER-VAL	-0,84
SER-THYR	0,95	SER-ILE	-0,85
SER-PHE	0,87	SER-LEU	-0,87
SER-NH ₃	0,95	GLN-ALA	0,92
GLU-GLN	0,80	GLN-VAL	-0,82
GLU-ILE	-0,79	GLN-ILE	-0,82
GLU-LEU	-0,93	GLN-LEU	0,87

Control		Test	
Clustered indicators	r	Clustered indicators	r
GLU-LYS	-0,77	GLN-ORN	0,86
GLY-THYR	0,89	GLN-LYS	-0,88
GLY-PHE	0,79	GLY-MET	0,81
GLY-NH ₃	0,89	ALA-BAL	-0,89
MET-ILE	0,82	ALA-ILE	-0,90
MET-PHE	0,86	ALA-LEU	-0,94
MET-NH ₃	0,89	ALA-ORN	0,82
MET-ORN	0,97	ALA-LYS	-0,81
ILE-LEU	0,95	VAL-ILE	0,99
ILE-THYR	0,83	VAL-LEU	0,98
ILE-PHE	0,83	MET-LEU	0,99
ILE-ORN	0,80		
ILE-LYS	0,81		
LEU-LYS	0,78		
THYR-PHE	0,86		
THYR-NH ₃	0,90		
PHE-NH ₃	0,80		
PHE-ORN	0,83		
PHE-LYS	0,82		
ORN-NH ₃	0,82		

Table 7.6

Relationships characterizing links between amino acids and their derivatives in tissues of skeletal muscles during incorporation of radionuclides based on correlation factors (r)

Control		Test	
Clustered indicators	r	Clustered indicators	r
CA-VAL	0,77	CA-NH ₃	0,79
CA-ILE	0,80	CA-methIS	0,75
CA-THYR	0,88	TAU-MET	0,79
CA-PHE	0,75	THRE-THYR	0,85
CA-LYS	0,90	THRE-PHE	0,83
CA-HIS	-0,87	THRE-NH ₃	0,87
ASP-THRE	0,91	THRE-ORN	0,96
ASP-SER	0,88	THRE-LYS	0,81
ASP-GLU	0,98	THRE-methIS	0,91
ASP-GLN	0,75	SER-GLY	0,91
ASP-VAL	0,80	SER-THYR	0,77
ASP-LEU	0,95	SER-PHE	0,86
THRE-GLU	0,81	SER-NH ₃	0,83
THRE-GLN	0,89	SER-ORN	0,84
THRE-PRO	0,84	SER-methIS	0,85
THRE-LEU	0,96	GLU-GLN	0,96

Control		Test	
Clustered indicators	r	Clustered indicators	r
SER-GLU	0,79	GLU-THYR	0,81
SER-GLN	0,94	GLU-PHE	0,78
SER-PRO	0,84	GLN-THYR	0,82
SER-LEU	0,96	GLN-PHE	0,84
GLU-LEU	0,90	GLY-PHE	0,80
GLN-PRO	0,88	GLY-NH ₂	0,85
GLN-LEU	0,90	GLY-ORN	0,87
PRO-GLY	0,88	GLY-metHIS	0,86
VAL-THYR	0,93	ALA-MET	0,90
VAL-HYS	-0,85	ALA-ILE	0,76
MET-ILE	0,79	MET-EA	0,80
THYR-LYS	0,76	ILE-LEU	0,89
THYR-HIS	-0,83	ILE-THYR	0,88
PHE-LYS	0,83	ILE-PHE	0,79
ORN-HIS	-0,86	ILE-ORN	0,86
HYS-metHIS	-0,82	LEU-THYR	0,85
		LEU-ORN	0,77
		THYR-ORN	0,92
		ORN-metHIS	0,86

Experiments with male rats of the "Wistar" line have revealed that consumption of radioactive grain during 10 days leads to the reduction of concentration of glycine and non-substitutable amino acids, such as threonine and lysin, in the blood plasma (Table 7.7) against a slight (10%) reduction of the total pool of free amino acids in the plasma. Such reduced concentration of non-substitutable amino acids is accompanied by a greater ratio between substitutable and non-substitutable amino acids and between the amino acids with the ramified carbon chain and aromatic amino acids (from 3.00 to 3.33 and 2.71 to 3.13, respectively). The liver tissue manifests a lesser total amount of amino acids (27% less) due to the reduced concentration of substitutable (aspartate, glutamate, thyroxine, ornithine) and non-substitutable (valine, leucine, ethionine, phenylalanine, lysin) amino acids as well as their derivatives (taurine, phosphor ethanol amine, ethanol amine). Depletion of the aminoacidic pool of the liver tissue is accompanied by a greater ratio between the substitutable and non-substitutable amino acids and between the amino acids with the ramified carbon chain and aromatic amino acids from 10.6 to 11.8 and 1.40 to 2.40, respectively (Table 7.8).

In addition to the reduced concentration of non-substitutable amino acids (threonine, valine, isoleucine, leucine, methionine) and glycine in the myocardial tissue of the "Wistar" line male rats the concentration of glutamine would validly increase (Table 7.9) indicating activation of the glutamine synthesis reaction and withdrawal of glutamate from active involvement in the energy exchanges in cardiomyocytes [10]. No significant disorder of the total aminoacidic pool has been detected in the myocardial tissue after 10 days of incorporal exposure. However, similarly with the data for other tissues, the ratio between the substitutable and non-substitutable amino acids would increase.

