

Oligonucleotide Fingerprints of RNA Species Obtained from Rhabdoviruses Belonging to the Vesicular Stomatitis Virus Subgroup

JON P. CLEWLEY, DAVID H. L. BISHOP,* CHIL-YONG KANG, JOHN COFFIN,
W. M. SCHNITZLEIN, M. E. REICHMANN, AND ROBERT E. SHOPE

Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294;
Department of Microbiology, University of Southwest Texas Health Science Center, Medical School, Dallas,
Texas 75235; Tufts Medical School, Boston, Massachusetts 02111; University of Illinois, Department of
Microbiology, Urbana, Illinois 61801; and Yale School of Medicine, Department of Epidemiology and Public
Health, New Haven, Connecticut 06510*

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The relationships among the genomes of various rhabdoviruses belonging to the vesicular stomatitis virus subgroup were analyzed by an oligonucleotide fingerprinting technique. Of 10 vesicular stomatitis viruses, Indiana serotype (VSV Indiana), obtained from various sources, either no, few, or many differences were observed in the oligonucleotide fingerprints of the 42S RNA species extracted from standard B virions. Analyses of the oligonucleotides obtained from RNA extracted from three separate preparations of VSV Indiana defective T particles showed that their RNAs contain fewer oligonucleotides than the corresponding B particle RNA species. The fingerprints of RNA obtained from five VSV New Jersey serotype viruses were easily distinguished from those of the VSV Indiana isolates. Three of the VSV New Jersey RNA fingerprints were similar to each other but quite different from those of the other two viruses. The RNA fingerprints of two Chandipura virus isolates (one obtained from India and one from Nigeria) were also unique, whereas the fingerprint of Cocal virus RNA was unlike that of the serologically related VSV Indiana.

The rhabdoviruses are a group of viruses obtained from vertebrates, invertebrates, and plants (6, 51). Forty rhabdovirus isolates of vertebrate and invertebrate origin have been categorized into 21 serological subgroups with little or no evidence of any serological relationship among the members of one subgroup as compared with the members of another subgroup (6, 45). Two of these rhabdovirus subgroups that have been studied serologically and biochemically are the rabies subgroup (rabies, Lagos bat virus, Mokola, kotonkan, Obodhiang, Bolivar, and Dunvenhage viruses) and the vesicular stomatitis virus (VSV) subgroup (VSV Indiana, VSV New Jersey, Cocal, VSV Brazil [Alagoas], Chandipura, and Piry viruses).

Although, by certain serological criteria, the VSV subgroup viruses are interrelated, the exact antigenic sites that are shared are not known. With use of undissociated virus preparations and sera raised against infectious virus, cross-neutralization of infectivity has been demonstrated by Cartwright and Brown (8), who used reciprocal tests between the sera and

viruses of VSV Indiana (C), Cocal, and VSV Brazil; however, they observed little cross-neutralization of infectivity between these viruses and VSV New Jersey (M), Chandipura, or Piry virus. In reciprocal tests, substantial cross-neutralization of infectivity has been observed by R. Shope, not only for some of these viruses, but also for Chandipura and Piry viruses (2, 45), although the latter finding has not been confirmed in similar experiments conducted by Cartwright and Brown (8).

With dissociated virus preparations and the respective antisera, substantial reciprocal cross-neutralization has been reported for VSV Indiana (C), Cocal, VSV Brazil, and VSV New Jersey (M) viruses, suggesting that their infectious nucleocapsids possess common antigenic determinants (8). Complement fixation studies with these viruses confirm (although to differing extents) the presence of common antigenic determinants for members of the VSV subgroups (2, 8, 45).

The serological evidence which indicates that certain VSV subgroup members are more

closely related to each other than to other members of the subgroup has been confirmed, in part, by defective particle interference studies and by RNA hybridization studies (15, 39; W. M. Schnitzlein and M. E. Reichmann, *Virology*, in press). By annealing viral complementary RNA isolated from virus-infected cells to labeled viral RNA extracted from purified virions, it has been shown that some RNA homology exists between VSV Indiana (Birmingham) and Cocal viruses, but there is little homology between either virus and VSV New Jersey (Concan), Chandipura, or Piry virus (39). Various VSV New Jersey isolates exhibit differing extents of RNA homology to each other (Schnitzlein and Reichmann, in press; *vide infra*). These results have been reinforced by enzyme studies, whereby it has been shown that the dissociated transcriptase of VSV Indiana (Birmingham) can transcribe (and render infectious) Cocal enzyme-stripped nucleocapsids but not those of Chandipura or VSV New Jersey (Concan) (4).

Complementation between temperature-sensitive mutants of Cocal and VSV Indiana (C) has also been reported (35), although none was observed between mutants of VSV Indiana (C) and VSV New Jersey (M) or between VSV New Jersey (M) and Cocal virus (34, 35). An ability of wild-type VSV New Jersey (Concan) to rescue only the *in vivo* primary transcription capability of the VSV Indiana (C) *ts* mutant G I-114 at nonpermissive temperatures has, however, been demonstrated (38).

Taken together, all the available evidence indicates that although there are some serological and functional relationships among the members of the VSV subgroup, many of the isolates are serologically and biochemically distinguishable.

The technique of oligonucleotide fingerprinting of a viral RNA (16) allows a further probing of the relationships among various viruses. In addition, it allows one to determine the stability of a virus *vis à vis* its passage history in various laboratories. Since many different isolates of VSV subgroup viruses have been collected (17, 18-20, 22, 43, 44, 47, 50, 51), it is possible to determine by this technique the relationship of one strain of virus to other isolates of the same virus. We report here analyses of some 18 VSV subgroup viruses (including 10 VSV Indiana viruses) obtained from various sources. The divergence that has occurred between these viruses in nature, but their relative stability through passage in many laboratories, is documented by the analyses presented.

MATERIALS AND METHODS

Materials. Acrylamide and bisacrylamide were obtained from Serva laboratories through the Accurate Chemical Corp., Hicksville, N.Y. Sankyo RNase T₁ was obtained through Calbiochem, La Jolla, Calif. [³²P]sodium orthophosphate came from International Chemical and Nuclear, Irvine, Calif.

Viruses. The virus strains used in these studies have each been cloned in BHK-21 cell monolayers and are designated by their serotype and origin, e.g., VSV Indiana (San Juan), or laboratory, e.g., VSV Indiana (Birmingham), where the origin is not known. VSV Indiana ATCC 158, lot 5, was obtained from the American Type Culture Collection (ATCC), Rockville, Md., and is reported by the ATCC to have come from the 1925 VSV Indiana strain isolated by W. E. Cotton from cattle in Richmond, Ind. (14). It will be referred to in this communication as VSV Indiana (standard) strain. VSV Indiana (Birmingham) is the strain that we have maintained in our laboratories for 6 years (1); it originally came from R. Simpson (Rutgers University, New Brunswick, N.J.), who received it (46) from P. W. Choppin (Rockefeller University, New York), who received it (10) from E. D. Kilbourne (Mount Sinai School of Medicine, New York). We have not been able to trace its previous history. The VSV Indiana (HR mutant) was given to us by L. Prevec (McMaster University, Hamilton, Ontario, Canada), who originally received (28) the prototype (designated here as the Toronto strain, from which the heat-resistant HR mutant was developed) from W. Henle (Childrens Hospital, Philadelphia, Pa.). W. Henle obtained the virus (30) from Lederle Laboratories (Pearl River, N.Y.). We have been unable to trace its prior history.

VSV Indiana (San Juan) is the strain provided by L. O. Mott and associates (Agricultural Research Service, Beltsville, Md.) to R. R. Wagner (University of Virginia, Charlottesville, Va.) in 1960 (52). It was originally obtained from vesicular tongue epithelium of an infected steer on the San Juan Indian reservation near Espanola, N.M., in 1956. It has subsequently been passaged once in a cow and then six times in embryonated eggs before being sent to R. R. Wagner (R. R. Wagner, personal communication). An earlier passage of the virus (second egg passage) was received by the Yale Arbovirus Research Unit (YARU). Viruses from both sources have been cloned in BHK-21 cells.

