According to the current Transplant Registry statistics, approximately 50 percent (*8*) of all renal transplants performed in the United States and Europe during the last year were of living related donor origin. Hypertrophy of the donor's kidney raises glomerular filtration rate to about 140 percent of the predicted normal value (*9*). Furthermore, about one in 800 people are born with a solitary kidney and live normal lives (*10*). Nevertheless, whether a kidney donor will fare as well with one as with two kidneys, should parenchymal renal disease develop, is uncertain.

We explored this question, utilizing the NZB/NZW mouse model of a human disease, systemic lupus erythematosus, which can lead to uremic death. Male mice were chosen, since their disease is milder in onset and their life expectancy greater than that of females. It was felt that differences in mortality over time would therefore be accentuated.

The finding that unilaterally nephrectomized NZB/NZW mice had a significantly shorter survival than did sham-operated controls raises questions regarding people with immune complex disease and one kidney. Studies in humans will be required to determine whether there is increased morbidity from immune disease if only one kidney is present.

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Metal Mutagens and Carcinogens Affect RNA Synthesis Rates in a Distinct Manner

Abstract. *Five metal salts (lead, cadmium, cobalt, copper, and manganese), which are mutagenic or carcinogenic, decreasing the fidelity of DNA synthesis in vitro, stimulated chain initiation of RNA synthesis at concentrations that inhibited overall RNA synthesis. In contrast, other metal salts (zinc, magnesium, lithium, sodium, and potassium) not in this category inhibited chain initiation of RNA synthesis at concentrations that inhibited overall RNA synthesis.*

The ability of metal ions to react with a variety of electron donor sites on polynucleotides as well as to provide optimal conditions for the RNA polymerase reaction has received considerable attention (*1-8*). The presence of 0.2*M* KCl has been shown to be essential for the rate of phage T4 DNA transcription in vitro to approach the rate of chain growth in vivo (*6*) and is believed to increase the rate of RNA chain initiation by promoting the activity of sigma factor, the initiation subunit of *Escherichia coli* RNA polymerase, as well as to permit the release of template-bound RNA and enzyme (*3, 4, 7*). Certain metals have been identified as potential environmental carcinogens through occupational exposure as well as in the laboratory (*9*). Metal ions in this category as well as metal mutagens have recently been shown to inhibit and decrease the fidelity of DNA synthesis in vitro (*10*).

It was our purpose in the study reported here to examine the effects of metal ions on the rate of overall RNA synthesis and on the rate of initiation of RNA synthesis, using *E. coli* RNA polymerase with calf thymus DNA (not sigma factor-dependent) and phage T4 DNA (sigma factor-dependent) as templates. We examined the effects of ten different metal salts, five of which are considered to be metal mutagens or carcinogens, having been shown to decrease the fidelity of DNA synthesis in vitro. We present evidence that the five metals which are in this category stimulate the rate of initiation of RNA synthesis at concentrations that diminish overall RNA synthesis with either DNA template. In contrast, other metal salts which do not fall into this category inhibit the rate of initiation of RNA synthesis at concentrations that inhibit overall RNA synthesis.

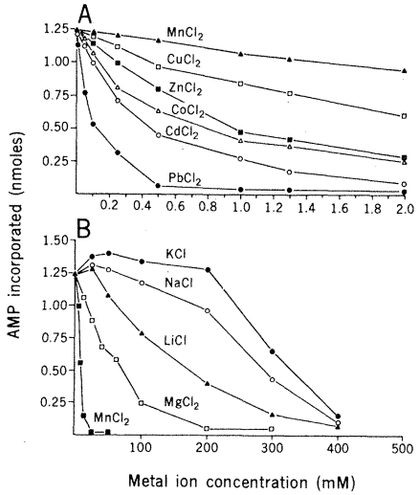

Fig. 1. Effect of increasing concentrations of metal salts on calf thymus DNA-directed overall RNA synthesis: (A) $CdCl_2$, $CoCl_2$, $CuCl_2$, $MnCl_2$, $PbCl_2$, and $ZnCl_2$; (B) KCl, LiCl, $MgCl_2$, $MnCl_2$, and NaCl. Reaction conditions were as described in Table 1.

