Cd^{2+}, Mn^{2+} AND Zn^{2+} INDUCED SYNTHESIS OF NUCLEAR RNA IN THE LIVERS OF NORMAL AND ADRENALECTOMIZED RATS

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1. Introduction

Zn^{2+} has been shown to markedly affect the biosynthesis of nuclear polynucleotides in rat liver [1–4]. However, the mode of action is unknown and no conclusions can be drawn as to whether Zn^{2+} complexes with proteins or nucleic acids or both. In this study we report to what extent Zn^{2+} could be replaced by other transition metals such as Mn^{2+}, Co^{2+}, Cr^{3+}, Hg^{2+}, Cu^{2+} and Cd^{2+}. Fractionation of newly synthesized nuclear RNA following the treatment of rats with these metal ions, was expected to reveal whether or not the synthesis of a specific RNA fraction had been stimulated. The possibility that the reactivity of metal ions could be ascribed to increased secretion of corticoid hormones known to stimulate nuclear RNA synthesis [5–8] was excluded by using adrenalectomized rats.

2. Material and methods

2.1. *Animals*

Female albino rats (Wistar) weighing 120 ± 20 g were obtained from Ivanovas Co., Germany. They received Altromin-R-10 stock diet and water *ad libitum*. If necessary bilateral adrenalectomy was performed under Evipan narcosis by the dorsal approach [9]. Metal salts were injected intraperitoneally 10 hr before the rats were decapitated. Generally metal concentrations between 10^{-4} and 10^{-6} moles per kg rat were used. Zn^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+} were injected (0.5 ml) as the dichlorides, Cr^{2+} and Hg^{2+} as acetates. Control animals received 0.15 M NaCl solution.

2.2. *Chemicals*

All chemicals were obtained from the sources already described [3].

2.3. *RNA labeling*

^{14}C-Labelled nuclear RNA was obtained by injecting intraperitoneally 2 μCi 6-^{14}C-orotate to each rat 10 min before decapitation. Liver nuclei from 3–4 rats were combined and the RNA was extracted using phenol-dodecylsulfate at 65° [5, 10]. The RNA was fractionated employing a linear sucrose gradient centrifugation at pH 5.1 [5, 11]. Aliquots of each fraction were subjected to readings of A_{260} (Zeiss PMQ II) and to radioactive counting (Packard Tri Carb, model 3003) using 10 ml of a polar liquid scintillator of dioxane base [12].

2.4. *DNA-dependent RNA polymerase*

RNA polymerase assay was carried out as described earlier [1]. The incubation mixture was slightly modified by using HEPES buffer. This precaution proved necessary to avoid chelation of metal ions with the buffer [13]. The isolated nuclei displayed a high purity with regard to RNA/DNA ratio of 0.25 ± 0.01, microscopic integrity (optical and electron microscopy) and ability to synthesize RNA

* Where reprint requests should be sent.

[1], DNA [14] and nuclear protein [15]. In all experiments the assay mixture contained approximately the same concentration of rat liver nuclei (usually 0.6 mg nuclear DNA). This was achieved by suspending the nuclei in sucrose-magnesium buffer (0.25 M sucrose plus 1 mM $MgCl_2$) and measuring the absorbance at 600 nm. Nuclear suspensions from different animals were adjusted to the same A_{600}. Later DNA analysis employing diphenylamine [16] yielded similar results.

3. Results

3.1. *RNA synthesis in vitro*

A study of DNA-dependent RNA polymerase employing enzymatically active whole liver nuclei in presence of different transition metal ions appeared appropriate to obtain information on the reactivity of metal ions on RNA biosynthesis. The rate of nuclear RNA synthesis was inhibited after the addition of Co^{2+}, Cr^{3+}, Hg^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. Only Mn^{2+} stimulated RNA polymerase activity (fig. 1).

