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# EFFECTS OF ORAL ADMINISTRATION OF CADMIUM TO PIGLETS ON ITS CUMULATION IN THE TISSUES AND ON CATALYTIC ACTIVITIES OF ASPARTATE AMINOTRANSFERASE, ALANINE AMINOTRANSFERASE AND LACTATE DEHYDROGENASE IN THE BLOOD SERUM

L. MALOTA, T. RUSEK and O. SYNEK

Department of Chemistry, Physics and Biochemistry, University of Veterinary Science, 612 42 Brno

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**Abstract**

M a l o t a L., T. R u s e k, O. S y n e k: *Effects of Oral Administration of Cadmium to Piglets on Its Cumulation in the Tissues and on Catalytic Activities of Aspartate Aminotransferase, Alanine Aminotransferase and Lactate Dehydrogenase in the Blood Serum.* Acta vet.Brno,56,1987: 79-85.

Cadmium was administered to experimental weaned piglets in feed at two different concentrations (50 and 200 mg Cd.. $kg^{-1}$) to study its effects on catalytic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) and on cadmium ion levels in the blood serum and tissues. The cadmium exerted an activating effect on LHD and inhibitory effect on AST catalytic activity at both concentrations. The catalytic activity of ALT, however, was affected by each of the two concentrations in a different way: in animals treated with cadmium at the lower rate it decreased beginning day 7 and fell to the nadir on day 14, whereas in animals treated at the higher rate it rose from day 7, peaking on day 10 of the experiment. Cadmium ion concentration of the blood serum was highest on days 2 and 10, this effect being particularly marked in piglets receiving cadmium ions at the lower rate. The bulk of cadmium was cumulated in the kidney and liver.

*Aspartate aminotransferase, alanine aminotransferase, liver, kidney, brain, lactate dehydrogenase, cadmium, piglet.*

The toxicity of cadmium after single or prolonged administration becomes manifested, owing to its considerable cumulation in the body, by impairment of the kidney. In man the biological half-life of cadmium is particularly long. As much as 50 % of cadmium cumulates mainly in the liver and kidney. Its cumulation is particularly high, in the renal cortex, causing damage to the tubules (N o r d b e r g 1978). Sublethal doses generally produce non-specific disturbances characterized by anaemia, proteinuria and impaired mineralization of the bones (H a s t i n g s 1978). Cadmium has not been demonstrated to constitute an essential part of an enzyme, but in vitro it

can replace, e.g., zinc in certain Zn-metalloenzymes which, in consequence, may show a change in specifity and catalytic activity.

The object of the present study was to find what effects different cadmium additions to the ration would have on catalytic activities of lactate dehydrogenase (LDH) EC 1.1.1.27 , aspartate aminotransferase (AST) EC 2.6.1.1 and alanine aminotranferase (ALT) EC 2.6.1.2 in experimental piglets and to determine cadmium levels in the blood serum and tissues of these animals.

## Materials and Methods

Landrace weaned piglets averaging 17 kg in body mass were used. They were divided into groups of 3 or 6 animals and were fed ČOS II, a Czechoslovak commercial starter, supplemented with cadmium salt at two different concentrations (50 and 200 mg $Cd.kg^{-1}$) in the form of 2 $CdCl_2.5\ H_2O$ as shown in Table 1. The supplements were added to weighed quantities of feed by mixing in a blender. The piglets were fed ad libitum, the mean daily ration available being 1 kg per animal per day. They were kept in cages under good hygienic conditions for 32 days. Piglets receiving cadmium at the higher rate and half of the animals in the other two groups were killed after 14 days. The design of the experiment is shown in Table 1.

Blood samples were withdrawn from the cranial vena cava into sterile test tubes. Serum obtained after blood clotting was used directly for determination of LDH, AST and ALT catalytic activities and for assessment of cadmium ion levels by atomic absorption spectrometry. Catalytic activity of LDH was determined by the method following the development of pyruvate from lactate (D z ú r i k 1967). Catalytic activities of AST and ALT were determined by means of Bio-La Test kits, Lachema. Optical measurement was carried out in a Varian Techtron apparatus, model UV VIS 635.

