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# Long-Term Treatment with Cobalt Chloride Affects Mouse Development

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The study aims to determine the influence of long-term cobalt chloride treatment on mouse development. Treatment for 60 or 90 days with $CoCl_2$ including late embryogenesis induced significant body and organ weight changes in the developing mice compared to control animals. Mice exposed to $CoCl_2$ for two or more than 10 days before birth were affected to a different degree. Hematological parameters such as hemoglobin content and plasma iron concentration were also affected. Mice treated with $CoCl_2$ showed reduced body weight and increased spleen, liver and kidney weight indices compared to age-matched controls. Long-term embryonical exposure increased spleen and liver weight in day 60 mice and increased spleen and kidney weight in day 90 mice. Their hemoglobin content was lower than that of mice with short embryonic exposure. Plasma iron concentration was higher in day 90 mice with long embryonic treatment. Results indicate that $CoCl_2$ treatment during pregnancy affects the offspring.

*Key words:* cobalt chloride, developing mice, *in vivo* treatment, body and organ weight, hematological parameters

## Introduction

Cobalt (II) bioaccumulates in different organs and can further cause deleterious damage [8]. Cobalt chloride ($CoCl_2$) treatment was shown to improve hematological parameters - red blood cell count, hemoglobin content, hematocrit on one hand and to reduce body and organ weight, on the other [2, 7]. Treatment with $CoCl_2$ is shown to develop oxidative stress in rat liver [5]. Garoui et al. [4] find that exposure of rats to the compound during late pregnancy and early postnatal period affects antioxidant enzyme activities and lipid peroxidation indicating liver damage in treated mothers and their pups. Kidneys are very sensitive to hypoxia responding to changes in oxygen delivery with altered erythropoietin production, thus affecting erythropoiesis [6]. As hypoxia mimicking agent $CoCl_2$ will affect iron metabolism as well. Studies show strong relationship between cobalt blood and serum concentrations and iron status [1]. There are insufficient data regarding the influence of $CoCl_2$ on the offspring of pregnant mice.

The ***aim*** of the study was to determine the influence of long-term cobalt chloride treatment on mouse development.

## Material and Methods

### Animal model

Pregnant ICR mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) treatment at daily dose of 125 mg/kg which continued until day 60 or day 90 of the newborn pups. The compound was dissolved and obtained from drinking tap water. Animals were fed a standard diet and had access to food *ad libitum*. The newborn pups were sacrificed on days 60 and 90 which correspond to different stages of development. Organ weight indices of spleen (SI), liver (LI) and kidneys (KI) were calculated as a ratio of organ weight to body weight. Whole blood samples were obtained, centrifuged and plasma was stored at -20°C until further analysis. Blood plasma samples were used for measuring hemoglobin (Hb) and iron (Fe) concentration. Hb concentration was determined by hemiglobincianide method (HiCN) [3]. Plasma iron concentration (transferrin-bound iron) was measured using "Iron Liquid" analytical kit based on Ferene-S as a chromogen (Sentinel Diagnostics, Italy).

### Statistical analysis

The obtained results are presented as mean value ± SD. Statistical significance between the experimental groups was determined using Student's *t*-test. Difference was considered significant at $p<0.05$.

## Results and Discussion

Long-term treatment (for 60 or 90 days) with $CoCl_2$ including during late embryogenesis induced significant body and organ weight changes in the developing mice compared to control animals. Mice exposed to the compound for two and/or more than 10 days before birth were affected to a different degree. Animals treated with $CoCl_2$ showed reduced body weight [Fig.1]. Day 60 mice with longer embryonical treatment had lower body weight compared to those exposed to the compound for two days before birth. Surprisingly day 90 mice with longer embryonical treatment were heavier than those in the other experimental group which could be due to the small number of animals in the group. Hematological parameters such as hemoglobin content (Hb) and plasma iron (Fe) concentration were also affected [Figs. 2, 3]. Hemoglobin was increased in day 60 and day 90 mice treated with $CoCl_2$ with short embryonical exposure compared to controls. Longer treatment before birth led to decreased Hb values in both day 60 and day 90 mice. The latter showed the lowest content. The result indicates that prolonged embryonical treatment disturbs erythropoiesis and it is not restored even in adulthood. Plasma Fe concentration was decreased in the treated animals except in day 60 mice with short embryonical treatment. The results are in agreement with those of Barany et al. [1] showing that cobalt treatment affects iron metabolism. Increased spleen, liver and kidney weight compared to age-matched controls were found [Figs. 4-6]. Long-term embryonically exposed mice showed increased spleen and liver weight in day 60 mice and increased spleen and kidney weight in day 90 mice. Histological studies are required to elucidate the changes induced by $CoCl_2$ in order to explain the observed hematological alterations. Morphological changes in the spleen and liver could indicate altered enzyme activities thus affecting iron metabolism. Changes in the kidneys will alter erythropoietin production which will affect hemoglobin synthesis and thus oxygen supply.

