

## Influence of Ionising Radiation on the Activity of Selected Adaptive Enzymes in Chickens and Rats

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### Abstract

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The effects of ionising radiation on the activity of two adaptive enzymes in the serum of poultry and laboratory rats were investigated. Chickens were divided into three groups. Those aged 28 days were gamma-irradiated with either a single whole-body dose of 2.0 Gy (output 0.27 Gy/min) (group 1, n = 6), or a single whole-body dose of 4.5 Gy (output 0.33 Gy/min) (group 2, n = 6). Rats and chickens (group 3, n = 6) aged 37 days were exposed to a single whole-body dose of 5.0 Gy (output 0.27 Gy/min). Analyses were carried out at 6, 24 and 48 h after irradiation.

Chickens irradiated with the 2.0 Gy dose showed a significant decrease in the activity of ALT at 6, 24 and 48 h after irradiation compared to controls. Chickens irradiated with the 4.5 Gy and 5.0 Gy dose showed a significant decrease at 6 and 24 h after irradiation. Rats irradiated with the 5.0 Gy dose showed a significant decrease in the activity of ALT at 24 and 48 h after irradiation compared to controls.

The activity of aspartate aminotransferase was increased in all observation periods in chickens from the group irradiated with 2.0 Gy. The chickens irradiated with 4.5 Gy showed a decrease in this parameter, significant ( $p < 0.05$ ) at 6 and 48 h after irradiation. The activity of AST in chickens irradiated with 5.0 Gy was increased in all periods of investigation. A non-significant decrease in the activity of AST was detected in rats irradiated with 5.0 Gy in all periods of observation compared to controls.

The results of this study show different effects of ionizing radiation on two adaptive enzymes, both in chickens and rats.

*Gamma radiation, rat, chick, alanine aminotransferase, aspartate aminotransferase*

There is no doubt that use of nuclear power is one of the important discoveries of the 20<sup>th</sup> century. However, this discovery poses risks to humans and other living systems, whether in the form of nuclear weapons, increasing radioactive background, or accidents with technical equipment intended for peaceful use of nuclear energy. This indicates a need for continuous study of harmful effects of ionising radiation not only on mammals (Flory and Neuhaus 1976; Konečná and Kalina 1987; Maruyama and Feola 1987; Kalina et al. 1994; Staníková et al. 1994; Cigánková et al. 1996) but also on other species (Sesztáková 1996; Škardová et al. 1994).

At the same time, new radioprotective substances are searched for and tested with the aim to decrease the radiation damage (Fedoročko et al. 1992, 1994).

Adaptation of organisms to changes in the external environment is achieved through a sequence of very closely related adaptive processes which result eventually in a new balance between an organism and its environment. With respect to this it is important that individual changes do not necessarily result from the original stimulus but they constitute most frequently a response to the changes in the preceding link of the adaptive chain.

Adaptation may be induced by prolonged continuous or intermittent stimuli. They may include, besides cell plasma-nucleus relations, also such regulatory mechanisms that

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affect the organism as a whole. Adaptation involves almost all physiological functions. However, their final impact is manifested on a cellular level. "The adaptive enzymes" such as ALT and AST are of importance for diagnosis of diseases. Their activities change under the influence of various effects of environment and therefore they are still the aim of research work.

Enzymes play one of paramount roles in metabolic processes of the body. They attracted considerable attention both under physiological conditions and after the action of some pathogenic factors (Kottferová et al. 2001), e.g. ionising radiation (Toropila et al. 1995). The character of changes in the activity of enzymatic systems, either in cells or in animal tissues, is important for elucidation and understanding of the effect of ionising radiation on biological material.

Thus a question comes to the fore, whether radiation damage to cells, tissues or organisms, accompanied by the damage to their function and destruction and disintegration of many biologically important substances, is not caused by the damage to these metabolic regulators (Ševčíková et al. 2000).

The present study investigated changes in the serum activity of alanine aminotransferase and aspartate aminotransferase in laboratory rats and domestic fowl subjected to single, whole-body irradiation with gamma rays at doses of 2.0, 4.5 and 5 Gy.

#### Materials and Methods

The experiment was carried on 26 chickens - broiler hybrids 37-day-old (6 birds per each of three experimental groups, 8 birds in control group) and 45 chickens 28-day-old (6 birds per each experimental groups, 9 birds in the same control group). After previous disinfection of experimental facilities the chickens were allowed to acclimatize to experimental conditions. They were fed a granulated BR II diet and water *ad libitum*. In the period of acclimatization, they were preventively administered an intestinal disinfectant in the form of Cholesol tablets a.u.v., at a dose of 1 tablet per litre of water per day.