To determine cadmium concentration of the feed mixture, a 2 g feed sample was oven-dried at 105°C to a constant mass. Afterwards 5 ml of concentrated sulphuric acid was added and the sample was allowed to stand for two hours. Then 20 ml of concentrated nitric acid was added and the sample was heated in a Gorsuch apparatus for two hours and subsequently cooled. The clear mineralizate was then made up to 100 ml volume with deionized water. The blood sera and tissues were processed in the same way except that drying was omitted. The determination itself was made by flameless atomization in a Varian Techtron CRA-90 in connexion with a Varian Techtron AAG apparatus. The detection limit of the method was 0.005 $mg.kg^{-1}$.

## Results

The initial blood serum cadmium ion levels in the experimental groups as well as the cadmium ion levels found in the serum of control animals were at the limit of measurability. In Group C 50 animals receiving cadmium at the lower rate the blood serum cadmium ion level rose to 0.220 µg/ml on day 2, declined to 0.01 µg/ml on day 4, reached 0.695 µg/ml on day 10 and declined afterwards, falling to 0.006 µg/ml on day 26 (Fig. 1).

In Group C 200 piglets receiving cadmium at the higher rate the blood serum ion level rose, amounting to 0.403 µg/ml on day 4 and to 0.545 µg/ml on day 10. After reaching the second peak, it fell, declining to the final value of 0.018 µg/ml on day 14 of the experiment (Fig. 1).

The catalytic activity of AST was reduced in both experimental groups throughout the experiment as compared with the controls. This decrease was

Table 1
**Design of the experiment and cadmium concentrations determined in the feed mixture**

| Group | No. animals No. days of administration | $Cd^{2+}$ ion concentration added $mg.kg^{-1}$ | determined $mg.kg^{-1}$ $\bar{x}$ | $s_{\bar{x}}$ |
|---|---|---|---|---|
| Controls | 6/14<br>3/32 | 0 | 0.62 | 0.1 |
| C 50 | 6/14<br>3/32 | 50 | 42.35 | 1.47 |
| C 200 | 3/14 | 200 | 130.60 | 5.97 |

Table 2
Cadmium concentrations in the liver, kidney and brain ($mg.kg^{-1}$).

| Group (days) | | Liver | Kidney | Brain |
|---|---|---|---|---|
| Controls (14) | $\bar{x}$ | 0.145 | 0.141 | 0.161 |
| | $s_{\bar{x}}$ | 0.015 | 0.023 | 0.013 |
| Controls (32) | $\bar{x}$ | 0.120 | 0.213 | 0.059 |
| | $s_{\bar{x}}$ | 0.018 | 0.016 | 0.009 |
| C 50 (14) | $\bar{x}$ | 1.114 | 5.532 | 0.082 |
| | $s_{\bar{x}}$ | 0.093 | 0.342 | 0.007 |
| C 200 (14) | $\bar{x}$ | 2.177 | 18.350 | 0.093 |
| | $s_{\bar{x}}$ | 0.084 | 0.380 | 0.015 |

more pronounced in the group receiving cadmium at the higher rate (Fig. 2). The catalytic activity of ALT was affected in a different way. During the first 6 days of the experiment the values recorded in the two experimental groups showed little difference from those found in the controls. Beginning day 7 the catalytic activity of ALT declined in the group receiving cadmium at the lower rate, falling to the nadir on day 14, but rose in Group C 200, peaking there on day 10 (Fig. 3).

The catalytic activity of LDH rose in both experimental groups, peaking on days 8 and 10 respectively (Fig. 4).

The cadmium concentrations in the liver, kidney and brain are shown in Table 2.

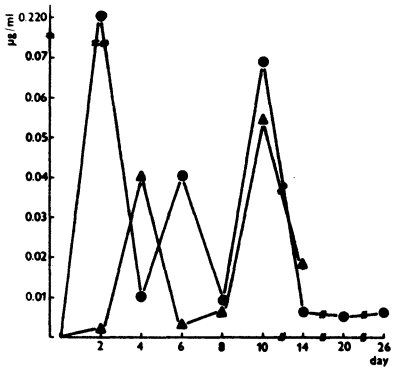


Fig. 1. Blood serum cadmium levels in the groups of piglets given cadmium at the lower (closed circles) and higher rate (triangles).

