

Proteins of Vesicular Stomatitis Virus

II. Immunological Comparisons of Viral Antigens

C. Y. KANG AND L. PREVEC

Department of Biology, McMaster University, Hamilton, Ontario, Canada

Received for publication 11 March 1970

The antigens of the nucleoprotein core and the coat of vesicular stomatitis virus (VSV) particles of the Indiana serotype were prepared and purified by sucrose gradient fractionation. Antibody was prepared separately to each of the two antigen fractions. By immunological procedures, it was shown that soluble antigens of VSV preparations sedimenting at 20S and in the leading edge of the 6S region are antigenically related to VP3, the protein of the virus core, whereas the 6S soluble antigen cross-reacts only with viral coat antibodies. These results confirm previous results obtained by polyacrylamide gel analysis of the antigens. It has further been demonstrated that the 6S antigen is a glycoprotein. Comparing antigens of the New Jersey and Indiana serotype showed that the coat antigens of virus particles and the 6S antigen are immunologically distinct in the two serotypes. In complement-fixation tests, the core antigens and the soluble 20S antigens from one serotype showed a cross-reaction with antiserum prepared against core proteins of the other serotype.

Infection of cells in culture with the Indiana serotype of vesicular stomatitis virus (VSV) results in the release, into the extracellular fluid, of an infectious B particle, a shorter defective T particle, and at least two smaller antigens characterized by their sedimentation coefficients of 20S and 6S (2, 4, 5). It has previously been shown (7, 13) that both B and T particles contain identical protein components and that one of these proteins (VP3 in our nomenclature) arises from the viral nucleoprotein core whereas the two other major proteins (VP2 and VP4) are components of the virus particle coat (7, 14). The 20S antigen contains a single protein species which is indistinguishable in its mobility on polyacrylamide gels from VP3 (7). The 6S antigen, on the other hand, contains a single protein possessing a mobility on polyacrylamide gels which, although close to that of VP2, suggested that it was distinct from any of the major viral proteins. [The protein of the 6S antigen will be designated VP2a to distinguish it from VP2 (see Discussion).]

The New Jersey and Indiana serotypes of VSV can be distinguished by the fact that specific antibody will neutralize the infectivity of the homologous serotype only (6). The two serotypes do, however, share a common complement-fixing (CF) antigen (9; M. E. McClain et al., unpublished data). In a comparison of the protein components in the virus particles of the two serotypes, Wagner et al. (13) showed that the only

apparent difference resides in the slightly greater mobility on polyacrylamide gels of VP4 from the New Jersey serotype as compared with the corresponding protein from the Indiana serotype.

MATERIALS AND METHODS

Cells and virus. The growth and maintenance in suspension culture of a subline of Earle's L cell have been previously described (7). The source, growth, plaque assay, and characteristics of the Indiana serotype of VSV, strain HR-LT, have also been described (7; M. Petric and L. Prevec, *Virology*, *in press*). Virus of the New Jersey serotype was obtained from J. Campbell, School of Hygiene, University of Toronto, and was grown and assayed by procedures identical to those used for the Indiana serotype.

Preparation of antisera. Purified antigens for rabbit inoculation were prepared by treating purified virus with sodium deoxycholate and centrifuging the resultant material through a sucrose gradient. As previously shown (7), the nucleoprotein of the virus containing VP3 moves down the gradient, and the viral coat components containing proteins VP2 and VP4 remain at the top of the gradient. The core fractions and the coat fractions were separately collected. They were dialyzed and concentrated by pressure dialysis against phosphate-buffered saline, and were then inoculated into rabbits. The first rabbit inoculation consisted of 0.4 ml of antigen with Freund's adjuvant given intraperitoneally and intramuscularly. Three subsequent injections at weekly intervals consisted of 1.0 ml of antigen given intraperitoneally and intravenously. A total amount of 1 mg of core and coat protein was administered. The rabbit was bled 1 week after the

last injection. Procedures for the preparation of antisera to whole virus particles have been described (7).

Production of labeled antigens. The methods of labeling infected cell proteins with ^{14}C - or ^3H -amino acid mixtures, as well as the purification of labeled virions or viral antigens from the extracellular fluid, have been previously described (7). Tritium-labeled proteins were prepared identically, except that ^3H -amino acid mixtures (26 mc/mg) purchased from New England Nuclear Corp., Boston, Mass., were used at final concentrations of $1\ \mu\text{c}/\text{ml}$. The radioactive labeling of viral antigens with ^{14}C -glucosamine was done in the same way, except that ^{14}C -glucosamine ($32\ \mu\text{c}/\text{mg}$) was added to the infected cells along with or in the absence of radioactive amino acids.