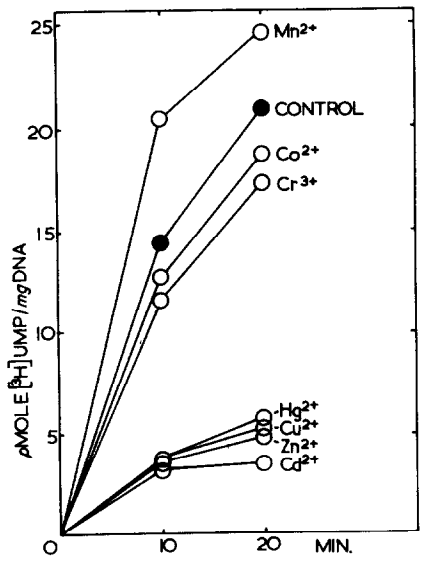

Fig. 1. DNA-dependent RNA polymerase activity of freshly isolated rat liver nuclei in presence of different transition metal ions. All compounds used were chloride salts except for $Hg(CH_3COO)_2$ and $Cr(CH_3COO)_3$ which were added directly to the assay mixture. The complete incubation medium contained (in 0.8 ml): HEPES buffer, 50 mM, pH 8.4; spermidine-3HCl, 5.6 mM; NaF, 8 mM; phosphoenolpyruvate, 2.5 mM; $MgCl_2$, 1.5 mM; GTP, CTP, ATP, 45 μM each; ^{3}H-UTP, 0.45 μM (∿ 1 μCi); pyruvate kinase (1–2 U.); liver nuclei (= 0.8 mg nuclear DNA); added metal salts (shown on the curves) 10^{-4} M; temperature 37°.

If the rats received the metal ions intraperitoneally 10 hr prior to the isolation of liver nuclei a completely different picture is obtained (fig. 2). Practically no inhibition occurred, even after the injection of a higher dose of metal ions (up to 10^{-4} moles per kg rat). To avoid the severe toxic effects of Cd^{2+} and Hg^{2+} no higher concentrations were used. It is interesting that Cd^{2+}, Mn^{2+}, and to some degree Hg^{2+}, could stimulate the rate of RNA synthesis *in vitro*. This effect is very similar to that previously observed with Zn^{2+} [1, 2]. Furthermore, it was intriguing that Cu^{2+}, Co^{2+} and Cr^{3+} remained virtually inactive.

3.2. *RNA synthesis in vivo*

Cd^{2+}, Mn^{2+} and in an earlier study Zn^{2+} [1–3] are able to stimulate markedly the rate of DNA-dependent RNA polymerase activity provided that these metal ions are injected 10 hr before the liver nuclei are isolated. ^{14}C-Pulse labeling of nuclear RNA under exactly the same experimental conditions, therefore, seemed of importance. 3 groups of 4 rats each received a 10 hr pulse of Cd^{2+}, Zn^{2+} and Mn^{2+}. The control group was treated with 0.15 M NaCl solution. 10 min before the experiment was terminated each rat was injected intraperitoneally with 2 μCi 6-^{14}C-orotate. Radioactivity of the isolated ^{14}C-labelled RNA is expressed relative to A_{260} (table 1).

Table 1
Incorporation of 6-^{14}C-orotate into nuclear liver RNA following the intraperitoneal injection of Mn^{2+}, Zn^{2+} and Cd^{2+}. Control rats received 0.15 M NaCl. The standard error was 6–8%.

Group	$\frac{A_{280}}{A_{260}}$	Incorporation (cpm/A_{260}/ml)
1. Control	0.491	2934
2. + $MnCl_2$ (10^{-4} moles/kg rat)	0.512	2556
3. + $ZnCl_2$ (10^{-4} moles/kg rat)	0.512	4714
4. + $CdCl_2$ (10^{-5} moles/kg rat)	0.513	5951

Injections of Zn^{2+} caused a 61% stimulation of the ^{14}C-orotate incorporation which was very similar as determined in a preceeding experiment [2]. Surprisingly Cd^{2+} increased the rate of nuclear RNA synthesis up to 116% compared to the control group. Again Cd^{2+} concentrations higher than 10^{-5} moles per kg rat were not used to avoid severe toxic symptoms. Apparently only control incorporation of ^{14}C-orotate could be observed following Mn^{2+} injections. The fractionation of pulse labelled RNA performed to reveal whether or not the synthesis of a specific RNA fraction was attributable to the metal induced stimulation of RNA synthesis. Freshly isolated RNA obtained under the same experimental conditions was subjected to sucrose gradient centrifugation (fig. 3).