The amount of free amino acids in skeletal muscles does not undergo significant variations during this term of incorporation of radionuclides. Still the concentration of glycogen amino acids aspartate and glutamate does increase and the quantity of methionine and phenylalanine decrease (Table 7.10).

Table 7.11 shows the correlation matrix for the blood plasma with various correlation links in the experimental and control groups of animals. The linear discriminatory analysis yields the highest

Table 7.7

Concentration of free amino acids in blood plasma of rats during incorporation of radionuclides (nmol/ml)

Amino acid	Control	Test
Cystic acid	17±1	13±3
Taurine	563±38	496±14
Aspartate	110±4	115±2
Threonine	710±34	532±27*
Serin	645±15	612±25
Glutamate	220±16	265±13
Glutamine	640±12	592±29
Amino adipinic acid	30±3	31±1
Glycine	755±15	690±12*
Alanine	840±17	678±31
Valine	261±20	266±5
Methionine	90±12	78±2
iso-Leucine	150±10	145±2
Leucine	222±19	214±4
Thyrosine	102±9	105±7
Phenylalanine	132±17	95±2
Ethanol amine	62±10	49±0,5
Ammonia	2742±608	2361±602
Ornithine	213±19	228±6
Lysin	777±45	627±35*
Histidine	84±9	86±3

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.8

Concentration of free amino acids in liver tissue of rats during incorporation of radionuclides (nmol/g)

Amino acid	Control	Test
Cystic acid	228±20	183±11
Taurine	6810±693	3480±315*
Phosphoethanol amine	2359±370	1192±43
Aspartate	4144±333	2288±668*
Threonine	1241±184	881±121
Serin	2091±338	1625±132
Glutamate	4790±338	2696±893*
Glutamine	5747±594	4000±664
Glycine	3533±490	2857±570
Alanine	2334±287	2854±333
Valine	483±40	273±55*
Methionine	213±12	93±20*
Leucine	579±75	719±112
Thyrosine	451±58	237±55*
Phenylalanine	307±56	174±25*
Ethanol amine	722±59	521±31*
Ammonia	7637±554	8015±569
Ornithine	752±100	510±24*
Lysin	1172±111	741±75*
Histidine	699±66	678±48

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.9

Concentration of free amino acids in myocardial tissue of rats during incorporation of radionuclides (nmol/g)

Amino acid	Control	Test
Cystic acid	137±4	151±6
Taurine	21485±251	21357±950
Aspartate	3727±76	3559±590
Threonine	1077±31	693±57
Serin	1022±40	865±63
Glutamate	9534±310	9942±482
Glutamine	5601±265	6530±290
Glycine	1219±63	941±51
Alanine	3032±237	2692±158
Valine	473±34	325±44
Methionine	220±28	132±14
IsoLeucine	317±18	200±11
Leucine	570±81	353±8
Thyrosine	227±42	211±9
Phenylalanine	306±35	280±25
Ethanolamine	390±10	342±96
Ammonia	9504±218	8627±422
Lysin	1603±164	1121±132
Histidine	275±40	321±60

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.1

Concentration of free amino acids in the tissue of skeletal muscles of rats during incorporation of radionuclides (nmol/g)

Amino acid	Control	Test
Taurine	14547±110	15683±450
Aspartate	820±26	961±54*
Threonine	1038±75	1062±33
Serin	1310±29	1345±47
Glutamate	1617±75	2351±237*
Glutamine	2906±185	2665±182
Glycine	5728±371	5368±407
Alanine	3911±76	3824±202
Valine	218±15	184±12
Methionine	102±10	71±6*
Leucine	173±11	149±8
Thyrosine	274±18	254±6
Phenylalanine	136±8	97±5
Ammonia	8739±359	9320±254
Lysin	741±102	838±27*
Hystidine	237±5	271±14

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.11

Relationships characterizing links between amino acids and their derivatives in the blood plasma of the "Wistar" line rats during incorporation of radionuclides

Control		Test	
Clustered indicators	r	Clustered indicators	r
CA-THRE	-0,83	CA-NH ₃	-0,97
CA-SER	-0,97	CA-LYS	0,95
CA-MET	-0,93	TAU-ASP	0,89
CA-EA	0,88	TAU-THRE	0,90
CA-HIS	-0,94	TAU-SER	0,99
TAU-GLN	0,82	TAU-GLY	0,91
TAU-AAaA	0,97	TAU-ALA	0,99
TAU-ALA	0,88	TAU-MET	0,88
TAU-VAL	0,99	ASP-SER	0,90
TAU-THYR	0,94	ASP-GLU	0,92
TAU-NH ₃	0,83	THRE-GLY	0,91
ASP-THYR	0,88	THRE-ALA	0,91
SER-HIS	0,86	THRE-ALA	0,95
GLN-MET	-0,92	THRE-ILE	0,94
AAaA-ALA	0,93	THRE-LEU	0,99
AAaA-VAL	0,99	THRE-EA	-0,80
AAaA-ILE	0,99	SER-GLY	0,84
AAaA-LEU	0,99	SER-ALA	0,98
AAaA-THYR	0,99	SER-ILE	0,95
GLY-EA	0,89	SER-LEU	0,95
GLY-NH ₃	0,87	GLU-GLN	-0,94
GLY-ORN	0,80	GLU-AAaA	0,96