Polyacrylamide gel electrophoresis. The disruption of purified labeled viral antigens with acetic acid, urea, mercaptoethanol, and sodium dodecyl sulfate (SDS) according to the procedure of Summers et al. (11), as well as the conditions of polyacrylamide gel electrophoresis, have been described (7). When dual label (^3H and ^{14}C) was employed in one gel, the gel slices were depolymerized with hydrogen peroxide (12, 13) and were counted after the addition of Bray's scintillation fluid (3). In some cases, the following procedure was employed to recover over 70% of protein from the gel. The gel slices were individually incubated for 3 days at 4 C in 0.5 ml of 1 mM ammonium bicarbonate (unbuffered). Samples of 0.1 ml were removed from each tube and spotted on glass-fiber filters in scintillation vials. The filters were dried, toluene scintillation fluid was added, and radioactivity was determined.

Immunological procedures. The CF test employed in our laboratory has been described (7). The neutralization titer of antiserum was determined by incubating standard amounts of virus with various antiserum dilutions for 30 min at 37 C. The residual infectivity was determined by plaque assay procedures. Serum blocking power was measured by mixing dilutions of antigen with a known titer of virus-neutralizing antiserum.

After incubation at 37 C for 6 hr, a standard amount of virus was added to each tube. Incubation was continued at 37 C for 30 min, and the residual infectivity in each tube was then determined by plaque assay. Immune precipitates of viral antigens were prepared by mixing antigen with an equal volume of rabbit antiserum and incubating the mixtures at 4 C for 2 days. The precipitates that formed were collected by centrifugation at $7,000 \times g$ for 30 min and were washed twice with 95% ethyl alcohol containing 0.1 M ammonium acetate prior to preparation for polyacrylamide gel analysis.

RESULTS

Neutralizing activity of core and coat antibodies. The virus-neutralizing activities of antibody preparations directed against virus nucleoprotein only and against virus coat components only were determined. For this test, a standard amount of HR-LT virus was mixed with serial dilutions of both types of antibody. After incuba-

tion of the mixtures at 37 C for 30 min, residual viral infectivity was determined by plaque assay. As can be seen in Fig. 1, antibody directed against the viral nucleoprotein core had no virus-neutralizing activity. Antibody against virus coat, however, possessed a high degree of neutralizing activity ($k = 60\ \text{min}^{-1}$).

Antigenic relationships of 20S and 6S antigens. The low molecular weight antigens present in cell-free, virus-free lysates of the Indiana serotype were concentrated by ammonium sulfate precipitation and were fractionated on sucrose gradients, as previously described (7). Successive 1-ml fractions were collected, and a portion of each fraction was separately tested for CF ability with antisera prepared against the nucleoprotein portion (core antibody) and coat protein (coat antibody) of Indiana virions, as described in Materials and Methods (Fig. 2). The optical density profile along the gradient in Fig. 2 is due to components of fetal calf serum present in lysate fluids; the absorbance peaks probably

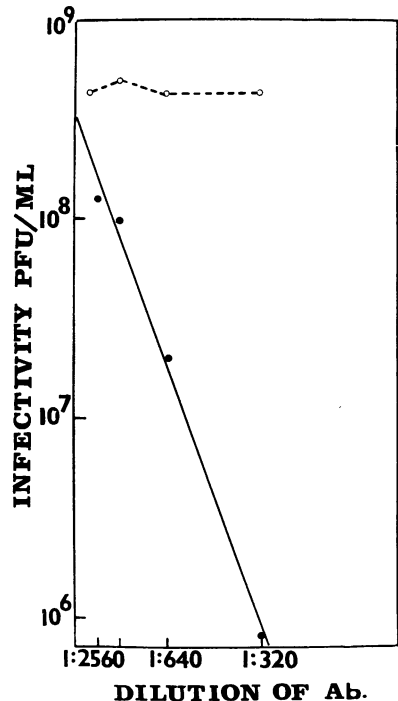


FIG. 1. Virus neutralization by use of antibody to viral nucleoprotein core and antibody to viral coat. To serial dilutions of either Indiana core antibody (O) or Indiana coat antibody (●) was added an equal volume (0.5 ml) of stock Indiana VSV containing 5×10^8 plaque-forming units (PFU). The mixtures were incubated at 37 C for 30 min, at which time samples were removed. The samples were appropriately diluted, and the residual PFU were determined.