A rather similar ^{14}C-labelling pattern could be observed after the fractionation of each RNA from the control group, the Zn^{2+} and Cd^{2+} treated rats. High molecular weight and/or ribosomal RNA are the preferentially labelled fractions and Cd^{2+} induced the highest ^{14}C-incorporation (note the different cpm scale). With Mn^{2+}, on the other hand, maximum radioactivity was shifted towards RNA fractions of lower molecular weight.

3.3. *RNA synthesis in adrenalectomized rats*

The possibility that this metal induced stimulation of nuclear RNA synthesis may result from higher secretion of corticoid hormones which stimulate nuclear RNA synthesis [5–8] was investigated by using rats which had been totally adrenalectomized (fig. 4). There was virtually no difference in the mode of metal action in normal and adrenalectomized animals (see also fig. 2). The injected metal ions seemed slightly more active in operated rats.

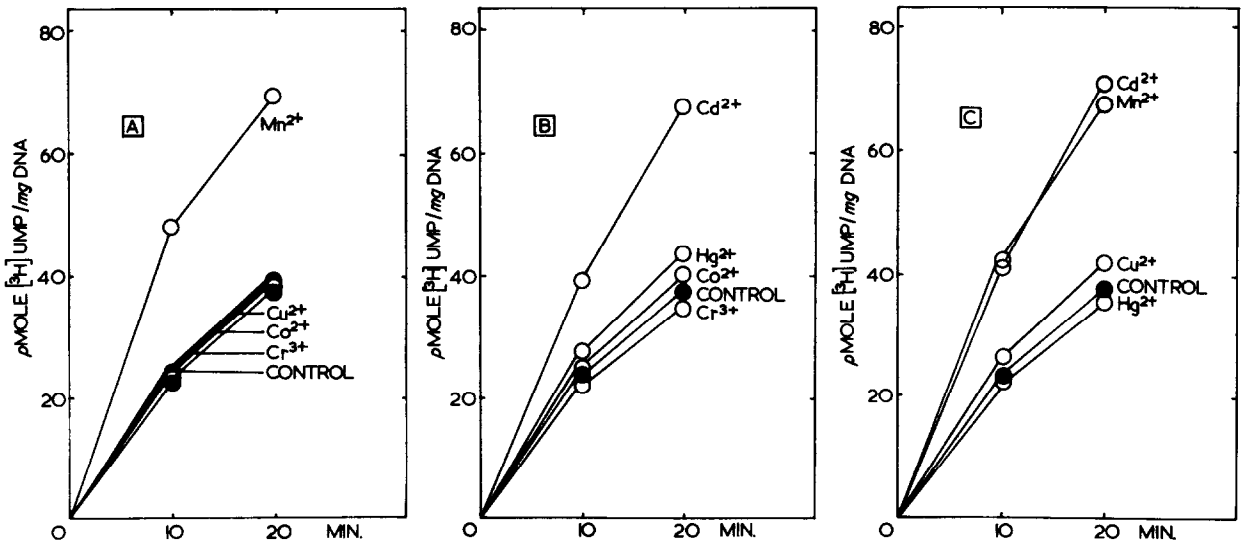

Fig. 2. DNA-dependent RNA polymerase activity of liver nuclei from rats receiving a 10 hr pulse of some transition metal ions. The metal ions were injected intraperitoneally in a volume of 0.5 ml; (A) 10^{-4} moles per kg rat; (B) 10^{-5} moles per kg rat; (C) 10^{-6} moles per kg rat. Each point represents the mean value of the nuclear RNA polymerase activity from three separately injected rats. The complete incubation mixture contained (in 0.8 ml): liver nuclei (= 0.6 mg nuclear DNA); HEPES buffer, 50 mM, pH 8.4; spermidine-3HCl, 5.6 mM; NaF, 8 mM; phosphoenolpyruvate, 2.5 mM; $MgCl_2$ 1.5 mM; GTP, CTP, ATP, 45 μM each; ^3H-UTP, 0.45 μM (∿1 μCi); pyruvate kinase (1–2 U.); temperature 37°.

