zero field (shielding effect), and of cooling the sample in 100 Oe (Meissner effect). In both of these curves, superconductivity begins at a temperature (39.5 K) that is within ±1 K of that seen in resistance or a-c susceptibility, but the transition is considerably broader. For all the samples measured, the Meissner signal is 50 to 60% of the shielding signal, which is evidence for bulk superconductivity. These measurements were repeated in fields up to 5 T. At this flux density a reduction of T_c along with a broadening of the transition is observed. From our measurements, we estimate dH_c/dT = 1.3 T/K, which extrapolates to a critical field of 40 T at 4.2 K. Preliminary critical field measurements at high fields support this value (*18*).

Superconducting transitions are sensitive to changes in composition, vacancy ordering, and phase segregation. The composition x = 0.15 has a sharp transition as measured by d-c resistance, a-c mutual inductance, and Meissner expulsion. These three measurements show this composition to be a bulk superconductor. At other compositions the transitions are broader, and some show structure. It is possible that all our samples have comparable inhomogeneities in the strontium content. A maximum in T_c as a function of x would mean that the minimum in the transition width simply reflects a maximum in T_c versus x near x = 0.15. We do not believe the structure in the transition is due to impurity phases, since none are observed by x-ray diffraction to 5% accuracy. It is also possible that the material is a single phase for x = 0.15, but not for other compositions, and that the phase separation is not complete for our cooling speed. However, a sample with x = 0.20 cooled in 16 hours instead of 1 hour from 1100°C showed features in the superconducting transition similar to those in samples with the same x cooled in 1 hour or quenched. Sharp transitions over a narrow range in composition also occur in $BaPb_{1-x}Bi_xO_3$ (*19*). It has been suggested that inhomogeneities are responsible for the broadening at other compositions (*20*).

The superconducting transition is sensitive to sample composition and preparation, and it is possible that higher T_c will be found. Oxygen vacancies are important, as shown by the shift in T_c with the atmosphere used for annealing. We have also found evidence for T_c = 40 K in these oxides where calcium replaces strontium, but in this case the superconducting transition was more than 10 K wide, and furthermore the Meissner effect indicated that only a small fraction of the sample was superconducting. We are presently studying other compounds in these series.

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Ribavirin Antagonizes the Effect of Azidothymidine on HIV Replication

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Azidothymidine and ribavirin both inhibit replication of human immunodeficiency virus in vitro and show promise of clinical utility in patients infected with this virus. In this study, the possible interactions of these drugs were examined in vitro, and a reproducible antagonism between azidothymidine and ribavirin was found to occur under a variety of experimental conditions. The mechanism responsible for this antagonism appeared to be inhibition of azidothymidine phosphorylation by ribavirin. Because similar effects may occur in vivo, clinical trials of these two drugs in combination must be performed only under carefully controlled conditions.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) causes a variety of clinical syndromes including the acquired immune deficiency syndrome (AIDS) and chronic neurological disorders (*1–5*). Several antiviral agents inhibit the replication of HIV in vitro (*6*). One, 3′-azido-3′-deoxythymidine (AZT, N_3dThd) (*7*), reduces morbidity and mortality in patients with AIDS (*8*), and another, ribavirin (*9*), has been reported to delay or prevent development of AIDS in patients with HIV-induced persistent generalized lymphadenopathy (*10*).

Control of HIV infections in patients for prolonged periods may require a combination of antiviral agents. We have shown that AZT and recombinant interferon-α A synergistically inhibit HIV replication in vitro, presumably by acting at different sites of virus replication (*11*). On the basis of these studies, clinical trials of this combination of drugs are planned. Because of the activity of AZT and ribavirin when administered separately, the combination of these drugs is one that deserves further study, particularly since both drugs are bioavailable when administered orally, cross the blood-brain barrier, and have acceptable toxicity (*6, 12, 13*). Furthermore, the mode of antiviral action of each is different: the triphosphate of AZT acts as an inhibitor of HIV DNA synthesis (*14*) and the triphosphate of ribavirin interferes with post-transcriptional processing (*15*). Therefore, we evaluated the effects of various dose combinations of AZT and ribavirin on HIV replication in vitro. To our surprise, the combination proved antagonistic. The mechanism of antagonism appears to be inhibition of AZT phosphorylation by

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metabolic changes induced by ribavirin.