Control		Test	
Clustered indicators	r	Clustered indicators	r
ALA-VAL	0,93	GLU-THYR	-0,76
ALA-THYR	0,89	GLU-HIS	-0,90
ALA-PHE	-0,92	GLU-LYS	-0,85
ALA-NH ₃	0,97	GLN-AAaA	-0,82
ALA-ORN	0,79	GLN-THYR	0,90
VAL-ILE	0,99	AAaA-HIS	-0,98
VAL-LEU	0,98	GLY-ALA	0,90
VAL-THYR	0,96	GLY-MET	0,99
VAL-NH ₃	0,89	GLY-ILE	0,97
VAL-LYS	-0,84	GLY-LEU	0,92
MET-LYS	0,85	ALA-MET	0,89
MET-HIS	0,81	MET-ILE	0,95
ILE-THYR	0,96	MET-LEU	0,91
ILE-NH ₃	0,88	THYR-ORN	-0,87
ILE-LYS	-0,85	THYR-LYS	0,86
LEU-THYR	0,96	PHE-HIS	-0,84
LEU-PHE	-0,82	NH ₃ -ORN	0,89
LEU-NH ₃	0,96	NH ₃ -LYS	-0,94
THYR-NH ₃	0,91	ORN-LYS	-0,95
PHE-EA	-0,87		
PHE-NH ₃	-0,88		
PHE-ORN	-0,96		
EA-ORN	0,96		
EA-HIS	0,86		
ORN-HIS	0,82		

Fisher constants for threonine, serin, methionine, alanine, taurine, aspartate and ammonia within the range from 52.3 (thionine) to 5.88 (taurine).

The linear discriminatory analysis of the data has yielded the magnitude D2-Mahalabonis equal to 48 governing space arrangement of all the indicators in the study for the control and experimental groups of animals. It confirms the assumption about a pronounced aminoacidic disbalance under the effect of a disturbing factor, in this case the incorporated radionuclides.

It is an essential problem of the duration of rehabilitation and possibilities to restore the affected functions and biochemical indicators in tissues after incorporation of radionuclides with food is discontinued. A group of animals from a consecutive series was used in another experiment. They were kept on a common vivarium diet during 7 days, i.e. they received the food free from radionuclides. Though this "clean" interval of time was rather long, the animals manifested valid modifications in concentrations of individual amino acids. So, the liver tissue had the total concentration of free amino acids 22% less. A higher ratio between the substitutable and non-substitutable amino acids persisted due to the low level of leucine, isoleucine and phenylalanine. Reduced quantities of serin, ornithine and phosphor ethanol amine were detected. The drop of thyroxin concentration was apparently caused by the reduced concentration of its prerunner phenylalanine in the liver tissue (Table 7.12). Lack of valid changes in the myocardial and drop of just lysin concentration in the tissue of skeleton muscles compared with the control group allow to assume a rapid normalization of biochemical indicators in the muscular tissues. Still, changes in the hormone responsive tissues persist under the effect of incorporated radionuclides. So, we have detected reduced concentrations of thionine, glutamate, thyroxin and cysta thionine in the thymus tissue (Table 7.13).

The thymus is a highly sensitive tissue in respect to ionizing radiation. Depression of the thymus endocrine function during an acute radiation diseases plays an important role in the mechanism of disorders of the immune system. It can be caused both by direct and indirect effects of radiation through activation of the glucocorticoid function of the adrenal cortical layer.

Table 7.12

Concentration of free amino acids in the liver tissue of the "Wistar" line rats 7 days after incorporation of radionuclides (nmol/g)

Amino acid	Control	Test
Cystic acid	322±24	269±22
Taurine	3195±732	2232±295
Phosphor ethanol amine	2081±282	1175±189*
Aspartate	4621±671	4354±509
Threonine	1148±173	741±117
Serin	4045±530	2576±209
Glutamate	3976±428	2928±200*
Glutamine	4071±319	2789±426*
α-aminoadinine acid	1254±97	1291±175
Glycine	3739±542	3335±343
Alanine	3276±490	3547±546
α-aminobutyric acid	406±143	200±35
Valine	315±36	236±25
Methionine	85±19	89±11
iso-Leucine	203±28	147±18*
Leucine	362±28	247±30*
Thyrosine	418±52	179±20*
Phenylalanine	303±51	151±52*
Ethanolamine	1341±162	1132±55
Ammonia	4257±305	3604±304
Ornithine	379±46	195±41*
Lysin	864±228	459±83
Hystidine	905±103	845±118
TOTAL	41567±238	32721±170
SU/NSU	11,67	14,81
ARCC/AA	1,22	1,91

Note: * — valid ($p < 0,05$) in respect to the control animal group

Table 7.13

Concentration of free amino acids in the thymus tissue of rats 7 days after incorporation of radionuclides (nmol/g)

Amino acid	Control	Test
Cystic acid	77±8	73±2
Taurine	9355±395	9435±266
Phosphor ethanol amine	8109±387	8748±460
Aspartate	3903±229	3719±272
Threonine	866±31	693±28*
Serin	922±99	1110±28
Glutamate	7574±952	4748±213*
Glutamine	1404±111	1436±87
Glycine	2945±222	2954±138
Alanine	2482±219	2416±284
Valine	234±24	269±11
	322±21	222±11*
Methionine	50±7	64±5
Isoleucine	114±11	162±25
Leucine	191±34	216±23
Thyrosine	285±34	161±25*
Phenylalanine	191±16	221±15
α-Alanine	129±23	231±18*
α-isoaminobutyric acid	245±27	309±78
γ-aminobutyric acid	132±12	173±68
Ethanol amine	393±23	350±14
Ammonia	2053±214	4264±1174
Ornithine	86±12	84±15
Lysin	348±53	437±38
Histidine	123±31	177±28

Note: * — valid ($p < 0,05$) in respect to the control animal group

In our experiments the test animals would have a persistent hypertrophy of adrenal glands, however, the concentration of corticosterone in the adrenal tissue and in the blood plasma did not differ from the control levels. Restoration of the structure and metabolism of nucleic acids in the thymus after a protracted introduction of tritium oxide into the organism of animals is known to have a transient nature. Later compensatory mechanisms become damaged resulting in a repeated damage of the structure and metabolism of nucleic acids in the thymus and induction of the synthesis of a great range of polypeptides [5]. Thymocytes of irradiated rats lose potassium, it may result from the reduction of the total concentration of amino acids in the cell and its greater need for taurine [2, 11].