VSV Indiana (C) *ts* G I-114 and *ts* G I-11 are the temperature-sensitive group I mutants of VSV Indiana (C) isolated by C. Pringle (34) in Glasgow, Scotland, from a wild-type VSV Indiana (C) he obtained (34) from J. B. Brooksby of the Animal Virus Research Institute, Pirbright, Surrey, England. We presume that this virus originally came from the 1942 Colorado outbreak of the disease from which VSV Indiana (C) (7, 17) was isolated by A. H. Frank from equine tissue (44). Its origins and subsequent history are described by Shahan et al. (44), Skinner (47), and Federer et al. (17). VSV Indiana (standard) strain, *ts* O I-5, was isolated by A. Flamand (Ph.D. thesis, Université de Paris-Sud, Centre d'Orsay,

France, 1973) in Orsay, France, from a wild-type strain of VSV Indiana that P. Printz (Université de Paris-Sud, Orsay, France) obtained from J. Peries, Hôpital St. Louis, Paris (A. Flamand, personal communication). J. Peries received the virus in 1965 as a reference VSV Indiana A305 strain from C. Chagny (A. Flamand, personal communication). C. Chagny received the virus from H. Levy and S. Baron (National Institutes of Health, Bethesda, Md.), who obtained it from A. Glasgow (National Institutes of Health) in 1961. He received it from the ATCC as the ATCC 158 VSV Indiana (standard) (H. Levy, personal communication).

VSV Indiana (New Mexico) was kindly provided by C. H. Calisher, Center for Disease Control, Fort Collins, Colo., as an isolate obtained from an *Aedes* species at Rancho de Abiquin, N.M., in 1966 and subsequently passaged in suckling mouse brains (49). We have cloned the virus in BHK-21 cell monolayers from the third suckling mouse brain passage received from C. H. Calisher.

Chandipura (Nagpur) was isolated from the serum of a female patient from the Chandipura locality of Nagpur, Maharashtra, India, in 1965 (3). The patient was suffering from a febrile illness during a period when there were Chikungunya and dengue virus epidemics in the locality. A sample of the isolate was received by YARU. We have cloned the virus in BHK-21 cells from a second suckling mouse brain passage made at YARU. Chandipura (Ibadan) was received by YARU as a third mouse brain passage from an isolate obtained from a hedgehog (*Atelexis* sp.) by the Ibadan virus laboratory, University of Ibadan, Nigeria (2). With use of BHK-21 cells, this virus has been cloned from a fourth suckling mouse brain passage of the virus.

VSV New Jersey (Hazelhurst) was obtained from the ATCC (ATCC 159). It is a strain that originated from epithelial tissue taken from infected swine in Hazelhurst, Ga., in 1952 (20).

VSV New Jersey (Concan) was obtained by us from R. Simpson, who received it from R. R. Wagner (R. Simpson, personal communication), who originally obtained the virus from L. O. Mott and associates (R. R. Wagner, personal communication). This strain was isolated in Concan, Tex., in 1949 from material harvested from the dental pad of an infected cow (29). The strain was subsequently passaged three times in cows and then four times in embryonated eggs before being received by R. R. Wagner in 1960 (R. R. Wagner, personal communication). VSV New Jersey (M) was obtained from C. R. Pringle who received it from J. F. Brooksby (34). We believe that this strain is the New Jersey (M) isolated from infected swine in Missouri in 1943 (46). The history of this strain is described by Holbrook et al. (20), Shahan et al. (44), and Skinner (47). VSV New Jersey (Guatemala) was obtained from C. H. Calisher, Center for Disease Control, Fort Collins, Colo. (50). It had initially been isolated in 1970 from a pool of 95 unfed *Culex nigripalpus* mosquitoes collected in 1970 in Montufar, Guatemala (50), and we have cloned it from the third suckling mouse brain passage with BHK-21 cell monolayers.

VSV New Jersey (Ogden) was obtained from J. Holland (University of California at San Diego, La Jolla, Calif.), who received it through S. H. Madin from F. Schaffer (Naval Biomedical Laboratory, Oakland, Calif.), who in turn received it from L. O. Mott (F. Schaffer, personal communication). Originally, the virus was isolated from vesicular epithelium taken from a cow in Ogden, Utah, in 1949 (R. Hanson, University of Wisconsin, personal communication).

Cocal virus was obtained by us from F. Murphy (Center for Disease Control, Atlanta, Ga.), who obtained it from C. H. Calisher (F. Murphy, personal communication), who received it from YARU (C. H. Calisher, personal communication). The virus was originally isolated from *Gigantotaelaps* mites removed from rice rats in Bush Bush Island near Trinidad (23).

Infection of cells and virus purification. Confluent monolayers of 10^7 BHK-21 cells were infected at room temperature for 30 min with a virus stock at an input multiplicity of infection of 0.001 PFU/cell. The cultures were overlaid with 25 ml of minimal essential medium supplemented with 5% (vol/vol) fetal calf serum and 250 μ Ci of [32 P]sodium orthophosphate per ml. The infected cells were incubated at 33°C for a further 36 to 48 h, and the supernatant fluids were recovered and clarified by low-speed centrifugation at 4°C for 30 min at $8,000 \times g$ in a Sorvall RC-5 centrifuge to remove cell debris. Virus was purified from these fluids by polyethylene glycol-NaCl precipitation followed by centrifugation first in a combination potassium tartrate-glycerol gradient and then in a sucrose gradient as described previously (29). Virus bands recovered from the sucrose gradients were diluted fivefold with TSE buffer (0.01 M Tris, 0.15 M NaCl, and 0.002 M EDTA, pH 7.4) and then pelleted through a cushion of 1 ml of 30% (wt/vol) sucrose in TSE buffer by centrifuging at 35,000 rpm for 120 min at 4°C in a Spinco SW41 rotor. Virus pellets were finally resuspended in TSE buffer at protein concentrations of about 1 mg/ml, clarified by centrifugation at $5,000 \times g$ for 10 min, and stored at 4°C until required.

Purification of labeled viral RNA species. Purified, 32 P-labeled virus was adjusted to 1% (wt/vol) sodium dodecyl sulfate (SDS) and immediately layered on preformed linear gradients of 30 to 15% (wt/vol) sucrose containing 0.1% SDS, 0.02 M Tris-hydrochloride, 0.1 M NaCl, and 0.002 M EDTA, pH 7.4. The gradients were centrifuged at 40,000 rpm in a Spinco SW41 rotor at 20°C for 4 h. Fractions (0.3 ml) were collected into siliconized, sterile glass scintillation vials by dripping out from the bottom of the gradients. The distribution of radioactivity was then determined, and the fractions containing the viral RNA species were pooled. Carrier unlabeled chicken embryo RNA (100 μ g) was added to the viral RNA, which was then phenol extracted. After two successive alcohol precipitations to remove all traces of SDS, the RNA samples were freeze-dried, dissolved in 15 μ l of 0.02 M Tris-hydrochloride-0.002 M EDTA, pH 7.4, and stored at -20°C.

Nuclease digestion of RNA and separation of oli-

gonucleotides by two-dimensional gel electrophoresis. The procedures used for RNase T₁ digestion of samples of ³²P-labeled viral RNA containing 2 × 10⁶ to 6 × 10⁶ cpm and the resolution of the resulting oligonucleotides by the two-dimensional polyacrylamide gel electrophoresis system of de Watcher and Fiers (16) have been described (11). Gels were radioautographed as reported previously (11).

RNA annealing and determination of RNase resistance. The methods used to determine the resistance of RNA to digestion by pancreatic and T₁ RNases before or after annealing have been described (39, 42; Schnitzlein and Reichmann, in press).

Preparation of antisera and plaque reduction tests. Antiviral antisera were prepared against VSV Indiana (Toronto) and VSV New Jersey (Concan). Both serotypes of VSV were grown in mouse L cells and partially purified by sucrose gradient centrifugation. The virus band in the sucrose gradient was collected and treated with sodium deoxycholate. The material was then dialyzed and concentrated by pressure dialysis against phosphate-buffered saline and then inoculated into rabbits. The first inoculation into rabbit footpads consisted of 0.4 ml of an antigen-Freund adjuvant mixture. Two subsequent intravenous injections given at weekly intervals consisted of 1 ml of antigen. The total amount of protein administered was approximately 2 mg. The antisera were collected 5 weeks after the initial immunization, and all the antisera were heat inactivated at 56°C for 30 min. Antisera prepared against VSV Indiana (Toronto) G and M proteins were obtained as described previously (25). Antisera raised against other strains of undissociated virus were prepared as described elsewhere (45).

To perform the plaque neutralization tests, 0.5 ml of a 1:8 dilution of antiserum was added to an equal volume of a virus stock. The mixtures were incubated at 37°C for 45 min, and the residual numbers of PFU were determined by plaque assay with BHK-53 or BHK-21 monolayer cultures. The control virus preparations were treated similarly except that normal rabbit serum was used.

Complement fixation and neutralization of infectivity tests with infant mice. Complement fixation tests used 2 U of complement and grid titrations of antibody and antigen incubated for 18 h at 4°C as described elsewhere (9). For the neutralization tests, antibody-virus mixtures were incubated for 1 h at 37°C before intracerebral inoculation in infant mice as described previously (9). Reference hyper-immune mouse antisera were made against VSV Indiana (standard) strain, New Jersey (Hazelhurst), and immune mouse antiserum against VSV New Jersey (Guatemala).

