chromosomes and 6.4 pg or more DNA per nucleus. The simplest interpretation of these findings, in view of the albumin phylogeny, is that there was a duplication of the genome (accompanied by a small number of chromosomal rearrangements) in the common ancestor of the *X. laevis* group, as indicated in Fig. 1 (*25*). Thus the *Xenopus* species commonly used for molecular studies of development and genome organization probably are tetraploid, that is, have two pairs of genes for every function. Although this finding may surprise those who study genome organization in *Xenopus*, it will not surprise herpetologists, who are beginning to recognize that polyploidy is rather common in frogs of several families (*26*).

The duplication event referred to in Fig. 1 evidently occurred so long ago that evolutionary divergence has taken place between the duplicated genes. The complex electrophoretic patterns observed for the enzymes lactate dehydrogenase, malate dehydrogenase, and superoxide dismutase in *X. laevis* and *X. borealis* (*27*) are consistent with this possibility. While the existence of duplicate genes may complicate attempts to study the molecular basis of development in *Xenopus*, biologists now have a good opportunity for the molecular study of evolutionary aspects of gene duplication in a laboratory animal.

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23. We designate the central highlands populations, formerly known as *X. l. borealis*, as *X. borealis*, because hybrids between these frogs and *X. l. laevis* are not fertile (*20*), and because *X. borealis* and *X. l. laevis* differ greatly at the macro molecular level, not only in albumin structure but also in immunoglobulin structure (*9*) and at the nucleic acid level [I. B. Dawid, *Dev. Biol.* **29**, 139 (1972); G. A. Galau, M. E. Chamberlin, B. R. Hough, R. J. Britten, E. H. Davidson, in *Molecular Evolution*, F. J. Ayala, Ed. (Sinauer Associates, Sunderland, Mass., 1976), pp. 200–224]. Our designation of this species as *X. borealis* is consistent with that of J. Tymowska and M. Fischberg (*20*). For details regarding the identification and systematics of these and other clawed frogs, see M. Fischberg, in preparation.
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25. Following this ancient development of tetraploidy, additional duplications of the genome occurred in some species of the *X. laevis* group. Thus *X. vestitus* is tetraploid with respect to *X. laevis* while *X. ruwenzoriensis* is hexaploid with respect to *X. laevis* (*20, 24*).
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Ribavirin: Efficacy in the Treatment of Murine Autoimmune Disease

Abstract. *Ribavirin, a drug with known antiviral activity, was given to mice with established lupus nephritis. Ribavirin was effective in prolonging survival, reducing the titer of antibodies to DNA, and reversing proteinuria. Other antiviral agents were not effective in the dosages used.*

Female New Zealand black by New Zealand white F_1 (NZB/W) mice develop an autoimmune disease characterized by immune complex glomerulonephritis, antibodies to DNA and RNA, lupus erythematosus (LE) cells, and shortened life expectancy (*1*). These findings are also seen in human systemic lupus erythematosus (SLE). Although genetic, immunologic, and hormonal factors have been implicated in the pathogenesis of this disorder, recent studies suggest that viruses may be causative agents of autoimmune disease in both animals and man (*2*). These studies indicate that a new approach to the therapy of immune complex disease might be directed against viral infection. We therefore studied the effect of several antiviral drugs and an immunostimulatory drug on the natural history of NZB/W lupus.

NZB/W female mice (152, aged 20 weeks), already manifesting autoimmunity were randomly selected to receive intraperitoneal injections of one of the following drugs: ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) at a dose of 250 mg/kg twice weekly; ara-A (9-β-D-arabinofuranosyladenosine) at a dose of 600 mg/kg twice weekly; phosphonoacetic acid at a dose of 750 mg/kg five times weekly; and levamisole (L-2,3,5,6-tetrahydro-6-phenylimidazo-[2,1*b*]-thiazole·HCl) at a dose of 10 mg/kg twice weekly, or normal saline twice weekly.

