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INTRODUCTION

Discoverers of radioactivity as W. C. Röntgen, H. Becquerel, M. Sklodowská-Curie, P. Curie, resp. their followers surely unsuspected what energy of ionising radiation may cause – whether for military or pacificatory purposes. In the history of mankind the real experiment with this energy in praxis in its larger sense was realized more than 20 years ago.

One o'clock in the morning in 26th April 1986, the operators of the fourth and newest atomic pile of electric power station Chernobyl finished only the first day of a special test. In this test they wanted to recognize whether residual (supplementary) energy of rotating turbine may provide sufficient power in case of emergency gate and loss of external energy. For this purpose security systems were unplugged, mode of operation was counteracted to perform this test.

From available data follow that within 4.5 seconds power potention of reactor increased 2 000 times up to its 120 fold of projected capacity. This reality can be simply described as "slow nuclear explosion". One tonne thousand cover above reactor overrode and "rare fireworks" could be seen, hot fuel and graphite rushed to night sky.

In 1979 in Three Mile Island a nucleus of reactor melt partly and contaminated power station, cover of reactor remained and only small part of dangerous materials was relased into environment in Pennsylvania. But the Ukrainians had not this luck. For the first time in history a lethal radioactive content of huge reactor got into atmosphere. Local representatives had not suspicion or vision about magnitude of beginning tragedy. Thus another reactor next to damaged one was closed out only 16 hours after accident and other two reactors were operated 24 hours longer. In the fight with fire and radioactive material helicopters threw down 40 tonnes of boron carbide, 800 tonnes of limestone, 2 400 tonnes of lead and thousands of sand and clay on the reactor. This effort was only partly successful. Despite of certain weather assistance, Ukraine, but many other states, suffered health and ecological catastrophe. Scientists estimated that about 3 or 4 percentages of radioactive isotopes from reactor got to the environment that represented about 7 000 kilogrammes of material containing from 100 to 120 curie of radioactive substances, i.e. 1 000 fold of the amount released during accident in the U.S.A. This material contained more than 50 radioactive isotopes with half-time from two hours to 24 000 years and with various biological activity.

Chernobyl cloud left extremly complex pattern of radioactive fall-out that may be never exactly understood. Chernobyl tragedy as a warning indicator showed and reminded mankind of risk hidden in nuclear energy, even though its pacificatory use. It showed that it is necessary to be prepared to rescue human lives, animals, whole biocoenosis from harmful effects of ionising radiation and in a broad sense to understand behaviour of single radionuclides in biosphere. It showed visible effects what would happen after exposure to invisible – radioactive irradiation.

In former Czechoslovakia an attention has been paid to the questions of the effects of radiation on organism from the 1950s. After discharge of Commonwealth the Radiobiological Association, that facilitated interchange of the results of research work among various national and foreign institutions, was also abolished.

After establishment of the autonomous Slovak Republic UVM in Košice in collaboration with UPJŠ in Košice linked to tradition of radiobiology and organized the 1st Radiobiological Conference in Slovakia. At this conference 37 lectures of home and 9 of Czech authors were presented. At the end of conference the agreement was made to held this conference every second year. The 2nd Radiobiological Conference was held in 2004 at the occasion of the 55st anniversary of UVL establishment in Košice.

The 3rd Conference held on 25th May 2006 in UVL in Košice was inscribed to the 20th anniversary of Chernobyl disaster. Selected articles dealing with this problem create contents of Supplementum inscribed to the 50th anniversary of the scientific journal *Folia Veterinaria.*

Prof. RNDr. Michal Toropila, CSc.
Scientific guarantee of conference

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THE EFFECT OF IONIZING RADIATION ON THE COLOUR AND THE ACTIVITY OF LACTATE DEHYGROGENASE IN PORK

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ABSTRACT

The effect of the irradiation of pork was studied in relation to colour changes. Samples of *m. longissimus lumborum et thoracis* were obtained from pork carcasses 24 hours *post mortem*. Samples were irradiated using a ^{60}Co source, at a dose of 2.5 and 5 kGy; a dose rate of 2.86 kGy.h^{-1}. Non-irradiated controls were stored in the same condition as irradiated samples. The colour of a freshly cut interior surface of control and irradiated pork was measured (portable spectrophotometer Superchroma S-Spex in CIELAB system) before and after irradiation. L* and b* values of controls and irradiated pork did not change after irradiation. The a* values (red colour) of irradiated pork were significantly higher than non-irradiated pork. The activity of lactate dehydrogenase of pork was measured and did not change after irradiation.

Key words: enzyme; gamma radiation; meat; pig

INTRODUCTION

The technology of food irradiation is most widespread in the U.S.A. where there is an effort to enforce irradiation not only for health but also for technological purposes, for example, to improve the colour of meat products (2).

Even if meat irradiation is a promising technology, on the other hand the treatment brings about physical-chemical and biochemical changes (3), which can damage nutritional value and the sensory properties of irradiated meat (5). Colour belongs among the most significant sensory and quality factors, which are important for the customer purchasing meat (4). No changes in colour have been observed in the exposed meat during an exposure to 5 kGy (7). Similarly, N a n k e (8) has described that after the meat exposure to doses up to 5 kGy certain changes in particular colour parameters are observed, but the resultant meat colour is not affected.

The other sensory and temperature changes are not noticeable for a low-dose exposure of meat (up to 5 kGy), but for the doses higher than 5 kGy the temperature of the exposed meat is increased and sensory changes can be observed, for example, an undesirable smell or turning brown (1). Enzyme-catalyzed changes are one of the main causes of the dissociative processes after the exposure of fresh meat (6). In addition to meat colour, the activity of lactate dehygrogenase, which has been monitored in this paper belongs to the indicators of ripening.

MATERIAL AND METHODS

Fifteen samples were taken from pork meat *(m. longissimus lumborum et thoracis*). The samples were taken within 24 hours *post mortem* and then were cut and divided into three groups: 1. the test group was exposed to 2.5 kGy, 2. the test group was exposed to 5 kGy, both at a dose rate of 2.86 kGy.h-^{1}. ^{60}Co was used as the ionizing radiation source. The colour was measured before and after irradiation in the CIELAB system by means of the Superchroma S-Spex spectrophotometer.

The samples in the control group were not irradiated and were measured at the same time as the test samples. Colour was measured on fresh slices of muscle, always on five points of the slice and the results were averaged from five measurements. Data were statistically processed by the paired *t*-test. The samples for the determination of lactate dehygrogenase were taken, divided and exposed to 2.5 and 5 kGy as well as samples for colour measurement. The enzyme activity of lactate dehygrogenase was determined in tissue homogenates by means of the commercial BIO-LA-TEST set produced by Lachema (CzR).

RESULTS AND DISCUSSION

As seen from Tab. 1, a statistically significant difference between the exposed groups (2.5 a 5 kGy) and the control group was not found for the L^* and b^* parameters while the a^* parameter, which represents the change in the red wavelength range, manifested a statistically significant difference between the test and the control groups. For the exposed samples (2.5 kGy and 5 kGy) the a^* parameter values were higher compared to non-exposed samples ($\alpha < 0.01$). The intensive red colour of the exposed meat samples was already evident during the visual inspection. Similarly, N a n k e (8) has stated that the L^* values after exposure to the dose of 4.5 kGy do not change. He has also stated that the a^* parameter due to the exposure of the dose of 1.5 and 3.0 kGy is reduced ($\alpha < 0.01$) and the a^* parameter, due to a dose of 4.5 kGy, is increased while these values were lower than for the non-exposed control group. As with the values of the b^* parameter, N a n k e has stated that for the dose of 1.5 and 3.0 kGy the values were reduced and for a dose above 4.5 kGy the values were again increased.

Table 1. The relationship of the parameters of pork meat colour *versus* the exposure to a dose of 2.5 kGy (exposure time of 0.88 h) and 5 kGy (exposure time of 1.75 h), at a dose rate of 2.86 kGy.h^{-1}

COLOUR PARAMETER			L^*	a^*	b^*
0 kGy	before exposure	$\bar{x}$	52.58	0.95	7.42
		$s_{\bar{x}}$	1.258	0.289	0.360
	after exposure	$\bar{x}$	52.28	1.13	7.48
		$s_{\bar{x}}$	1.093	0.349	0.353
2.5 kGy	before exposure	$\bar{x}$	51.60	0.80	6.92
		$s_{\bar{x}}$	1.320	0.253	0.400
	after exposure	$\bar{x}$	52.44	2.74^{++}	7.00
		$s_{\bar{x}}$	1.363	0.221	0.289
5 kGy	before exposure	$\bar{x}$	51.90	0.75	7.11
		$s_{\bar{x}}$	1.161	0.248	0.298
	after exposure	$\bar{x}$	51.74	3.05^{++}	7.17
		$s_{\bar{x}}$	1.394	0.449	0.413

$\bar{x}$ — arithmetic mean, $s_{\bar{x}}$ — standard deviation of the mean

On the other hand L u c h s i n g e r (7) has stated that the a^* parameter values do not changed after exposure. A remarkable difference in the a^* parameter values is seen in the Tab. 1 for the control non-exposed group where the meat colour was measured in the same time as the test groups before irradiation and after radiation. However, this difference is not statistically significant and it is brought about by the dynamics of meat ripening.

In references, inconsistent data have been found in connection with the problems of enzymes of exposed food. It was stated previously that through irradiation the meat can be kept fresh due to the enzyme inhibition which affects cellular integrity. However, the enzymes of Ca^{2+} dependent protease and D katepsine during exposure of the meat to doses of 10 kGy have a certain resistance grade (6). During the monitoring of lactate dehydrogenase it was found that its content in tissue does not change after exposure of 2.5 kGy and 5 kGy (Tab. 2).