In studies of AZT-ribavirin interactions, we used different cell types, different modes of HIV inoculation, and different times of drug addition. Four different target cell types, including phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBL) from various healthy seronegative donors, acutely and persistently infected H9 cells (CD4 positive), and a human monocyte cell line (BT4) were used. Experiments were performed in which virus was added before, simultaneously with drugs, or after addition of drugs. In all experiments we used concentrations of AZT and ribavirin that are achievable in human serum and serial two- to fourfold dilutions of these concentrations. In all experiments, AZT alone inhibited HIV replication. Ribavirin's antiviral effect was variable, perhaps reflecting different sensitivities of cell populations from different individuals.

Initially, we used 3-day-old, PHA-stimulated PBL from a healthy HIV-seronegative donor. The cells were cultured at an initial concentration of 1×10^6 per milliliter in 5 ml of culture medium. Cell-free HIV, 5000 tissue culture infectious doses ($TCID_{50}$), was added to each flask. Appropriate concentrations of AZT (0.01, 0.04, 0.08, and 0.16 μM) and ribavirin (4.1, 8.2, 16.4, and 32.8 μM), either alone or in combination, were added to the cultures simultaneously. In all the experiments, the culture medium was changed at 3- or 4-day intervals, and drugs were added at each medium change so that the original drug concentrations were maintained. In this first experiment we measured only viral reverse transcriptase (RT) activity (*16*) as an indicator of HIV replication, and found on days 7 and 10 that the activity was elevated 1.5 to 5 times in cultures that received the drug combination compared to cultures that received only AZT. Cell viability, assessed by trypan blue exclusion, was similar among the cultures studied. Correction of the RT values for viable cell numbers did not affect the results. For those data showing a dose-related inhibitory effect with each drug, interactions were mathematically evaluated by the median effect principle and the isobologram technique according to a computer program (Table 1) (*17, 18*). Strong antagonism between AZT and ribavirin was found for the combined treatment by both methods.

In experiment 2 we sought to confirm these preliminary results by a more detailed analysis (Fig. 1). PBL from a different donor were exposed to 500 $TCID_{50}$ of HIV per 1×10^6 cells for 60 minutes at 37°C and then washed three times to remove any residual extracellular virus. Cells plus AZT or ribavirin, or both, were added to duplicate culture flasks. On days 7, 10, 14, and 18, cultures were assessed for RT activity, for HIV antigens by indirect immunofluorescence (IFA) (*16, 19*), and for virus yield (Fig. 1.) In addition to these analyses, expressed HIV antigen was also assayed by a p24 competition radioimmunoassay (described in legend to Fig. 3). Results were mathematically evaluated after correction for viable cell numbers. Antagonism was observed at all time points (Table 1).