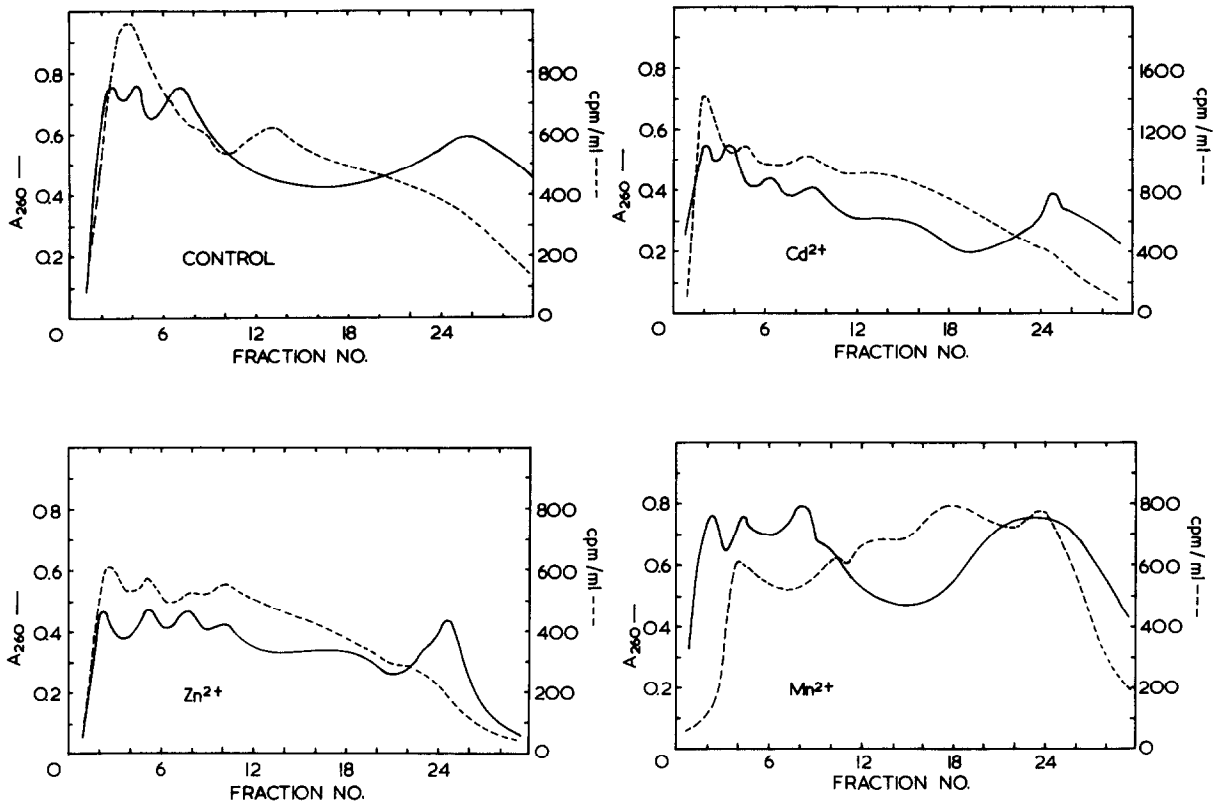

Fig. 3. Linear sucrose density gradient centrifugation of isolated ^{14}C-labelled (10 min pulse of 2 μCi 6-^{14}C-orotate) nuclear RNA from the livers of rats receiving 10 hr before the end of the experiment: 0.5 ml 0.15 M NaCl (control); 10^{-4} moles $ZnCl_2$ per kg rat (Zn^{2+}); 10^{-4} moles $MnCl_2$ per kg rat (Mn^{2+}); and 10^{-5} moles $CdCl_2$ per kg rat (Cd^{2+}). In addition to sucrose (5–20% w/v) the gradient contained sodium acetate (0.01 M); NaCl (0.1 M) and EDTA (1 mM). Centrifugation in a SW 25.1 rotor (Spinco-L-2 ultracentrifuge) at pH 5.1, 24,000 rpm, 3–5° for 16 hr. Fraction volume 1 ml; fraction no. one contained 20% sucrose.

