Effect of cadmium on polyribosome structure and function in mouse liver

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Summary. Cadmium chloride injected to mice (20 μmoles/kg) provokes in the livers a degradation of polyribosomes and diminishes their protein synthetic ability measured in vitro. $CdCl_2$ added in a final concentrations between 30 and 100 μM to the protein synthetic cell-free system derived from livers of control mice inhibits its activity.

Cadmium is becoming an increasingly important environmental toxic agent. It produces a wide range of toxic effects in man and animal[2–4]. Some effects of cadmium on protein and nucleic acid biosynthesis have been described recently. After administration of cadmium, cadmium-binding protein appears in the cytosol of rat liver, and it is proposed that cadmium induces its synthesis, stimulating specific mRNA synthesis[5,6]. At the same time, cadmium inhibits temporarily the overall RNA-polymerase activity and diminishes the incorporation of a radioactively labelled aminoacid into liver proteins[7]. In partially hepatectomized rats, cadmium decreases DNA synthesis[8]. Most conditions in which the rate of protein synthesis is decreased are accompanied by a disaggregation of polyribosomes and by an increase in the proportion of monomeric ribosomes in the total ribosomal population of the cell[9]. In this paper we shall present a similar effect provoked by cadmium in mouse liver.

Materials and methods. Male albino mice weighing 25–30 g maintained on standard 'Pliva-Zagreb' diet ad libitum were used. The polyribosome fraction and cytosol were isolated from pooled livers of 3 mice as described previously[10] using the α-amylase treatment of postmitochondrial supernatant for isolation of polyribosomes. In the present work, 'Boehringer' α-amylase was used.

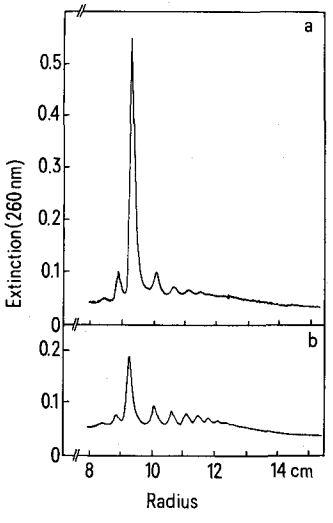

Fig. 1. Sedimentation pattern of polyribosome fraction isolated from livers of *a* cadmium-treated and *b* control mice. The treated mice were injected i.p. with $CdCl_2$, 20 μmoles/kg 1 h before killing. In three separate experiments similar results were obtained.

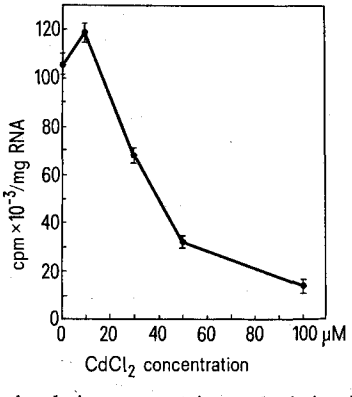

Fig. 2. Effect of cadmium on protein synthesis in vitro. $CdCl_2$ was added to cell-free systems derived from livers of control mice in the final concentrations indicated. The systems were incubated for 30 min at 37 °C. The results are the means and SE of values obtained in three separate incubations.

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Protein synthesis in cell-free systems derived from the livers of cadmium-treated and control mice

Group	Cell fraction		Incorporation of ^{14}C-leucine into proteins cpm × 10^{-3}/mg RNA
	Polyribosome	Cytosol	
1	Cadmium-treated	Cadmium-treated	39.64 ± 7.29*
2	Cadmium-treated	Control	43.31 ± 5.90*
3	Control	Cadmium-treated	80.64 ± 6.59
4	Control	Control	83.52 ± 13.02

The cell-free systems were composed from the cell fractions derived from livers of cadmium-treated or control mice. The mice were treated as described in figure 1. Cell-fractions were incubated for 30 min in a system composed as described previously[9]. Results are the means and SE of values obtained in 7 separate experiments; *indicates significant difference for Student's t-test with $p < 0.05$ between a particular group and group 4.

To analyze polyribosome sedimentation pattern, aliquots of polyribosome fractions containing approx. 0.05 mg of RNA were layered on 14.5 ml of 15–35% (w/w) convex sucrose density gradient[11]. After centrifugation in a 6×16.5 ml MSE swingout rotor for 4 h at 4 °C and $110{,}000 \times g$ (r_{av}. 10.97 cm) the gradients were displaced upwards through a Perkin-Elmer 124 spectrophotometer and extinction at 260 nm was continuously recorded. Protein synthesis in a cell-free system was measured as described previously[10], except that cytosol was used (1.5 mg of protein per 0.5 ml of incubation mixture) instead of pH 5 fraction.