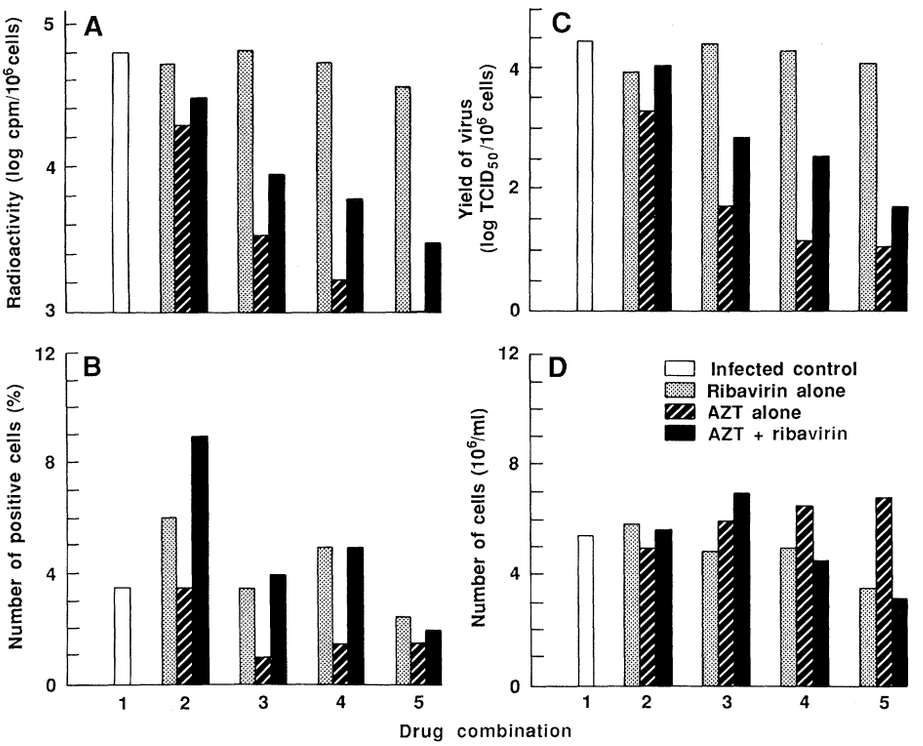

Fig. 1. HIV replication in 7-day PBL cultures from a single donor treated with various combinations of ribavirin and AZT: 1, infected control; 2, 4.1 μM ribavirin and 0.01 μM AZT; 3, 8.2 μM ribavirin and 0.04 μM AZT; 4, 16.4 μM ribavirin and 0.08 μM AZT; 5, 32.8 μM ribavirin and 0.16 μM AZT. All cultures were maintained in 5 ml RPMI-1640 medium supplemented with 20% fetal bovine serum, antibiotics and 10% interleukin-2. Viral replication was assessed by (**A**) RT activity, (**B**) IFA, (**C**) virus yield, and (**D**) cell numbers, and all bars represent the mean value of duplicate cultures. Assays for RT and IFA with patient serum were performed as described (*16, 19*). Virus yield assays, on cell-free culture supernatants, were performed in quadruplicate in 96-well plates with the use of uninfected H9 cells as described (*11, 24*).

In experiment 3 (Fig. 2), uninfected H9 cells were incubated with AZT, ribavirin, or both drugs for 60 minutes before 500 $TCID_{50}$ of HIV were added to all duplicate cultures except the negative controls. Antagonistic interactions were documented on day 7 by virus yield, RT activity, and indirect immunofluorescence.

In experiment 4, we evaluated the anti-HIV effect of a fixed concentration of ribavirin (32.8 μM) with variable concentra-

Table 1. Combination indices for AZT and ribavirin calculated for RT and virus yield (VY) assay data.

Experiment	Day	Assay	Combination molar ratio (AZT: ribavirin)	Combination indices at different percentages of inhibition*		
				ED_{50}	ED_{90}	ED_{95}
1	7	RT	1:200	1.36	2.34	2.92
2	7	RT	1:200	2.89	3.13	3.28
2	10	RT	1:200	2.48	1.40	1.20
2	7	VY	1:200	2.36	3.10	3.42
2	10	RIA	1:200	3.49	3.93	4.12

*Combination indices (CI) were determined by the median effect plot parameters and the multiple drug effect equation as described (*17, 18*). CI values of <1, =1, and >1 indicate synergism, summation, and antagonism, respectively. Analyses were carried out on the assumption of a mutually exclusive drug effect, with the CI being calculated according to the formula $(D)_1/(D_x)_1 + (D)_2/(D_x)_2$, where D_x is the dose required to inhibit a system x %, such as ED_{50}, ED_{90}, and so on, and the combination $(D)_1$ and $(D)_2$ also inhibits x %. If a mutually nonexclusive assumption is used, a third term, which is the product of the first two terms, should be added for CI calculation (*18*). As a consequence, slightly higher CI would be obtained. The CI method also gives isobolograms (*17*) that similarly indicate antagonism between AZT and ribavirin. Peripheral blood mononuclear cells in experiments 1 and 2 were from two different healthy HIV-seronegative donors. Experiment 2 was performed in duplicate.