Table 2. The activity of lactate dehygrogenase in pork muscles after exposure to a dose of 2.5 kGy (exposure time of 0.88 h) and 5 kGy (exposure time of 1.75 h), at a dose rate of 2.86 kGy.h^{-1}

	Lactate dehygrogenase [µkat.g^{-1}] 0 kGy	2.5 kGy	5 kGy
$\bar{x}$	805	795	793
$s_{\bar{x}}$	148	254	186

$\bar{x}$ — arithmetic mean, $s_{\bar{x}}$ — standard deviation of the mean

As a total activity lactate dehydrogenase is given by the sum of activities of particular isoenzymes (9) and as the tissue isoenzyme pattern of tissue is changed in consequence of the external effects (10), we believe that irradiation has no significant influence on isoenzymes of muscle lactate dehydrogenase. Its high resistance to irradiation is important, because lactate dehydrogenase is a key enzyme in the ripening of the meat. From this point, it would be worth verifying the effect of ionizing radiation on isoenzymes of other organs and other species.

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*A Contribution on **The Third Radiobiologic Conference** held at the University of Veterinary Medicine Košice on May 25, 2006 on occasion of 20 years anniversary of the tragic accident in Chernobyl (Ukraine)*

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THE 30th AND 29th ANNIVERSARIES OF THE REACTOR ACCIDENTS IN THE A-1 NUCLEAR POWER PLANT AT JASLOVSKÉ BOHUNICE

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SUMMARY

The facts about the reactor accidents in the A-1 Nuclear Power Plant at Jaslovské Bohunice, Slovakia are presented. There was a reactor KS150 (HWGCR) cooled with carbon dioxide and moderated with heavy water. A-1 NPP was commissioned on December 25, 1972. The first reactor accident happened on January 5, 1976 during fuel loading. The second serious accident in A-1 NPP occurred on February 22, 1977 also during fuel loading. This INES level 4 of reactor accident resulted in damaged fuel integrity with extensive corrosion damage of the fuel cladding and release of radioactivity into the plant area. The A-1 NPP was consecutively shut down and is being decommissioned at the present time. Both reactor accidents are described briefly.

Key words: Bohunice A-1 reactor; INES; reactor accident; reactor decommissioning; surface contamination

INTRODUCTION

In this year we are commemorate the thirtieth anniversary of the first incident and the twentieth-ninth anniversary of the second accident in the nuclear reactor KS 150 in A-1 NPP. The prototype nuclear power plant A-l located at Jaslovské Bohunice was a HWGCR with channel type reactor KS 150 with natural uranium (refuelling during operation) and a capacity of 143 MWe. The steam rising from the primary circuit of the reactor with a temperature of 410 °C moved ahead into six modules of steam generators and then into the turbines with generators. Fuel change was realised during the operation of the reactor. The construction of the NPP started in 1958, it was commissioned on December 25, 1972. This NPP produced 916.1 MWh of electric energy during quadrennial operation (9).

THE FIRST INCIDENT ON A-1 NPP JASLOVSKÉ BOHUNICE

Thirty years ago the first incident (the failure of the closing mechanism of the technological channel) happened on January 5, 1976 (Nuclear Regulatory Authority of the Slovak Republic (6)). Fresh fuel assembly (together with the technological plug) ejected into the reactor hall. Coolant (carbon dioxide) flowed out from the reactor for a short time until the refuelling machine was reconnected to the open technological channel and stopped the coolant leakage. Immediate personnel were not irradiated. Two people out of the hall, who did not respond to the warning system, were suffocated by carbon dioxide. No radioactive substances escaped into the atmosphere. The public was not informed about the progress of the incident, while the population was not endangered. This incident has been described in detail in F r i š o v á' s paper (3). This incident to the reactor A-1 NPP has not been evaluated according to the INES scale up to the present time (INES, The International Nuclear Event Scale User's Manual (4)). In our opinion the incident to the reactor A-1 of 1976 year should be classified at least at the third level of the INES scale. The reactor was consecutively deplanned, shut down and reconstructed by the end of 1976.

ACCIDENT ON A-1 NPP JASLOVSKÉ BOHUNICE

During refuelling, the insufficiently transmissive fuel assembly was charged into the reactor core on February 22, 1977. Local overheating of the fuel, the technological channel and heavy water circuit tube caused a loss of barrier integrity between the heavy water moderator and fuel with cooling gas. Cladding and steam generator tube corrosion under water saturated by carbon dioxide occurred and resulted in a contamination of the primary and secondary circuit (2). In 1979 a final decision was made to decommission this plant. There were 439 from a total of 571 spent fuel assemblies transported to the former Soviet Union from 1984 to 1990. 132 damaged fuel assemblies were sent to PA Mayak (Russia) in 1999 (6).

In 1991 the International Atomic Energy Agency introduced the INES scale (The International Nuclear Event Scale (10)) with 7 levels. Subsequently, the second accident on A-1 NPP was classified as an accident at the fourth level of the INES scale. During the accident it was alleged that no leakages of radioactivity into the environment occurred (Nuclear Regulatory Authority of the Slovak Republic – (6)).

Abnormal rainfall on A-1 NPP site and insufficient countermeasures against flooding led to a flooding of rooms in the plant-controlled area in June 1978. A huge amount of contaminated water was produced. The contaminated water subsequently was released into the reservoir of the Dudvah River and then into the Vah River. Notwithstanding the increased radioactivity of the effluents, no immediately countermeasures for the mitigation of consequences were taken. Water from these rivers is used for the irrigation of fields (8).

After the second accident the Government of the CSSR decided on the decommissioning of A-1 NPP with the resolution No. 135 of May 17, 1979. The actual decommissioning started in 1995. Around eight billion Slovak crowns (~ 267 million $) were spent on the decommissioning of A-1 NPP from 1995 to the end of 2005 (1). It is necessary to add also the finances spent up to 1995 to the expenses for the liquidation of A-1 NPP.

Some papers have been published about the radiation consequences of the accident to A-1 NPP and about the monitoring of the environment. Their short review was published in the *Proceedings of the Third Radiobiological Conference* (5).

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THE REDUCTION OF THE ACTIVITY CONCENTRATION OF RADIOCESIUM IN MEAT BY HEAT-PRESSURE BOILING

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ABSTRACT

Boar meat (*Sus scrofa*) was salted (5 g NaCl to 100 g meat) before preparation by heat pressure boiling – fifteen minutes. A reduction in activity concentration of radiocesium of about 50 % (from 42.7 % to 58.3 %) was achieved. The meat had an activity concentration of radiocesium 106 Bq.kg^{-1}. An activity concentration of radiocesium was reduced on average to 53 Bq.kg^{-1}. An activity concentration of ^{137}Cs was measured by gamma-spectrometry.

Key words: 137Cs; food safety; pig meat; *Sus scrofa*

INTRODUCTION

In the last century, radionucleides were widely distributed in natural systems either as the by-products of nuclear tests or as the result of nuclear accidents. One of the most important radionucleides surviving in the environment is cesium (^{137}Cs) with a half-life of 30.2 years and having similar chemical properties to potassium.

The committed dose of radionucleides from plant and animal food can be reduced by different countermeasures, which are based on the elimination of contaminated food (or fodder) in the food chain if a sufficient amount of non-contaminated food (fodder) is available. However, preparation and decontamination itself are time-demanding and complex from a technical and economic viewpoint and furthermore the subsequent disposal of contaminated waste is difficult. Hence, the chemical and physical properties of specific radionucleides as well as the food composition, knowledge of physiology and biological processes are considered in the alimentary human/animal tract in an effort to reduce the committed dose.

Different complex or chelate additives with an absorptive ability to bond radionucleides and form the chemicals are successfully applied, i.e. additives, which are not absorbed in the alimentary tract but more easily secreted from the organism. Radiocesium forms the most effective bond with the compounds of a hexacyanferrate type, for example, with Prussian blue whose ammonium salts are widely used (1, 2).

Good results have been achieved during the reduction of radiocesium activity concentration when additives of clay minerals were applied to fodder (3, 5).

In the case of radiocesium contamination of free-living animals protective prevention cannot be applied as with livestock. The only solution is to check each captured animal and in the case of increased cesium contamination the game shall be post-treated if the radiocesium level exceeds the international level for post-Chernobyl contamination (600 Bq.kg^{-1}) laid down in Regulation No. 307/2002 Sb. in the Czech Republic.

The possibilities of radiocesium reduction in meat have been studied sporadically so far. However, heat treatment of meat by boiling in salt water (1 % solution) can reduce the radiocesium activity by 80 % (4).

MATERIAL AND METHODS

Refrozen meat from the wild-boar rump (*Sus scrofa*), in fact a piglet with a weight of fifteen kilograms coming from Staré Ransko (near Havlíčkův Brod, the Czech Republic), was cut into the small cubes approximately 1.5 cm × 1.5 cm × 1.5 cm and put into a Marinelli beaker (450 ml). Before subsequent treatment radiocesium activity was determined. Then, the meat was dry-salted (five grams of sodium chloride per 100 grams of meat) and put into a pressure cooker with 400 ml of water. The water in the pressure cooker was boiled

Table 1. Activity concentration reduction in the meat after heat-pressure boiling. The activity concentration ^{137}Cs in meat was 106 Bq.kg^{-}1 before treatment

Item	^{137}Cs (Bq.kg^{-1}) Meat	^{137}Cs (Bq.kg^{-1}) Bouillon	^{40}K (Bq.kg^{-1}) Meat	^{40}K (Bq.kg^{-1}) Bouillon	^{137}Cs (%) Meat
1	43	57	84	82	58.3
2	59	69	102	95	42.7
3	67	90	147	129	34.9
4	55	66	122	107	46.6
5	43	63	105	90	58.3
$\bar{x}$	53	69	112	101	48.2
s	10.3	14.2	27.1	20.2	10.1
s_r (%)	19.33	20.57	24.19	20.09	20.89
$s_{\bar{x}}$	4.6	6.3	12.1	9.0	4.5

$\bar{x}$ — arithmetic mean, s — standard deviation, sr — relative deviation,
$s_{\bar{x}}$ — standard deviation of the mean

and after pressurization meat was treated for fifteen minutes in this way. After cooling the meat samples were separated from the bouillon. The meat samples and the bouillon were separately measured in the Marinelli beaker geometers on a gamma-spectrometric system.