Results and discussion. Sedimentation pattern of the polyribosome fraction derived from the livers of mice killed 1 h after i.p. injection of 20 μmoles/kg cadmium chloride shows a high increase of the monomeric ribosome peak and a partial disaggregation of heavy polyribosome aggregates (figure 1).

The cell-free system composed of polyribosomes and cytosol isolated from the livers of cadmium-treated mice has a reduced ability to incorporate ^{14}C-leucine into proteins (table). The cytosol derived from control mice added to the cell-free system containing polyribosomes prepared from cadmium-treated mice did not alter the activity of the system. Also the cytosol of cadmium-treated mice did not affect the activity of the polyribosomes derived from control animals. The results indicate that cadmium affects the polyribosome fraction rather than the cytosol. Cadmium chloride added to the cell-free system composed from polyribosomes and the cytosol derived from control mice in concentrations between 30 and 100 μM decrease the ability of the system to incorporate ^{14}C-leucine into proteins (figure 2). The results presented indicate that cadmium inhibits protein synthesis, apparently by producing a defect in the polyribosome fraction, possibly damaging either the ribosomes or some ribosome-bound factor or factors engaged in protein synthesis. In addition to the references cited[5–8], the described effect of cadmium on polyribosome structure and function indicates a complex effect of this toxic element on nucleic acid and protein biosynthesis. More detailed studies of the effects of cadmium on polyribosome structure and function and RNA biosynthesis could lead to a better understanding of the mechanism of cadmium-produced cell damage. This is the subject of our further investigations.

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Mechanism of potassium deficiency-induced retardation of chlorophyll biosynthesis in Zea mays

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Summary. Potassium deficiency decreased the formation of protochlorophyll and retarded the rate of transformation of protochlorophyll to chlorophyll in maize seedlings.

The retardation of chlorophyll formation during potassium deficiency is well-documented[1,2], but the mechanism of this effect is not fully understood[2,3]. Such an effect may be due, amongst other things, to a decreased formation of protochlorophyll, or to a decreased rate of conversion of protochlorophyll to chlorophyll. In this report, investigations are described which were carried out to test these hypotheses.

Materials and methods. Seedlings of Zea mays Linn. cv NS1 were raised under potassium deficiency and full nutrient regimes as previously described[4]. On the 7th day, and at intervals of 3 or 4 days thereafter, chlorophylls were extracted from shoots harvested at random from

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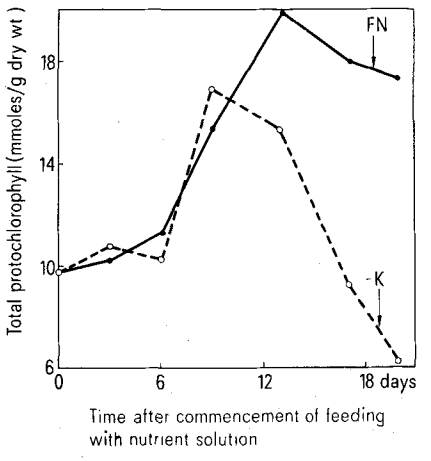

Fig. 1. Time-course of protochlorophyll formation in seedlings of Zea mays maintained under potassium deficiency (–K) and full nutrient (FN) conditions. SD varied between 0.1 and 0.5.

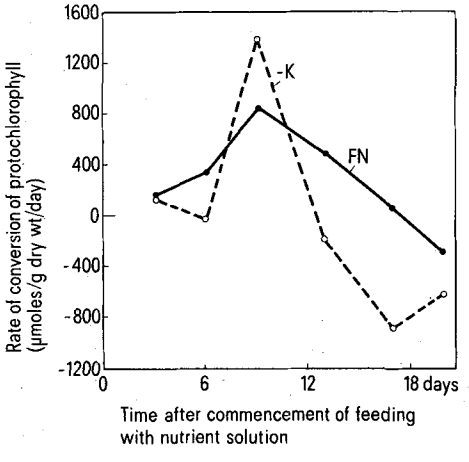

Fig. 2. Rate of conversion of protochlorophyll to chlorophyll in seedlings of Zea mays maintained under potassium deficiency (–K) and full nutrient (FN) conditions.