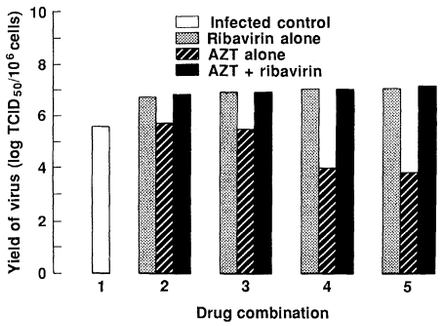

Fig. 2. HIV replication as measured by virus yield ($TCID_{50}$ per 10^6 cells) in H9 cells after 7 days of exposure to different concentrations of ribavirin and AZT: 1, infected control; 2, 4.1 μ*M* ribavirin and 0.01 μ*M* AZT; 3, 8.2 μ*M* ribavirin and 0.04 μ*M* AZT; 4, 16.4 μ*M* ribavirin and 0.16 μ*M* AZT; 5, 32.8 μ*M* ribavirin and 0.64 μ*M* AZT. Duplicate cultures were maintained in RPMI-1640 medium supplemented with 20% fetal bovine serum. Similar results were obtained by measuring RT activity and antigen-positive cells by IFA. All bars represent the mean value of duplicate cultures.

tions of AZT (0.04 to 10 μ*M*) in PBL cultures from a third donor. The use of a fixed concentration of ribavirin precluded the same mathematical evaluation, but the data in Fig. 3 demonstrate that HIV p24 expression was constantly increased in cultures treated with both AZT and ribavirin. Furthermore, the Lineweaver-Burk plot (double reciprocal plot) of the data (Fig. 3) indicates that the anti-HIV effect of AZT is competitively reduced by ribavirin. HIV replication was no longer detectable when concentrations of 10 μ*M* AZT were achieved, either alone or in combination with 32.8 μ*M* ribavirin. This concentration of AZT may be unachievable for prolonged periods in humans.

In experiment 5, we exposed an acutely infected ($CD4^+$) human monocyte cell line (BT4) to various concentrations of AZT, ribavirin, or both drugs in combination. Cell-free supernatant fluid was evaluated on day 7 and an antagonism between AZT and ribavirin was again observed by measuring RT activity and p24 expression.

In experiment 6, persistently infected H9 cells (0.2×10^6 per milliliter) were cocultivated with the same number of uninfected H9 cells and immediately exposed to AZT (0, 0.1, 1.0, or 10 μ*M*) or ribavirin (0, 8.2, 16.4, or 32.8 μ*M*), or both drugs at these concentrations. At days 4 and 7 the culture supernatants were evaluated for HIV replication by radioimmunoassay (RIA) and RT, respectively, and all combination indices indicated strong antagonism in cultures treated with the drug combination.

Thus, antagonism was demonstrated in six separate experiments under different conditions. AZT was nontoxic at the concentrations used. The cytotoxicity of the drug combination was equivalent to that observed with ribavirin alone, and generally became apparent with ≥16.4 μ*M* ribavirin, especially in experiments with prolonged culture time (≥14 days).

Because both AZT and ribavirin are phosphorylated intracellularly (*14, 15*), we surmised that ribavirin or one of its phosphorylated derivatives might inhibit the phosphorylation of AZT. To evaluate this, we exposed uninfected and infected H9 cells and PBL to either AZT alone (0.4 μ*M*) or to a combination of AZT (0.4 μ*M*) and ribavirin (32.8 μ*M*). The concentration of phosphorylated derivatives of AZT was lower in those cultures incubated with the combination of drugs than in control cultures exposed to AZT alone (Table 2). In additional experiments we found consistently a three- to fivefold diminution in azidothymidine triphosphate (AZTTP) when ribavirin was present. This effect was not the result of ribavirin directly inhibiting the phosphorylation of AZT by the host cell cytosolic thymidine kinase (TK). Purified TK (*14*) was not detectably inhibited (<8%) by ribavirin at concentrations up to 20 μ*M* when the concentration of deoxythymidine (dThd) was at its apparent K_m [Michaelis constant, 3 μ*M*; see (*14*)]. For competitive inhibition by ribavirin, the inhibition constant would thus be >100 μ*M*.