Activity concentrations of ^{137}Cs and ^{40}K were determined with the Canberra gamma-spectrometry system which consists of a Desktop Inspector MCA and HPGe GC2020 semiconductor germanium detector (20 % efficiency and 1.8 keV resolution). The high-resolution gamma-spectrometry system was certificated by the Czech Metrological Institute, Certificate No. ČMI – Praha No.911 – OL – Z 2888b/2003. The measurement time was eighteen hours (T = 64 800 sec) in this geometer. The minimum detectable activity (MDA) reached 0.4 Bq.kg^{-1} for 137Cs in the Marinelli beaker. For spectra processing, Genie 2000 (Canberra) and Gamat (PK-Servis Praha) routines were used.

RESULTS AND DISCUSSION

The measurement results of radiocesium activity reduction in meat by heat pressure boiling are shown in Tab. 1. The average radiocesium activity concentration in the meat before treatment was 106 Bq.kg^{-1}. The average radiocesium activity concentration in the meat after heat pressure boiling was 53 Bq.kg^{-1} and the average radiocesium activity concentration in the bouillon was 69 Bq.kg^{-1}. This treatment reduced radiocesium activity concentration in the meat by 50 % on average.

As mentioned before activity concentration was reduced on average by 50 % by the heat-pressure treatment of meat in our work. On the other hand, activity concentration in the meat by boiling in the salt solution was reduced by 80 % (see: No. 4) if the salt solution is replaced repeatedly. Even the different kinds of meat used by the author could not remarkably change the activity concentration in such a way. It is likely that a single radiocesium application could have a definite significance in this experiment.

CONCLUSION

The reduction of the radiocesium activity concentration by 50 % is highly significant and hence this method of meat treatment is very suitable providing the bouillon after treatment is not consumed. This method is not applicable for soup cooking as well as for other culinary specialties.

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THE INFLUENCE OF IONIZING RADIATION ON THE ENZYMATIC METABOLISM AND TRIACYLGLYCEROL SERUM LEVEL IN IRRADIATED CHICKENS

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SUMMARY

Investigation of the influence of ionizing radiation in experimental animals helps to develop methods of protection against its undesirable effects. The study investigated the influence of low doses of ionizing radiation on the organism of the domestic fowl. We observed changes in the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triacylglycerols (TG) in time intervals of 1, 3, 14 and 25 days after a single, whole body irradiation with gamma rays at a dose of 3 Gy. The activity of AST differed significantly on day 1 post-irradiation and the concentration of TG was increased significantly on days 14 and 25 post-irradiation, in comparison with the control.

Key words: amino transferase; chickens; gamma radiation; triacylglycerols

INTRODUCTION

All live organisms are constantly exposed to ionizing radiation. Alterations in biochemical parameters allow us to observe metabolic changes after irradiation and, eventually, to estimate the degree of damage to some organs.

MATERIAL AND METHODS

The experiment was carried out on 60 broiler chickens, 28 days old at the beginning of the experiment.

From their first day the chickens were kept in an experimental facility which was disinfected prior to stocking (4). They were supplied water and feed *ad libitum*.

The chickens were irradiated at PF UPJŠ in Košice using an apparatus CHISOSTAT. Irradiation was carried out in plastic cages.

In our experiment the chickens were exposed to gamma rays using a single, whole body dose of 3.0 Gy at input power 0.079558 Gy.min^{-1}. After 1, 3, 14 and 25 days, analyses were carried out. The animals were decapitated (2), mixed blood was collected in Petri dishes placed on ice and then centrifuged to obtain blood serum for analyses. The activity of ALT, AST and concentration of TG was determined using Bio-La test kits (Lachema-Brno, CzR).

Results obtained in the experimental and control groups at different intervals after irradiation were evaluated statistically by a non-paired *t*-test. Five to ten birds from individual groups were evaluated on average. The study was conducted in autumn.

Table 1. Changes in the activity of enzymes and concentration of triacylglycerols after a single, whole body irradiation with gamma rays at a dose of 3 Gy

Time	ALT ($\mu kat.l^{-1}$)		AST ($\mu kat.l^{-1}$)		TG ($mmol.l^{-1}$)	
	control	irradiated	control	irradiated	control	irradiated
Day 1	0.1004 ± 0.01921	0.348 ± 0.008442	0.686 ± 0.08710	**0.869 ± 0.03535***	3.364 ± 0.3705	2.93 ± 0.2852
Day 2	0.1124 ± 0.01497	0.1319 ± 0.006614	0.886 ± 0.03669	0.914 ± 0.02945	1.606 ± 0.1321	1.567 ± 0.08927
Day 14	0.1552 ± 0.01972	0.1191 ± 0.01416	1.29 ± 0.07404	1.395 ± 0.08891	1.019 ± 0.1325	**1.579 ± 0.06635*****
Day 25	0.1232 ± 0.01866	0.1461 ± 0.01488	1.237 ± 0.04587	1.288 ± 0.04522	0.7475 ± 0.08365	**1.327 ± 0.1153****

Values are presented as arithmetic mean ± S.E.M.
Significance of differences between irradiated and control group: * — $p < 0.05$ (Bold numerals)

RESULTS AND DISCUSSION

The activity of ALT increased on days 1 and 3 after irradiation and decreased on day 14 post-irradiation in comparison with the control. The differences were insignificant. The activity of AST increased significantly on day 1 and insignificantly on days 3 and 14 post-irradiation in comparison with the control. The concentration of TG in blood serum increased insignificantly on days 1 and 3 and significantly on days 14 and 25 post-irradiation in comparison with the control (Table 1).

The irradiation of organisms with sub-lethal and lethal doses of ionizing radiation increases transaminase activity. The increase in the activity of the so-called adaptive enzymes points to predominantly katabolic processes in the irradiated body related not only to changes in cell permeability and the development of serious histological changes in hepatocytes soon after irradiation but also to the stimulation of the synthesis of *de novo* transamination enzymes induced by an increased secretion of the adrenal cortex after irradiation (3).

A typical picture of animal organisms after exposure to ionizing radiation consists of marked changes in the concentration of serum lipids, which include glycerols, lipoproteids, phospholipids and cholesterol (7). With regard to the effect of radiation in the later period, we could call the respective changes unspecific. It is due to enhanced lipolysis and lipomobilisation, which lead to an increased supply of non-esterified fatty acids to various tissues. Besides this, the increasing level of cholesterol in the liver may be the consequence of its increased concentration in the blood serum (5, 6, 1).

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THE ELIMINATION OF THE NEGATIVE EFFECTS OF CADMIUM IN *Artemia franciscana* BY EXPOSURE TO IONIZING RADIATION

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ABSTRACT

Nuclear reactor failures present a risk of global contamination, which can be affected by other environmental factors, such as chemical elements. The present study investigated the effect of low doses of gamma radiation in relation to the presence of low levels of Cd. We used an alternative biotest of IInd generation on *Artemia franciscana*. Interaction between irradiation and the action of cadmium chloride could result in a protective effect of ionizing radiation at some concentrations of cadmium chloride.

Key words: *Artemia franciscana*; cadmium; *hormesis*; ionizing radiation

INTRODUCTION

Industrialisation of animal production results in an increased load on the ecosystem and considerable economic problems. With an increasing environmental load, the interest in the consequences of the action of xenobiotics, including harmful chemical elements, on living systems increases with regard to both the general whole-body response and individual organ systems.

The effects of higher doses of ionizing radiation, which are harmful to organisms have been described by a number of authors. On the other hand, low doses can have also positive effects (what is known as *radiation hormesis*) (7).

The present policy is to decrease the number of experiments on animals to a minimum. Because of that our investigations were carried out by means of an alternative biotest of IInd generation on *Artemia franciscana* (4).

MATERIAL AND METHODS

The experiment was carried out on *Artemia franciscana*, hatched in sea-water (6). Ten freshly hatched nauplii stages were placed into sea-water in polystyrene Petri dishes, 60 mm in diameter on average (the total volume of liquid with sample was 10 ml). Cadmium chloride ($CdCl_2.2H_2O$) solutions of concentrations 5, 10, 25 and 50 mg.l^{-1} in sea-water were prepared.

The nauplii stages were irradiated with gamma rays at a dose of 25 Gy (^{60}Co-source, CHISOSTAT) with a dose input of 11.36 Gy.min^{-1}.

Nine experimental and one control group were formed. The Petri dishes were positioned with a thermostat set to 20 ± 1 °C.

At the time intervals of 24, 48, 72, 96 and 120 hours we counted all live *Artemia*. Results obtained in experimental groups were compared with the control group. We also compared pairs of groups, each one consisting of a group given a certain concentration of cadmium chloride and another exposed to cadmium chloride and gamma radiation. The results were evaluated statistically. The remote values were eliminated by means of the Dean-Dixon test (5). The significance of differences between the groups were tested by the method described by Wayland and Hayes (9).

RESULTS

Figure 1 and Table 1 show differences between the investigated groups.

