for DES. With methanol-water as the solvent system, isotopic dilution (10 μg of ^{14}C-labeled material and 400 mg of ^{3}H-labeled DES) showed after two recrystallizations that nearly 60 percent of the labeled material was not [^{14}C]DES.

GC-MS analysis of material obtained from an unlabeled reaction conducted on a larger scale, showed the formation of approximately 2 percent hexestrol, suggesting that a stoichiometry problem, caused by the highly reactive nature of $TiCl_3$, may have caused the concurrent formation of hexestrol. A repetition of the labeled reactions yielded DES with little hexestrol formation. This problem emphasizes the need for caution in the use of isotope dilution as a means of determining purity and emphasizes the need for intensive and varied purity determinations on labeled chemicals prior to their use in studies involving residue determinations. Attention has been drawn to other purity problems involving labeled DES (*3*); however, hexestrol was probably not a problem in previous studies in that we analyzed by GC-MS the ethyl-labeled DES used in a study by Aschbacher *et al.* (*4*) and found no hexestrol.

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Unilateral Nephrectomy: Effect on Survival in NZB/NZW Mice

Abstract. *Male F_1 New Zealand Black × New Zealand White mice, which spontaneously develop immune complex renal disease, underwent unilateral nephrectomy at 3 months of age and were compared with sham-operated controls. At 12 months of age only 24 percent of mice with a single kidney were alive, while 85 percent of sham-operated controls survived to the same age. Unilaterally nephrectomized mice had more severe renal histologic changes, as shown by light and immunofluorescence microscopy.*

Helyer and Howie (*1*) reported a spontaneously occurring glomerulonephritis which progressed to renal failure in the F_1 generation of New Zealand Black and New Zealand White (NZB/NZW) mice. Burnet and Holmes (*2*), Hicks and Burnet (*3*), and McGiven and Lynraven (*4*) subsequently described the anticipated life expectancy and the gross and microscopic changes in NZB/NZW kidneys, which are now accepted as an animal analog of systemic lupus erythematosus. There is a marked sex-related difference in survival of NZB/NZW mice. Although close to 100 percent of these mice die in uremia, females develop renal failure sooner and die months earlier than males. All female animals are dead by about 1 year of age, whereas 60 to 70 percent of males live longer than 1 year.

The pathologic picture in the kidney is one of severe glomerulonephritis, thought to be secondary to immune complex deposition. Renal function begins to deteriorate at approximately 5 months of age in female and 7 months in male NZB/NZW mice. This is reflected in elevations of blood urea nitrogen (BUN) and creatinine concentration. Proteinuria may or may not be present early in the disease, but is usually present in the two months preceding death (*5*).

Fifty male NZB/NZW mice from colonies maintained at the National Institutes of Health (produced by NZB female × NZW male matings) were randomized at 10 weeks of age. Mice were housed five to a cage and were given standard mouse chow (Ralston Purina Co.) and water without restriction. After allowing 2 weeks for adjustment to their new environment, all mice were weighed and bled for baseline renal function values. Weights (mean, 41 ± 2 g) and BUN (27 ± 2.3 mg/100 ml) were comparable in all animals and well within normal limits for that age.

Table 1. Antibodies to DNA and mean BUN in unilaterally nephrectomized and sham-operated male NZB/NZW mice, at 8 and 10 months of age. Abbreviations S.E.M., standard error of the mean: *P*, probability.

Age of mice (mo.)	Mice with unilateral nephrectomy	Sham-operated controls	*P*
Mean percent of DNA bound (± S.E.M.)			
8	28.8 ± 1.2	36.7 ± 2.0	< .005
10	32.5 ± 2.0	43.8 ± 2.4	< .005
Mean BUN (± S.E.M.)			
8	26.3 ± 1.9	19.9 ± 0.8	< .05
10	47.3 ± 11.4	22.6 ± 1.1	< .05

All BUN determinations were done with a Beckman BUN analyzer in duplicate on blood from the tail vein.