Drug dosages were based on previous short-term studies that suggested that the above schedule would be relatively nontoxic while still demonstrating antiviral activity (*3*). Levamisole was included in the therapy groups because of its ability to stimulate immune responses and the suggestion of usefulness in rheumatological disorders (*4*). Treatment was started at 20 weeks of age when demonstrable immune-complex deposition in the kidneys and circulating antibodies to nucleic acid are present, thus making the experimental system analogous to clinical situations.

All mice were followed for the development of antibodies to DNA, proteinuria, and for survival. The protein con-

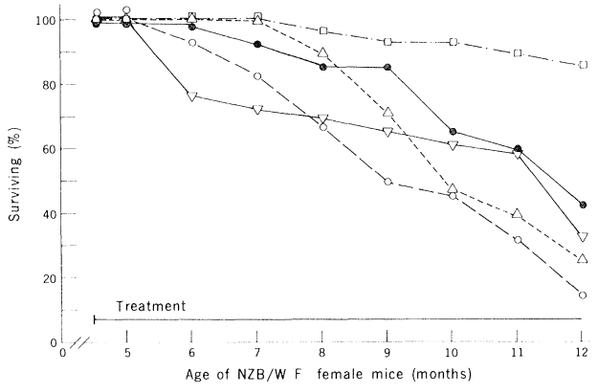

Fig. 1. The effect of antiviral therapy on survival of NZB/W F_1 female mice. □, Ribavirin treatment; ●, phosphonoacetic acid treatment; ▽, levamisole treatment; △, saline control; ○, ara-A treatment.

Table 1. Effect of antiviral agents on proteinuria and antibodies to DNA in NZB/W F_1 mice.

Age (months)	Therapy group: Ara-A	Levamisole	Phosphonoacetic acid	Ribavirin	Saline
			*Proteinuria**		
5	0/30 (0%)	1/30 (3%)	0/30 (0%)	0/29 (0%)	0/32 (0%)
7	0/25 (0%)†	4/22 (18%)†	13/28 (46%)	10/29 (34%)†	21/31 (68%)
9	0/15 (0%)†	9/20 (45%)	14/26 (53%)	4/27 (15%)†	17/24 (71%)
11	0/7 (0%)†	11/17 (65%)	11/17 (65%)	0/26 (0%)†	8/13 (62%)
12	0/5 (0%)†	5/10 (50%)	6/13 (46%)	0/25 (0%)†	5/9 (56%)
			Antibodies to DNA‡		
7	64 ± 3%	55 ± 3%	52 ± 2%	44 ± 2%§	59 ± 2%

*Animals with urinary protein concentration greater than 100 mg/dl per number surviving. †$P < .05$ compared to the saline control by chi-square analysis. ‡Expressed as percent binding ± S.E.M. §$P < .05$ compared to saline control by Student's t-test.

centration of freshly expressed urine was measured with tetrabromphenol paper (Albustix). Serum antibodies to native DNA were detected by a modified Farr assay (*5*) at 7 months of age.

Median survival was prolonged in mice receiving ribavirin as compared to groups of mice on other treatments and control mice (Fig. 1). In control NZB/W mice the median survival was 10 months, with a 26 percent survival at 12 months. Ribavirin therapy was associated with a 12-month survival of 86 percent ($P < .01$ by Wilcoxon analysis). Levamisole and phosphonoacetic acid both modestly prolonged median survival to 11.4 and 11.6 months, respectively. However, this prolongation was not statistically significant by Wilcoxon analysis. Treatment with ara-A shortened median survival to 9 months and reduced 12-month survival to 17 percent.

The development of proteinuria with time is shown in Table 1. Animals receiving ara-A remained without proteinuria throughout the entire study. The incidence of proteinuria in the ribavirin-treated group was 34 percent in animals at 7 months, and there was no proteinuria at 11 months; between 7 and 12 months the percentage of control mice with proteinuria was constant, whereas the groups treated with levamisole and phosphonoacetic acid showed increased protein excretion.

Determination of antibodies to native DNA revealed a significant decrease in titer in the ribavirin-treated mice at 7 months of age (Table 1). Other treatment groups showed no consistent change in antibodies from 7 to 11 months. Control animals had the characteristic increase in binding levels of antibody to DNA until 8 months of age; after this time there was a decrease in mean titer among the survivors (*6*).