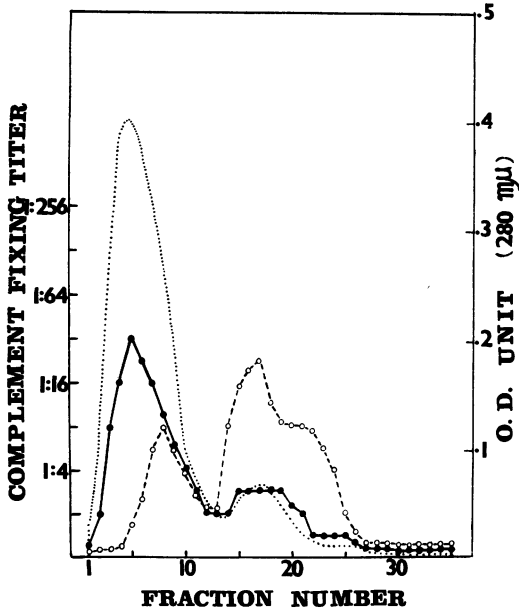


FIG. 2. Complement-fixing (CF) activity of Indiana 20S and 6S antigens with homologous coat and core antibody. The lower molecular weight antigens were prepared from virus lysates by differential centrifugation and ammonium sulfate precipitation and were fractionated by centrifugation for 17 hr at $51,000 \times g$ on sucrose gradients, as previously described (7). The optical density (280 nm) was monitored continuously (dotted line), and 1.0-ml gradient fractions were collected. The CF activity of each gradient fraction was tested against Indiana core antibody (○) and Indiana coat antibody (●). Four units of antibody, with four units of complement and one unit of hemolysin, were used in a microtiter CF test as described by Sever (10). The bottom of the gradient is to the right.

represent the 7S and 19S immunoglobulin fractions.

When core antibody was used to test each fraction of the gradient, CF activity was observed in the 20S antigen region (fractions 17 and 18, Fig. 2). The asymmetrical shape of the CF activity curve in the region of the 20S antigen suggests that this fraction may be composed of structures of various sizes, all of which contain VP3 as a basic protein subunit. The high CF activity exhibited by the 20S antigen with core antibody supports the hypothesis that the protein subunit of the 20S antigen is identical to the protein of the viral nucleoprotein core, VP3 (7). The core antibody unexpectedly showed CF activity with an antigen located in the leading edge of the 6S antigen region (fractions 7 to 10). The peak fractions of 6S antigen contain only one virus-specific protein (VP2a) which differs from VP3 (7). In an effort to reconcile the observed CF

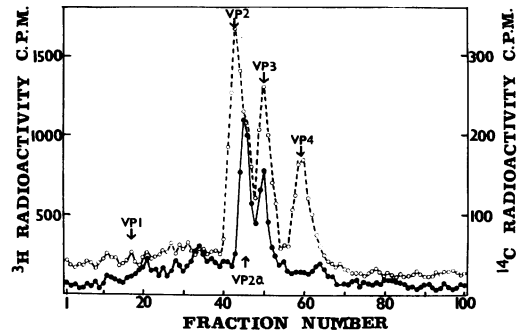


FIG. 3. Polyacrylamide gel analysis of the proteins from the leading edge of the 6S antigen fraction. ^{14}C -labeled low molecular weight viral antigens were prepared and purified on sucrose gradients as in Fig. 2. Fractions corresponding to fractions numbered 7 to 10 of Fig. 2 were combined, and the protein was collected by ethylalcohol precipitation. The precipitated protein was treated with urea, mercaptoethanol, and SDS, and was analyzed by polyacrylamide gel electrophoresis as previously described (7). ^3H -labeled viral structural proteins were added to the sample as markers prior to electrophoresis. The gels were sliced, and the ^3H -radioactivity (○) and ^{14}C -radioactivity (●) were determined after depolymerization of the slices with peroxide as described in Materials and Methods.

activity of core antibody with the leading edge of the 6S antigen, we reexamined the proteins of this particular region on polyacrylamide gel. The results of this analysis (Fig. 3) show that this region not only contains protein characteristic of the 6S antigen but also contains some VP3. This result is in agreement with the observations of Brown and Cartwright (4), who, employing immunodiffusion techniques, suggested that the 6S antigen fraction is composed to two partially separable antigens, one of which is antigenically similar to the 20S antigen.