It is apparent that cessation of incorporation of cesium and strontium radionuclides with food is not accompanied by any rapid normalization of the disorders of aminoacidic metabolism in the tissues of animal organisms. Such disorders are most persistent in the organs most sensitive to radiation, such as the thymus and the liver. Our linear discriminatory analysis confirms this assumption and demonstrates the reduction of the D3-Mahalanobis constant for the myocardial tissue and skeleton muscles to 0.8, it remains equal 7 for the liver and thymus tissues.

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Chapter 8

EFFECT OF ENTEROSORPTION UPON INDICATORS OF METABOLISM AMONG EXPERIMENTAL ANIMALS UNDER EFFECT OF RADIONUCLIDES

The exposure dose in the disaster region combines two components - external exposure to radionuclides which fell out on the soil surface and internal exposure through incorporation of radionuclides with food and potable water and through inhalation (to a lesser extent) [1].

The incorporated radioactive substances are absorbed by the organism and circulated by blood flow. The absorbability of these substances is a factor of their physical and chemical behavior and physiological condition of the organism [2].

During continuous incorporation of radionuclides by the human organism a syndrome of endogenous intoxication develops, initially without any clinical signs. It accelerates the processes of oxidation of free radicals, accumulation of incompletely oxidized metabolism products, inhibits immunity and nonspecific protection factors, affects the functional status of organs and systems. Later pathological conditions-evolve which aggravate various chronic diseases, the acute disorders develop more pronounced symptoms of the endogenous intoxication syndrome, autoimmune processes expand, the enzyme spectrum becomes distorted [3, 4].

The problem of developing means of mass prevention of possible medical effects of radiation exposure becomes prominent as radionuclides affect the metabolism of substances.

One of the most effective ways of removing the toxic products from the organism is to detoxify them by sorption. Decorporation of radionuclides both reduces the level of internal exposure and the probability of various disorders of stochastic and nonstochastic nature.

Preparations adsorbing radionuclides in the gastrointestinal tract lumen are among the means stimulating elimination of radioactive isotopes and their excretion from the human organism.

The enterosorption method is based on the ideas about four mechanisms of reducing the systems of toxic substances and metabolites:

1. A possibility to revert toxic substances from blood into the intestine and their further binding by sorbents.
2. Purification of digestive juices of the gastrointestinal tract.
3. Modification of the lipid and amino acid spectrum in the intestine.
4. Expulsion of toxic substance originating in the intestine (15).

Enterosorbents are appealing practically and scientifically during endogenous incorporation of radionuclides by the organism (with food) both for their expulsion and reparation of the metabolic disorders.

The objective of the present study is to investigate the status of metabolism of lab animals during endogenous incorporation of ^{137}Cs and ^{90}Sr radionuclides with food components and performance of various enterosorbents. The experiment was conducted on 116 animals in two series. The first series included 63 male rats of the "Wistar" line weighing 290—410 grams; the second series included 53 female rats of the same line weighing 210—250 grams. Three groups of sorbents were tested:

- (1) organic silicon sorbent grade "OK-M";
- (2) sorbent containing 60% modified clay and 40% dextrin;
- (3) coal sorbent "KAY-2".

The test animals of the first series received daily with oats 373 Bq/kg of ^{137}Cs during three weeks and the second series during five weeks. Enterosorbents were introduced intraintestinally 8 days before decapitation in the amount 100 mg one hour before feeding. The metabolism was assessed based on the following indicators: activity of choline esterase (CHE), aspartate and alanine amino transferase (AST, ALT), concentration of creatinine (CREA), urea (BUN), uric acid (URIC), total protein (TP) and phosphates (PHOS).

A biochemical analyzer "Synchron" CX-4 of Beckman Co. was used for biochemical studies. In addition variations in the rate of accumulation of radionuclides as a factor of sex, hematological parameters were investigated, the myocardial, liver, lungs, intestine tissues were evaluated histologically.

The obtained data were analyzed statistically.

RESULTS AND DISCUSSION

The study has revealed that a number of indicators characterizing metabolism among male and female rats in control groups differ significantly. Specifically the concentration of urea and uric acid in the blood of female rats exceeds that in male rats with a much lesser concentration of protein (Fig. 8.1—8.5).

Introduction of sorbent No.1 increased urea concentration in the blood of female rats (Fig. 8.6, 8.7). Sorbent No.2 did not change the indicators, sorbent No. 3 induced a higher urea concentration (Fig. 8.6).

Among male rats sorbent No.1 increased the concentration of creatinine, total protein and phosphorus in the blood (Fig. 8.7—8.9), sorbent No. 3 induced a higher urea, creatinine, total protein, phosphorus concentration and activated ALT. Sorbent No. 2 did not produce any significant changes of the above indicators (Fig. 8.6—8.10).

