before washing, the IC_{50} increased significantly ($P < .05$) to $19.7 \times 10^{-8}M$, which is reflected in Fig. 4 as a shift of the concentration-response curve to the right. Gyang and Kosterlitz (*6*) have reported similar results after phenoxybenzamine treatment of guinea pig ileum. However, a 15-minute incubation with $2 \times 10^{-8}M$ CNA had no effect on the norepinephrine IC_{50}. After these same treatments, opposite results were seen in the responsiveness of muscle strips to morphine; phenoxybenzamine treatment had no effect but CNA treatment significantly inhibited the morphine response. These results indicate that CNA selectively inhibits the depressant effects of morphine on the ileal muscle strips but does not affect the norepinephrine depression, which is not mediated by opiate receptors.

Our studies suggest that the sustained effects of COA and CNA are due to receptor alkylation. The ability of naloxone to block these effects and the absence of similar effects with phenoxybenzamine or chlorambucil demonstrate the specificity of action of COA and CNA. Moreover, the ability of both COA and CNA to act in a nonequilibrium fashion indicates that the aziridinium intermediates derived from these ligands are alkylating a nucleophilic group that is located in an identical or similar receptor environment during the equilibrium phase of binding. Thus we expect these compounds will be of value as pharmacologic and biochemical probes of opioid receptors.

Finally, the nonequilibrium behavior of COA is particularly relevant to the mode of interaction of narcotic agonists with receptors, since this indicates that receptor occupation rather than the rate of ligand-receptor association (*7*) is important for agonist activity.

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8. A computer program written for the parallel line assay [D. J. Finney, *Statistical Methods in Biological Assay* (Hafner, New York, 1964)] was used to analyze the data from at least three different preparations and to generate a concentration-response curve with three or four concentrations of drug. The program was also used to statistically compare two or more curves and to generate IC_{50}'s with a 95 percent confidence interval. These values were also used as a statistical comparison for concentration-response curves.
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Evolutionary Relatedness of Viper and Primate Endogenous Retroviruses

Abstract. *A retrovirus previously isolated from a tumored Russell's viper is shown by molecular hybridization to be an endogenous virus of this reptilian species. Radioimmunologic techniques revealed that the viper retrovirus is immunologically and, hence, evolutionarily related to endogenous type D retroviruses of Old World primates. These findings extend the number of vertebrate classes possessing endogenous retroviruses and suggest that type D retroviruses may even be more widely distributed in nature than type C retroviruses.*

Retroviruses have been isolated from species representing several classes of vertebrates including mammals, birds, and reptiles. In many instances, it has been possible to show that retroviral genomes are endogenous to their species of origin. Moreover, the evidence indicates that at least some endogenous viruses have been genetically transmitted within a vertebrate family for many millions of years (*1, 2*). One major group of endogenous retroviruses of mammals includes type C virus isolates from species as diverse as rodents and primates. These viruses have been shown to share antigenic determinants among each of several of their respective translational products (*3*). More recently, morphologically distinguishable viruses, designated type D retroviruses, have been isolated from species of New and Old World primates (*4*). Evidence of the immunologic relatedness of their major structural proteins has implied that these viruses constitute a distinct group of evolutionarily related retroviruses (*5*).

The only known retrovirus of reptilian origin was isolated from spleen tissue of a tumor-bearing Russell's viper (*Vipera russellii*) (*6*). Initial evidence indicated that the viper retrovirus (VRV) morphologically resembled type C viruses. Yet, VRV was found to be biochemically and immunologically distinguishable from a number of mammalian and avian retroviruses (*7*). We have examined the origin of VRV and determined its relation to retroviruses of other vertebrate species. Viper retrovirus is shown to be genetically transmitted within the viper. Moreover, the demonstration of immunologic relatedness of the viper retrovirus to endogenous type D retroviruses of Old World primates establishes the widespread distribution of this virus group among vertebrates.