Referring again to Fig. 2, it should be noted that core antibody did not show any CF activity with the major portion of the 6S antigen region. On the other hand, when viral coat antibody was used to check the fractions for CF activity, the only significant activity was found in the 6S antigen region. This result demonstrated that the 6S antigen is immunologically similar to one of the proteins present in the coat of the virus particle.

Immune precipitation of 6S antigen. Our first concern was to establish beyond doubt that the radioactivity associated with the 6S antigen is indeed present in the virus-specific protein and not in co-sedimenting cellular protein. For this purpose, we prepared and purified ^{14}C -labeled 6S antigen by methods previously described (7). It should be stressed that the radioactive material

used in this study consisted solely of 6S antigen and was not contaminated with VP3 from the leading edge of this peak. Equal quantities of radioactive 6S antigen were mixed with 10 volumes of serial twofold diluted Indiana whole virus antibody. The mixtures were incubated at 37 C for 1 hr and then were stored at 4 C for another 48 hr. The immune precipitates were collected by centrifugation, and the radioactivity remaining in the supernatant fluid was determined (Table 1).

The immune precipitates were pooled, and, after treatment with urea, mercaptoethanol, and SDS, were subjected to polyacrylamide gel analysis. The results (Fig. 4) demonstrated that VP2a is indeed a virus-specific antigen and not a cellular protein.

Serum blocking power of the 6S antigen. Because the 6S antigen cross-reacts with coat antibody, and coat antibody is responsible for the neutralization of viral infectivity, it was of some interest to determine whether absorption of coat antibody with 6S antigen could remove neutralizing activity. For this experiment, a constant amount of antibody was mixed with serial fourfold dilutions of 6S antigen, and the mixtures were incubated at 37 C for 6 hr. The residual neutralizing activity of the antiserum

was tested by adding 2×10^7 plaque-forming units per 0.1 ml of VSV to each reaction mixture. After incubation at 37 C for 30 min, the residual infectivity in each tube was checked by plaque assay. As indicated in Table 2, 6S antigen completely blocked the neutralizing activity of the virus antiserum. This observation very strongly suggests a close relationship between protein VP2a and one of the viral coat proteins.

Of some importance in this regard is the recent finding by Burge and Huang (*personal communication*) that VSV virions contain a glycoprotein which migrates in polyacrylamide gels to the region of VP2.

Demonstration that 6S antigen is a glycoprotein. Radioactive viral antigens were prepared by growing virus in the presence of ^{14}C -glucosamine and then purifying virions, 20S antigens, and 6S antigens on sucrose gradients (7). The radioactive glucosamine was found in virus particles and in the 6S antigen; no label was detected in the 20S antigen. When the labeled virions and 6S antigen were treated with urea, mercaptoethanol, and SDS, and examined on polyacrylamide gels with the use of ^3H -amino acid-labeled protein from virus particles as marker, the glucosamine in virus particles and in the 6S antigen was found to be

TABLE 1. Immune precipitation of 6S antigen^a

Dilution of antibody	6S fraction from infected culture			6S fraction from noninfected culture		
	Counts per min per 0.5 ml		Radioactivity in immune precipitate ^b	Counts per min per 0.5 ml		Radioactivity in immune precipitate
	In total mixture	Remaining in supernatant fluid		In total mixture	Remaining in supernatant fluid	
			%			%
1:2	1,350	810	40.0	880	890	0
1:4	1,234	827	33.1	827	798	3
1:8	1,256	885	29.6	821	802	2
1:16	1,081	926	14.3	855	860	0
1:32	1,140	1,034	9.3	800	795	0
1:64	1,191	1,092	8.3	783	803	0
1:128	1,130	1,083	5.1	790	795	0
1:256	1,132	1,104	2.3	781	780	0
1:512	1,060	1,085	0	770	750	3
1:1,024	1,070	1,074	0	720	733	0
No antibody	1,050	1,055	0	790	785	0