Accumulation of radioactive elements by male rats in the test group amounted to 100.37 ± 7.94 Bq/kg or validly higher than 5.36 ± 1.88 ($p < 0.001$) in the control. It is peculiar that the organisms of female rats during a longer period of time accumulated much less radionuclides or 48.14 ± 4.99 Bq/kg (10.42 ± 2.72 in the control, $p < 0.001$).

Radioactive substances significantly altered the metabolism among rats compared with the control in respect to creatinine, total protein, phosphorus and choline esterase activity in the peripheral blood, urea concentration increased and the activity of aspartate amino transferase reduced among female rats (Fig. 8.1—8.5).

Comparing the changes of the metabolic indicators among male and female rats a significant increase of creatinine, total protein, aspartate amino transferase concentration and phosphates should be noted among males under the effect of radionuclides (Table 8.1).

Enterosorbent No. 1 did not change noticeably the concentration of radioactive substances among male rats (Fig.8.11), major metabolic indicators (Fig. 8.12—8.17) among female rats under similar conditions manifested valid reduction of radionuclides in the organism (Fig. 8.11) compared with the test group. The blood serum demonstrated a valid increase of aspartate amino transferase (Fig. 8.16).

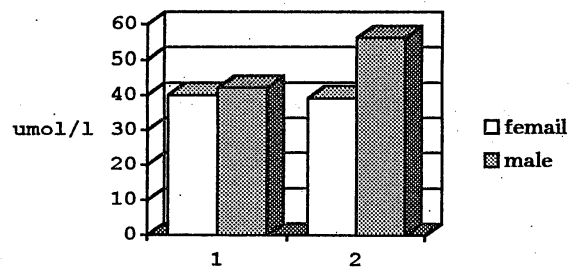


Fig. 8.1. Concentration of creatinin (CREA) in the peripheral blood of male and female rats of the "Wistar" line: 1 — control, 2 — test.

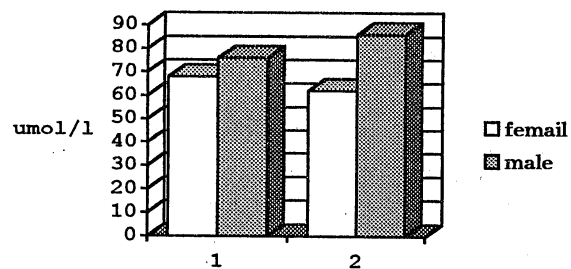


Fig. 8.2. Concentration of total protein (TP) in the peripheral blood of male and female rats of the "Wistar" line: 1 — control, 2 — test.

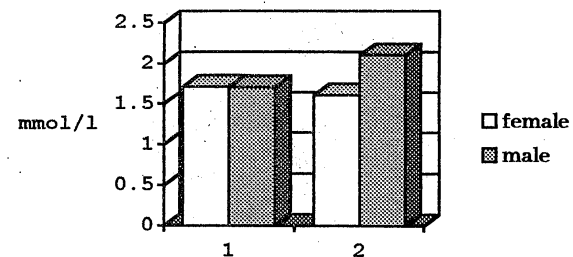


Fig. 8.3. Concentration of total phosphorus (PHOS) in the peripheral blood of male and female rats of the "Wistar" line: 1 — control, 2 — test.

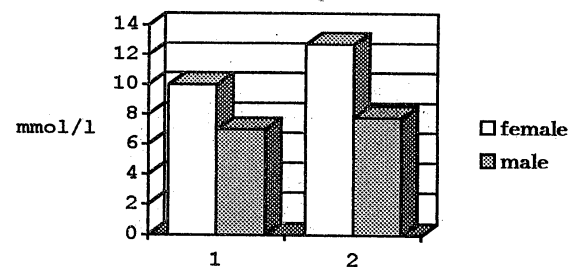


Fig. 8.4. Concentration of urea (BUN) in the peripheral blood of male and female rats of the "Wistar" line: 1 — control, 2 — test.

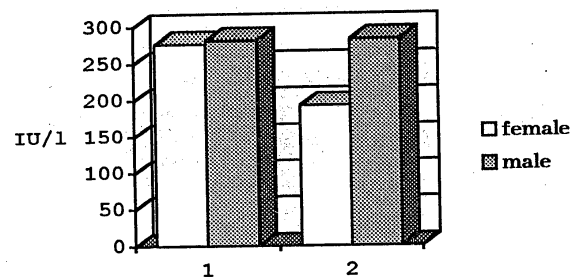


Fig. 8.5. Activity of aspartate amine transferase (AST) in the peripheral blood of male and female rats of the "Wistar" line: 1 — control, 2 — test

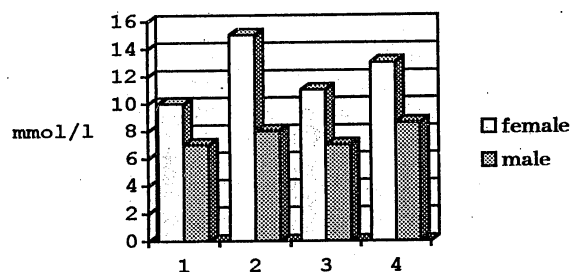


Fig. 8.6. Concentration of urea (BUN) in the peripheral blood of male and female rats of the "Wistar" line during introduction of enterosorbents: 1 — control, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

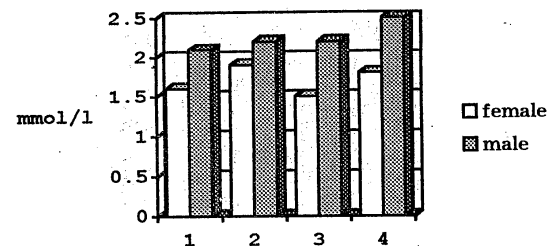


Fig. 8.7. Concentration of phosphorus (PHOS) in the peripheral blood of male and female rats of the "Wistar" line during introduction of enterosorbents. 1 — control, 2 — the first sorbent (control), 3 — the second sorbent (control), 4 — the third sorbent (control)