Mice were randomly assigned to one of two groups: animals in group 1 underwent unilateral nephrectomy and those in group 2 were used as sham controls. The operative procedure was similar in each group. Animals were anesthetized with ether and their ventral surfaces were shaved. After the abdominal skin was cleaned with Betadine, a midline incision was made and the left kidney freed from its perinephric fat. All unilaterally nephrectomized animals then had the renal artery, renal vein, and ureter ligated with 3-0 silk suture and, when hemostasis was complete, the abdomen was closed with interrupted 4-0 silk sutures. Those animals serving as sham controls underwent the same operative procedure, but after mobilization of the renal pedicle, the incision was closed as above. After intraoperative mortality, in each case secondary to anesthesia, there were 21 mice in the experimental and 19 mice in the sham control groups.

Animals were bled every other week from the retro-orbital fossa. Blood was allowed to clot at 0° for 1 hour, and serum was obtained by centrifugation to remove cellular debris. The BUN determinations were made immediately; the remaining serums were frozen for future serological study. Antibodies to native DNA were measured by an ammonium sulfate precipitation assay as previously described (*6*), in which 10 μl of serum and 50 ng of ^{14}C-labeled native human KB DNA were used.

Mice in either group were counted as renal deaths if they had concentrations of BUN exceeding 100 mg/100 ml when they were killed or if found dead with a previous BUN concentration of more than 80 mg/100 ml. Renal tissue was removed when the animals were killed or at the time of spontaneous death and was fixed in 10 percent formalin for histologic sectioning. Additional kidney tissue was frozen for study by immunofluorescence microscopy.

Sections for light microscopy were cut and stained according to standard tech-

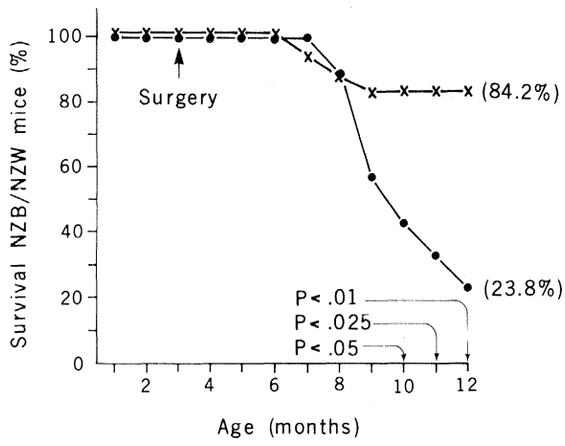

Fig. 1. Survival curves for male NZB/NZW mice. Mortality values become statistically significant at 10 months of age; $P > .01$ at the end of observation period when all animals surviving were killed. ×—×, Sham-operated controls; ●—●, unilaterally nephrectomized mice.

niques. Histologic evaluation was carried out on hematoxylin-eosin–, trichrome-, PAS- (periodic acid–Schiff), and amyloid-stained sections. Formalin-fixed tissue was also studied by electron microscopy. Frozen sections were cut and stained with fluorescein-labeled antiserum to mouse immunoglobulin (Hyland Labs.). Unfortunately, tissue was not available for study from all animals, as several mice were cannibalized by cagemates or tissue that had been removed had undergone postmortem autolysis.

At 18 weeks of age (6 weeks after surgery), before disease is manifest, the two groups did not differ significantly with regard to mean concentrations of BUN (36.5 ± 3.6 mg/100 ml in the experimental group and 31.2 ± 2.8 mg/100 ml in the sham controls). There were no deaths from any cause until one sham-operated animal died in renal failure at 7 months of age. Thereafter, a progressive loss of mice occurred in both groups. Without exception, deaths during the 12 months of observation were attributable to renal failure with BUN's of 80 to 180 mg/100 ml recorded in each case. There was no significant difference in the two mortality curves until the 10th month of life, when 12 of 21 nephrectomized animals and 3 of 19 controls had died ($P < .05$ by chi-square analysis). From this point on the statistical significance increased to $P < .01$ at 12 months of age (Fig. 1). All animals remaining alive were then killed, with 5 of 21 experimental and 16 of 19 controls still alive ($P < .001$).