4. Discussion

The inhibition produced by the metal ions added *in vitro* to the RNA polymerase mixture is in close agreement with the results reported by Novello and Stirpe [17]. It is interesting to note that those metal ions which were preferentially coordinated with SH-groups are the strongest inhibitors in this enzymic reaction. Co^{2+} and Cr^{3+} cause standard kinetics but their sulfide complexes are much less stable than those of the other metal ions. Their reactivity observed here is in contrast to their ability to stimulate nuclear protein biosynthesis *in vitro* [15, 18]. Mn^{2+}, which like Co^{2+} and Cr^{3+} is preferentially coordinated with oxygen atoms, stimulated RNA polymerase activity. However, this Mn^{2+} induced stimulation is a well studied phenomenon [19, 20]. Recently the identification [21] and the isolation a Mn^{2+} dependent nuclear RNA polymerase has been achieved [22, 23] but no data on the reactivity of Mn^{2+} based on a precise molecular mechanism is available. It appears that the metal ions added *in vitro* react non-specifically. The metal concentrations in the incubation mixture cannot be compared with the metal concentrations injected *in vivo*. Further, specific metal transport mechanisms have to be considered [26]. *In vivo* experiments, revealed a completely different reactivity of metal ions – with the exception of Mn^{2+}. No decreased RNA polymerase activity was seen in the liver nuclei from metal treated rats. In previous

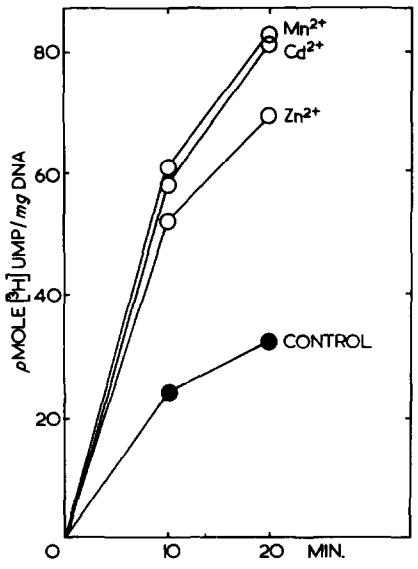

Fig. 4. DNA-dependent RNA polymerase activity of liver nuclei from totally adrenalectomized rats. Bilateral adrenalectomy was performed 65 hr prior to decapitation. 3 rats per group. Metal ion concentrations (moles/kg rat): Mn^{2+} and Zn^{2+}, 10^{-4}; Cd^{2+}, 10^{-5}. Intraperitoneal injections 10 hr before nuclei were isolated. Further experimental details as described in fig. 2.

studies where Zn^{2+} was injected *in vivo*, there was no inhibition of RNA synthesis [1–3, 25] and this is confirmed by the present investigation.

Zn^{2+} [6], Cd^{2+} and Mn^{2+} enhance the formation of nuclear RNA indicating a specific reactivity of these transition metal ions in nucleic acid biosynthesis. Cu^{2+}, Hg^{2+}, Co^{2+} and Cr^{3+} were virtually inactive while similar experiments with Cu^{2+} have led to the same result [17]. Experiments with ^{14}C-orotate revealed that Zn^{2+} and Cd^{2+} stimulate preferential synthesis into high molecular weight and/or ribosomal RNA in a way similar to the Mg^{2+} induced synthesis [24]. By contrast, low molecular weight RNA appears after Mn^{2+} treatment. We still do not know the molecular mechanism of metal ion induced stimulation. This reactivity cannot be ascribed to high ionic strength because the concentrations of metal ions producing the effects recorded here were several orders of magnitude lower than those used in other laboratories [23, 27–32].

Experiments with adrenalectomized rats exclude secondary effects attributable to higher levels of corticoid hormones [5–8]. The intracellular distribution of $^{65}Zn^{2+}$ [26, 33] suggests that the site of metal ion activity is located in the nucleus since $^{65}Zn^{2+}$ is highly concentrated in this cellular compartment while only traces of $^{203}Hg^{2+}$ are detectable. Further results, to elucidate whether complexes of metal ions with polynucleotides or proteins, or both, are necessary since the biosynthesis of polynucleotides will be awaited with interest.

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