The second experiment was conducted with laboratory female rats of Wistar SPF breed, obtained from the breeding station VELAZ Prague, CR. Their initial mean body weight was 180 g. The experimental facilities were disinfected and the rats were kept for one week before the experiment in plexit cages on wooden shavings at 22-24 °C, relative humidity 60-70%, and natural illumination (Ondrašovič et al. 1997) to adapt to the experimental conditions. They had free access to pellets of LD diet and water (Toropila et al. 1996).

The animals were irradiated directly in adjusted plexit cages out at the Faculty of Natural Sciences of UPJŠ in Košice, using the CHISOSTAT<sup>60</sup>Co-Chirana apparatus. Chickens were divided into 3 groups according to the dose. At the beginning of the experiment the first group of chickens, 28-day-old, was irradiated with a single whole-body dose of gamma rays of 2.0 Gy, at the output of 0.27 Gy/min. The second group of 28-day-old chickens was irradiated with a single whole body dose of 4.5 Gy, at the output of 0.27 Gy/min. The rats and 37-day-old chickens were exposed to a single whole-body irradiation at a dose of 5.0 Gy of gamma rays and the output of 0.27 Gy/min.

Sham irradiation of the controls was carried out in parallel, i.e. the animals were exposed to the same treatments as the experimental ones except for the irradiation with gamma rays. Analyses were carried out at 6, 24 and 48 h post-irradiation. The animals were decapitated after an overnight fast (Bugarský et al. 1995). The mixed blood and lymph were collected in Petri dishes, centrifuged, and the serum obtained was used for determination of AST and ALT activities by means of Bio-la-test kits (Lachema-Brno). Measurements were carried out on a spectrophotometer Spekol 11. Statistical significance of differences between experimental groups and control (not irradiated) was determined by the unpaired *t*-test.

#### Results

The activity of alanine aminotransferase (Fig. 1) decreased in all experimental groups and periods of observation compared to controls. The difference was significant ( $p < 0.05$ ) in chickens irradiated with a dose of 2.0 Gy in all periods of observation. The chickens exposed to doses of 4.5 and 5.0 Gy exhibited a significant decrease ( $p < 0.05$ ) after 6 and 24 h. The rats exposed to a dose of 5.0 Gy showed significantly decreased activity of ALT at 24 and 48 h post-irradiation in comparison with the control.

The activity of aspartate aminotransferase (Fig. 2) increased in all periods of observation in chickens exposed to the 2.0 Gy dose. The difference was significant 24 h post-irradiation.

The chickens exposed to irradiation with 4.5 Gy had a decreased activity of AST. The difference was significant at 6 and 48 h post-irradiation. The chickens irradiated with 5.0 Gy showed an increased activity of AST in all experimental periods, significant at 6 h post-irradiation. Activity of AST decreased, though non-significantly, compared to controls in all periods under study in rats exposed to the dose of 5.0 Gy.

### Discussion

After irradiation of live systems the enzyme activity may either decrease or increase contrary to irradiation of pure isolated enzymes which results in no such changes (Toropila 1993). The damage to enzymes in the irradiated organisms may occur in the active protein centre, in the prosthetic components of enzymes, or enzymatic bonds. The increased activity, particularly of the adaptive enzymes, points to predominance of catabolic processes in the irradiated organisms related not only to changes in their cellular permeability and development of serious histological changes in hepatocytes early after irradiation but also to stimulation of *de novo* synthesis of transamination enzymes induced by increased secretion of the irradiated adrenal cortex.

When evaluating the changes that occur in the irradiated organism one must consider both external and internal factors that can affect the response to radiation (stress, species, age, sex). One of the factors may be the stress related to the handling of animals during irradiation. Therefore all control animals were exposed to the same handling procedures as the irradiated ones with the exception of irradiation with gamma rays.

The first symptoms of acute disease caused by irradiation include loss of appetite, nausea, vomiting and diarrhoea, i.e. symptoms associated with the gastrointestinal tract, i.e. the intestinal syndrome (Wisecup 1962). For this reason the animals were fasted overnight before the analysis. The differences in the activities of AST, observed after irradiation with doses of 4.5 Gy and 5.0 Gy, may be explained by different age of chickens.