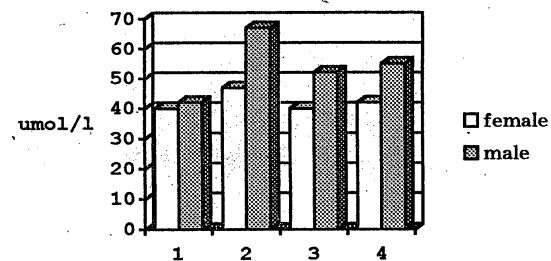


Fig. 8.8. Concentration of creatinin (CREA) in the peripheral blood of male and female rats of the "Wistar" line during introduction of enterosorbents: 1 — control, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

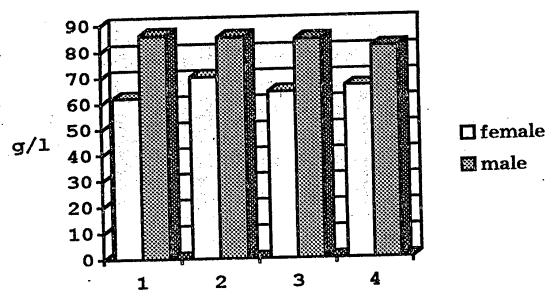


Fig. 8.9. Concentration of total protein (TP) in the peripheral blood of male and female rats of the "Wistar" line during introduction of enterosorbents: 1 — control, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

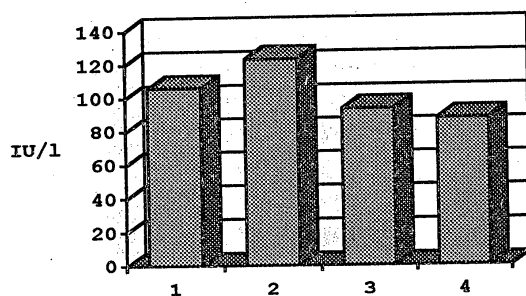


Fig. 8.10. Concentration of alanine aminotransferase (ALT) in the peripheral blood of male rats of the "Wistar" line during introduction of enterosorbents: 1 — control, 2 — the first sorbent (control), 3 — the second sorbent (control), 4 — the third sorbent (control)

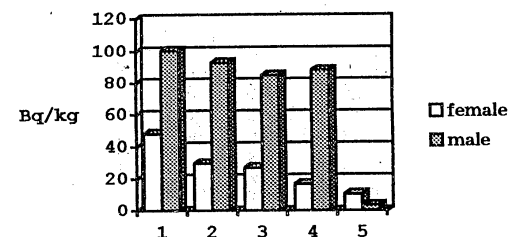


Fig. 8.11. Accumulation of radionuclides in the "Wistar" line rat organism under the effect of radionuclides: 1 — effect of radionuclides, 2 — effect of radionuclides and the first sorbent, 3 — effect of radionuclides and the second sorbent, 4 — effect of radionuclides and the third sorbent, 5 — control; $p < 0.05$ in respect to the control, $p < 0.001$ in respect to the control

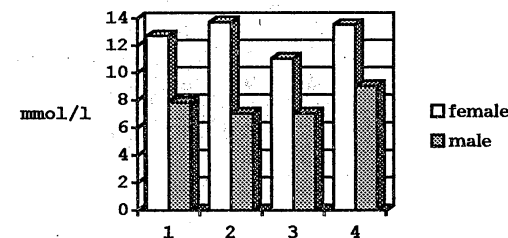


Fig. 8.12. Concentration of urea (BUN) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

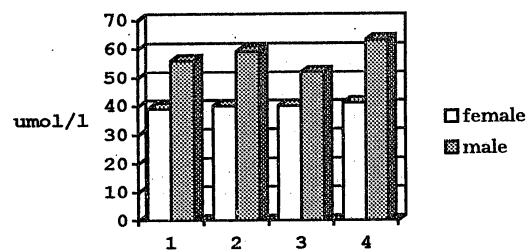


Fig. 8.13. Concentration of creatinin (CREA) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbents

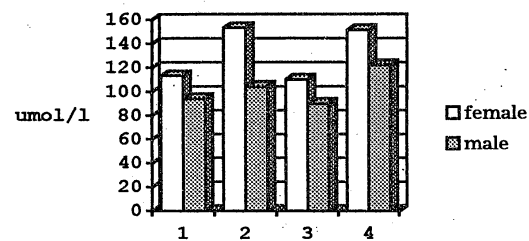


Fig. 8.14. Concentration of uric acid (URIC) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

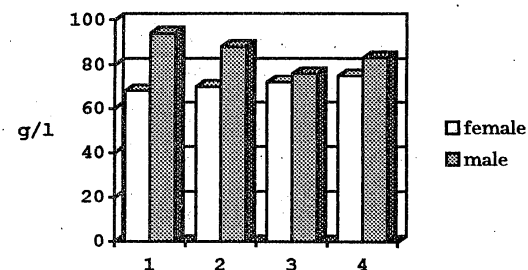


Fig. 8.15. Concentration of total protein (TP) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