RESULTS

Oligonucleotide fingerprint of VSV Indiana (Birmingham). Purified 42S RNA obtained from virions of VSV Indiana (Birmingham) was digested with RNase T₁, and the resulting oligonucleotides were separated by two-dimen-

sional polyacrylamide gel electrophoresis (Fig. 1A). Two reference dye markers were included in the sample, and their final positions are indicated (bromophenol blue, top center, and xylene cyanol FF, lower center, left). We have found that the oligonucleotide fingerprint is highly reproducible between duplicate analyses of the same RNA preparation or among various preparations of the same virus strain. Although we do not know how many of the spots on the autoradiograph represent multiple oligonucleotides, a nomenclature system for the oligonucleotides is proposed (Fig. 1B). This nomenclature was arrived at by comparing the radioautograms of samples run for different lengths of time in the first or second dimension to produce slightly better resolution of some of the groups of oligonucleotides seen in Fig. 1A. Based on the different intensities of the various spots and the relative radioactivity in the oligonucleotide gel plugs, it is likely that certain spots represent multiple oligonucleotides; consequently if, at some later date, this is shown to be true, then we suggest that they be assigned letters (e.g., 50A, 50B, etc.). The resolution of the faster-moving oligonucleotides in the top half of the second-dimension gel is not sufficient to permit identification of the individual species. These oligonucleotides probably represent non-unique nucleotides or nucleotides with similar electrophoretic mobilities (16). In Fig. 1B, their positions are indicated by the broken lines.

By computing the total radioactivity present in the 71 oligonucleotides identified in the gel and relating this to the amount of ³²P-labeled RNA initially digested, we have determined that these 71 oligonucleotides represent approximately 10% of the VSV genome. Subsequent sequence analyses will be needed to further characterize the oligonucleotides and to determine the exact proportion of the genome that they represent.

Oligonucleotide fingerprint of other laboratory strains of VSV Indiana. The origin of the VSV Indiana strain that D. H. L. Bishop and associates have used over the past 6 years [VSV Indiana (Birmingham) is not known. It has been traced back to a VSV Indiana stock obtained from E. Kilbourne (see Materials and Methods). Although we do not know its earlier passage history, it is of interest to know the genomic relationship of this strain to strains used in other laboratories as well as to recent isolates of the virus.

The VSV Indiana (Toronto) virus used in the laboratory of C.-Y. Kang is believed to have come from Lederle Laboratories, although where Lederle obtained the virus is also not

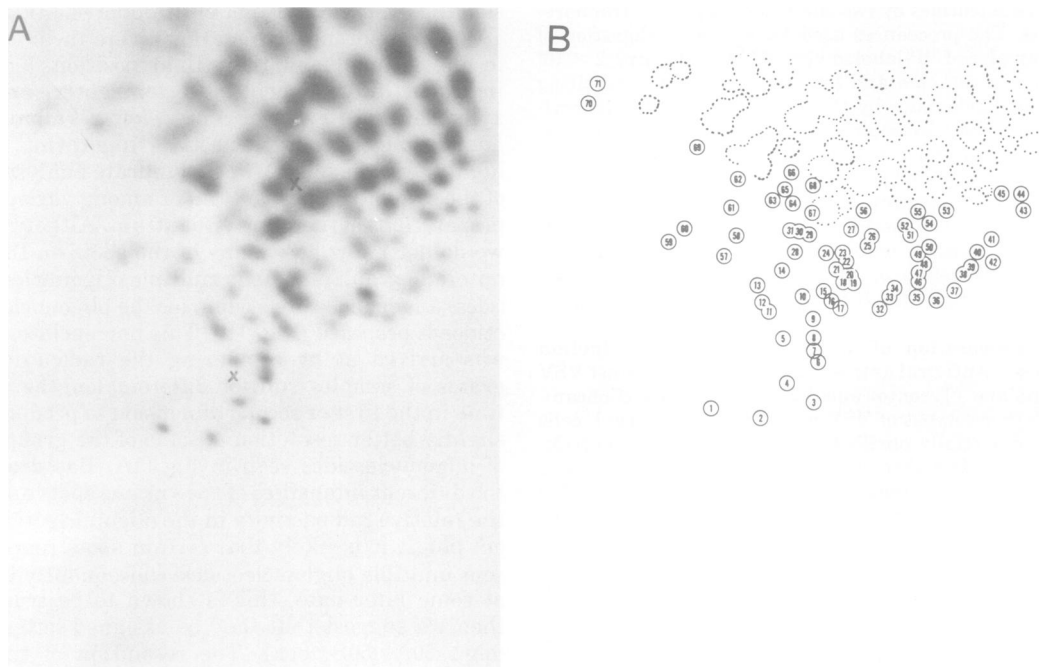


FIG. 1. Oligonucleotide fingerprint of VSV Indiana (Birmingham) B particle RNA. The radioautograph of the oligonucleotide fingerprint of 4×10^6 cpm of VSV Indiana (Birmingham) B RNA is shown (A). A nomenclature system for 71 oligonucleotides resolved in the fingerprint pattern is given in (B). The positions of two dye markers are indicated (\times).

known (see Materials and Methods). Its oligonucleotide fingerprint is indistinguishable from that of VSV Indiana (Birmingham) presented in Fig. 1A (data not shown). The fingerprint of the heat-resistant VSV Indiana mutant derived from VSV Indiana (Toronto) virus lacks only oligonucleotide no. 17 (data not shown). We presume that the absence of this oligonucleotide is a result of the selection procedure used to derive the HR mutant (28). The oligonucleotide fingerprint of the VSV Indiana (standard) strain obtained from the ATCC is also indistinguishable from that of the Birmingham and Toronto viruses (data not shown), suggesting that both viruses may have originated from the standard strain. Although we cannot be certain of this, for the purposes of convenience we propose that the ATCC strain, since it was derived from W. E. Cotton's original isolate, be named VSV Indiana (standard) strain.

We have obtained the oligonucleotide fingerprint of a temperature-sensitive VSV Indiana mutant isolated in the Orsay laboratory of A. Flamand [VSV Indiana (standard) *ts* O I-5]. Its fingerprint was also identical to that of the Birmingham, Toronto, and ATCC viruses (Fig. 2A). Since this virus has been traced back to a

sample obtained from the ATCC over 16 years ago and has subsequently been passed through six laboratories, this result suggests that the genome of the virus has not undergone many changes over that time period.

The oligonucleotide fingerprint of VSV Indiana (San Juan) is given in Fig. 2B. Clearly, the fingerprint differs from those shown in Fig. 1: 11 oligonucleotides (no. 1, 2, 3, 16, 26, 37, 57, 58, 62, 63, and 69) are missing (indicated by an asterisk in Fig. 2B), and 11 new oligonucleotides are present (indicated by arrows in Fig. 2B). A VSV Indiana (San Juan) obtained from YARU, which was received by them after one cow and two egg passages of the original isolate, was found to have an oligonucleotide fingerprint indistinguishable from that shown in Fig. 2B (data not shown).

The oligonucleotide fingerprint of the VSV Indiana (C) *ts* G I-114 mutant isolated in Glasgow is shown in Fig. 2C. The fingerprint bears some resemblance to that of the other strains, although there are several noticeable differences. By comparison with the oligonucleotides identified in Fig. 1B, it appears that oligonucleotides no. 9, 42, 46, 57, 59, and 61 (and possibly oligonucleotide no. 51) are missing (indicated

by asterisks in Fig. 2C), whereas six new oligonucleotides are present (indicated by arrows in Fig. 2C). We do not have, in our laboratories, the parental VSV Indiana (C) from which this *ts* mutant was isolated; however, an analysis of VSV Indiana (C) *ts* G I-11 gave the same fingerprint as that shown in Fig. 2C (data not shown). This result suggests that, unless the mutagenesis procedure used to obtain these two

mutants caused the same genomic changes, the parental strain probably has a fingerprint like that of the two mutants.