The effects of the chloride salts of Pb^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, and Mn^{2+} on overall RNA synthesis, as reflected by incorporation of [^{14}C]adenosine monophosphate (AMP), were examined by using calf thymus DNA and phage T4 DNA as templates. These five metal salts are considered to be mutagens or carcinogens, decreasing the fidelity of DNA synthesis in vitro (*10-12*). Rates of RNA chain initiation were measured by incorporation of [γ-^{32}P]adenosine triphosphate (ATP) and of [γ-^{32}P]guanosine triphosphate (GTP), since it has been established that in RNA polymerase reactions with a variety of DNA templates, there is incorporation of these groups at the 5′ ends of the RNA chains formed (*13*). The reaction mixture was maintained at 10 m*M* $MgCl_2$, an optimum concentration for the RNA polymerase reaction (*14*). Overall RNA synthesis was inhibited by all five of these metal salts with the concentration-dependent order of inhibition $Pb^{2+} > Cd^{2+} > Co^{2+} > Cu^{2+} > Mn^{2+}$, and was the same with either template (Table 1 and Fig. 1). However, the degree of inhibition varied with the template. Stimulation of RNA chain initiation was apparent, as reflected by increased incorporation of [γ-^{32}P]ATP and of [γ-^{32}P]GTP in the presence of all five of these metal salts at concentrations that inhibited overall RNA synthesis with either template. Stimulation of incorporation was more pronounced with [γ-^{32}P]ATP than with [γ-^{32}P]GTP. The extent of stimulation of RNA chain initiation is shown in Table 1

at metal salt concentrations that inhibited overall RNA synthesis by 40 to 60 percent. However, stimulation of RNA chain initiation became apparent at concentrations that inhibited overall RNA synthesis by 10 to 15 percent.

The effects of the chloride salts of Zn^{2+}, Mg^{2+}, Li^+, Na^+, and K^+ were examined in a similar manner. These five metal salts are not generally considered to be mutagenic or carcinogenic (*15*) and do not decrease the fidelity of DNA synthesis in vitro (*10*). At concentrations that inhibited overall RNA synthesis by about 40 to 60 percent there was no evidence of stimulation of RNA chain initiation (Table 1 and Fig. 1). Instead, the incorporation of [γ-^{32}P]ATP and of [γ-^{32}P]GTP decreased, with a predominant effect on [γ-^{32}P]ATP, indicating diminished chain initiation in the case of both templates. Decreased chain initiation became apparent at concentrations that inhibited overall RNA synthesis by 10 to 15 percent. However, lower concentrations of $ZnCl_2$ resulted in [γ-^{32}P]GTP incorporation above the control level, although [γ-^{32}P]-ATP incorporation was inhibited quite effectively.

In general, the effects on [γ-^{32}P]ATP incorporation were more pronounced than the effects on [γ-^{32}P]GTP incorporation, which was also found in studies with phage T7 DNA and varied $MgCl_2$, NaCl, or KCl concentrations (*16, 17*). Our results suggest, but do not prove, that the metal mutagens and carcinogens promote RNA initiation at new sites on the DNA template, whereas other metal salts are unable to do so. Further experiments are required to test this possibility.

The order of concentration-dependent inhibition of overall RNA synthesis by all metals studied was $Pb^{2+} > Cd^{2+} > Co^{2+} > Zn^{2+} > Cu^{2+} > Mn^{2+} > Mg^{2+} > Li^+ > Na^+ > K^+$ and was the same for both templates. This order of inhibition may be partially explained on the basis of the affinities of these metal ions for nucleic acid bases compared to phosphates—namely, $Pb^{2+} > Cd^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+} > Li^+$, Na^+, K^+ (*1, 2*). Donnan equilibrium data are consistent with the phosphate-binding order of $Mg^{2+} > Li^+ > Na^+ > K^+$, which is also consistent with calculated energies of formation (*18, 19*).