Retroviruses that are transmitted within the germ line of a species exhibit extensive nucleotide sequence homology with cellular DNA of their species of origin. To investigate whether or not VRV was endogenous to viper cells, a complementary DNA (cDNA) probe was synthesized in an endogenous reaction with the use of a sucrose gradient-purified VRV. In order to demonstrate that probe sequences were virus-specific, attempts were made to propagate the virus in cells unrelated to Russell's viper. Several mammalian and reptilian cell lines were tested, but VRV grew only in a cell line established from a timber rattlesnake (*8*). As is shown in Fig. 1A, VRV cDNA was hybridized to an extent of more than 70 percent by cellular DNA of rattlesnake cells chronically infected with VRV. In contrast, there was only about 15 percent hybridization with the DNA of uninfected rattlesnake cells. These results demonstrated that the VRV cDNA probe was composed substantially of VRV-specific nucleotide sequences.

In order to ascertain the representation of VRV sequences within viper cellular DNA, the VRV cDNA probe was tested for its ability to hybridize with normal tissue DNA of *V. russellii*. The maximum extent of annealing achieved was 70 percent with half-maximal hybridization of the cDNA probe occurring at a C_0t of 85 (Fig. 1B). These findings demonstrated that the VRV was endogenous to *V. russellii* cells. By comparison with the $C_0t_{1/2}$ for unique sequence *V. russellii* DNA (1500), there were multiple

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(15 to 20) copies of viper retroviral gene sequences within normal *V. russellii* DNA.

Molecular hybridization studies were performed in an effort to detect sequence homology between VRV and other known retroviruses. Under conditions where a given viral cDNA was hybridized to an extent of at least 90 percent by its homologous viral RNA, there was no genetic relatedness demonstrable between VRV and representative type C viruses including Rous sarcoma virus, Rauscher murine leukemia virus, feline leukemia virus, simian sarcoma associated virus, and the baboon endogenous virus. Similarly, there was no detectable sequence homology of VRV with type D viruses including Mason-Pfizer monkey virus (MPMV), squirrel monkey retrovirus (SMRV), and the langur (*Presbytis obscurus*) endogenous virus.

Immunologic techniques can be used to show genetic relatedness between retroviruses that have no detectable nucleotide sequence homology as tested by molecular hybridization. Thus, we undertook the isolation and immunologic characterization of the major structural protein of VRV by sequential phosphocellulose and gel filtration chromatography (*9*). The molecular weight of the major protein of VRV was about 24,000. Antiserum prepared against detergent-disrupted VRV precipitated ^{125}I-labeled VRV p24 at a titer of 320,000 (Table 1). The maximum extent of precipitation was greater than 95 percent. Antiserum to VRV was next tested for precipitation of the major structural proteins of several other retroviruses. There was no significant binding of the proteins of avian or mammalian type C retroviruses. Unexpectedly, antiserum to VRV recognized antigenic determinants associated with the major structural protein (p26) of MPMV, a prototype type D virus. Antiserums directed against either MPMV or the closely related langur endogenous retrovirus also bound VRV p24 (Table 1). In contrast, there was no demonstrable relatedness of SMRV and VRV as determined by immunoprecipitation.

A variety of mammalian type C viruses showed cross-reactivity among their major structural proteins (Table 1). Yet, none of these antiserums recognized the major structural protein of VRV. Similarly, no evidence of cross-reactivity was observed between VRV p24 and the major structural proteins of either avian myeloblastosis virus or mouse mammary tumor virus (Table 1). These results suggested an immunological relationship between VRV and type D viruses of Old World monkeys.

In order to assess the specificity of the immunoprecipitation reactions, a series of competition radioimmunoassays, utilizing various combinations of antibodies and labeled viral proteins, were performed (Table 2). In a homologous immunoassay, in which limiting antibody to VRV was used to precipitate [^{125}I]VRV p24, only VRV competed. In heterologous immunoassays utilizing antiserums directed against either VRV or MPMV to precipitate the reciprocal labeled major structural protein, these viruses, as well as the langur endogenous virus, competed completely. In contrast, none of the type C viruses tested demonstrated detectable cross-reactivity. Finally, VRV failed to compete in an interspecies immunoassay for known type D viruses of New World and Old World primates (Table 2). Thus, the viper retrovirus shares a subset of antigenic determinants with type D viruses of Old World monkeys. Moreover, these determinants are distinct from the subset shared between the latter viruses and SMRV.