Oligonucleotide fingerprint of recent isolates of VSV Indiana. A relatively recent isolate of VSV Indiana has been analyzed by the oligonucleotide fingerprinting technique. The VSV Indiana (New Mexico) isolate (Fig. 2D) bears substantial resemblance to those of the

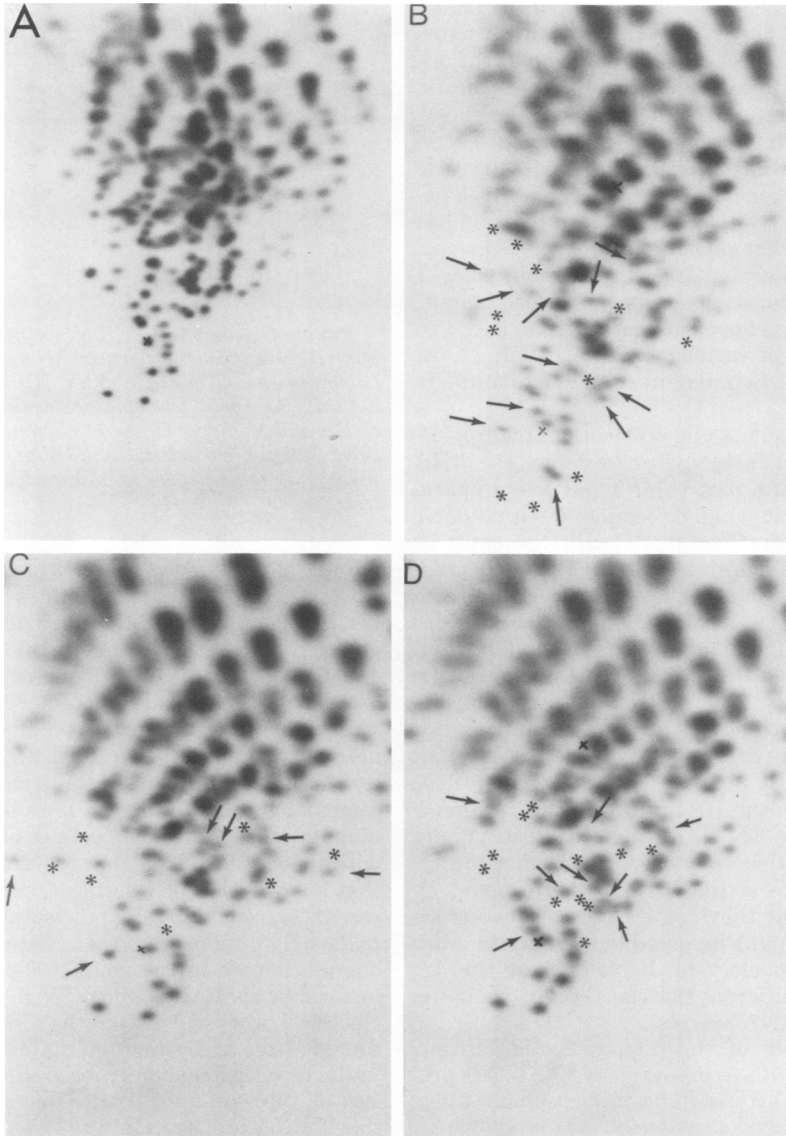


FIG. 2. Oligonucleotide fingerprints of VSV Indiana (Orsay) *ts* O I-5 (A), VSV Indiana (San Juan) B particle RNA (B), VSV Indiana (C) *ts* G I-114 B particle RNA (C), and VSV (New Mexico) B particle RNA (D). Those oligonucleotides in these digests not found in the digest of VSV Indiana (Birmingham) RNA are indicated by arrows, and the oligonucleotides in the VSV Indiana (Birmingham) pattern not found in this pattern are indicated by (*). (See Fig. 1 and text.)

other VSV Indiana strains. However, 11 oligonucleotides are absent from the New Mexico isolate (no. 7, 10, 15, 16, 24, 25, 49, 57, 58, 63, and 65, indicated by stars in Fig. 2D), whereas eight new oligonucleotides are present (indicated by arrows in Fig. 2D).

Oligonucleotide fingerprints of T particle RNAs of VSV Indiana strains. The ability of infectious B particles of VSV Indiana to produce various defective interfering, truncated T particles has been well documented (5, 12, 15, 21, 26, 27, 31, 32, 36, 40–42, 48; Schnitzlein and Reichmann, in press). What determines the origin or induction of T particles is not known. Different types of T particles have been shown to contain RNA species of different sizes. In some cases it has been shown that T particle preparations contain complementary RNA sequences (26, 34, 41), whereas in others it has been shown that no complementary RNA species can be detected (40). In addition, some T particle preparations appear to be unique, whereas others appear to contain particles and RNA species of various sizes (5, 15, 21, 27, 31, 36, 40–42, 48; Schnitzlein and Reichmann, in press).

The technique of oligonucleotide fingerprinting allows an investigator to define the RNA sequence(s) of a particular T particle preparation in terms that can be compared with other T particle preparations made with the same or another virus strain. Moreover, T particle preparations made in other laboratories can also be compared. In addition, if oligonucleotides are found in the T RNA digest that are not present in the digest of the B RNA, this will raise the possibility that the T RNA may contain sequences not present in the presumed progenitor B RNA (e.g., positive-strand RNA sequences). Although most T RNA species (except those obtained from the HR mutant of VSV Indiana) anneal to the 30S viral mRNA (42), it has not been proven that they originate from a contiguous sequence of the B genome. However, by assuming that they come from contiguous sequences, it might be possible to begin to order certain oligonucleotides by comparing the fingerprints of different size classes of overlapping sequence T RNA species.

An example of the oligonucleotide fingerprint of a T RNA preparation (VSV III) prepared from a VSV Indiana (Birmingham) infection as described previously (40) is shown in Fig. 3. Two types of spots on the radioautogram of the T RNA are evident: faint spots similar in position and number to those seen in the fingerprint of the complete B RNA (Fig. 1A) and major spots identical in position to some, but

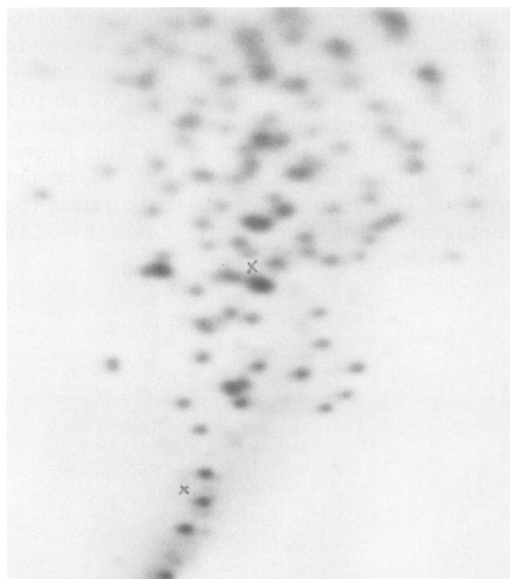


FIG. 3. Oligonucleotide fingerprint of VSV Indiana (Birmingham) T particle RNA. VSV Indiana (Birmingham) T particle (VSV III) RNA oligonucleotide fingerprint was obtained as described in Materials and Methods. Of the 71 oligonucleotides identified for the B particle RNA, 24 oligonucleotides are present as major spots in this radioautogram (see text).

not all, of the oligonucleotides of the B particle RNA. Of the 71 oligonucleotides identified in Fig. 1B, 24 oligonucleotides are present as major spots in the T RNA fingerprint (Fig. 3: no. 2, 4, 6, 8, 10, 14, 18, 21, 22, 25, 30 [or 29 or 31], 36, 37, 38, 44, 47, 50, 54, 56, 59 [or 60], 64, 66, 67, and 70). Although further analyses will be needed to resolve the ambiguities, this tentative assignment has been confirmed in part by comparing the counts per minute in the gel plugs of various oligonucleotides of a B pattern with those of this T pattern (data not given), so that a conclusion of whether certain spots represent one or two oligonucleotides could be made (e.g., indicating that there is only one oligonucleotide in the no. 59, 60 pair, etc). No major new spots (i.e., non-VSV B type oligonucleotides) are evident on the fingerprint, although some underdigested material is present in the lower left-hand corner of the electropherogram. The self-annealing RNase resistance of this T RNA preparation was less than 5%. It is of interest to note that nucleotide complexity analyses of VSV III RNA have given molecular weight estimates of 1.23×10^6 , i.e., also approximately one-third that of the B genome (37).

The fingerprint of a T RNA preparation ob-

tained from an infection with VSV Indiana (standard) strain was identical to that shown in Fig. 3, indicating that the production of this T particle type is a common occurrence.

Oligonucleotide fingerprints of VSV New Jersey strains. Unfortunately, we have been unable to obtain a sample of the original VSV New Jersey isolated by W. E. Cotton (13). However, we have obtained and analyzed five other VSV New Jersey strains (Concan, Guatemala, Ogden, Hazelhurst, and New Jersey M) (Fig. 4

and 5). The fingerprints of the Concan, Ogden, and Guatemala viruses differ by either a few or several oligonucleotides (Fig. 4). The radioautograms of the M and Hazelhurst RNA digests are quite different from those of the Concan, Ogden, and Guatemala viruses (Fig. 5). Coelectrophoresis of digests of the various RNA species and subsequent sequence analyses will be required to determine whether these viruses have any identical oligonucleotides.