^a ^{14}C -labeled 6S antigen was prepared and purified from Indiana VSV-infected cells by procedures previously described (7). A parallel fraction from ^{14}C -labeled but noninfected cell cultures was also obtained. Serial twofold dilutions of Indiana whole virus antiserum were prepared (0.5 ml each), and to each was added an equal volume of either infected or uninfected 6S material. A sample, 0.05 ml in volume, was immediately removed from each tube, and the radioactivity was determined. The tubes were incubated at 37 C for 1 hr and then stored at 4 C for a further 48 hr. The precipitate was then removed by centrifugation at $1,500 \times g$ for 10 min, 0.05 ml was removed from the supernatant fluid and its radioactivity was determined. The difference between the radioactivity in the initial sample and that remaining in the supernatant fluid after immune precipitation is expressed as a percentage of the initial radioactivity.

^b Percentage of total counts per minute.

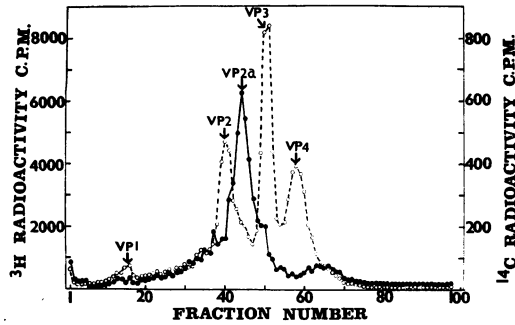


FIG. 4. Polyacrylamide gel analysis of proteins in immune precipitate of 6S antigen. The ^{14}C -labeled protein of the immune precipitate described in Table 1 was washed with ethyl alcohol, mixed with marker ^3H -labeled viral structural protein, denatured, and analyzed on acrylamide gels. The ^3H radioactivity (\circ) and ^{14}C -radioactivity (\bullet) were determined as described in Fig. 3.

TABLE 2. Serum blocking power of 6S antigen^a

Dilution of 6S antigen	Surviving infectivity (PFU/ml)
Undiluted	2.3×10^7
1:4	1.5×10^7
1:16	3.1×10^5
1:64	4.4×10^3
1:256	3.4×10^2
1:1,024	2.1×10^2
Antibody without 6S antigen	1.4×10^2
Control virus titer	2.1×10^7

^a Sucrose gradient purified 6S antigen was prepared from Indiana VSV lysates as previously described (7). Serial fourfold dilutions of 6S antigen (0.5 ml each) were prepared. To each antigen dilution was added 0.5 ml of a standard dilution of anti-Indiana VSV antiserum. The antigen-antiserum mixtures were incubated at 37 C for 6 hr. Each mixture then received 2×10^7 plaque-forming units (PFU) of Indiana VSV in 0.1 ml. Incubation was continued at 37 C for 30 min, at which time the mixture was appropriately diluted and the residual viral infectivity was determined (PFU/ml). Controls showing the virus titer after the addition of antiserum alone and the virus titer in the absence of antiserum are included.

associated with a component which migrates with a mobility slightly greater than that of VP2 (Fig. 5).

In an effort to clarify the relationship between the viral and 6S glucosamine-containing components, infected cells were labeled simultaneously with ^{14}C -glucosamine and ^3H -amino acids between 1 and 10 hr postinfection. Both virus particles and 6S antigen were purified by sucrose gradient centrifugation from the extracellular