Alterations in RNA synthesis rates characterized by stimulated chain initiation under conditions of diminished overall RNA synthesis, as observed in this study, could serve to complement present in vitro assays for screening metal compounds for suspected mutagenicity or carcinogenicity. It is also possible that other chemical carcinogens which require activation by host enzymes (*20*) could be tested in a similar manner if first coupled with a microsomal enzyme preparation (*21*).

Table 1. (A) Effect of metal salts on RNA synthesis, using calf thymus DNA as a template. Each metal salt was tested at a minimum of six different concentrations for effects on overall RNA synthesis. The percentage incorporation of [γ-^{32}P]ATP and of [γ-^{32}P]GTP is shown for metal concentrations that inhibited [^{14}C]AMP incorporation by about 50 percent. Calf thymus DNA was obtained from Worthington Corporation and dialyzed against 0.02*M* tris (*p*H 7.8) before use. The purification of RNA polymerase (E.C. 2.7.7.6) *E. coli* B was done as described by Stevens (*14*), except that the final sucrose density gradient centrifugation step was replaced by the glycerol gradient centrifugation procedure of Burgess (*22*). The reaction mixture for measuring the overall rate of RNA synthesis (0.2 ml) contained 20 m*M* tris (*p*H 8.1); 20 m*M* 2-mercaptoethanol; 10 m*M* $MgCl_2$; ATP, GTP, cytidine triphosphate, and uridine triphosphate [ATP was labeled with ^{14}C (1×10^3 counts per minute per nanomole)] at 0.25 m*M* each; calf thymus DNA, 10 μg; and RNA polymerase, 10 μg. The reaction mixture (0.2 ml) for measuring the initiation rate of RNA synthesis was the same, except that ATP or GTP was labeled with ^{32}P (1×10^5 counts per minute per nanomole) as specified. The remaining procedure was as reported in (*23*), and background levels were determined by using reaction mixtures lacking DNA or enzyme (*5*). All assays were performed at least four times and averages were determined. Average control values of incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP were 2.9 and 2.2 pmole, respectively. (B) Effect of metal salts on RNA synthesis, using phage T4 DNA as a template. Reaction conditions were as described above, except that 10 μg of phage T4 DNA was used instead of calf thymus DNA. Bacteriophage T4 DNA was isolated by phenol extraction as described by Thomas and Abelson (*24*), then dialyzed against and stored in 0.02*M* tris (*p*H 7.8). Incorporation of [^{14}C]AMP by controls was similar to that of calf thymus DNA shown in Fig. 1. The average control values for incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP were 2.6 and 1.4 pmole, respectively.

Metal salt	Concentration (m*M*)	Incorporation (% of control) [^{14}C]-AMP	[γ-^{32}P]-ATP	[γ-^{32}P]-GTP
A. Calf thymus DNA				
Control		100.0	100	100
$CdCl_2$	0.35	50.4	176	127
$CoCL_2$	0.75	43.8	319	150
$CuCl_2$	2.00	49.6	220	103
KCl	300.00	55.4	58	79
LiCl	150.00	47.1	51	73
$MgCl_2$	40.00	54.5	68	71
$MnCl_2$	5.00	47.9	194	168
NaCl	250.00	57.0	60	82
$PbCl_2$	0.10	46.3	163	140
$ZnCl_2$	1.00	40.5	37	97
B. Phage T4 DNA				
Control		100.0	100	100
$CdCl_2$	0.35	46.3	149	123
$CoCl_2$	0.75	48.1	236	141
$CuCl_2$	2.00	44.4	164	120
KCl	550.00	58.3	45	77
LiCl	400.00	52.8	41	69
$MgCl_2$	30.00	39.8	44	63
$MnCl_2$	4.00	43.5	202	146
NaCl	500.00	63.0	40	75
$PbCl_2$	0.10	62.0	135	118
$ZnCl_2$	1.00	58.3	59	94

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