Relationship of VSV New Jersey strains as

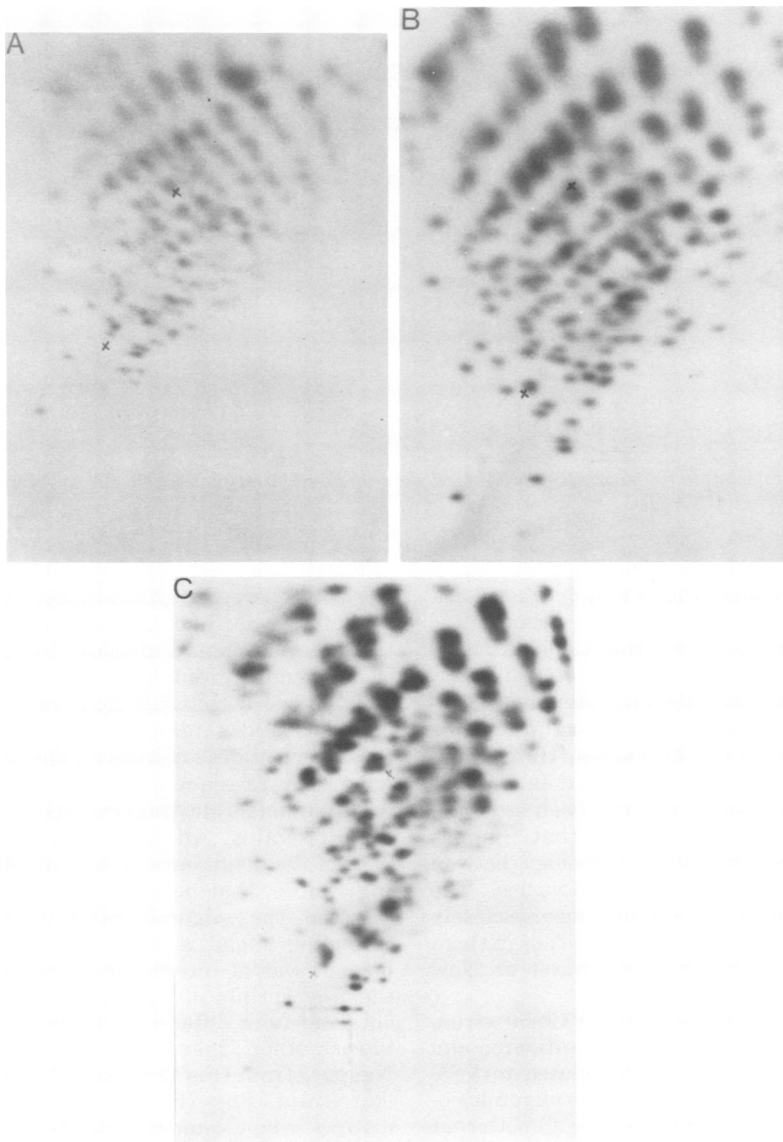


FIG. 4. Oligonucleotide fingerprints of VSV New Jersey (Concan) B particle RNA (A), VSV New Jersey (Guatemala) B particle RNA (B), and VSV New Jersey (Ogden) B particle RNA (C).

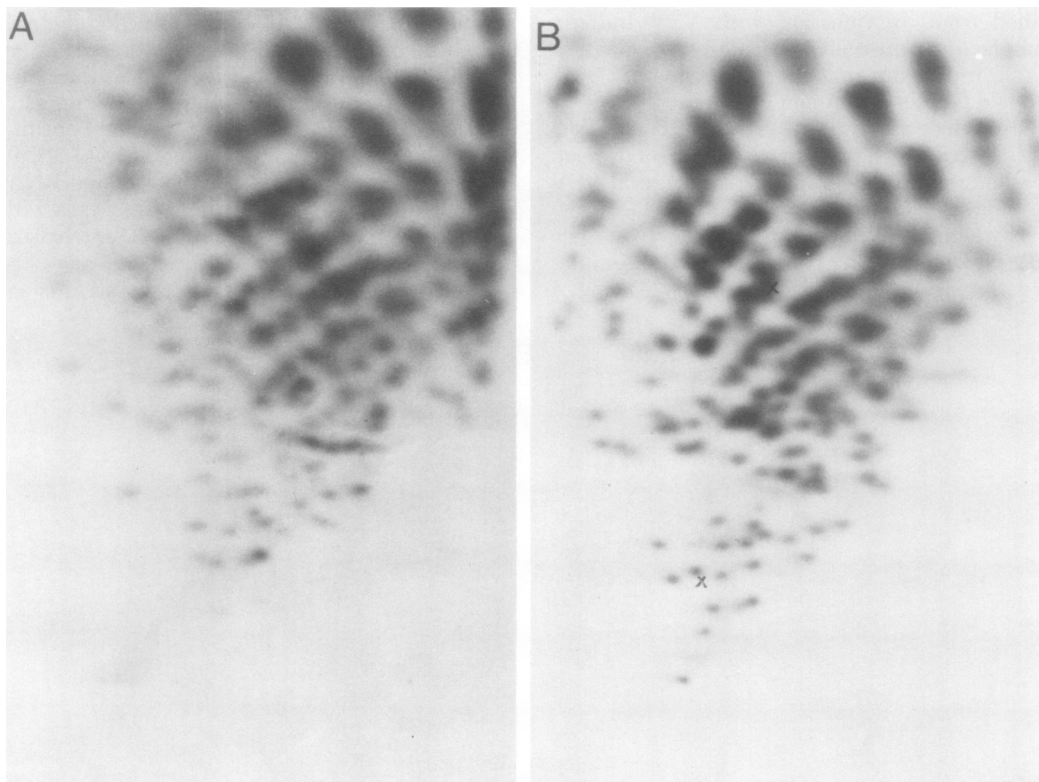


FIG. 5. Oligonucleotide fingerprints of VSV New Jersey (Hazelhurst) B particle RNA (A) and VSV New Jersey (M) B particle RNA (B).

shown by RNA hybridization studies. The results obtained above (Fig. 4 and 5) are consistent with a recent report concerning the limited RNA homology between the Ogden and M strains of VSV New Jersey as determined by hybridization studies (Schnitzlein and Reichmann, in press). Data obtained for the genomic homology between the M, Ogden, Hazelhurst, and Concan strains are presented in Table 1 (the Guatemala isolate has not been studied). From these results, it is clear that there is substantial RNA sequence homology between the Hazelhurst and New Jersey M viruses. The Ogden and Concan viruses are likewise closely related to each other; however, neither of them is closely related to the Hazelhurst or New Jersey M virus (Table 1).

Oligonucleotide fingerprint of Cocal virus. Cocal virus has been shown, by antigenic and RNA homology studies, to be related to VSV Indiana (23, 39). Comparison of the oligonucleotide fingerprint of Cocal virus (Fig. 6) with that of the VSV Indiana (Birmingham) virus (Fig. 1) indicates that there are few large oligonucle-

otides that are identical for the two virus types. Coelectrophoresis of RNA digests of Cocal and VSV Indiana (Birmingham) viruses confirms this observation and indicates that, of oligonucleotides no. 1 through 50 of VSV Indiana (Fig. 1B), no more than 10 comigrate with Cocal oligonucleotides (data not shown). Whether these nucleotides are in fact identical will again have to await sequence analyses.

Oligonucleotide fingerprints of two Chandipura strains. Although it was unlikely, in view of the divergence seen in all of the foregoing studies, that homology would be observed between the oligonucleotide fingerprints of Chandipura and other VSV subgroup members, it was of interest to determine whether the fingerprints of Chandipura virus isolates obtained from different continents are similar to each other. The fingerprints of Chandipura, Nagpur strain (Fig. 7A), and Chandipura, Ibadan strain (Fig. 7B), were found to be quite distinct when compared with each other (or with other VSV subgroup members). No coelectrophoresis studies have been undertaken, so

we do not know how many of the oligonucleotides of the two Chandipura strains comigrate.

Serological identification of the virus isolates. The viruses used in these studies were tested against antiserum raised against VSV Indiana (Toronto), VSV Indiana (Birmingham), or VSV New Jersey (Concan), in order to confirm their serotypes. In plaque reduction tests conducted in C.-Y. Kang's and D. H. L. Bishop's laboratories, all the viruses were shown to belong to the expected serotype, with the exception that, in one series of tests, VSV New Jersey (Guatemala) was neutralized by both VSV Indiana and VSV New Jersey antisera (Table 2). A second batch of VSV Indiana antisera raised against VSV Indiana (Toronto) G and M proteins gave the same results. In another set of analyses conducted in the laboratory of D. H. L. Bishop, only VSV New Jersey (Concan) antiserum neutralized the VSV New Jersey (Guatemala) virus. An independent study in a third laboratory was undertaken, and the ability of antisera to protect mice against VSV New Jersey (Guatemala)

was determined. Only the VSV New Jersey antiserum neutralized VSV New Jersey (Guatemala) in mice (Table 3). Complement fixation studies also documented the relationship of the Guatemala virus to VSV New Jersey (Table 4).