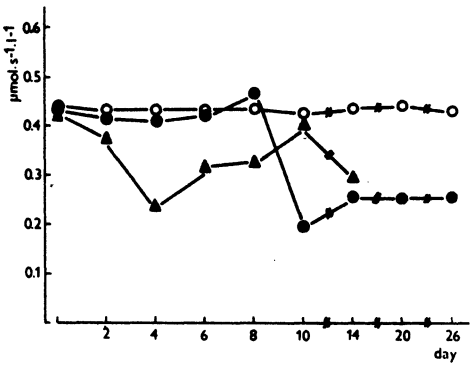


Fig. 2. Catalytic activity of AST in control piglets (open circles) and in the groups given cadmium at the lower (closed circles) and higher rate (triangles).

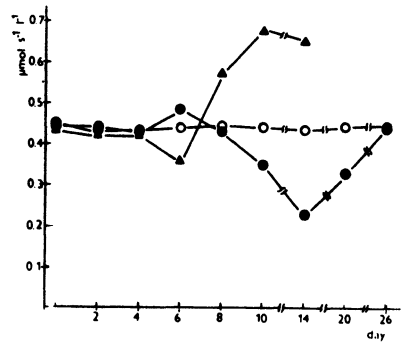


Fig. 3. Catalytic activity of ALT in control piglets (open circles) and in the groups given cadmium at the lower (closed circles) and higher rate (triangles).

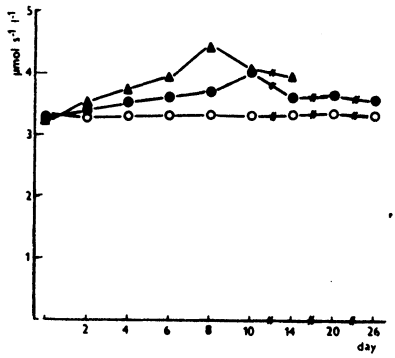


Fig. 4. Catalytic activity of LDH in control piglets (open circles) and in the groups given cadmium at the lower (closed circles) and higher rate (triangles).

## Discussion

In the present study the blood serum cadmium ion levels of piglets receiving starter supplemented with 2 $CdCl_2.5\ H_2O$ showed a manifold rise, compared with that of the controls which was at the limit of measurability. In the first 4 days the capacity of the body to resorb cadmium ions is apparently different or, possibly, the resorption is affected by the size of the intake of cadmium ions themselves. The catalytic activity of enzymes in the blood serum

depends on inhibitory effects of cadmium ions and not on the extent of damage to the body, particularly cell membranes, by these ions. Also the results reported by other writers concerned with this problem are differing (Wachsmut and Thorhorst 1974; Jacobson and Turner 1980; Kačmár et al. 1978). Our results agree with the data obtained upon parenteral administration of cadmium in that the LDH catalytic activity rose at both cadmium treatment levels used in our orally treated animals. $Cd^{2+}$ has apparently an activating effect on LDH activity, similar to that demonstrated for $Zn^{2+}$ in vitro by Musilová and Malota (1970). The differing results for AST and ALT catalytic activity can presumably be accounted for by different modes of administration of $Cd^{2+}$ ions and by oral long-term exposure as against single parenteral administration used by the aforementioned authors. Upon oral administration more regulating mechanisms are involved in the entry of individual ions. The most important of these mechanisms can presumably be seen in mutual ion effects on the resorption of individual ions and in subsequent induction of the production of specific proteins of differing affinity to individual ions mainly in the kidney and liver. The data reported by Synek et al. (1980) provided evidence to indicate that the interaction between cadmium, copper and zinc occurs already during resorption from the digestive tract. Zinc supports the resorption of cadmium and promotes its cumulation in the kidney. According to Sato and Nagai (1978), Cherian and Goyer (1978) and Korkeala and Sankari (1980) the toxicity of cadmium is considerably reduced by production of highly active catalase in microsomes of the liver tissue which breaks down aerobically produced hydrogen peroxide that, together with cadmium, is capable to induce destabilization and destruction of membrane complexes. Although piglets are susceptible to toxic effects of cadmium ions, our results support the observations that well-balanced representation of macro- and microelements in a ration as is the case with ČOS II enables the animal body, owing to operation of a number of adaptive mechanisms, to prevent a toxic element from manifesting its negative action for a certain length of time. The values obtained for catalytic activities of the enzymes under study do not provide unequivocal evidence of injury to the tissues, particularly to the liver. They demonstrate, however, that cadmium may be the cause of impaired homeostasis, presumably by destabilization of cell structures.

