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# A preliminary study of the submandibular gland of the rat after long-term cadmium intoxication

Elżbieta Czykier[1], Janusz Dzięcioł[2], Anna Zalewska[3], Krzysztof Zwierz[3]

[1]*Department of Histology and Embryology, Medical University, Białystok, Poland*
[2]*Department of Anatomy, Medical University, Białystok, Poland*
[3]*Department of Pharmaceutical Biochemistry, Medical University, Białystok, Poland*

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*The aim of the study was to establish to what degree the 24-week exposure of a rat to 5 and 50 mg Cd/dm$^3$ affects the proliferating activity of cells with PCNA and Ki-67 nuclear immunoreactivity in the submandibular gland cells. The control animals received only redistilled water to drink. The group I rats were given 5 mg Cd/dm$^3$, while the group II animals were given 50 mg Cd/dm$^3$. The highest concentration of cadmium was observed in group II, with a concomitant increase in the number of PCNA-positive cells. In group I, cadmium concentration was significantly less compared to group II, and there were fewer PCNA-positive cells. The reaction for Ki-67 in both experimental groups was negative.*

**key words: rat, submandibular gland, cadmium intoxication**

## INTRODUCTION

Cadmium reaches all organs of the body, including the salivary glands [3, 4]. The accumulation of this metal results in the synthesis of metallothionein (MT) in the cells of the salivary glands [7]. Moreover, experimental animals show a decrease in amylase activity, a drop in protein content and fluctuations in saliva calcium levels [1, 2]. These observations seem to confirm that cadmium substantially disturbs the functioning of the salivary glands. However, the available literature provides no information concerning human Ki-67 antigen (Ki-67) and proliferating cell nuclear antigen (PCNA) expression in the salivary glands of rats subjected to chronic exposure to cadmium. We therefore decided to determine to what degree a 24-week exposure to cadmium in a dose of 5 and 50 Cd/dm$^3$ affects the proliferating activity of cells with PCNA and Ki-67 nuclear immunoreactivity in the submandibular glands of the rat.

## MATERIAL AND METHODS

The study used 21 young male Wistar rats, allocated to 3 groups of 7 animals each. 7 control rats received redistilled water to drink. 14 experimental rats were given an aqueous solution of cadmium chloride ($CdCl_2$): 7 rats in group I received a dose of 5 mg Cd/dm$^3$ and the remaining 7 in group II — 50 mg Cd/dm$^3$. The animals were sacrificed in pentobarbital narcosis. Both submandibular glands were weighed and the cadmium concentration was measured in one of them by the atomic absorption spectrometry method. The other salivary gland was fixed in Bouin's fluid. Specific monoclonal mouse antibodies against PCNA 1:50 and anti human Ki-67 1:50 were employed for immunocytochemical reaction. The sections were counterstained with haematoxylin. Analysis of variance was used to determine the statistical significance of the results. The package STATISTICA 5.0 was applied for the calculations. The

Address for correspondence: Elżbieta Czykier, Department of Histology and Embryology, ul. Kilinskiego 1, 15–089 Białystok, e-mail: czykier@amb.edu.pl