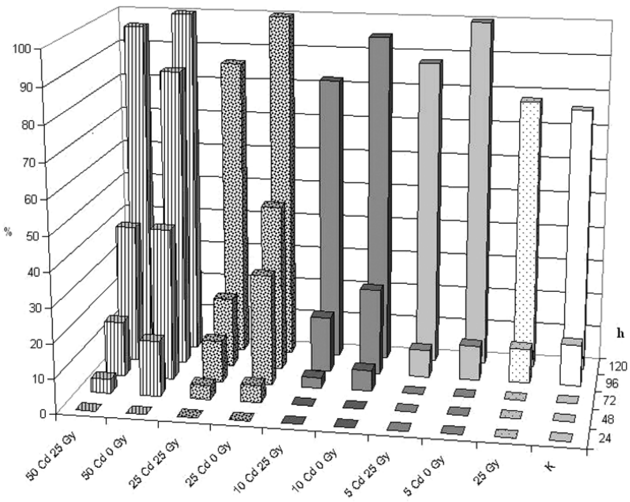

Fig. 1. Fatalities resulting from interaction of cadmium chloride and gamma radiation

When comparing the groups exposed to cadmium chloride and those exposed to the same level of cadmium chloride but also irradiated with gamma rays at a dose of 25 Gy we observed a decreased fatality in the irradiated groups.

The group exposed to 25 mg.l^{-1} cadmium chloride and irradiation, showed a significantly decreased fatality 72 and 96 hours after the beginning of the experiment in comparison with the non-irradiated group.

When the level of cadmium chloride reached 50 mg.l^{-1}, irradiation resulted in a significant decrease in fatality after 48, 72 and 96 hours of the experiment in comparison with the non-irradiated group.

DISCUSSION

The decrease in fatality after irradiation can be explained by *hormesis*, i.e. the protective action of low doses of ionising radiation on the action of other toxic factors. In medicine and biology *hormesis* is most frequently mentioned in connection with the action of antibiotics and other pharmaceutics, heavy metals, selenium, hormones, vitamins, some minerals and chemicals (2, 3).

Many authors speak about *hormesis* in connection with the action of ionizing radiation. Favourable effects of low doses of ionising radiation have been described and discussed in relation to the growth and development of organisms, reproduction, immune system but *hormesis* has been most frequently considered in connection with the development of malignant tumours (1, 8).

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Table 1. Fatalities resulting from interaction of cadmium chloride and gamma radiation (h)

CROUPS	24			48			72			96			120		
	x	n	s	x	n	s	x	n	s	x	n	s	x	n	s
control	**0**	5	0.0	**0**	5	0.0	**0**	5	0.0	**10**	5	8.6	**76**	5	17,2
25 Gy	**0**	5	0.0	**0**	5	0.0	**0**	5	0.0	**10**	5	8,6	**78**	5	17,2
5 Cd 0 Gy	**0**	5	0.0	**0**	5	0.0	**0**	5	0.0	**10**	5	12.9	**100**	4	0.0
5 Cd 25 Gy	**0**	5	0.0	**0**	5	0.0	**0**	5	0.0	**8**	5	8,6	**88**	5	12.9
10 Cd 0 Gy	**0**	5	0.0	0	5	0.0	**6**	5	8.6	**25**	4	4.9	**95**	4	4.9
10 Cd 25 Gy	**0**	5	0.0	**0**	5	0.0	**3**	4	0.0	**16**	5	8.6	**82**	5	12.9
25 Cd 0 Gy	**0**	5	0.0	**5**	4	4.9	**32^{*}**	5	21.5	**48^{*}**	5	21.5	**100**	4	0.0
25 Cd 25 Gy	**0**	5	0.0	**4**	5	4.3	**12^{*}**	5	12.9	**20^{*}**	5	8.6	**86**	5	12.9
50 Cd 0 Gy	**0**	5	0.0	**16^{+}**	5	8.6	**44^{+}**	5	17.2	**86^{+}**	5	12.9	**100**	5	0.0
50 Cd 25 Gy	**0**	5	0.0	**4^{+}**	5	4.3	**16^{+}**	5	8.6	**40^{+}**	5	8.6	**96**	5	4.3

$^{+\,*}$ — the difference between the values was significant (α = 0.05)

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THE HEALTH AND ENVIRONMENTAL EFFECTS OF USING DEPLETED URANIUM

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SUMMARY

In the 1970s, a core of depleted uranium (DU) began to be introduced into the break through anti-tank munitions to enhance their effectiveness. The health and environmental threats of DU stem from the pyrophoric character of the core, burnt when penetrating armour to an aerosol of uranium oxides deposited in tissues after inhalation or ingestion. Their delayed effects are due to internal alpha irradiation by daughter products and toxicity of uranium.

Key words: anti-tank munitions; depleted uranium; radiotoxicity; toxicity; uranium oxides

INTRODUCTION

The current trend in anti-armour ordnance represents "arrow" munitions with a significant increase in mouth velocity and thus kinetic energy of penetrator in target. In order to enhance the effects on the target of previous "sub-calibre" munitions, using hard steel for the core that carries kinetic energy for breakthrough, new materials for the penetrator with higher density were looked for. Beside tungsten, depleted uranium (DU), the waste in nuclear fuel cycle was found suitable for its high density (19 × 103 kg.m^{-3}) and mechanical properties. DU contains about 0.2—0.4, exceptionally up to 0.7 per cent of ^{235}U and a negligible amount of ^{234}U (6). A typical DU composition is shown in Table 1. Apart from manufacturing munitions, there are no too serious risks in handling. The main problems begin when they are used, i.e. in striking a target. Due to friction, the DU core usually burns when piercing armour to highly a breatheable aerosol of uranium oxides (5). Th current problem is the vertical and horizontal proliferation of DU weapory and dissemination of huge amounts (of order 10^{3}.t^{-1}) of DU already used in the armed conflicts of the 1990s and later with adverse health and environmental consequences (5).

Table 1. Typical composition of depleted uranium

Component	Per cent	Half-life [years]	Energy [MeV]	Activity [kBq.kg^{-1}]
^{238}U	99.7	4.47 × 109	4.17	12.40
^{235}U	ca 0.3	7.04 × 108	4.40	0.16
^{234}U	ca 0.001	2.25 × 105	4.70	2.26

Note: All three radionuclides emit alpha-radiation.
– In some cases, traces of Pu were found, witnessing that such "DU" was by-product of reactor fuel reprocessing rather than of primary uranium enrichment

HEALTH EFFECTS OF DU

The chemical toxicity of uranium (not depending on its isotopic composition) is due to the chemical damage of kidney tubular cells, causing thus *nephritis*. While water-soluble uranium salts are renal and systemic toxicants, insoluble compounds are in the firstly lung toxicants. This relates mainly to the products of burning (depleted) uranium, i.e. UO_2, UO_3 and U_3O_8.

Studies in humans have shown: a) respiratory effects (degeneration of lung epithelium, haemorrhagic lungs; uranium oxides dust has a biological half-life in lungs of about one year); b) gastrointestinal effects (anorexia, abdominal pain, diarrhoea, tenesmus or ineffective straining, and pus and blood in stool); c) renal effects (proteinuria, elevated levels of NPN, amino acid nitrogen/creatinine, increased urinary catalase, *diuresis*, and abnormal phenolsulphophthalein excretion) (5).

Radiotoxicity seems to be more relevant. Because of the slow absorption of DU aerosol particles through the lungs and its long retention in body tissues, its primary effect is its radiological damage to internal organs rather than the chemical damage of renal system. DU is composed of more than 99 % of U-238, where the specific activity 12.4 kBq means 12,400 transformations per second – each releases one energetic alpha particle and each atomic transformation produces another radionucleide: Th-234 (half-life 24.1 days) >> Pr (half-life 6.75 hours) >> U-234 (half-life 2.25 × 105 years). The delayed effects of the deposited particles of DU oxides are caused by the internal irradiation of sensitive organs by daughter products at a cellular level leading to the enhanced incidence of carcinoma and leukaemia (3).

Environmental consequences of using DU

The aerosol of highly breatheable particles (70 % of the smoke contains particles in order of micrometers) contaminates the immediate neighbourhood and can be moved downwind hundreds of meters. Field studies by UNEP (7, 8) from Kosovo and Montenegro did not detect significant contamination but stated that due to the corrosion of yet undetected buried penetrators missing targets, future environmental problems might emerge. None of the studies by intergovernmental agencies assessed the delayed health effects on exposed military personnel and civilians. A prestigious study (1) has stated a significant increase of carcinoma incidence on the sites of the NATO air strikes.

CONCLUSIONS

DU munitions are conventional weapons with dangerous side effects. Their extreme mass use in the armed conflicts of the 1990s, declared as peace-keeping or peace-making (Iraq, former Yugoslavia) and enhanced use in recent missions (Iraq, Afghanistan) should have been avoided or sharply diminished. Even peacetime testing and training should take into account health and environmental risks (2), as also our experience with testing DU munitions in the 1980s showed. The basic military technological and legal problems of using DU have been elucidated elsewhere recently (4).

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THE INFLUENCE OF IONIZING RADIATION ON THE MORPHOLOGY OF *Poecilia reticulata*

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SUMMARY

Our investigations focused on morphological changes in *Poecilia reticulata* irradiated with gamma rays at a dose of 30 Gy. The histological findings corresponded with the clinical symptoms. Lethargy and uncoordinated movements indicated changes in the brain, such as swelling and necrosis of nerve cells. Changes in the small intestine mucosa involved particularly the intestinal villi and enterocytes. These changes resulted in absorption disorders and subsequent emaciation.

Key words: gamma radiation; histological changes; *Poecilia reticulata*

INTRODUCTION

Ionizing radiation is an inseparable component of the environment. The human population is constantly exposed to the effects of natural ionizing radiation emitted by internal and external sources (6). At the same time, ionizing radiation as one of the environmental stress factors has affected life on our planet for hundreds of millions of years.

With the development of nuclear energy, protection of the environment against radiation, particularly protection of humans against the negative effects of ionizing radiation, has been increasingly investigated.

The effect of gamma radiation on fish, contrary to that on mammals and birds, has been less intensively studied.

Recently, alternative model systems have been introduced into biomedicinal research (4, 5). Aquarium fish, the undemanding guppy *Poecillia reticulata* and *Danio retio* in particular have been used widely (8).