The cadmium levels in the tissues of control animals are in agreement with relevant published data (Salmi and Hirn 1984). The fact that the kidney cadmium level of control animals rose with time confirms that cadmium tends to cumulate in the body even under conditions of its negligible intake. In Group C 50 piglets receiving $Cd^{2+}$ ions at the lower rate this trend is still more pronounced, being observed also in the liver. Administration of cadmium at the higher rate in Group C 200 resulted in more cadmium accumulation in the kidney than in the liver tissue, which suggests a higher cumulation capacity of the former upon an acute attack. From the course of changes in blood serum cadmium concentration in the two experimental groups and in cadmium levels of the liver and kidney it can be concluded that under conditions of short-term low cadmium intake this metal is absorbed rather easily from the digestive tract and circulates in the blood stream, being bound to albumin or other non-specific proteins or blood plasma complexes. Prolonged low cadmium intake or a heavier cadmium load gives rise to a specific protein, metallothionein, in the liver. Some of this protein is deposited there in non-toxic form but the bulk of it is transported to the kidney where specific metallothionein is induced by the action of both cadmium from degradated liver metallothionein and loosely bound $Cd^{2+}$ ions transported by plasma proteins.

In the kidney cadmium is then deposited in non-toxic binding to metallothionein from which it can be only very slowly released and excreted from the body (C o u s i n s 1983).

**Vliv orální aplikace kadmia na jeho kumulaci ve tkáních a katalytickou aktivitu aminotransferáz a laktátdehydrogenázy v séru selat**

U selat po odstavu byl sledován účinek iontů kadmia v přijímaném krmivu ve dvou rozdílných koncentracích (50 resp. 200 mg $Cd.kg^{-1}$) na katalytickou aktivitu aminotransferáz a laktátdehydrogenázy, hladinu iontů kadmia v krevním séru a tkáních pokusných zvířat. Byl prokázán aktivační účinek na katalytickou aktivitu LDH a inhibiční účinek na AST u obou koncentrací. U ALT nižší dávka způsobuje pokles její aktivity od 7. dne pokusu a vyšší dávka vzestup katalytické aktivity s maximem ve 14. resp. 10. dnu pokusu. Obsah iontů kadmia v krevním séru byl nejvyšší ve 2. a 10. dnu, především při podávání nižší dávky kademnatých iontů. Největší množství kadmia je kumulováno v ledvině a játrech.

Влияние орального применения кадмия на его кумуляцию в тканях и каталитическую активность аминотрансфераз и лактатдегидрогеназы в сыворотке поросят

У отъемных поросят проводились исследования воздействия ионов кадмия в потребляемых кормах в двух разных концентрациях (50 или 200 мг кадмия . $кг^{-1}$) на каталитическую активность аминотрансфераз и лактатдегидрогеназы, уровень ионов кадмия в кровяной сыворотке и тканиях подопытных животных. Было установлено активирующее воздейсвие на каталитическую активность LDH и ингибирующее воздействие на AST у обеих концентраций. У ALT более низкая доза вызывает понижение активности с 7 суток эксперимента и более высокая доза - увеличение каталитической активности с максимумом на 14 или 10 сутки эксперимента. Максимальное содержание ионов кадмия в кровяной сыворотке было установлено на 2 и 10 сутки, прежде всего при даче более низкой дозы ионов кадмиа. Самое большое количество кадмия накапливается в почках и печени.

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