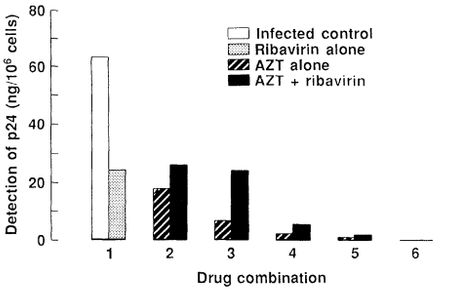

Fig. 3. HIV expression of PBL exposed to a fixed concentration of ribavirin (32.8 μ*M*) and variable concentrations of AZT for 11 days: 1, infected control with ribavirin alone; 2, ribavirin and 0.04 μ*M* AZT; 3, ribavirin and 0.16 μ*M* AZT; 4, ribavirin and 0.64 μ*M* AZT; 5, ribavirin and 2.5 μ*M* AZT; 6, ribavirin and 10 μ*M* AZT. PBL cultures from a single donor were infected with 500 $TCID_{50}$ cell-free HIV and immediately exposed to the drugs. A competition radioimmunoassay (DuPont Corp.) was run in duplicate to detect HIV p24 in cell-free supernatant fluids from all cultures as described in (*11*) and (*25*). Antagonism was confirmed by RT activity.

The effect of ribavirin on AZT phosphorylation is probably due to the well-established ribavirin-induced increase in deoxythymidine triphosphate (dTTP) levels that results in a feedback inhibition of the TK by dTTP (*20, 21*). Furthermore, increasing the concentration of dTTP could reduce the interaction of AZTTP with the HIV reverse transcriptase.

In a 6-month clinical trial, AZT reduced mortality in patients with AIDS and *Pneumocystis carinii* pneumonia (*8*). Ribavirin has been reported to reduce or delay the progression of HIV-associated lymphadenopathy to AIDS in a placebo-controlled clinical trial (*10*). Both drugs would thus appear to be logical candidates for use in combination. Although the results of studies in vitro must be interpreted cautiously, the results we report here suggest that clinical trials of AZT and ribavirin in combination should be attempted only under carefully controlled conditions. Animal studies of the interactions between these compounds might answer the questions of whether phosphorylation inhibition and antagonism occur in vivo.

Our observation of AZT-ribavirin antagonism has another more general implication for the evaluation of antiviral compounds. Other combinations of drugs that undergo intracellular phosphorylation, even when the phosphorylation is catalyzed by different enzymes, may show similar antagonism. It will, therefore, be important to study the interactions between any drugs that utilize similar metabolic pathways, and may be used in combination, such as 2′,3′-dideoxycytidine and other nucleoside analogs (*22*).

Table 2. Effect of ribavirin on AZT phosphorylation. Uninfected and infected H9 cells (20×10^6 H9) and PBL (20×10^6 PBL) were cultured for 24 hours in 25 ml of medium (see Figs. 2 and 3) in the presence of 0.4 μ*M* [5′-^{3}H]AZT (2.44 Ci/mmol) (Burroughs Wellcome) or 32.8 μ*M* ribavirin, or both drugs at these concentrations. Neutralized perchloric acid extracts of the treated cells were analyzed by high-pressure liquid chromatography as described (*14, 23*).

Cells	Treatment	Azidothymidine (picomoles per 10^6 cells)		
		Monophosphate	Diphosphate	Triphosphate
PBL	AZT	29	0.4	0.4
PBL	AZT + ribavirin	5.8	0.1	0.1
HIV-PBL	AZT	39	0.5	0.6
HIV-PBL	AZT + ribavirin	6.3	0.1	0.2
H9	AZT	6.5	0.2	0.2
H9	AZT + ribavirin	2.9	0.04	0.04
HIV-H9	AZT	7.1	0.1	0.1
HIV-H9	AZT + ribavirin	3.0	0.01	0.02

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Region-Specific Expression of Two Mouse Homeo Box Genes

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Mammalian homeo box genes have been identified on the basis of sequence homology to *Drosophila* homeotic and segmentation genes. These studies examine the distribution of transcripts from two mouse homeo box genes, Hox-2.1 and Hox-3.1, throughout the latter third of prenatal development. Transcripts from these genes are regionally localized along the rostro-caudal axis of the developing central nervous system, yielding expression patterns very similar to patterns of *Drosophila* homeotic gene expression.