To identify conclusively the virion proteins responsible for the immunologic cross-reactivity between VRV and MPMV, detergent-disrupted viruses were subjected to gel filtration chromatography according to the methods previously described (*5*). In each, the antigenic reactivity detected in the interspecies immunoassays cochromatographed with the respective major structural protein, as determined by both molecular size and antigenic reactivity in the appropriate homologous immunoassay. These findings establish that the major internal proteins of VRV and MPMV share interspecies antigenic determinants.

The immunologic relatedness of several respective proteins of mammalian type C viruses (*3*) has provided the strongest evidence in support of the hypothesis that these viruses are all evolutionarily related and, thus, arose from a common

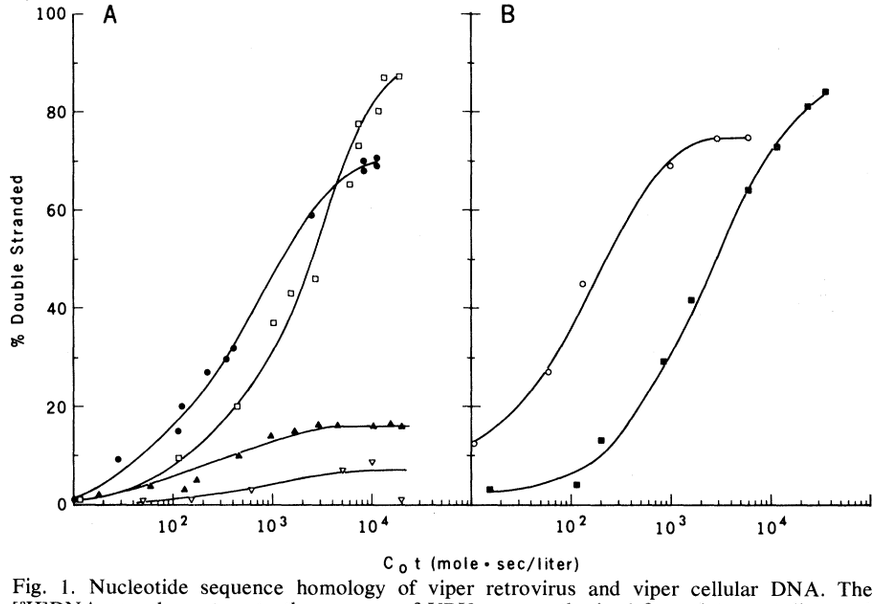

Fig. 1. Nucleotide sequence homology of viper retrovirus and viper cellular DNA. The [^{3}H]DNA complementary to the genome of VRV was synthesized from detergent-disrupted VRV propagated in a line of Russell's viper spleen cells (*6*). The reaction mixture contained 50 m*M* tris-HCl (*p*H 7.5); 60 m*M* KCl; 1 m*M* dithiothreitol; 5 m*M* $MgCl_2$; 2 m*M* each deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanosine triphosphate; 0.01 m*M* [^{3}H]thymidine triphosphate (50 Ci/mmole, New England Nuclear); 0.1 mg/ml actinomycin D (Calbiochem); 0.1 percent Triton X-100; oligonucleotide primers (0.8 mg/ml) prepared by exhaustive digestion of calf thymus DNA by deoxyribonuclease (*13*); and viral protein (0.075 mg/ml). After incubation at 37°C for 8 hours, the cDNA was purified (*14*). Ninety-five percent of the VRV cDNA was rendered acid-soluble after incubation with single-strand specific nuclease (S1); more than 90 percent of the cDNA was protected from digestion by S1 after annealing with excess VRV 70*S* RNA. The $C_0t_{1/2}$ was 2.0×10^{-2} mole · sec/liter, a value consistent with the sequence complexity of the retroviral genome. Total cell DNA and [^{3}H]thymidine-labeled unique sequence cell DNA were prepared as described (*15*). Cell DNA's were fragmented to an average size similar to that of VRV cDNA (6 to 8*S*) by partial depurination (*16*). The DNA-DNA hybridization reactions were performed at 62°C in the presence of 0.6*M* NaCl as described (*1*). Data are presented as a function of C_0t (moles of nucleotide × seconds per liter) corrected to a monovalent cation concentration of 0.18*M*[15]. (A) Hybridization of VRV-cDNA with DNA isolated from uninfected (▲) and VRV-infected (●) rattlesnake cells, and with calf thymus DNA (▽). The rate of annealing of tracer amounts of rattlesnake unique sequence cell [^{3}H]DNA with rattlesnake DNA was also determined (□). (B) Hybridization of VRV [^{3}H]cDNA with DNA isolated from tissues of a normal Russell's viper (○). The rate of the reaction was compared with the rate of hybridization of tracer amounts of Russell's viper unique sequence cell [^{3}H]DNA by its homologous unlabeled cell DNA (■).