DISCUSSION

The VSV subgroup of rhabdoviruses have been shown to be antigenically related to each other to greater or lesser extents (2, 3, 6, 8, 17, 20, 23, 45). Although these viruses have similar types of virion structural polypeptides (L, G, N, NS, and M), serological, genetic, and molecular studies have indicated that there is considerable divergence among the group (see review by Bishop and Smith [6]). The present investigation was designed to determine whether the oligonucleotide fingerprints of the different members of the subgroup confirmed that divergence and whether different isolates of one serotype were unique or could be shown by this procedure to be identical or similar to each other.

TABLE 1. RNA hybridization analyses of VSV New Jersey strains^a

Probe (VSV New Jersey strain)	RNA species	RNA sample	Probe annealed (%)
(M)	13-18S	None	1
(M)		BHK-21 ribosomal RNA	1
(M)		VSV N.J. (M) viral RNA	93
(M)		VSV N.J. (Hazelhurst) viral RNA	83
(M)		VSV N.J. (Concan) viral RNA	25
(M)	28S	None	15
(M)		BHK-21 ribosomal RNA	14
(M)		VSV N.J. (M) viral RNA	88
(M)		VSV N.J. (Hazelhurst) viral RNA	77
(M)		VSV N.J. (Concan) viral RNA	15
(Ogden)	13-18S	None	4
(Ogden)		BHK-21 ribosomal RNA	2
(Ogden)		VSV N.J. (Ogden) viral RNA	100
(Ogden)		VSV N.J. (Hazelhurst) viral RNA	30
(Ogden)		VSV N.J. (M) viral RNA	26
(Ogden)		VSV N.J. (Concan) viral RNA	91
(Ogden)	28S	None	5
(Ogden)		BHK-21 ribosomal RNA	5
(Ogden)		VSV N.J. (Ogden) viral RNA	93
(Ogden)		VSV N.J. (Hazelhurst) viral RNA	25
(Ogden)		VSV N.J. (M) viral RNA	15
(Ogden)		VSV N.J. (Concan) viral RNA	80

^a Preparations of approximately 10^8 cpm of ³H-labeled mRNA species (13-18S or 28S) were annealed to unlabeled viral RNA, and the percent RNase resistance was determined as described previously (42; Schnitzlein and Reichmann, in press). VSV New Jersey (N.J.) (Concan) viral RNA annealed 97 and 90%, respectively, to VSV N.J. (Concan) 13-18S and 28S mRNA species. VSV N.J. (M) is the VSV N.J. G (Glasgow) referred to previously (Schnitzlein and Reichmann, in press), whereas VSV N.J. (Concan) is the VSV N.J. P (Prevec) referred to previously (Schnitzlein and Reichmann, in press).

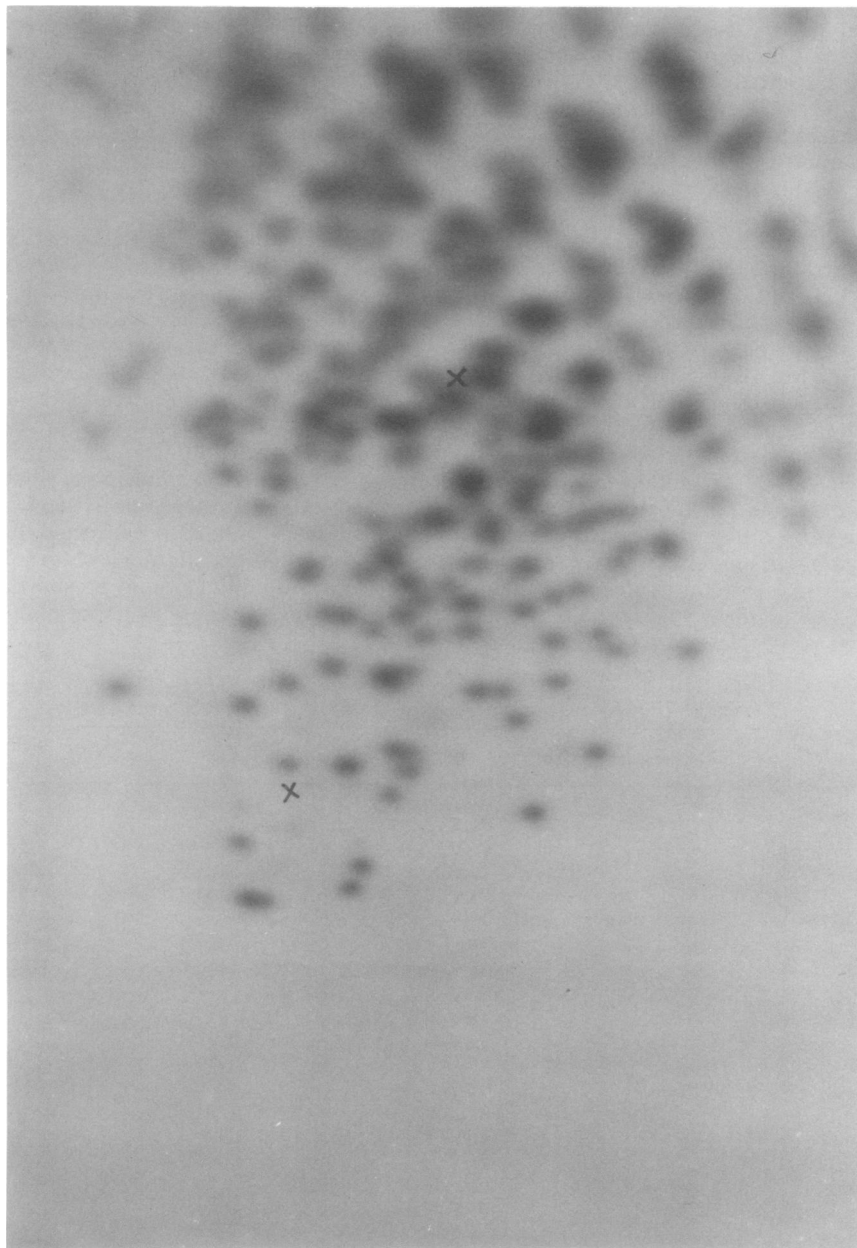


FIG. 6. *Oligonucleotide fingerprint of Cocal B particle RNA.*

The results obtained indicate that, although different isolates of VSV Indiana are distinguishable by the oligonucleotide fingerprinting technique, there are a considerable number of oligonucleotides that appear to have been conserved. Thus, the ATCC VSV Indiana (standard) strain isolated in 1925 from a cow has a very similar oligonucleotide fingerprint to that

of VSV Indiana (San Juan) isolated in 1956 from a steer, as well as those of VSV Indiana (New Mexico) isolated in 1966 from insects and VSV Indiana (C) that was originally isolated in 1942 from a horse. By contrast, the fingerprint of Cocal virus is quite distinct from the VSV Indiana patterns, indicating the divergence that has occurred between the genomes of these two

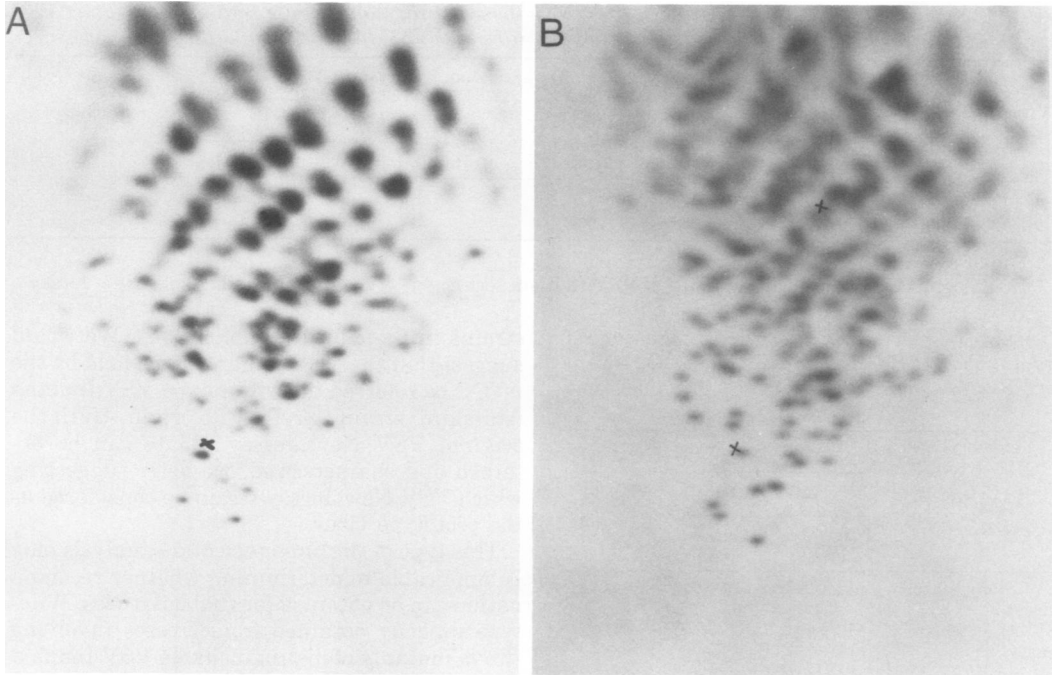


FIG. 7. Oligonucleotide fingerprints of Chandipura (Nagpur) B particle RNA (A) and Chandipura (Ibadan) B particle RNA (B).