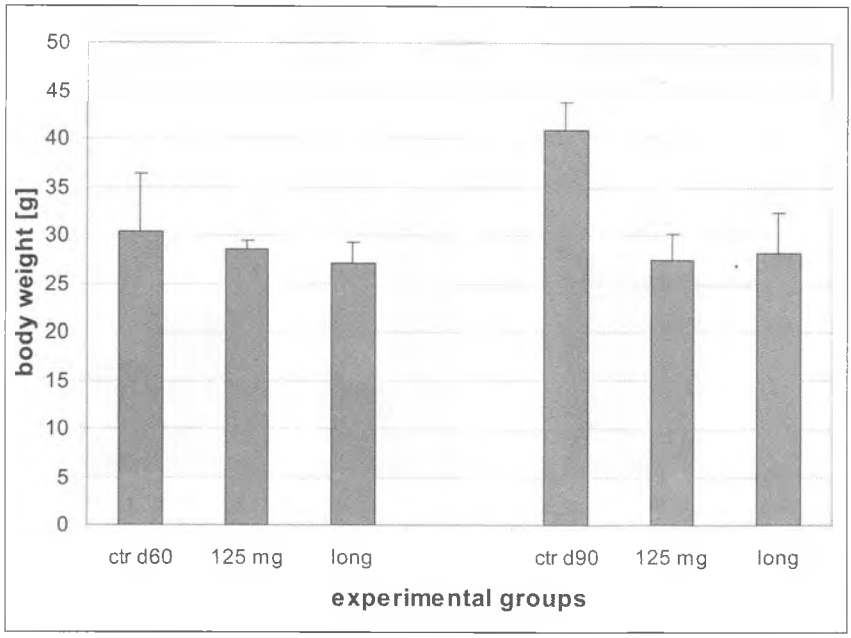


Fig. 1. Body weight in control and treated mice d 60 and d 90 mice. "Long" indicates long pregnancy treatment

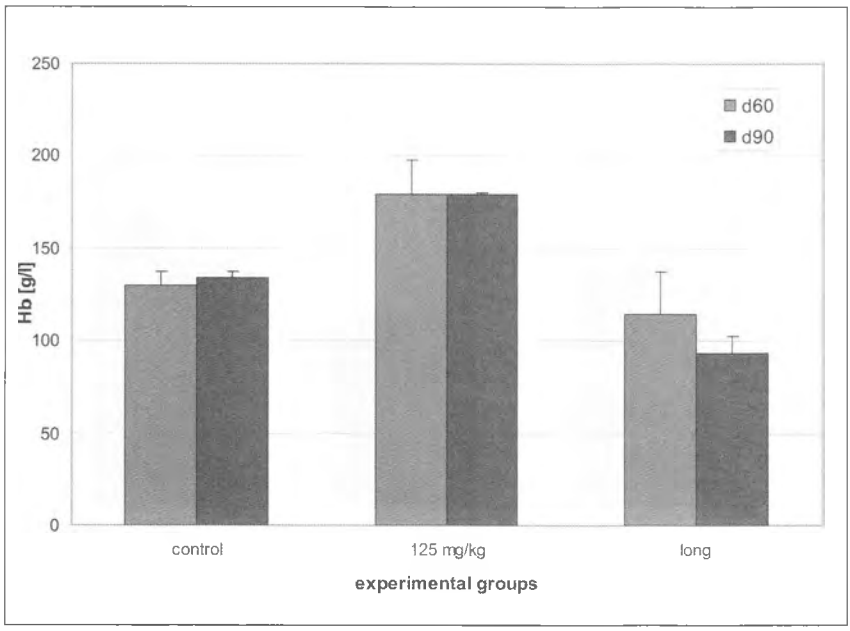


Fig. 2. Hemoglobin content in control and treated mice d 60 and d 90 mice. "Long" indicates long pregnancy treatment