progenitor (*10*). Endogenous retroviruses of avian cells have been classified as type C viruses on the basis of their morphogenesis. As yet, however, there is no evidence immunologically linking endogenous avian and mammalian type C retroviruses (*3*). Thus, the evolutionary relatedness of these major groups of endogenous retroviruses of mammals and birds remains to be established.

Our studies establish that a retrovirus initially isolated from a tumor-bearing Russell's viper (*6*) is an endogenous virus of this reptilian species. We were able to demonstrate antigenic relatedness of VRV to mammalian type D retroviruses, some of which are known to be genetically transmitted in New and Old World primates (*2, 11*). Although it cannot be excluded that the shared antigenic determinants have resulted from the convergent evolution of proteins with a similar function, the specificity of the results suggests that VRV and type D viruses of Old World primates are evolutionarily related. As such, this would represent the first known example of evolutionarily linked retroviruses that are genetically transmitted within more than one class of vertebrates.

There are several possible mechanisms by which immunologically related retroviruses might exist within the germ lines of species representing primates and reptiles. The progenitor of present-day endogenous type D viruses of reptiles and primates may have undergone interspecies transfer relatively recently in evolution. This may have occurred by virus transmission from one line to the other, or through infection of both lines by a progenitor retrovirus from a third

Table 1. Antigenic relationships among the major structural proteins of retroviruses as determined by immunoprecipitation. Viper retroviral p24 protein was purified by ion exchange and gel filtration chromatography (*9*) from 10 mg of double sucrose-banded viper retrovirus. The purification of other retroviral structural proteins including Mason-Pfizer monkey virus p26, squirrel monkey retrovirus p35, baboon (*Papio cynocephalus*) endogenous virus p30, simian sarcoma associated virus p30, Rauscher-MuLV p30, avian myeloblastosis virus p27, and mouse mammary tumor virus p26 have been reported (*17*). Each viral protein (1 to 3 μg) was labeled to high specific activity (50 to 80 μCi/μg) by the chloramine T method (*18*). Immunoprecipitation reactions were performed by incubating each of the above ^{125}I-labeled retroviral proteins (10^4 count/min) with twofold serial dilutions of the appropriate antiserum (*19*).