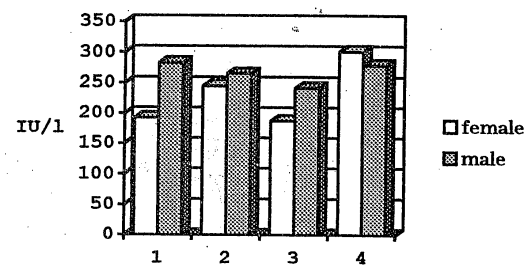


Fig. 8.16. Activity of aspartate amine transferase (AST) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

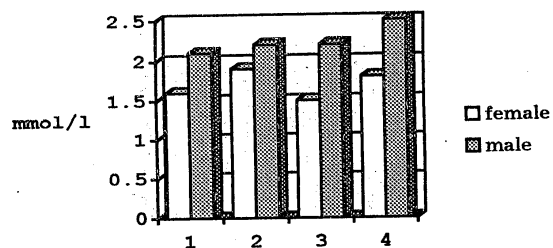


Fig. 8.17. Concentration of phosphorus (PHOS) in the peripheral blood of male and female rats of the "Wistar" line under the effect of radionuclides and introduction of enterosorbents: 1 — test, 2 — the first sorbent, 3 — the second sorbent, 4 — the third sorbent

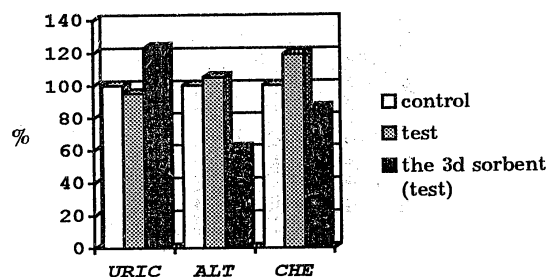


Fig. 8.18. Relative concentration of uric acid (URIC), alanine aminotransferase (ALT), choline transferase (CHE) in the peripheral blood of the "Wistar" line male rats under the effect of radionuclides and during introduction of the third enterosorbent

Table 8.1

Comparison of metabolic indicators in the organisms of male and female rats of the "Wistar" line under the effect of radionuclides

Indicators	sex	
	male	female
Creatinine (CREA, umol/l)	56,38±2,78	39,17±2,18
Phosphorus (Phos, umol/l)	2,15±0,10	1,62±0,22
Total protein (TP, g/l)	86,75±1,69	62,50±6,80
Aspartate amino transferase (AST, IU/l)	283,00±14,35	197,00±36,25

Note: valid if $p < 0.05$

Enterosorbent No. 2 did not produce any valid changes either in the concentration of radionuclides compared with the test group (Fig. 11), yet a valid reduction of urea concentration and the activity of aspartate amino transferase and choline esterase in the blood was noted (Fig. 8.12—8.16).

Females under similar experimental conditions demonstrated reduction of concentration of radionuclides (Fig. 8.11), while the above metabolic indicators did not change.

Enterosorbent No. 3 did not reduce significantly the amount of incorporated radionuclides among male rats (Fig. 8.11), it induced a greater concentration of uric acid and reduced activity of alanine amino transferase and choline esterase (Fig. 8.18). Female rats demonstrated a pronounced reduction of radionuclides in the organism (Fig. 8.11) with the growing activity of aspartate amino transferase in the blood compared with the test group (Fig. 8.16).

Microscopic studies of histological preparations of kidneys of male and female lab animals revealed a pathological modification of

glomerules in the form of proliferation of mesangial cells, infiltration of loops, lymphoid histocytary elements, fragmentation, death of glomerules.

The latter manifested a granular and hyaline drop dystrophy of the epithelium of straight and twisted canals.

Administration of sorbents No. 1 and No. 3 to intact animals caused a number of destructive processes in the glomerule and canal apparatus in the form of disintegration of loops of some glomerules, granular dystrophy and necrotic nodes on the epithelium of canals.

The above pathological changes were the most pronounced in the kidneys of the animals which received these sorbents after dieting with the grain containing radionuclides. Infiltrates containing lymph histocytary, plasmatic cells and neutrophil leucocytes in various portions of kidneys, specifically in the region of accumulating tubules and the epithelium lining of renal pelvises.

Sorbent No. 2 did not produce any significant changes to be visible under the microscope among the intact animals nor did it aggravate pathological modifications caused by the grain with radionuclides, moreover the pathological process evolved in a smoother manner.

The study has indicated that accumulation of gamma-sources during enteral incorporation of radionuclides takes place differently between male and female animals. Male animals accumulate radioactive elements quicker. Both sexes manifest strong injuries of the kidney tissues together with a significant accumulation of protein metabolism products, specifically, urea, creatinine, phosphorus. Also, significant differences in the concentration of major indicators of metabolism in the blood of male and female animals should be remembered.

Sorbents No. 1 (the OK-M organic silicon sorbent) and No. 3 (KAY-2) would stimulate accumulation of urea, uric acid, creatinine, phosphorus, these processes among male rats were accompanied by a higher concentration of total protein in the blood.

Considering significant pathological changes in kidneys in the form of injury of the glomerule and canal apparatus it can be assumed with full justification that they cause the increasing concentration of nitrogen-containing products of the metabolism of protein and nucleic acids.

Thus, it can be concluded with full justification that the concentration 400 Bq/kg of radionuclides in the grain affects metabolism by in-

curing a severe injury of kidneys. Sorbents containing the organic silicon and coal produce a similar effect.

The sorbent containing 60% modified clay and 40% dextrin does not produce any side effect and is suitable for enterosorptional therapy considering its capability to eliminate radionuclides from the organism.