MATERIAL AND METHODS

The experiment was carried out on the fish *Poecilia reticulata* from laboratory rearing, established two years ago using individuals from a special rearing of guppies, intended for laboratory purposes. They were kept under constant conditions: water temperature 24 °C, content of Ca + Mg 0.7 mmol.l^{-1}, pH 7.4, artificial aeration and filtration of water, photoperiod twelve hours per day (9). The fish were fed live, flaked and granulated food. Ten control and twenty experimental fish of both sexes were selected at random and used in the experiment.

The fish were irradiated with gamma rays using a dose of 30 Gy produced by a ^{60}Co-Chisostat with a dose input of 11.36 Gy.min^{-1}. Gravid females were also irradiated. The fish were irradiated in glass Petri dishes in aquarium water at a water column height of one centimetre. The control fish were subjected to sham irradiation, i.e. to all processes and manipulations as the experimental fish with the exception of irradiation with gamma rays.

The samples for histological examination were processed by common methods. Whole killed fish were fixed in neutral formalin and embedded in paraffin. Histological sections of thickness 7 μm were stained with haematoxylin-eosin. The sections were examined under a light microscope, Jenamed, equipped for taking microphotographs.

RESULTS

In the first days post irradiation the fish were lethargic. They stayed close to the aquarium bottom, moved very little and their movements were uncoordinated. Microscopical changes in the brain pointed to swelling. The brain had a spongy structure. Karyolysis and karyorhexis was observed in nervous cells which resulted in their necrosis. No marked morphological changes were seen in the neuroglia cells.

The initial macroscopically visible changes in the body of the irradiated fish included the bilateral exophthalmus, which was frequently preceded by haemorrhagia of the eye. Microscopical observations showed retinal detachment. The intactness of the cornea and lens was not affected.

Starting from day 10 after irradiation we observed a decreased intake of food by the fish and their considerable emaciation. Morphological changes were observed in the intestine. The intestinal villi were relatively low and the epithelium consisted of enterocytes of a cylindrical to cubical shape. Microvilli were indistinctive. In some places delamination of epithelium occurred. Empty spaces were left after necrotized villi. The stroma of intestinal villi contained homogeneous material, which indicates intestinal wall necrotization.

DISCUSSION

Despite the fact that the effects of ionizing radiation on the organisms of mammals and birds have been studied and described in detail (1, 2, 7, 12), the information available on the effect of gamma radiation on fish is scarce.

Lethargy, loss of appetite and considerable emaciation are symptoms resembling those observed in mammals and birds. S e i d e l o v á (11) has described similar clinical symptoms in X-ray irradiated *Poecilia reticulata*.

Decreased intake or complete refusal of food has been described in mammals and birds as symptoms of a gastrointestinal syndrome of acute irradiation sickness (10). These symptoms corresponded to histological findings and the clinical symptoms described in our study. Changes in the gastrointestinal tract resulted in subsequent emaciation.

Bilateral exophthalmus in irradiated fish and microscopically visible retinal detachment can be the consequence of increased bulbar pressure.

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THE EFFECTS OF A HEAD-ONLY GAMMA-IRRADIATION ON THE LEARNING AND SPATIAL MEMORY AND ON OPEN FIELD BEHAVIOR IN RATS

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SUMMARY

The effects of a sublethal dose of gamma-rays applied to the head on selected behavioral parameters were investigated in this study. Adult male Sprague-Dowley rats (n = 9) were irradiated with a single dose of 20 Gy of gamma rays from a ^{60}Co radiation source. The irradiated animals as well as sham-irradiated controls were tested daily in a Morris water maze (MWM) and in the open field test. The ability of spatial learning given by latency time to find the hidden platform was followed in the MWM. Horizontal and vertical locomotion, the number of crossings of the center of the field and washing behavior were recorded in tests in the open field. The results obtained showed, that radiation did not significantly alter the time course of learning in MWM during the experiment. The levels of horizontal and vertical locomotory activity in open field were lower in the irradiated group in comparison with the controls. The number of the crossings of the field's center, related to the level of anxiety of the animals was not significantly lower in the irradiated animals, whereas no differences in the number of washings between both groups were detected. The results point to differences in radiosensitivity in various behavioral parameters in rats, maybe due to different levels of their control and coordination in the CNS.

Key words: gamma rays; ionizing radiation; Morris water maze; open field

INTRODUCTION

The results of studies on experimental animals, together with experience from irradiation of men by nuclear accidents implies, that neuronal tissue belongs to the relatively radioresistant parts of the body. To induce the CNS-syndrome, leading to the inevitable death of the animal some hours to some days after irradiation, doses as high as 50 Gy, overriding many times the value of $LD_{50/30}$, are needed (3). On the other hand, low doses of radiation caused changes in some neurophysiological and behavioral parameters (4). The goal of our work was to establish, whether the irradiation of the head with a relatively high dose of gamma rays would influence selected forms of innate and learned behavior in rats.

MATERIAL AND METHODS

Adult, male Sprague-Dowley rats, adapted three weeks to conditions of the animal house were used in the experiment. The animals were randomly separated in two experimental groups (n = 9 each). The first group was irradiated with a single dose of 20 Gy of gamma rays, delivered from a ^{60}Co source, on the head only (the rest of the body was shielded by lead). The second group was manipulated in the same manner, apart from irradiation. Thirty-minutes after irradiation (sham-irradiation) the animals were tested for five minutes in the open field test.

Table 1. Effects of irradiation with gamma-rays on selected behavioral parameters in the open field on rats. Data given as arithmetic mean ± SEM. ** — $P < 0.01$, * — $P < 0.05$

Parameter	Group	Days after irradiation 0	1	2	3	4
Horizontal locomotion	Control	129.8 ± 34.6	116.4 ± 15.6	152.1 ± 16.7	132.9 ± 14.4	103.3 ± 17.4
	Irradiated	100.2 ± 8.5	118.1 ± 13.1	111.1* ± 9.3	73.9** ± 12.4	54.9** ± 9.2
Rearing	Control	21.1 ± 1.7	24.1 ± 3.7	21.4 ± 2.9	15.1 ± 2.6	13.3 ± 2.7
	Irradiated	17.1 ± 5.7	22.8 ± 1.8	16.2 ± 1.9	12.0 ± 2.2	5.3* ± 1.1
Centercrossing	Control	2.2 ± 0.7	2.0 ± 0.6	2.0 ± 0.8	2.0 ± 0.5	1.1 ± 0.4
	Irradiated	1.2 ± 0.5	1.3 ± 0.4	1.2 ± 0.4	1.0 ± 0.5	0.4 ± 0.2
Washing	Control	1.6 ± 0.3	2.1 ± 0.3	2.7 ± 0.5	2.3 ± 0.5	3.1 ± 0.5
	Irradiated	2.4 ± 0.6	2.2 ± 0.4	2.1 ± 0.3	2.3 ± 0.4	2.4 ± 0.3

The open field sized 100 × 100 cm was divided into 25 squares and was surrounded by a 60 cm high white wooden wall. The behavior of the animal was recorded by video camera. The number of squares crossed, the number of rearings and "washings" and the number of the crossings of the center of the field were evaluated. The animals were tested then immediately in the Morris water maze for spatial memory and learning.

The maze consisted of a plastic basin one metre in diameter, filled with water (t = 23 °C) to a height of fifteen centimetres. The water was made opaque by the addition of milk. The time needed to reach a platform sized 15 × 15 cm placed two centimetres under the water surface was measured in rats, placed into the basin at random positions. The test was repeated after one hour. The animals in both groups were tested in the same way over four subsequent days. The test in the Morris water maze was repeated fourteen days after irradiation.

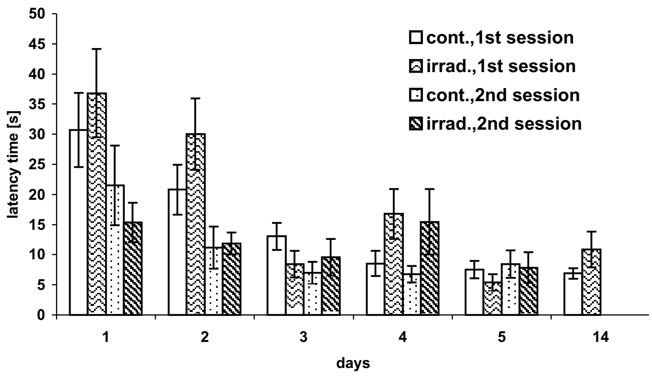

Fig. 1. Spatial learning in rats in the Morris water maze after irradiation with gamma rays. Data given as arithmetical mean ± SEM

RESULTS AND DISCUSSION

The irradiation caused a decrease in horizontal (statistically significant differences at days 2, 3 and 4), as well in vertical locomotor activity. The differences in washing and in crossing of the center were not statistically significant (Table 1). Radiation acts in general toward a decrease in locomotory activity in most species, e.g. in guinea pigs, hamsters, rats and primates (2). Landauer (5) has found a gradual decrease of locomotory activity in mice for a dose range from 0.5 to 7.0 Gy with a minimum on the 15th day after irradiation.

Our findings are in agree well with these results. Literary data reveals, that learning and memory are represented by relatively radioresistant functions of the CNS (1, 9). In our experiments a relatively high dose of 20 Gy did not significantly chang the ability of rats to learn the position of the hidden platform and remember this position for as long as two weeks (Fig. 1).

More recent studies show, that the impact of radiation on the CNS depends highly on radiation quality. Shukitt-Hale *et al.* (10) have found, that irradiation of rats with 4 Gy of 250 MeV neutrons had no effect on amphetamine-induced taste aversion and on learning in the Morris water maze. On the contrary, in another study (10) they established, that irradiation with a dose of only 1 Gy of high-energy $^{56}Fe^{2+}$ particles worsened the performance of rats in a radial maze.