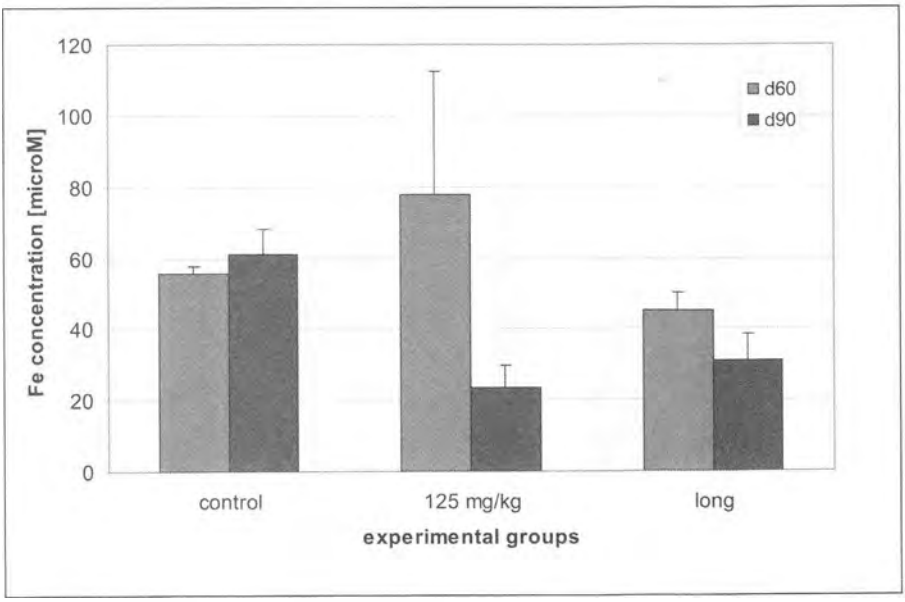


Fig. 3. Plasma iron concentration in control and treated mice d 60 and d 90 mice. "Long" indicates long pregnancy treatmen

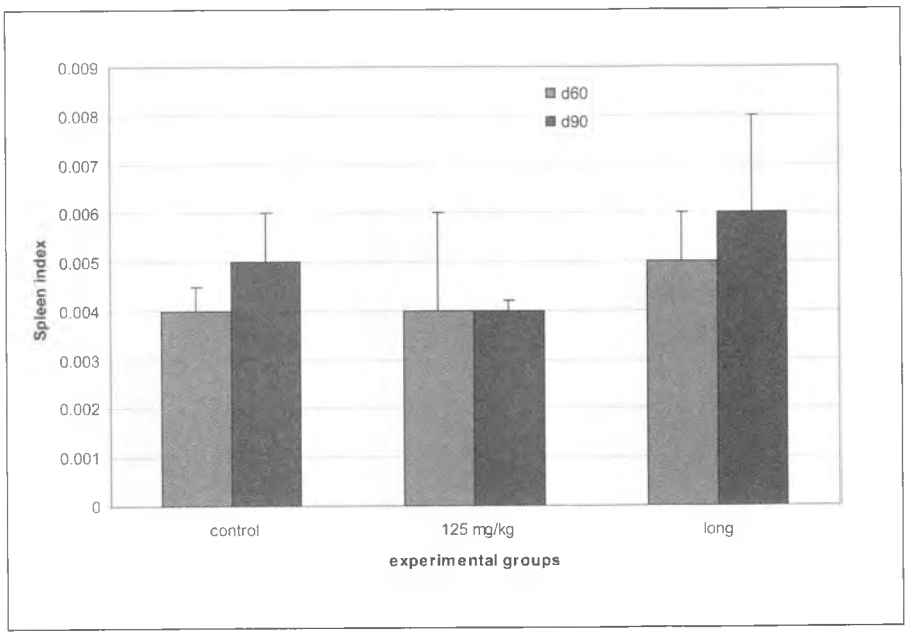


Fig. 4. Spleen index in control and treated mice. "Long" indicates long pregnancy treatment.