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CONCLUSIONS

The data in the present manuscript disclose the condition of a number of vital systems in response to accumulation of radioactive substances in the organism. Human organisms continuously incorporate radionuclides, primarily through alimentary tracts in the areas where the soil is contaminated. It is validated by experimental studies aimed at developing models of accumulation of radioactive elements with food by experimental animals (Yu. Bandazhevsky et al., 1993).

This study has resulted, from the necessity to examine the penetration of radioactive substances into the soil and their proliferation through biological chains (in human and animal organisms). The main idea of experimentation has been to utilize the same products widely consumed by humans. The concentration of ^{137}Cs in the majority of products just slightly exceeded permissible levels. For example, the ^{137}Cs concentration as a major radioactive element in grain flour is 370 Bq/kg (it equaled 373-446 Bq/kg in the experiments).

The ^{137}Cs and ^{90}Sr isotopes prevailing in the environment are absorbed by organic substances consumed by humans and animals modifying the structure and functions of organs and systems. Significantly smaller quantities of these substances cause stronger pathological effects compared with their artificial introduction.

The study has revealed that organisms of experimental animals accumulate relatively quickly radioactive elements with food (the peak accumulation is reached after 10-30 days on the average). This process depends upon a number of factors, sex, age, concentration of radioactive elements in food and duration of their incorporation, the organism functional condition being the major ones. Specifically, females accumulate ^{137}Cs in lesser quantities than males. Pregnancy stimulates the process.

A similar extent of ^{137}Cs accumulation by experimental animals (white rats) and human organisms should be emphasized.

Thus, development of the experimental model of incorporation and accumulation of radioactive substances by organisms of laboratory animals allows to extrapolate the obtained data upon humans adequately to the incorporated dose. Therefore, it was the amount of radionuclides in the organism which served to evaluate the vitality condition.

Numerous clinical and experimental investigations have revealed the relationship between the evolution of pathological conditions and the dose of accumulated radionuclides. It is most pronounced in the cardiovascular and nervous systems. Disorders of metabolic processes involved in energy cycles result in degenerative modifications of the myocardial cells. Remembering that the cardiac muscle has tropism in respect to ^{137}Cs , it can be assumed that it is capable to substitute K^+ ions in the electrolytic exchanges of the myocyte cellular membrane. It may be the cause of disorders of the electric pulse intracardiac conductivity. Similar processes may possibly occur in the nervous cell.

Disorders of enzyme systems under the effect of radioactive elements inhibit the phagocytic activity of macrophages and neutrophil leucocytes, differentiation of cells producing antibodies, as it is demonstrated by a lesser production of class A immunoglobulins.

Radioactive elements affect the female reproductive system causing disorders in the generation of sexual hormones by ovaries and it is undoubtedly influences the uterus morphofunctional condition. The extent to which these effects are pronounced is a factor of accumulation of radioactive elements by the organism.

The effect of radioactive substances upon the mother-fetus system results in a greater intrauterine death rate, primarily during the preimplantation stages of fetus development, in addition to disorders in the development of the skeletal system. It should be noted that ^{137}Cs almost does not penetrate into the fetus organism.

When excreted from the organism ^{137}Cs together with other radionuclides affects the kidney nephron causing a severe pathological process accompanied by the accumulation of protein metabolism product.

So, incorporation of radioactive elements by the organism results in a significant disorder of metabolism caused by the damage of energy-dependent mechanisms in the cells of various organs and tissues.

Such conditions have been experienced by a considerable proportion of the population in Belarus accumulating radionuclides primarily with food.

It is quite apparent that even slight doses of radioactive substance of the order of 50-80 Bq/kg of ^{137}Cs can cause significant pathological disorders both in human organisms and in the organisms of experimental animals. It is a natural question then how to remove these substances

from the organisms, what phenomena or mechanisms can be effective? Scrutinizing the data available so far, a number of substances can be useful for this purpose, like those exhibiting sorptional properties. The study has confirmed the fact. Still, it should be confirmed that these preparations are harmless for the organism and it is necessary to determine the terms of their application.

It is without doubt that evaluation of metabolism conditions in the organism based on the laboratory criteria, as well as the amount of accumulated radioactive elements should be assigned top priority in developing the strategy of health care for the people living in the contaminated areas. In a number of cases it will help prevent severe pathological processes with lethal outcomes.

LIST OF ABBREVIATIONS

CA	Cystic acid
TAU	Taurine
ASP	Aspartate
THRE	Threonine
SER	Serin
GLU	Glutamate
GLN	Glutamine
GLY	Glycine
ALA	Alanine
VAL	Valine
MET	Methionine
ILE	iso-Leucine
LEU	Leucine
THYR	Thyrosine
PHE	Phenylalanine
ORN	Ornithine
LYS	Lysin
HIS	Histidine
3-metHIS	3-methyl Histidine
PRO	Proline
EA	Ethanol amine
AAdA	Amine adynilic acid
iso-AOA	iso-Aminobutyric acid
AOA	Aminobutyric acid
AA	Aromatic amino acid
AST	Aspartate amino transpherase
MAO	Mono amine oxidase
LDG	Lactate dehydrogenase
PHEA	Phosphorus ethanol amine

SU/NSU	Substitutable/ non substitutable
ARCC	Acids with ramified carbon chains
ALT	Alanine amine transpherase
AcPH	Acid phosphotase
APH	Alkaline phosphotase
CPK	Creatine phosphokinase
T ₃	Triiodine thyronin
T ₄	Thyroxin
TG	Thireoglobulin
EFA	Esterified fatty acids
γ-GTP	γ- glutamate transpeptidase

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