CONCLUSIONS

The results have confirmed the low radiosensitivity of learning and spatial memory in rats. The effect of irradiation was most profound in influencing locomotory activities in the open field, but not other types of innate behavior (e.g. comforting behavior).

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INHIBITION OF COX-2 DOES NOT AFFECT THERAPEUTICAL RESULT OF PHOTODYNAMIC THERAPY WITH HYPERICIN DESPITE OF ITS INCREASED ACTIVITY AND EXPRESSION

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ABSTRACT

A photodynamic therapy (PDT) is a multifarious therapeutical approach based on administration of photosensitizer and light dose of appropriate wavelength. PDT with hypericin, activates p38 MAPK signalling pathway which induces expression of COX-2 and thereby increases concentration of its main product, PGE2, what is considered as contradictory to photocytotoxic effect and an efficacy of PDT with hypericin. In our experiment, effect of rofecoxib, a specific COX-2 inhibitor, as a post-treatment after PDT with hypericin in HeLa and HT29 cells have been evaluated. Surprisingly, 24 as well as 48 hour treatment with 1µM rofecoxib applied immediately after PDT did not induce significant decrease in cell proliferation, though gene expression and activity of COX-2 was elevated. Considering our results, we can predict, that activity of COX-2 and its inhibition does not play crucial task in PDT experiments *in vitro* however its importance manifests *in vivo* as it affects angiogenesis of tumour.

Key words: COX-2; hypericin; photodynamic therapy; rofecoxib

INTRODUCTION

Hypericin is a naturally-occurring photosensitive compound with properties suitable for PDT. However ROS production in cells induces activation of p38 MAPK and initiates cascade of processes leading to stimulation of cyclooxygenase-2 (COX-2) expression and therefore increases conversion of arachidonic acid towards eicosanoids hence negatively affects result of PDT (3). Employment of various photosensitizers and more or less specific COX-2 inhibitors manifested effectiveness of COX-2 pathway modulation in cell proliferation after PDT, whensoever often not dependent on inhibitor's concentration (1).

MATERIALS AND METHODS

A human colon adenocarcinoma cell line HT29 and human cervical adenocarcinoma cell line HeLa culture and irradiation procedures as well as MTT assay were mentioned elsewhere (4). RQ-RT-PCR have been performed on iCycler iQ (Bio-Rad) with forward/reverse primers according to Niikura *et al.* (7). Cyclooxygenase activity assay is based on conversion of fluoresecent dye Amplex Red (Molecular Probes).

DISCUSSION

In spite of increased COX-2 expression manifested by elevated mRNA concentration (Fig. 4) as well as by COX-2 activity (Fig. 3), our data suggest that modulation of COX-2 activity does not affect proliferation of cells intervened by photocytotoxic effect of PDT with hypericin *in vitro* (Figs. 1 and 2).

Since some experiments proved increased effectivity of PDT after *in vitro* as *in vivo* application of various COX-2 specific inhibitors (1) which are known to affect cell proliferation by mechanism independent from

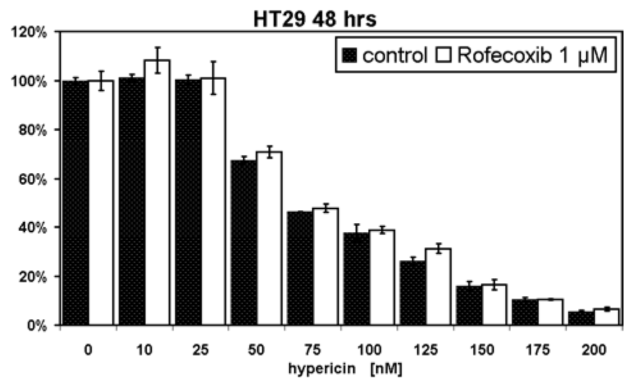

Fig. 1. MTT assay with colon adenocarcinoma cells HT29

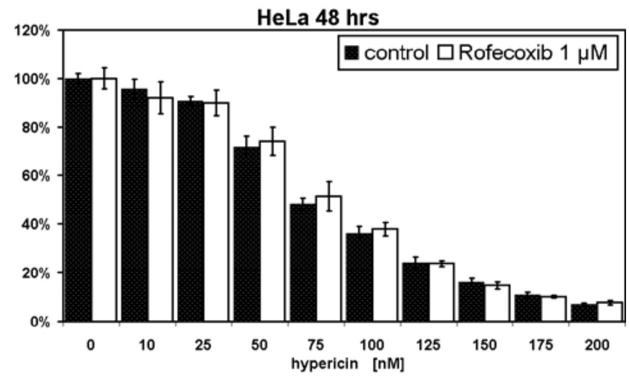

Fig. 2. MTT assay with cervical adenocarcinoma cells HeLa

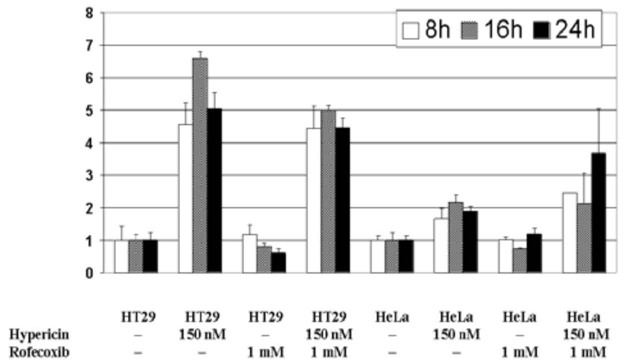

Fig. 3. Cyclooxygenase enzymatic activity in HT29 and HeLa cells

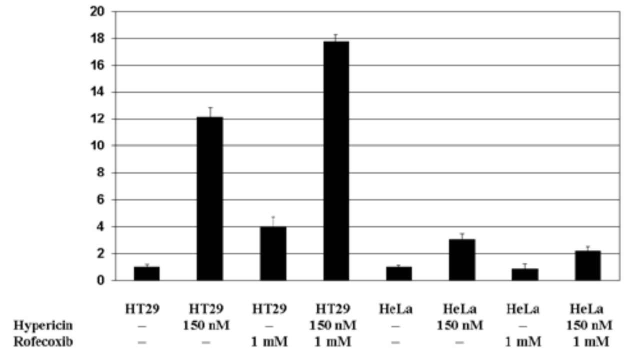

Fig. 4. Real-time RT-PCR analysis of COX-2 gene expression 16 h after PDT

COX-2 inhibition (5), we chose rofecoxib (commercially known as VIOXX®), a selective COX-2 inhibitor with no effect on cell proliferation or apoptosis incidence in HUVEC (6),or HT29 cells in spite of its ability to block PGE2 production (8).

The results of our experiment imply, that inhibition of COX-2 does not affect proliferation of colon adenocarcinoma cells HT29 and cervical adenocarcinoma cells HeLa *in vitro*. However considering the significance of this pathway and its products for angiogenesis (2), application of COX-2 inhibitors *in vivo* is not loosing its value.

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THE EFFECT OF ERYTHROPOIETIN ON THE PHOTODYNAMIC THERAPY OF OVARIAN ADENOCARCINOMA A2780 AND SKOV-3

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ABSTRACT

Erythropoietin is a cytokine with a pleiotropic effect. It is the principal haematopoietic growth factor, has a proangiogenic and protective effect in diverse nonhaematopoietic organs. In our experiment cell proliferation and/or survival assay was used to demonstrate Epo's cytoprotective effect against the photodynamic therapy of hypericin. Epo protected SKOV-3 cells against the cytotoxic effect of hypericin. These results advocate a restricted use of Epo on cancer pacients with EpoR positive tumors.

Key words: erythropoietin; Epo; EpoR; hypericin; photodynamic therapy

INTRODUCTION

The biological actions of Epo are not limited to the haematopoietic system, but Epo and EpoR have been localized in many nonhaematopoietic tissues and cells including brain, endotelial cells, solid tumors, liver and others (1). The expression of the Epo receptor in cancer cells has raised the possibility, that exogenous Epo can exert direct effects on tumor cells and stimulate their proliferation, inhibit apoptosis and modulate sensitivity to radio-, chemo- or photodynamic therapy. Photodynamic therapy (PDT) presents a new approach in the treatment of some types of cancer.

Among the most used photosensitizers for *in vivo* as well as *in vitro* PDT is hypericin (2). The goal of this work was to evaluate the impact of exogenous Epo on the response of EpoR positive ovarian adenocarcinoma cells A2780 and SKOV-3 in conditions of the photodynamic therapy of hypericin.

MATERIALS AND METHODS

A2780 and SKOV-3 cell lines were obtained from the ECAC (European Collection of Animal Culture, Salisbury, UK) and cultured according to ECAC directions. All culture media were purchased from Gibco (Grant Island, NY, USA). Erythropoietin-EPREX 10000 IU.ml^{-1} (Janssen-Cilag International N.V., Beerse, Belgium). Experimental procedures such as PDT treatment and assessment of cell proliferation and/or survival are essentially as described in K l e b a n *et al.* (3).