Antiserums elicited against	Antiserum titers* for precipitation of ^{125}I-labeled							
	VRV p24	MPMV p26	SMRV p35	BEV p30	SSAV p30	R-MuLV p30	AMV p27	MMTV p26
Viper retrovirus (VRV)	320,000	2,500	<20	<20	<20	<20	<20	<20
Type D retroviruses								
Mason-Pfizer monkey virus (MPMV)	320	600,000	5,000	<20	<20	<20	<20	<20
Langur endogenous virus (Po-l-Lu)	40	80,000	640	<20	<20	<20	<20	<20
Squirrel monkey retrovirus (SMRV)	<20	2,500	80,000	<20	<20	<20	<20	<20
Type C retroviruses								
Baboon endogenous virus (BEV)	<20	<20	<20	150,000	1,200	640	<20	<20
Simian sarcoma associated virus (SSAV)	<20	<20	<20	6,000	250,000	5,000	<20	<20
Gibbon ape leukemia virus (GaLV)	<20	<20	<20	2,500	250,000	5,000	<20	<20
Rauscher-murine leukemia virus (R-MuLV)	<20	<20	<20	2,000	2,500	250,000	<20	<20
Feline leukemia virus (FeLV)	<20	<20	<20	12,000	10,000	120,000	<20	<20
Avian myeloblastosis virus (AMV)	<20	<20	<20	<20	<20	<20	400,000	<20
Rous sarcoma virus (RSV)	<20	<20	<20	<20	<20	<20	200,000	<20
Type B retroviruses								
Mouse mammary tumor virus (MMTV)	<20	<20	<20	<20	<20	<20	<20	80,000

*Titers are expressed as the reciprocal of the highest serum dilution capable of binding 20 percent of the appropriate ^{125}I-labeled retroviral protein.

Table 2. Immunologic relationships between viper retrovirus and other retroviruses previously isolated from avian and mammalian species. Double sucrose-banded retroviruses (0.1 to 0.5 mg of total protein per milliliter) were tested at twofold serial dilutions for their ability to displace the ^{125}I-labeled retroviral protein (10,000 count/min per tube) for binding limiting amounts of antiserum to the corresponding retrovirus. The experimental conditions for the double antibody competition radioimmunoassay have been described (*19*).

Competing retrovirus	Percent competition* in the following radioimmunoassays							
	αVRV†	αVRV	αMPMV	αSMRV	αRD114	αGaLV	αFeLV	αR-MuLVp30
	VRV p24	MPMV p26	VRV p24	MPMV p26	BEV p30	SSAV p30	R-MuLV p30	RD114 p30
Viper retrovirus (VRV)	100	100	100	<5	<5	<5	<5	<5
Type D retroviruses								
Mason-Pfizer monkey virus (MPMV)	<5	100	100	100	<5	<5	<5	<5
Langur endogenous virus (Po-l-Lu)	<5	100	100	100	<5	<5	<5	<5
Squirrel monkey retrovirus (SMRV)	<5	<5	10	100	<5	<5	<5	<5
Type C retroviruses								
Baboon endogenous virus (BEV)	<5	<5	<5	<5	100	<5	<5	<5
RD114 endogenous cat virus	<5	<5	<5	<5	100	<5	<5	<5
Simian sarcoma associated virus (SSAV)	<5	<5	<5	<5	<5	100	100	100
Gibbon ape leukemia virus (GaLV)	<5	<5	<5	<5	<5	100	100	100
Rauscher-murine leukemia virus (R-MuLV)	<5	<5	<5	<5	<5	<5	100	100
Feline leukemia virus (FeLV)	<5	<5	<5	<5	<5	<5	100	100
Mink endogenous virus (MiLV)	<5	<5	<5	<5	<5	<5	70	100
Deer kidney virus (DKV)	<5	<5	<5	<5	<5	<5	10	100
Avian myeloblastosis virus (AMV)	<5	<5	<5	<5	<5	<5	<5	<5

*Relative displacement of the ^{125}I-labeled retroviral proteins achieved by 10 μg of double-sucrose density gradient purified retroviruses when tested as competing antigens in each of the indicated double-antibody radioimmunoassays. †Schematic representation of a competition radioimmunoassay in which limiting amounts of antiserum elicited against a given retrovirus (for instance, αVRV) are used to precipitate ^{125}I-labeled retroviral protein (10^4 count/min) (for instance, VRV p24).