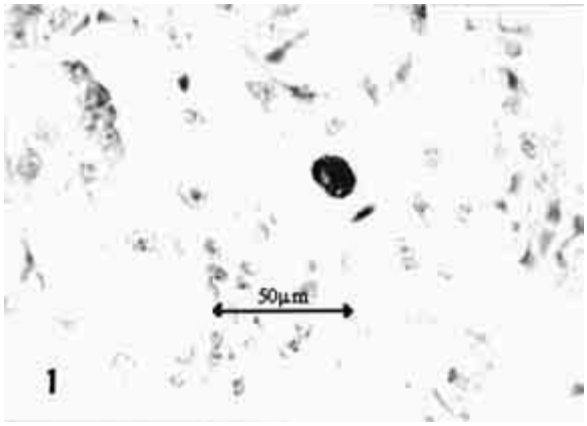


**Figure 1.** Control. Strong immunoreactivity for PCNA in the nucleus of the serous cell.

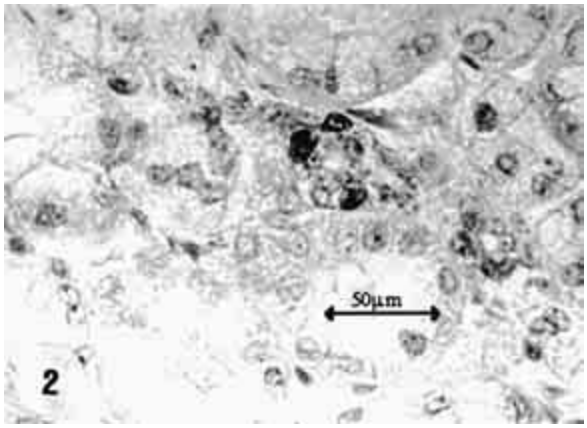


**Figure 2.** Group I. 5 mg Cd/dm$^3$. Immunostaining of PCNA is present in the nuclei of serous cells.

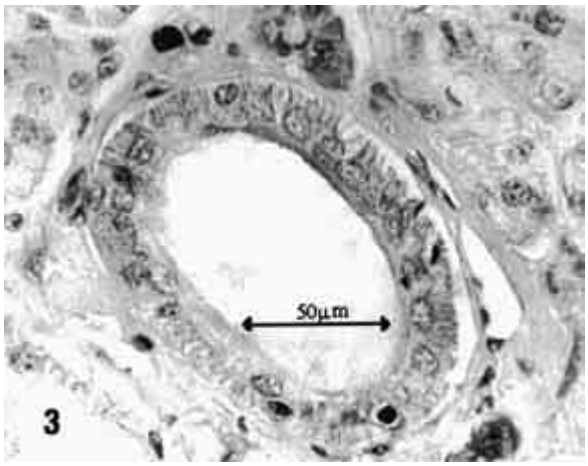


**Figure 3.** Group II. 50 mg Cd/dm$^3$. Positive reaction for PCNA in the nuclei of serous cells and in the ductal epithelial cells.

experiment received acceptance no 2001/44 from the Local Ethical Committee for Animal Testing in Białystok.

## RESULTS AND DISCUSSION

After 24 weeks of the experiment cadmium concentration in the submandibular glands of the control rats was 0.0293 $\mu$g Cd/g of wet tissue, in rats exposed to 5 mg Cd/dm$^3$ it was 0.119 $\mu$g Cd/g of wet tissue and in those exposed to 50 mg Cd/dm$^3$ it was 1.167 $\mu$g Cd/g of wet tissue. The cadmium concentration in the submandibular gland of the control rats was significantly lower compared to group I rats ($p = 0.00135$) and group II rats ($p = 0.003989$). We also observed statistically significant differences in the concentration of Cd in the submandibular glands between groups I and II ($p = 0.0054$). The immunocytochemical reactions against PCNA showed a very weak reaction in the submandibular gland in the control rats. Only the nuclei of single serous cells exhibited a positive reaction for PCNA. In the submandibular gland in group I, the PCNA-positive cells were more frequently observed than in the control. In some places agglomerations of PCNA--positive cells were seen. In the salivary glands of group II rats the number of PCNA-positive cells was substantially increased compared to group I and the control. Agglomerations of PCNA-positive cells were also observed in this group. The reaction for Ki-67 in the control salivary glands was very weak. A positive reaction was found only in a few cells. However, in groups I and II, the reaction for Ki-67 was negative. The present findings seem to prove that long-term administration of cadmium to rats results in its accumulation in the submandibular glands, its concentration in tissues being dose-dependent. The concomitant increase in the number of PCNA-positive cells in the salivary glands in group I and II rats may be associated both with enhanced proliferation of these cells and DNA repair processes. PCNA functions as a co-factor for DNA-polymerase in the S-phase and in DNA synthesis associated with DNA repair [6]. However, in our experiment the increase in the number of PCNA-positive cells in both experimental groups and the absence of Ki-67-positive cells suggest the predominance of DNA repair rather than proliferation, as it is Ki-67 that is indicative of proliferation [5]. Lack of this reaction seems to confirm the predominance of DNA repair in the submandibular glands in rats after chronic exposure to cadmium.

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