Our study was designed to determine the efficacy of selected antiviral agents and an immunostimulatory drug in prolonging life in NZB/W mice. The results demonstrate the usefulness of ribavirin in prolonging survival, reducing proteinuria, and decreasing the titer of antibodies against native DNA. Mice treated with ribavirin displayed a regression of established proteinuria in a significant number of animals; the explanation for this phenomenon remains to be determined. Levamisole and phosphonoacetic acid did not appreciably alter the natural history of immune-complex glomerulonephritis, and ara-A treatment was associated with shortened survival even though it prevented proteinuria. It is difficult to compare drug efficacy in long-term studies because of the absence of data on long-term toxicity and dosage. Therefore, the negative results in the other therapy groups may be the result of dosage schedules and not of drug activity.

Within the limits of our study, ribavirin is an effective drug in altering the natural history of immune-complex glomerulonephritis and prolonging survival in NZB/W female mice. It has been suggested that ribavirin may have direct antitumor properties (*7*) and may be immunosuppressive in other systems (*8*). However, treatment of both NZB/W and BALB/c mice with the above ribavirin schedule for 1 month failed to depress serum antibody response to sheep red blood cells or an aqueous solution of polyinosinic acid · polycytidylic acid, and also failed to alter primary rejection of skin allografts from C57B1/6 mice. Since the incidence of tumor development in our control animals is less than 5 percent (*9*), the prolonged survival seen with ribavirin cannot be attributed to antitumor action. The exact mechanism of ribavirin action in this disease process has not been demonstrated, but there is no evidence to support its role as an immunosuppressive agent. Because of ribavirin's definite antiviral activity in vivo and in vitro against both RNA and DNA viruses in other systems (*10*), its effectiveness in a disease having a suggested viral pathogenesis offers new investigational possibilities for SLE and other diseases with similar pathogenesis.

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Zinc Binding: A Difference Between Human and Bovine Milk

Abstract. *Gel chromatography indicated that most of the zinc in cow's milk was associated with high-molecular-weight fractions, whereas zinc in human milk was associated with low-molecular-weight fractions. A species difference in zinc-binding ligands may explain why symptoms of the genetic disorder of zinc metabolism, acrodermatitis enteropathica, can be alleviated by feeding human but not cow's milk.*

Acrodermatitis enteropathica (AE) is an autosomal recessive inherited disorder characterized by severe skin lesions on the extremities and around body openings, alopecia, and diarrhea (*1*). The onset of symptoms of AE usually occurs when such infants are weaned from human breast milk to cow's milk (*1–3*). The therapeutic value of human milk in this disorder has been known for a long time (*2*). Moynahan and Barnes (*4*) have reported low levels of zinc in the plasma of AE patients and the successful treatment of the disorder with oral zinc, resulting in an increase in the levels of zinc in the plasma and subsequent clearing of epidermal lesions.

Human milk generally contains less zinc than bovine milk, with the zinc concentrations of both decreasing progressively throughout lactation (*5, 6*). Since the zinc concentration of human milk is generally lower than that of cow's milk we postulated that the zinc in human milk must be present in a form different from that found in cow's milk, and predicted that it contains a specific zinc-binding ligand not present in the bovine milk. To test this hypothesis we have separated both human and bovine milk by gel filtration to determine the association of zinc with protein fractions in the two species.

Fresh samples of human (N = 5) and Holstein cow's (N = 5) milk were cooled at 4°C and centrifuged at 1000*g* for 5 minutes to separate the fat. The zinc content of the fat-free cow's milk was 4.22 ± 0.37 μg/ml (S.E.M.) with a range of 3.56 to 4.80 μg/ml compared to 0.97 ± 0.29 μg/ml with a range of 0.27 to 2.05 μg/ml for the human samples.

Fat-free samples were diluted with an equal volume of 13 m*M* tris buffer, *p*H 7.4, and chromatographed on a Sepharose 2B column (50 by 2.6 cm). Fractions obtained by gel filtration were assayed for protein by the method of Warburg and Christian (*6*) and for zinc by means of a Unicam SP 90 atomic absorption spectrophotometer.