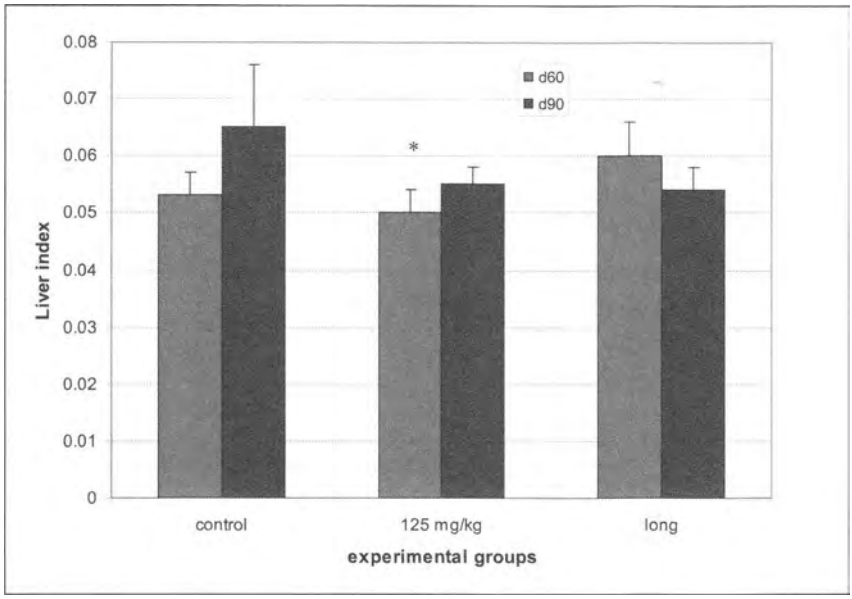


Fig. 5. Liver index in control and treated mice. "Long" indicates long pregnancy treatment, * $p<0.05$

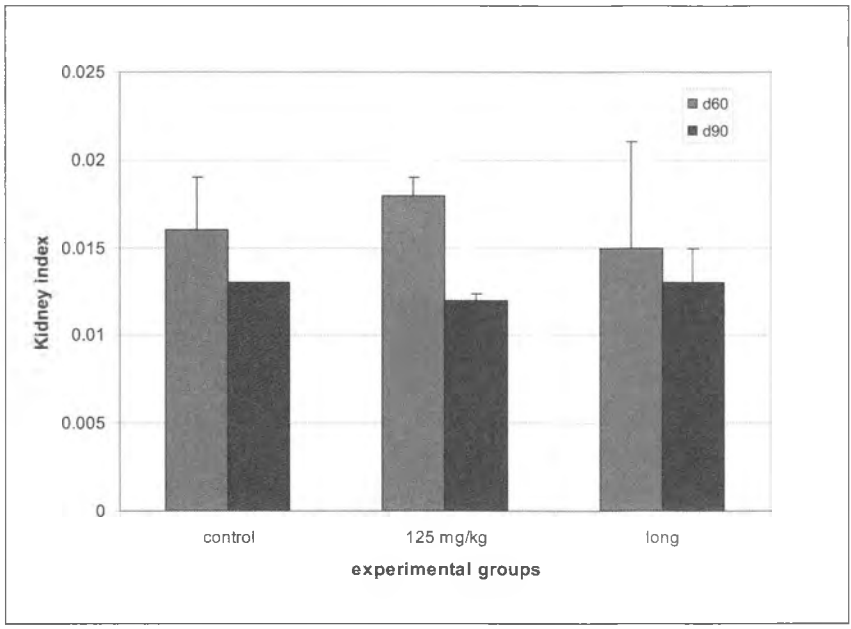


Fig. 6. Kidney index in control and treated mice. "Long" indicates long pregnancy treatment

## Conclusions

Long-term treatment with $CoCl_2$ induces changes in organ weight and some hematological parameters. Mice exposed to the compound show reduced body weight as well compared to untreated control animals. The duration of embryonical treatment is also an important factor. Results indicate that long-term $CoCl_2$ treatment during pregnancy affects the offspring by reducing hemoglobin content and plasma iron concentrations and these changes remain present even in adulthood.

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