Differences in the results obtained in broilers and laboratory rats exposed to the dose of 5.0 Gy may be ascribed to both differences in species and physiological-anatomical structure of the digestive system (Duke 1986). Differences in nitrogen metabolism between mammals

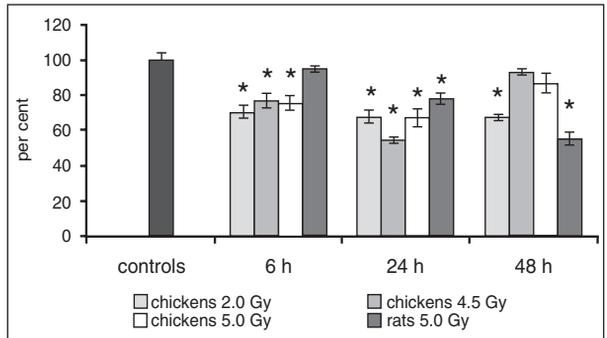


Fig. 1. Changes in the serum activity of ALT (%) after single whole-body irradiation. Significance of differences between irradiated and control groups is marked \* ( $p < 0.05$ ). The presented values are given against 100% level of the control values.

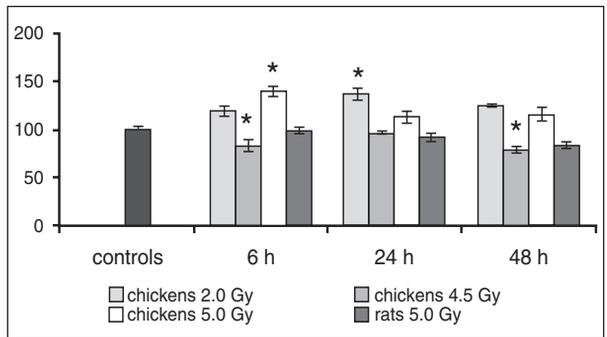


Fig. 2. Changes in the serum activity of AST (%) after single whole-body irradiation. Significance of differences between irradiated and control groups is marked \* ( $p < 0.05$ ). The presented values are given against 100% level of the control values.

and birds should also be considered. Uric acid is an important anti-oxidative metabolite and a scavenger of free radicals. Production of the complex of uric acid and iron inhibits considerably the oxidation of ascorbate and suppresses also peroxidation of lipids in liposomes and in the microsomal fraction of rat liver (Pecháň 1995). The results obtained contribute to the knowledge in the field of radiobiology in general particularly with respect to chickens for which they present new and original information.

### Vplyv ionizujúceho žiarenia na aktivitu vybraných adaptívnych enzýmov

Cieľom predloženej práce bolo sledovanie účinku ionizujúceho žiarenia na aktivitu niektorých adaptívnych enzýmov v sére kura domáceho a laboratórných potkanov.

Ožarovanie zvierat v upravených plexitových klietkach sme vykonali na Prírodovedeckej fakulte UPJŠ v Košiciach na prístroji CHISOSTAT  $^{60}\text{Co}$ -CHIRANA.

V experimente boli použitých 26 kurčiat vo veku 37 dní na začiatku experimentu a 45 kurčiat vo veku 28 dní na začiatku experimentu. Kurčatá podľa použitej dávky sme rozdelili do 3 skupín. Prvú skupinu kurčiat vo veku 28 dní na začiatku experimentu sme ožiarili jednorázovou celotelovou dávkou 2,0 Gy gama lúčmi pri príkone 0,27 Gy/min. Druhú skupinu kurčiat vo veku 28 dní na začiatku experimentu sme ožiarili jednorázovou celotelovou dávkou 4,5 Gy pri príkone 0,33 Gy/min. Potkany a tretiu skupinu kurčiat vo veku 37 dní na začiatku experimentu sme ožiarili jednorázovou celotelovou dávkou 5,0 Gy gama lúčov pri príkone 0,27 Gy/min.

Zdanlivo boli ožarované kontroly, t.j. podrobili sa všetkým procedúram manipulácie ako ožarované s výnimkou samotného ožarovania gama lúčmi. Analýzy sme vykonali 6, 24 a 48 hodín po ožarení.

Aktivita alanínaminotransferázy bola znížená vo všetkých sledovaných skupinách a obdobiach po ožarení. U potkanov ožarených dávkou 5,0 Gy bola aktivita ALT významne znížená 24 a 48 hodín po ožarení v porovnaní s kontrolou. Aktivita aspartátaminotransferázy bola zvýšená vo všetkých sledovaných obdobiach u skupiny kurčiat ožarených dávkou 2,0 Gy. U kurčiat ožarených dávkou 4,5 Gy bola aktivita znížená, štatisticky významne 6 a 48 hodín po ožarení. K zvýšeniu aktivity AST vo všetkých sledovaných obdobiach došlo u skupiny kurčiat ožarených dávkou 5,0 Gy. Nevýznamne znížená bola aktivita AST vo všetkých sledovaných obdobiach po ožarení v porovnaní s kontrolou u potkanov ožarených dávkou 5,0 Gy.

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