Representative elution patterns from gel filtration of bovine and human milk on Sepharose 2B are shown in Fig. 1, a and b. In cow's milk, zinc was associated with the fractions of higher molecular weight, whereas zinc in human milk was associated with the fractions of lower molecular weight. In order to observe whether the major zinc-binding peak of human milk was present in cow's milk but was masked by the large zinc-containing peak of the fractions of higher molecular weight, zinc-containing fractions (Fig. 1) were concentrated and chromatographed again on a Sephadex G-200 column (50 by 2.0 cm). The pooled gel-filtration fractions of cow's milk were eluted, resulting in a large zinc peak at void volume and four small zinc peaks (Fig. 1c). The major zinc-containing fraction of human samples eluted from Sepharose 2B were similarly chromatographed again on Sephadex G-200. Zinc was eluted in two broad peaks (Fig. 1d), but the second peak (fractions 130 to 150) varied directly with the amount of zinc in the original fat-free sample.

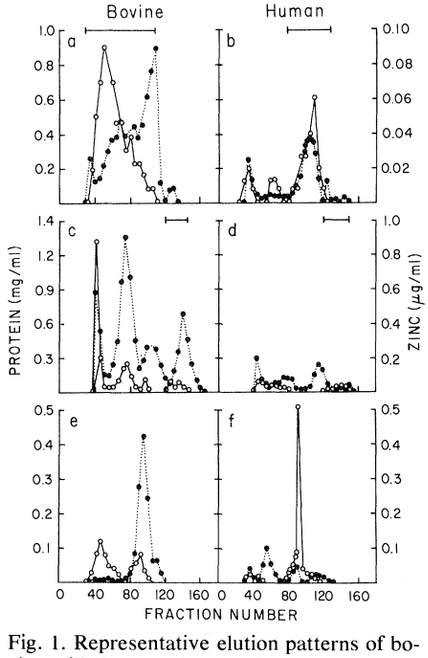

Fig. 1. Representative elution patterns of bovine milk (a, c, and e) and human milk (b, d, and f) separated by gel filtration. Fat-free samples were applied to a Sepharose 2B column, and fractions containing zinc (bracketed) (a and b) were pooled, concentrated by lyophilizing, and applied to a Sephadex G-200 column. Fractions containing zinc eluted from the Sephadex G-200 column (bracketed) (c and d) were pooled, concentrated by lyophilizing, and applied to a Sephadex G-75 column. (a and b) Sepharose 2B column (2.6 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 2 ml per fraction; (c and d) Sephadex G-200 column (2.0 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 1 ml per fraction; (e and f) Sephadex G-75 column (1.5 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 1 ml per fraction. Symbols: ○——, zinc; ●....., protein.

These zinc-containing fractions from human samples and the corresponding cow's milk fractions (Fig. 1, c and d) were further purified by gel filtration on a Sephadex G-75 column (50 by 1.5 cm). The zinc in both bovine and human samples separated into two peaks (Fig. 1, e and f). The elution patterns showed large peaks in both samples, but, surprisingly, they were of different composition. The large protein peak in cow's milk samples was associated with a small quantity of zinc, whereas, conversely, the large zinc peak in human samples, with a slightly smaller elution volume, was associated with only a small protein peak. To ensure that the zinc was tightly bound in samples from both human and bovine peaks, fractions were dialyzed for 22 hours against 13 m*M* tris buffer, *p*H 7.4, containing 10 m*M* EDTA and rechromatographed on Sephadex G-75. The zinc remained bound to both ligands, and elution volumes were nearly identical to those obtained prior to dialysis. After additional purification of the low-molecular-weight zinc-binding ligand on DEAE-cellulose, the molecular weight was estimated by gel filtration on Bio-Gel A-5m (6*M* guanidium chloride, *p*H 7.0) to be 8700. Insulin (chains A and B), trypsin inhibitor, ribonuclease, myoglobin, chymotrypsinogen, and ovalbumin were used as marker proteins (*7*).

The demonstration of a difference in zinc binding between human and cow's milk supports our hypothesis that there is a difference in the association of zinc to milk components in the two species.