DISCUSSION

In our experiments we did not find any significant difference in the response of A2780 and SKOV-3 cells to hypericin treatment (Figs. 1, 2). We tested three different concentrations of Epo: 10, 30 and 150 U.ml^{-1}. We did not observe any effect of Epo on cell proliferation and/or survival of A2780 cell after photodynamic therapy when the cells were pre-treated with 150 U.ml^{-1} of Epo (Fig. 1). On the other hand, a statistically significant difference in the survival of SKOV-3 cells pre-treated with 150 U.ml^{-1} of Epo followed by photodynamic therapy was detected (Fig. 2). We did not see any significant

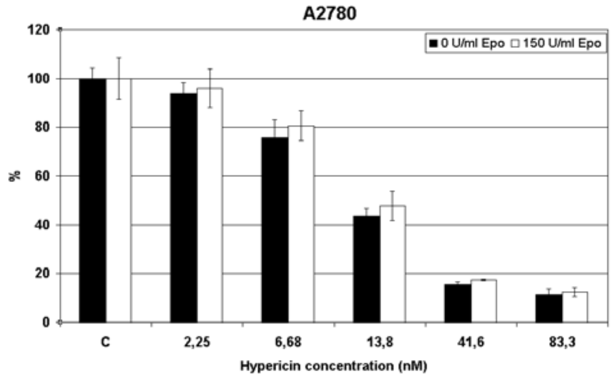

Fig. 1. A2780 MTT assay – mutual combination of hypericin and 150 U.ml^{-1} Epo

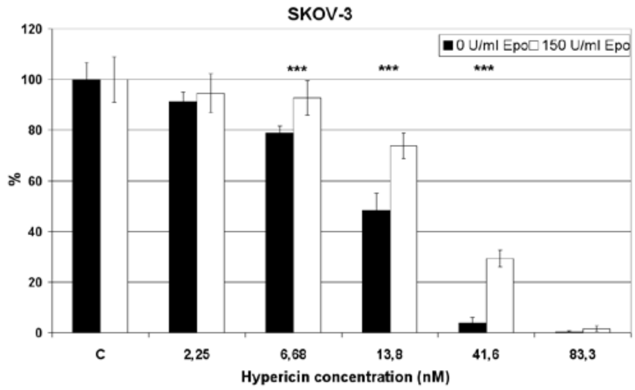

Fig. 2. SKOV-3 MTT assay– mutual combination of hypericin and 150 U.ml^{-1} Epo

effect of lower Epo concentrations on the survival of SKOV-3 cells (data not shown).

Recombinant Epo was introduced into clinical practice to treat anaemia, such as anaemia associated with cancer, to improve the response of tumor tissue to various types of treatment. Golab *et al.* (4) describes the renewal of anticancer activity of PDT in mice with chemically induced anaemia after Epo treatment. There are many papers showing the reduced apoptosis and developing resistance to ionizing radiation and chemotherapy after Epo administration. Epo treatment has resulted in the increase of resistance to cisplatin (5), to paclitaxel (6) and to ionizing radiation (5) in various types of cancer cells.

In our study, we could see the cytoprotective effect of Epo against the cytotoxicity induced by hypericin in SKOV-3 cells, but not in A2780 cells. In the future, our goal will be to study the mechanism of cytoprotective impact of Epo on SKOV-3 cells during the photodynamic therapy with hypericin. The results of this work indicate the possible cytoprotective effect of Epo on cancer cells. Further studies are necessary to uncover the molecular mechanisms of Epo actions on tumor cells.

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COUNTERMEASURES FOR THE REDUCTION OF THE RADIOACTIVE CONTAMINATION OF FARM ANIMALS AND ANIMAL PRODUCTS IN AGRICULTURAL ECOSYSTEMS

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SUMMARY

A wide variety of measures is available to reduce or prevent the transfer of radionucleides through the food-chain and hence reduce the radiation dose to the consumer. In this mini-review, both literature sources and the practice of applying agricultural countermeasures are summarized very shortly: Interventions at the soil-plant step, at the plant-animal step, and at the foodstuff-man step.

Key words: agricultural countermeasures; animal products; radionuclides; contamination

INTRODUCTION

Many studies have been conducted to investigate the effectiveness of various countermeasures reducing radionucleide contamination in the chain between soil and the final food consumed by the population in agricultural production. Countermeasures can be divided according to the state of deposition and distribution of radionucleides into short- and long-term, and according to the place of the depositions in the food chains into interventions at the soil-plant step, interventions at the plant-animal step and at foodstuff-man step. It is also important if such interventions are applied in natural or semi-natural agricultural ecosystems.

COUNTERMEASURES

Countermeasures at the soil-plant step

Once contamination has reached the soil of an agricultural system, these options can be summarized (12):

– to stop agricultural crop production,
– to grow alternative food crops,
– to apply physical or chemical methods to reduce the radionucleide soil-plant transfer.

The first two options are effective but expensive, and restrict highly or stop crop production. Methods for radionucleide transfer reduction, which were tested and/or applied after the Chernobyl accident in the former Soviet Union (1) and other countries such as Norway (13) are more suitable:

– removal of the radionucleides from the rooting zone of the plants (e.g. ploughing, leaching),
– dilution of radionucleides in the soil (e.g. ploughing, mixing),
– immobilization of radionucleides (e.g. precipitation),
– competition for root uptake (e.g. addition of potassium in the case of radiocaesium contamination),
– other treatments (e.g., application fertilisers, drainage).

Countermeasures at the plant-animal step

Interventions to reduce animal uptake, and then animal-derived food products, of any radionucleides can be divided into four basic approaches:

– reduction of the consumption of contaminated feed (management methods),
– reduction of gut absorption (use of chemical methods),

– blocking/reduction of radionucleide transfer across from body (addition of stable isotopes and/or chemical analogues),

– increase of the excretion rate of radionucleides.

Management methods have been summarized by Howard (5) and Segal (12). Many of them were tested and used after the Chernobyl accident; however some of them are speculative and require examination. These countermeasures include: destruction of crops or slaughtered animals; fertilizer application to reduce radionucleide uptake by vegetation; altering grazing pressure; reduction of consumption of contaminated feed; restricting access to highly contaminated areas; changing the animal species grazing in contaminated areas; selective culling of less contaminated animals; changing the hunting season or slaughter times (incl. *in vitro* monitoring); diverting animal food products from human to animal consumption.

Use of chemical methods is most effective to prevent radionucleide contamination of animals. Most of the methods described in the literature relate to radionucleides of caesium, strontium iodine and to lesser extend to actinides (e.g. Hove, – 4, Voigt, – 14). The effect of natural clay minerals (espec. bentonite) for the reduction of the radiocaesium uptake is in much more significance.

Application of potassium or stable caesium is only partially effective. Salt licks and rumen releasing boli with between 5 to 15 % of AFCF were successfully used for the reduction of the transfer of radiocaesium to animals in semi-natural environments. Prussian blue type compounds such as hexacyanoferrates (FeHCF, AFCF) can be even more effective than clay minerals (3, 14).

Commercially and locally produced caesium binders on the base of the hexacyanofferates, e.g. Ferrotcin (11), Radekont (10), or Bifezh (9), were also effective. Calcium was found as the best countermeasure for reduction of radiostrontium uptake. Administration of stable iodine is the most effective measure for the decrease of radioiodine input into the thyroid gland and also radionucleide transfer into milk.

Only in laboratory animals (rodents), was moderate effectiveness of polyaminocarbonacids (e.g. EDTA) on the reduction of uptake of actinides found.

Countermeasures at the foodstuffs – man step

Works on the decrease of radionucleide content directly in foodstuffs is rather scarce. A very significant reduction of radiocaesium content (in meat and mushrooms) was however achieved with macerating, marinating, soaking and/or boiling (6, 7, 8, 2).

CONCLUSION

The countermeasures, which can be applied to reduce radionucleide contamination of animals in agricultural ecosystems, will be obtained by both management changes and the use of chemical binders to prevent gut absorption. The use of effective interventions is possible at any step in the chain between soil and the final food consumed by the population.

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LATE METABOLIC CHANGES IN BROILER CHICKS AFTER IRRADIATION OF SETTING EGGS

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ABSTRACT

In our experiment setting eggs of broiler chicks were exposed to a single dose of gamma radiation (0.25 Gy) before putting into the hatcher. Analyses were performed in chicks, 51-days old, after hatching. A statistically significant decrease in glucose concentration and ALT activity and an increase in serum total proteins in chicks, that were exposed to irradiation during the embryonal stage of development, were recorded. Irradiation of chicks in this stage with low doses of ionising radiation may significantly influence the metabolism of individuals in subsequent developmental periods.

Key words: ALT; AST; cholesterol; gamma radiation; glucose; setting eggs; total proteins

INTRODUCTION

The observation of basic metabolic parameters after the impact of various pathogenic factors (5), to which ionising radiation belongs (8, 9) provides information about the character of the adaptation response of an animal organism on the effect of these factors (3). This information may be used for the prevention and therapy of irradiation sickness. The aim of this study was to observe the effect of sub-lethal doses of ionising gamma radiation on late metabolic changes in irradiated broiler chicks.

MATERIAL AND METHODS

The control group consisted of twenty, 51-day old, broiler chicks. In the control group there were 30 broiler chicks incubated under experimental conditions and included in the experiment as embryos.

The embryos were irradiated in the Faculty of Natural Sciences in Košice in a CHISOSTAT ^{60}Co-CHIRANA apparatus. The setting eggs were irradiated in a cardboard box. In the experimental group chick embryos were exposed to a dose of 0.25 Gy gamma rays at a power requirement of 0.295 Gy.min^{-1}. Then the eggs were put into a hatcher (21 days). Analyses were performed at the age of 51 days, i.e. 72 days after ambryo's irradiation.

The controls were submitted to the same procedures of manipulation expected in gamma irradiation.

After prior disinfection the chicks (4, 6) were kept in the experimental area from the first day after incubation. Water and food were offered *ad libitum*. The chicks were fed by feedstuffs BR I a BR II (granulated).

The chicks were sacrificed by vertebral cervical dislocation (1, 7). Mixed blood was captured in a Petri dish located on ice and after centrifuging serum was obtained for analyses.

The experiments were performed during summer-time.

The serum concentration of glucose, cholesterol, total proteins and activity of ALT and AST were determined by Bio-La tests (Lachema, Brno). The measurements were performed on the spectrophotomer Spekol 11.

Student's *t*-test was used to compare results between the treated and control chicks for statistical significance.

RESULTS AND DISCUSSION

Serum glucose concentration in experimental group was significantlly decreased in comparison with the control. Cholesterol concentration in the serum of the experimental group was not significantly decreased compared with the control. A statistically significant increase in the serum concentration of total proteins (Table 1) was observed in the experimental group against control. ALT activity was significantly decreased in exposed chicks against the control. AST activity was non-significantly decreased in the experimental group.

