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A MICRONUCLEUS TEST FOR THE DJUNGARIAN HAMSTER, *PHODOPUS SUNGORUS*, IN ENVIRONMENTAL MONITORING

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A micronucleus test for the Djungarian hamster, *Phodopus sungorus*, in environmental monitoring. – Udroiu I. – The frequencies of micronucleated erythrocytes in bone marrow were compared with those in peripheral blood of captive Djungarian hamsters. The results indicate that the spleen probably removes micronuclei from peripheral blood. In order to detect genotoxic damage in Djungarian hamsters, the micronucleus test can be applied on bone marrow but, on peripheral blood, it must be limited to polychromatic erythrocytes.

Key words: genotoxicity, micronuclei, peripheral blood, bone marrow, environmental monitoring.

Тестирование содержания микронуклеусов у джунгарского хомячка (*Phodopus sungorus*) в экологическом мониторинге. – Удрою И. – Проведено сравнение числа микронуклеусов эритроцитов в костном мозге и периферической крови джунгарского хомячка (*Phodopus sungorus*) для установления возможностей этого показателя при оценке уровня патогенности факторов окружающей среды. Показано, что селезенка эффективно очищает кровь от микронуклеусов в периферической крови и, таким образом, использование данного теста должно быть ограничено анализом эритроцитов костного мозга, либо только полихроматическими эритроцитами периферической крови.

Ключевые слова: генотоксичность, микроядра, периферийная кровь, костный мозг, экологический мониторинг.

Cytogenetic assays can be used to test the adverse effect of physico-chemical agents and to monitor the environment. For laboratory studies, rodents are appropriate as they can be managed and handled with ease. In field studies, feral small mammals are suitable biomonitors as they concentrate environmental pollutants, with different powers of magnification depending on their trophic level (Talmage, Walton, 1991).

Along with other cytogenetic assays, the micronucleus test is used for biomonitoring as a simple assay to detect genotoxic damage produced by environmental pollution (Ieradi et al., 2003). Among Cricetinae, this assay was used in laboratories studies with samples taken from *Mesocricetus auratus* (Matter, Schmid, 1971; Ormiston et al., 1989) and *Cricetulus griseus* (Matter, Schmid, 1971).

Until now, the Djungarian hamster, *Phodopus sungorus* Pallas, 1773, has been mainly used for studies in endocrinology (Niehaus et al., 1997; Kondratyuk et al., 2004), although it was indicated as a suitable tool for cancer research and cytogenetic studies (Pogosianz, 1975).

The aim of this work was to test the suitability of the micronucleus test in bone marrow and peripheral blood of Djungarian hamsters.

Six Djungarian hamsters (*Ph. sungorus*) from commercial stocks were used in the experiment. Bone marrow cells from the femur were flushed out with foetal calf serum with 25 mM EDTA. Cell suspensions were gently pipetted and centrifuged at 800 rpm for 5 minutes. The pellet was re-suspended and smeared on clean slides. Blood was taken by cardiac puncture. Smears were coded, air-dried, fixed in methanol for 5 minutes and stained with May-Grünwald and Giemsa method.

For each animal, 2000 polychromatic erythrocytes (PCE) and 2000 normochromatic erythrocytes (NCE) were analysed, both in bone marrow and peripheral blood. The percentage of PCE in peripheral blood was evaluated on the first thousand erythrocytes counted.

As the absolute values of micronuclei were low and not normally distributed, all the values have been transformed adopting Cox's formula: $x_{tras} = (x+0.5)^{1/2}$. The transformed data have been analysed with Student's *t*-test to compare micronucleated erythrocyte mean frequencies. A difference of $p < 0.05$ was considered statistically significant.

The results of the micronucleus test in bone marrow and peripheral blood of Djungarian hamsters are shown in the table. The percentage of PCE in the peripheral blood is comparable to those of mice and other rodents. In absolute terms, it is rather high, but this is a frequent characteristics for rodents as the short life-time of their erythrocytes determines fast erythropoiesis.

Frequencies of micronucleated erythrocytes in Djungarian hamsters

№	Bone Marrow		Peripheral Blood		
	MNCE / 1000 NCE	MPCE / 1000 PCE	MNCE / 1000 NCE	MPCE / 1000 PCE	% PCE
1	2.5	2	0	1	3.1
2	0.5	1.5	0	2	4.2
3	2.5	1.5	0	1	3.8
4	1	0.5	1.5	0.5	5.1
5	1.5	2	0	2.5	4.5
6	2	3	0.5	1.5	5.3
Mean ± SD	1.67±0.82	1.75±0.82	0.33±0.60	1.42±0.73	4.3±0.82

In the bone marrow, the mean frequency of MPCE (1.75±0.82) was higher than the frequency of MNCE (1.67±0.82), but the difference was not significant. In the peripheral blood, the difference between the frequency of MPCE (1.42±0.73) and the frequency of MNCE (0.33±0.60) was greater and this difference was significant ($t = 3.04$, $p = 0.012$). Comparing the two tissues, the difference between the mean frequency of MNCE in bone marrow and in peripheral blood was significant ($t = 3.41$, $p = 0.007$). The difference between the mean frequencies of MPCE in bone marrow and in peripheral blood was not significant.

In some species, the spleen selectively removes micronuclei from circulation (Cristaldi et al., 2004). As PCE have a life-time of 1 – 2 days before maturing in the spleen and becoming NCE (Blue, Weiss, 1981), most of PCE observed in peripheral blood have not yet passed through the spleen. Therefore, comparing the frequencies of MNCE and MPCE in bone marrow and peripheral blood can provide indications if the spleen interferes. The frequencies of MNCE in peripheral blood are significantly lower

than those of MPCE, both in blood and bone marrow. This clearly indicates the presence of splenic removal of micronuclei.

The Djungarian hamster is indicated as a new appropriate model for cytogenetic studies (Pogosianz, 1975) and, in particular, its karyotype with comparatively few and large chromosomes ($2n = 28$) makes micronuclei easier to be detected. This species may also be suitable for biomonitoring purposes, as its feeding habit, comprising seeds and invertebrates, makes it susceptible to contaminant exposure. Therefore, the micronucleus test can be proficiently used with Djungarian hamsters both in laboratory and field studies. However, if peripheral blood samples are used, only MPCE can be scored.

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