TABLE 2. Plaque reduction tests with rhabdoviruses and antisera raised against VSV New Jersey (Concan) or VSV Indiana (Toronto) or (Birmingham) strains

Virus	Titer after antiserum treatment			Serotype		
	Normal rabbit	VSV Ind. ^{a,b}	VSV N.J.	Ind.	N.J.	Other
VSV Ind. (New Mexico)	7.0×10^9	1.3×10^{2a}	1.1×10^{9b}	+		
VSV Ind. (Toronto)	1.5×10^8	$<10^a$	9.0×10^{7b}	+		
VSV Ind. (HR)	5.0×10^8	$<10^a$	7.4×10^{7b}	+		
VSV Ind. (standard) <i>ts</i> O I-5	1.6×10^9	$<10^a$	1.0×10^{9b}	+		
VSV Ind. (standard)	2.0×10^6	9.0×10^{3c}	2.0×10^{6d}	+		
VSV Ind. (Birmingham)	4.5×10^6	2.0×10^{4c}	4.8×10^{6d}	+		
VSV Ind. (C), <i>ts</i> G I-114	3.3×10^8	$<10^a$	2.9×10^{8b}	+		
VSV N.J. (Concan)	2.0×10^8	4.9×10^{7a}	$<10^b$		+	
VSV N.J. (M)	6.0×10^8	1.2×10^{8a}	1.2×10^{2b}		+	
VSV N.J. (Hazelhurst)	7.1×10^8	3.3×10^{8c}	2.0×10^{4d}		+	
VSV N.J. (Ogden)	5.2×10^8	2.9×10^{8c}	2.1×10^{4d}		+	
VSV N.J. (Guatemala)	3.3×10^8	$<10^a$	$<10^b$	+	+	
VSV N.J. (Guatemala)	8.5×10^4	8.0×10^{4c}	$<10^d$		+	
Cocal	1.0×10^9	2.0×10^{5a}	2.5×10^{8b}			+
Chandipura (Ibadan)	4.6×10^8	4.2×10^{8c}	4.4×10^{8d}			+
Chandipura (Nagpur)	1.3×10^9	3.7×10^{8c}	4.5×10^{8d}			+

^a Antisera prepared in one laboratory against (a) VSV Indiana (Toronto) and (b) VSV New Jersey (Concan) and in another laboratory against (c) VSV Indiana (Birmingham) and (d) VSV New Jersey (Concan) were mixed with virus, and the residual number of PFU was determined as described in Materials and Methods. Ind., Indiana; N.J., New Jersey.

virus types. The fingerprints of the Chandipura viruses, isolated in India or Nigeria, also appear to be quite distinct by comparison to each other. The conservation seen between the oli-

gonucleotide sequences of various VSV Indiana strains (and from the same strain passaged over many years in several laboratories) is much less evident from some of the VSV New

TABLE 3. Neutralization tests in mice with VSV New Jersey strains and mouse sera raised against VSV New Jersey, Hazelhurst, and Guatemala strains and VSV Indiana standard strain

Virus (VSV New Jersey strain)	Titer ^a after antiserum treatment				Serotype
	Normal mouse	VSV Ind. ^b	VSV N.J.		
			Hazelhurst	Guatemala	
Hazelhurst	9.5	7.2	<3.2	4.7	N.J.
Guatemala	7.4	6.1	2.2	3.4	N.J.

^a Log of mean lethal dose per gram.

^b Homologous log neutralization index of VSV Indiana serum was >4.7. Ind., Indiana; N.J., New Jersey.

TABLE 4. Complement fixation tests with antigens and antibody of Hazelhurst and Guatemala strains of VSV New Jersey and standard strain of VSV Indiana

Antigen		Antibody		
		VSV N.J. ^a		VSV Ind.
		Hazelhurst	Guatemala	
VSV N.J. Hazelhurst	512/1,024 ^b	16/1,024	<4/<4	
VSV N.J. Guatemala	512/64	32/64	<4/<4	
VSV Ind.	<4/<4	<2/<4	256/1,024	
Normal	<4/<4	<2/<4	<4/<4	

^a NJ, New Jersey; Ind, Indiana.

^b Reciprocal of antibody titer/reciprocal of antigen titer.

Jersey strains. The genomic RNAs of VSV New Jersey (Guatemala), (Ogden), and (Concan) strains have many oligonucleotides that appear to be identical. All three RNAs appear to have few oligonucleotides in common with VSV New Jersey (M) and VSV New Jersey (Hazelhurst). RNA homology studies confirm these differences (Schnitzlein and Reichmann, in press; Table 1). Why the VSV New Jersey isolates are so distinct, and yet are serologically related to each other (Tables 2 to 4), is an interesting question that should be investigated.

We have no explanation for the enigma of the VSV New Jersey (Guatemala) serological studies. From studies conducted in two laboratories, the virus appears to be a New Jersey serotype, whereas, from studies in a third laboratory, it appears to be substantially neutralized by both VSV Indiana and New Jersey antisera. Further analyses will be undertaken to resolve this question.

It is clear that different laboratories have been working with different strains of VSV Indiana. We have traced, as far as possible, the origins of all the strains we have analyzed in this study. This should allow many investigators to determine which virus strains they are working with and permit them to identify these

strains in their future publications. We would suggest that the VSV Indiana virus held by the ATCC be referred to as prototype VSV Indiana (standard) strain to reflect its origin. Until the original VSV New Jersey isolate can be obtained and characterized, we defer suggesting which VSV New Jersey strain be considered as the prototype virus.

This type of the oligonucleotide analysis may be applicable to determining whether recombination can be obtained for rhabdoviruses. Wild-type progeny obtained from crosses involving two *ts* mutants of distinguishable VSV Indiana strains could be screened for recombinant sequences. The limits of such an approach would be the revertant frequencies of the two *ts* mutants.

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LITERATURE CITED

1. Aaslestad, H. G., H. F. Clark, D. H. L. Bishop, and H. K. Koprowski. 1971. Comparison of the ribonucleic acid polymerases of two rhabdoviruses, Kern Canyon virus and vesicular stomatitis virus. *J. Virol.* 7:726-735.
2. Berge, T. O. 1975. International catalogue of arboviruses including certain other viruses of vertebrates. Department of Health, Education, and Welfare publication number CDC 75-8301. Center for Disease Control, Atlanta, Ga.
3. Bhatt, P. N., and F. M. Rodrigues. 1967. Chandipura: a new arbovirus isolated in India from patients with febrile illness. *Ind. J. Med. Res.* 55:295.
4. Bishop, D. H. L., S. U. Emerson, and A. Flamand. 1974. Reconstitution of infectivity and transcriptase activity of homologous and heterologous viruses: vesicular stomatitis (Indiana serotype), Chandipura, vesicular stomatitis (New Jersey serotype), and Cocal viruses. *J. Virol.* 14:139-144.
5. Bishop, D. H. L., and P. Roy. 1971. Properties of the product synthesized by vesicular stomatitis virus par-