MANY LABORATORIES ARE CURrently pursuing three general strategies that should help to define specific genes that regulate morphogenesis and cellular differentiation during vertebrate development. The first strategy is the isolation of region-, stage-, or tissue-specific embryonic gene products (RNAs or proteins), with the anticipation that these localized factors are involved in specific determinative events (*1*). The second uses insertional mutagenesis to induce developmental mutations that can be molecularly characterized after using the inserted sequences as a tag for the cloning of the disrupted genes (*2*). The third approach, and the one that we are pursuing, is the analysis of vertebrate genes containing sequences homologous to morphogenetic loci of the fruit fly, *Drosophila melanogaster* (*3*).

We and others (*4–11*) have previously reported the isolation of several murine genes containing sequences homologous to the "homeo box sequences" found in many of the homeotic and segmentation genes of *Drosophila* (*12–14*). On the basis of sequence conservation, homeo boxes are currently divided into two classes: the Antennapedia class and the Engrailed class.

In *Drosophila*, Antennapedia class homeo boxes have been empirically defined as highly conserved, 180-bp, open reading frames found in various genes of the Antennapedia and Bithorax gene complexes (*13*). Each of the homeotic genes of these complexes appears to be required for the proper morphogenesis of a distinct region along the anteroposterior axis of the fruit fly (*14, 15*). Mutant alleles of these genes can cause cells from one region of the embryo to form structures normally found elsewhere in the body. The region-specific functions of these genes are reflected by region-specific patterns of expression. To a first approximation, the expression of any one of these genes during embryonic and larval development appears to be spatially restricted to those cells that will give rise to the body segments most disrupted by mutations in that particular gene (*13, 16–19*).

We have previously proposed that mammalian homeo box genes of the Antennapedia class might perform similar region-specific determinative functions during mammalian development (*4, 10*). This hypothesis is supported by the spatially restricted expression of a murine homeo box gene, Hox-3.1, within the newborn and adult central nervous system (CNS) (*10*). Another possibility that has received some experimental support is that mammalian homeo box genes are involved in the differentiation of specific cell or tissue types (*5, 6, 8, 10, 11*). Although not mutually exclusive, these two hypotheses lead to different expected patterns of mammalian homeo box gene expression: the first to patterns that are region-specific, irrespective of cell or tissue type; and the second to patterns that are cell type–specific.

We recently reported that Hox-3.1 expression within the newborn mouse CNS is spatially restricted to cells posterior to the third cervical vertebra (*10*). We have now examined the distribution of Hox-2.1 transcripts within the CNS of the newborn mouse. The Hox-2 complex on chromosome 11 contains at least four homeo box sequences. The Hox-2.1 and Hox-3.1 probes used for Northern blot and in situ hybridization analysis are shown in Fig. 1a. The Hox-2.1 probe used was a 265-bp Hae III fragment that contains all but the first 10 bp of the homeo box. To date, only one homeo box sequence, Hox-3.1, has been identified in the Hox-3 locus on mouse chromosome 15 (*10*). The Hox-3.1 probe used was a 320-bp Hae III fragment that contains the entire homeo box sequence and 140 bp of flanking sequences (*10*). Hox-2.1 transcripts of between 1.7 and 1.9 kb were detected by Northern blot analysis with RNA samples from the brain (Fig. 1b, lane 1) and the cervical and thoracic regions of the spinal column (lanes 2 and 3, respectively). No transcripts were detectable in the RNA sample from the lumbar spinal column (lane 4). Figure 1c shows the results of a control hybridization of the same filter with a β_2-microglobulin probe, demonstrating comparable amounts of RNA in each track. When this same filter was hybridized with a probe from the Hox-3.1 gene (*10*), tran-

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