A significant increase in BUN was noted prior to death in the unilaterally nephrectomized group as compared with the control group (Table 1). After 10 months of age, individual mice in the experimental group had rapid increases in BUN. Antibodies to DNA are also shown in Table 1. No increase in DNA antibodies was found in the unilaterally nephrectomized group to explain their more rapid renal deaths. In fact, a small but significant decrease in DNA binding was observed in this group.

All kidneys removed at the time of unilateral nephrectomy were normal or showed mild focal proliferation. Table 2 gives data for all those animals from whom tissue was available when they were killed or at the time of spontaneous death. While 9 of 19 control animals had normal or focal histologic damage, none of 16 experimental animals fell into these categories. On the other hand, severe global sclerosis, frequently consistent with amyloid deposition, was seen in 8 of 16 kidneys from unilaterally nephrectomized mice, while none of 19 control animals showed this picture. The distribution of renal histologic abnormalities was significantly worse in the unilaterally nephrectomized group (Table 2).

While all but one animal in the study had positive mesangial immunofluorescence, only 35 percent of sham-operated control mice had positive immune-complex deposition within the glomerular capillary wall as compared with 74 percent of those animals that had previously undergone unilateral nephrectomy.

In this study we found a profound effect of single nephrectomy. Only 24 percent of unilaterally nephrectomized mice were alive at 12 months of age as compared with 85 percent of sham-operated controls. Nephrectomized mice had significantly higher BUN's and worse renal histology. There was no increase, but rather a small decrease, in antibodies to DNA. Thus, the accelerated renal lesion is not explainable on the basis of greater autoantibody production, but rather on the basis of reduced nephron mass.

This finding is consistent with the currently accepted pathogenesis of immune complex nephritis (*7*). Complexes of intermediate size trapped within the glomerular urinary space are incorporated into the glomerular basement membrane or within the mesangial region. Subsequent to complex deposition, activation of the complement cascade and ultimately the laying down of fibrin produces glomerular damage and obsolescence. By mass action when the number of circulating complexes is held constant and the number of glomeruli reduced by unilateral nephrectomy, a higher density of complexes per residual nephron must be present. That glomerular damage is proportionate to the concentration of immune complexes impinging on each glomerulus may be inferred from our results.

Table 2. Light microscopic histology and immunofluorescence of nephrectomized mice and the sham-operated controls.

Group	Glomerular histology					Immunofluorescence		Killed at 12 mo./ died in uremia
			Diffuse changes					
	Normal	Focal	Proliferative	Proliferative plus sclerosing	Sclerosing	Mesangium	Glomerular capillary wall	
Sham-operated controls	3/19*	6/19	3/19	7/19	0/19	17/17	6/17	16/3†
Unilaterally nephrectomized‡	0/16	0/16	0/16	8/16	8/16	15/16	12/16	5/11†

*Numerator represents number of mice with the finding; denominator, the total number of mice examined. †Data reflects all animals from whom tissues were available. ‡The distribution of abnormalities of glomerular histology was significantly different from that of sham-operated controls by chi-square analysis, $P < .05$.

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.

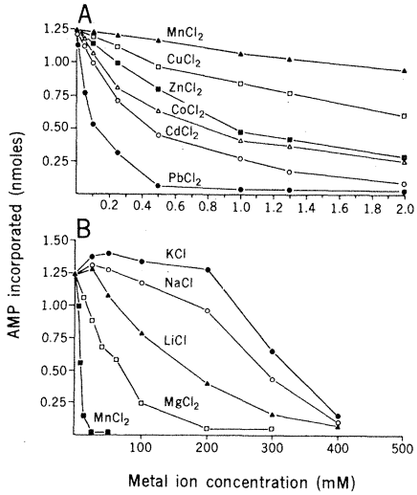

Fig. 1. Effect of increasing concentrations of metal salts on calf thymus DNA-directed overall RNA synthesis: (A) $CdCl_2$, $CoCl_2$, $CuCl_2$, $MnCl_2$, $PbCl_2$, and $ZnCl_2$; (B) KCl, LiCl, $MgCl_2$, $MnCl_2$, and NaCl. Reaction conditions were as described in Table 1.

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