Table 1. Late metabolic changes in broiler chicks after irradiation

Group	Control	Experimental
Glucose $mmol.l^{-1}$	12.93 ± 0.6211	**11.08 ± 0.5627***
Cholesterol $mmol.l^{-1}$	1.624 ± 0.1553	1.329 ± 0.06793
Total proteins $g.l^{-1}$	29.39 ± 4.251	**48.39 ± 7.925***
Alaninamino-transferase $mkat.l^{-1}$	0.08213 ± 0.004055	**0.0621 ± 0.006524***
Aspartatamino-transferase $mkat.l^{-1}$	0.8838 ± 0.06428	0.6850 ± 0.07116

* — $p < 0.05$

The present results showed that in the period of intensive growth the organism is more sensitive to ionising radiation than in adulthood. In the postnatal growth period irradiation causes growth retardation, resp. disorders in height because of the damage of differential cell processes in the growth zones (2).

There is little information regarding the effects of ionising radiation on bird embryos. It is known that mammalian embryos are the most sensitive to radiation during the pre-implantation period and the minimal lethal dose for a one-week old embryo is 0.25 Gy. In our experiment the same dose of single dose of gamma rariation in setting eggs was used.

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SOME CHANGES OF CHOLESTEROL, GLUCOSE AND TOTAL PROTEIN CONCENTRATIONS IN THE SERUM OF CHICKEN AFTER EXPOSURE TO A LOW DOSE OF IONISING RADIATION

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SUMMARY

The aim of this experiment was to investigate the effect of a low dose of ionising radiation on the organism of a chicken. We observed changes in the serum concentrations of cholesterol, glucose and total proteins on Days 1, 3, 14 and 25 after whole-body exposure to gamma irradiation of a single dose of 3 Gy.

Key words: gamma irradiation; cholesterol; glucose; total proteins

INTRODUCTION

Nowadays the human and animal organism is exposed to many negative factors in the environment, for example to chemical compounds, heavy metals, xenobiotics, irradiation and so on (6, 9). These factors can influence the health and life of organisms to a varying extent. Each organism is exposed to irradiation from natural sources. Furthermore, the use of ionising radiation in medical and technical applications increases this exposure from sources that have become a part of manufacturing processes and our lives. It suggests the need to study the harmful effects of ionising radiation on mammals (10, 11) and other species of animals even during an undisturbed lifetime (5, 2).

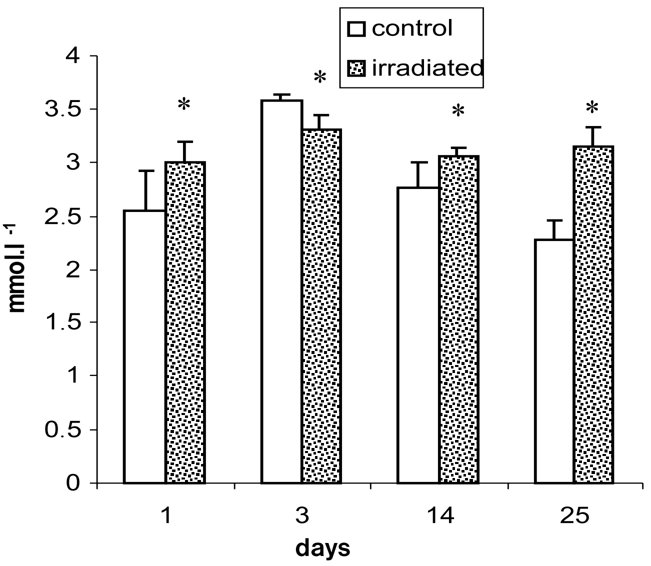
The aim of our study was to investigate the effect of a low dose of ionising radiation on the organism of broiler chicks. We also observed the changes in the serum concentrations of cholesterol, glucose and total proteins on Days 1, 3, 14 and 25 after a single whole-body irradiation at a dose of 3 Gy.

MATERIAL AND METHODS

In the experiment 60 broiler chicks, 28 days old, were used. From the first day of life animals were kept in an experimental compartment after a prior disinfection (7, 8). During the study, food and water were offered *ad libitum*. Granular feedstuffs BR I and BR II for chicks were used for feeding. The irradiation of animals was performed in PF UPJŠ, Košice with a CHISOSTAT apparatus. The animals were placed in plexi-glass cages and exposed to a single whole-body dose of 3 Gy of gamma rays with an input power of 0.079558 $Gy.min^{-1}$. Analyses were performed on Day 1, 3, 14 and 25 after irradiation. The animals were killed in accordance with the requirements of the Ethical Commission. Blood was collected in a Petri dish located on ice and after centrifugation the obtained sera were used for analyses. Cholesterol and triacylglycerol concentrations were determined by Bio-La tests (Lachema, Brno). Experiments were performed in the autumn. The unpaired Student's *t*-test was used to compare concentrations between the irradiated and control animals.

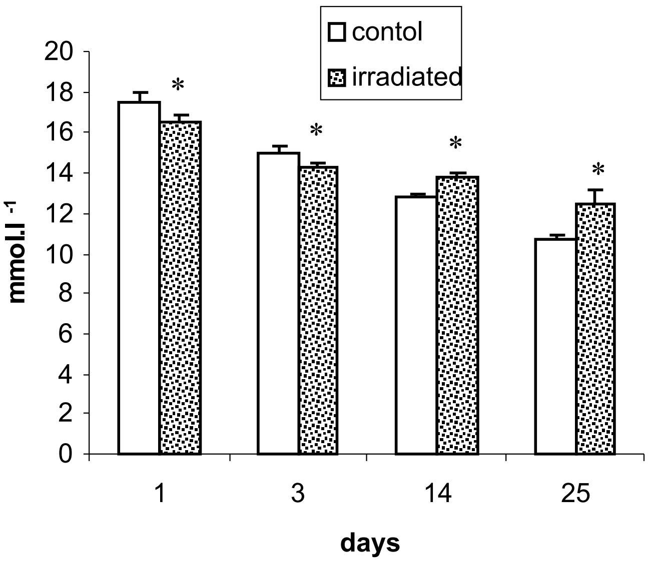
RESULTS AND DISCUSSION

In the irradiated groups the serum concentration of cholesterol (Fig. 1) had increased on Days 1, 14 and 25,



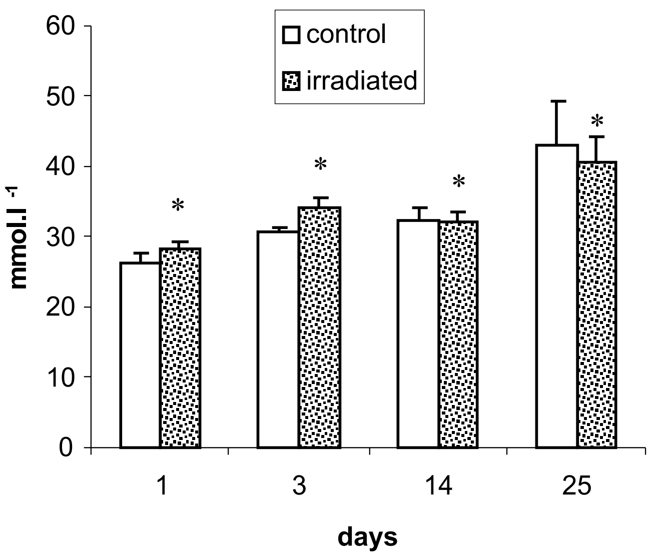
* — p < 0.05

Fig. 1. Changes in the serum concentrations of cholesterol in the control and irradiated groups of chicks on Days 1, 3, 14 and 25 after irradiation



* — p < 0.05

Fig. 2. Changes in serum glucose concentrations in the control and irradiated groups of chicks on Days 1, 3, 14 and 25 after irradiation



* — p < 0.05

Fig. 3. Changes in serum concentrations of total proteins in the control and irradiated chicks on Days 1, 3, 14 and 25 after irradiation

on Day 3 a decrease was recorded, but these changes were not statistically significant in comparison with the control group.

After exposure of the animal body to ionising irradiation significant changes in serum concentrations of lipids that include glycerols, lipoproteids, fosfolipids and cholesterol are usually observed. In the time after irradiation these changes are called non-specific. First of all, they are caused by enhanced lipolysis and lipomobilization leading to an increased supply of non-esterified fatty acids to various tissues. In addition, an enhanced concentration of cholesterol in the liver may also cause its increase in serum, which we observed in our experiment.

The serum concentration of glucose (Fig. 2) in the irradiated groups of chickens increased on Days 14 and 25 after exposure, a statistically significant increase was recorded on Day 14. On Days 1 and 3 a non-significant decrease in glucose concentration was observed.

After irradiation hyperglycaemia occurrs in an organism caused by gluconeogenesis from aminoacids, decreased effects of insulin and an increased effect of contrainsulin action (3). After irradiation the serum concentration of cholesterol is increased because of its increased formation and concentration in the liver. The activities of enzymes may increase or decrease. Increased activity of adaptive enzymes suggests catabolic processes in an irradiated organism.

The concentration of total proteins in serum (Fig. 3) increased non-significantly on Days 1 and 3 and then decreased on Day 14 and 25.

Through irradiation the structure of a protein molecule may be changed, eventually coagulation of proteins occurring. The compounds containing sulphhydryl groups are the most sensitive to irradiation causing a change into disulphites. Loss of protein function after irradiation is not obviously caused by a break of peptide linkage or disconnection of peptide chain structure. However, it may be caused by the change in the critical site of chain or hydrogen or disulphite chain discontinuance. This break causes an unrolling of closely rolled protein chains, a failure in space organisation of amino acid groups and relative chemical activity (1).

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