- ticles. *J. Mol. Biol.* 58:799-814.
6. Bishop, D. H. L., and M. S. Smith. 1977. Rhabdoviruses, p. 169-280. *In* D. Nayak (ed.), *The molecular biology of animal viruses*. M. Dekker, Inc., New York.
 7. Bradish, C. J., J. B. Brooksby, and J. F. Dillon. 1956. Biophysical studies of the virus system of vesicular stomatitis. *J. Gen. Microbiol.* 14:290-314.
 8. Cartwright, B., and F. Brown. 1972. Serological relationships between different strains of vesicular stomatitis virus. *J. Gen. Virol.* 16:391-398.
 9. Casals, J. 1967. Immunological techniques for animal viruses. *Methods Virol.* 3:113-198.
 10. Choppin, P. W. 1964. Multiplication of a myxovirus (SV5) with minimal cytopathic effects and without interferences. *Virology* 23:224-233.
 11. Clewley, J., J. Gentsch, and D. H. L. Bishop. 1977. Three unique viral RNA species of snowshoe hare and La Crosse bunyaviruses. *J. Virol.* 22:459-468.
 12. Cooper, P. D., and A. J. D. Bellett. 1959. A transmissible interfering component of vesicular stomatitis virus preparations. *J. Gen. Microbiol.* 21:485-497.
 13. Cotton, W. E. 1927. Vesicular stomatitis. *Vet. Med.* 22:169-175.
 14. Cotton, W. E. 1927. The causal agent of vesicular stomatitis proved to be a filter-passing virus. *Am. Vet. Med. Assoc. J.* 23:168-181.
 15. Crick, J., and F. Brown. 1972. Interference as a measure of cross relationship in the vesicular stomatitis group of rhabdoviruses. *J. Gen. Virol.* 18:79-82.
 16. de Watcher, R., and W. Fiers. 1972. Preparative two-dimensional polyacrylamide gel electrophoresis of ³²P-labeled RNA. *Anal. Biochem.* 49:184-197.
 17. Federer, K. E., R. Burrows, and J. B. Brooksby. 1967. Vesicular stomatitis virus—the relationship between some strains of the Indiana serotype. *Res. Vet. Sci.* 8:103-117.
 18. Hanson, R. P. 1952. The natural history of vesicular stomatitis. *Bacteriol. Rev.* 16:179-204.
 19. Hanson, R. P. 1968. Discussion of the natural history of vesicular stomatitis. *Am. J. Epidemiol.* 87:264-266.
 20. Holbrook, A. A., J. N. Geleta, and W. C. Patterson. 1956. A typing study of vesicular stomatitis virus field samples of swine origin, p. 293-301. *In* Proceedings of the U. S. Livestock Sanitation Association, 60th Annual Meeting.
 21. Huang, A. S., and R. R. Wagner. 1966. Defective T particles of vesicular stomatitis virus. II. Biologic role in homologous interference. *Virology* 30:173-181.
 22. Jenney, E. W., and L. O. Mott. 1963. Serologic studies with the virus of vesicular stomatitis. II. Typing of vesicular stomatitis convalescent serum by direct complement fixation. *Am. J. Vet. Res.* 24:874-879.
 23. Jonkers, A. H., R. E. Shope, T. H. G. Aitken, and L. Spence. 1964. Cocal virus, a new agent in Trinidad related to vesicular stomatitis virus, type Indiana. *Am. J. Vet. Res.* 25:236-242.
 24. Kang, C.-Y., and L. Prevec. 1969. Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. *J. Virol.* 3:404-413.
 25. Kang, C.-Y., and L. Prevec. 1970. Proteins of vesicular stomatitis virus. II. Immunological comparisons of viral antigens. *J. Virol.* 6:20-27.
 26. Lazzarini, R. A., G. H. Weber, L. D. Johnson, and G. M. Stamminger. 1975. Covalently linked message and anti-message (genomic) RNA from a defective vesicular stomatitis virus particle. *J. Mol. Biol.* 97:298-308.
 27. Leamson, R. N., and M. E. Reichmann. 1974. The RNA of defective vesicular stomatitis virus particles in relation to viral cistrons. *J. Mol. Biol.* 85:551-568.
 28. Nakai, T., and A. F. Howatson. 1968. The fine structure of vesicular stomatitis virus. *Virology* 35:268-281.
 29. Obijeski, J. F., A. T. Marchenko, D. H. L. Bishop, B. W. Cann, and F. A. Murphy. 1974. Comparative electrophoretic analysis of the virus proteins of four rhabdoviruses. *J. Gen. Virol.* 27:21-33.
 30. Paucker, K., Z. Skurska, and W. Henle. 1962. Quantitative studies on viral interference in suspended L cells. I. Growth characteristics and interfering activities of vesicular stomatitis, Newcastle disease, and influenza A viruses. *Virology* 17:301-311.
 31. Perrault, J. 1976. Cross linked double stranded RNA from a defective vesicular stomatitis particle. *Virology* 70:360-371.
 32. Petric, M., and L. Prevec. 1970. Vesicular stomatitis virus—a new interfering particle, intracellular structures, and virus-specific RNA. *Virology* 41:615-630.
 33. Pringle, C. R. 1970. Genetic characteristics of conditional lethal mutants of vesicular stomatitis virus induced by 5-fluorouracil, 5-azacytidine, and ethyl methyl sulphamate. *J. Virol.* 5:559-567.
 34. Pringle, C. R., I. B. Duncan, and M. Stevenson. 1971. Isolation and characterization of temperature-sensitive mutants of vesicular stomatitis virus, New Jersey serotype. *J. Virol.* 8:836-841.
 35. Pringle, C. R., and W. H. Wunner. 1973. Genetic and physiological properties of temperature sensitive mutants of Cocal virus. *J. Gen. Virol.* 12:677-683.
 36. Reichmann, M. E., C. R. Pringle, and E. A. C. Follett. 1971. Defective particles in BHK cells infected with temperature-sensitive mutants of vesicular stomatitis virus. *J. Virol.* 8:154-160.
 37. Repik, P., and D. H. L. Bishop. 1973. Determination of the molecular weight of animal RNA viral genomes by nuclease digestions. I. Vesicular stomatitis virus and its defective T particle. *J. Virol.* 12:969-983.
 38. Repik, P., A. Flamand, and D. H. L. Bishop. 1976. Synthesis of RNA by mutants of vesicular stomatitis virus (Indiana serotype) and the ability of wild-type VSV New Jersey to complement the VSV Indiana *ts* G I-114 transcription defect. *J. Virol.* 20:157-169.
 39. Repik, P., A. Flamand, H. G. Clark, J. F. Obijeski, P. Roy, and D. H. L. Bishop. 1974. Detection of homologous RNA sequences among six rhabdovirus genomes. *J. Virol.* 13:250-252.
 40. Roy, P., and D. H. L. Bishop. 1972. The genome homology of vesicular stomatitis virus and defective T particles and evidence for the sequential transcription of the virion ribonucleic acid. *J. Virol.* 9:946-955.
 41. Roy, P., P. Repik, E. Hefti, and D. H. L. Bishop. 1973. Complementary RNA species isolated from vesicular stomatitis (HR strain) defective virions. *J. Virol.* 11:915-925.
 42. Schnitzlein, W. M., and M. E. Reichmann. 1976. The size and cistronic origin of defective vesicular stomatitis virus particle RNAs in relation to homotypic and heterotypic interference. *J. Mol. Biol.* 101:307-325.
 43. Schoening, H. W. 1944. Vesicular stomatitis in swine, p. 85-86. *In* Proceedings of the U. S. Livestock Sanitation Association, 1944.
 44. Shahan, M. S., A. H. Frank, and L. O. Mott. 1946. Studies of vesicular stomatitis with special reference to a virus of swine origin. *J. Am. Vet. Med. Assoc.* 108:5-19.
 45. Shope, R. E. 1975. Rabies virus antigenic relationships, p. 141-152. *In* G. Baer (ed.), *The natural history of rabies*, vol. 1. Academic Press Inc., New York.
 46. Simpson, R. W., and R. W. Hauser. 1966. Structural components of vesicular stomatitis virus. *Virology* 29:654-667.
 47. Skinner, H. H. 1957. The virus of vesicular stomatitis in small experimental hosts. I. White mice, cotton rats, chick embryos and young chickens. *J. Comp. Pathol.*

- 67:69-86.
48. **Stamminger, G., and R. A. Lazzarini.** 1974. Analysis of the RNA of defective VSV particles. *Cell* 3:85-93.
 49. **Sudia, W. D., B. N. Fields, and C. H. Catisher.** 1967. The isolation of vesicular stomatitis virus (Indiana strain) and other viruses from mosquitoes in New Mexico. *J. Epidemiol.* 86:598-602.
 50. **Sudia, W. D., R. W. Lord, V. F. Newhouse, D. L. Miller, and R. E. Kissling.** 1971. Vector-host studies of an epizootic of Venezuelan equine encephalomyelitis in Guatemala. 1969. *Am. J. Epidemiol.* 93:137-143.
 51. **Wagner, R. R.** 1975. Reproduction of rhabdoviruses, p. 1-93. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 4: Reproduction of large RNA viruses. Plenum Press, New York.
 52. **Wagner, R. R., A. H. Levy, R. M. Snyder, G. A. Ratcliff, Jr., and D. F. Hyatt.** 1963. Biologic properties of two plaque variants of vesicular stomatitis virus (Indiana serotype). *J. Immunol.* 91:112-122.