source. In this regard, we have found no evidence of nucleotide sequence homology between VRV cDNA and cellular DNA's of a number of primate species. Similarly, cDNA probes synthesized from primate type D retroviruses have not hybridized with cellular DNA's of reptilian species so far examined. If such homology were demonstrated, it might provide evidence of relatively recent virus transmission from one line to the other. More extensive analysis will be necessary to determine whether information related to either virus group can be detected within the genomes of species representing other vertebrate families. It is also possible that type D viruses became endogenous to vertebrates prior to the divergence of lines leading to reptiles and mammals more than 300 million years ago. If so, the antigenic determinants shared by the major structural proteins of these viruses would have to be extremely well conserved (*12*). In any case, our studies establish type D retroviruses as a group whose distribution among vertebrates may even be wider than that of type C RNA viruses.

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Localization of the Neurally Mediated Arrhythmogenic Properties of Digitalis

Abstract. *Available evidence suggests that the propensity of digitalis glycosides to produce cardiac arrhythmias is due in part to their neuroexcitatory effects. We have performed experiments in cats which support the existence of a neurogenic component in the etiology of digitalis-induced ventricular arrhythmias. Our data further indicate that the locus of this neural effect lies within an area of the medulla 2 millimeters above to 2 millimeters below the obex. These findings, when considered with the effects of polar cardiac glycosides that do not cross the blood-brain barrier, suggest that the area postrema may be the site of neural activation.*

The cardiac glycosides possess the ability to enhance cardiac contractility and to control certain cardiac rhythm disturbances. These drugs also increase peripheral vascular resistance and, at high doses, may produce emesis, alterations in color vision, and hyperventilation (*1*). The latter phenomena are all thought to be due to the neuroexcitatory properties of digitalis (*2*). The most significant manifestation of digitalis toxicity is alteration of cardiac rhythm. Although digitalis clearly exerts arrhythmogenic effects by direct action on the heart, neural influences have been suggested to play a significant role in the facilitation of cardiac arrhythmias caused by digitalis (*3*).

Transection of the spinal cord at the atlanto-occipital junction (C-1) protects against digitalis toxicity, as judged by higher cumulative drug dose and myocardial content of glycoside at onset of cardiac toxicity. This has been cited as evidence for the nervous system's role in digitalis-induced cardiotoxicity in the cat (*4*). However, the resulting reduction in heart rate and arterial blood pressure by C-1 section causes uncertainty about the mechanism of this protection, since a decrease in myocardial blood flow could protect by decreasing myocardial drug delivery.

Evidence exists to indicate that inhibition of the monovalent cation transport enzyme sodium- and potassium-activated adenosinetriphosphatase is responsible for the direct toxic effects of digitalis on the heart (*5*). We have tried to define further the significance of the higher cumulative ouabain dose and myocardial content of ouabain found at onset of overt toxicity after spinal cord section by measuring active transport of the K^+ analog Rb^+ in myocardial samples from neurally intact and C-1 spinal cord–sectioned animals.

In pentobarbital-anesthetized (30 mg per kilogram of body weight) cats, C-1 spinal cord section increased the dose of the cardiac glycoside ouabain (infused at 1 μg per kilogram of body weight per minute) needed to produce ventricular tachycardia (VT). In a series of 12 experiments, C-1 section increased the dose of ouabain from 76 ± 4 (mean $\pm$ standard error) to 114 ± 8 μg per kilogram of body weight, and decreased the active transport of Rb^+ at onset of VT from 0.41 ± 0.04 to 0.24 ± 0.01 nmole per milligram of wet tissue weight in 30 minutes ($P < .01$). These data indicate that removal of neural influences on the heart by cord section at the level of the atlanto-occipital junction permits 50 percent more drug to be administered before onset of cardiac arrhythmias. Although spinal section caused a mean drop of arterial blood pressure of 57 percent, reduced myocardial blood flow and ouabain delivery do not account for this protective effect, since inhibition of active Rb^+ uptake at onset of VT was increased by 41 percent. A more marked inhibition

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