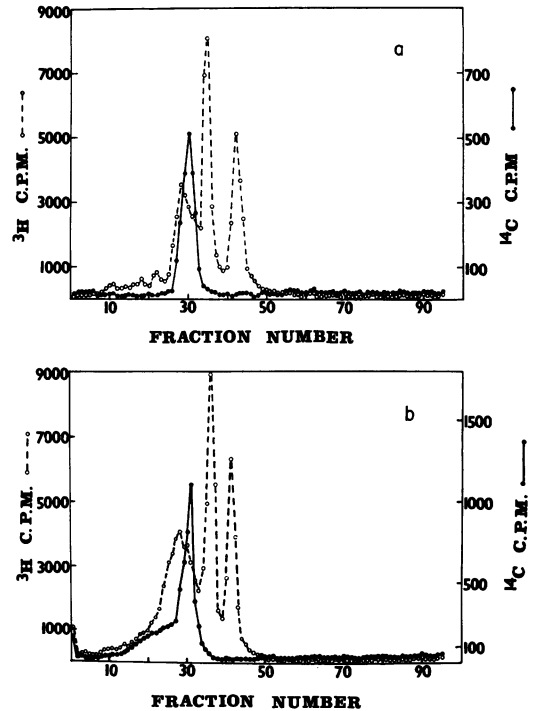


FIG. 5. Polyacrylamide gel analysis of glucosamine-containing fraction from 6S antigen and mature virions. ^{14}C -glucosamine-labeled virus lysate was prepared as described in Materials and Methods. Virus particles and free antigens were concentrated and purified on sucrose gradients as previously described (7). Both purified virus particles (a) and 6S antigen (b) were mixed with ^3H -labeled viral structural proteins as markers, denatured, and examined by polyacrylamide gel electrophoresis. The ^3H radioactivity (\circ) and ^{14}C radioactivity (\bullet) were determined.

fluid. The 6S antigen was further purified by immune precipitation with virus anti-coat antibody. Both virus and 6S antigen were disrupted and analyzed on polyacrylamide gels. In this analysis, the glucosamine in the virus appeared to coincide exactly with the position of VP2 (Fig. 6b). Both ^{14}C and ^3H radioactivity in the 6S antigen fraction were also coincident (Fig. 6a), suggesting that protein and carbohydrates are associated in one glycopeptide. Comparison of the ratio of ^{14}C to ^3H in Fig. 6a and 6b indicates that the 6S antigen contains approximately twice as much glucosamine per unit of protein as is present in VP2 of the virus.

Immunological comparisons of Indiana and New Jersey serotypes. As can be seen in Fig. 7, antibody made to one VSV serotype exhibited virtually no neutralizing capacity for particles of the heterologous serotype (6). On the other hand, it

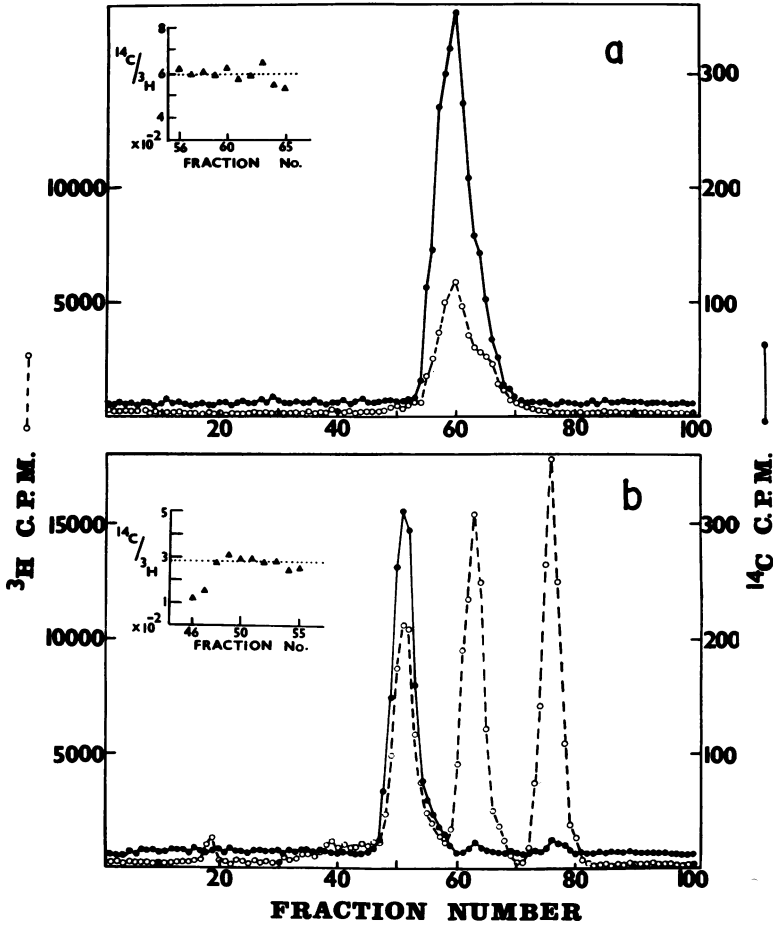


FIG. 6. Polyacrylamide gel analysis of ^{14}C -glucosamine- and ^3H -amino acid-labeled proteins in 6S antigen and matured virions. Virus particles and free antigens labeled with ^{14}C -glucosamine and ^3H -amino acids were prepared and purified from a single lysate. Purified virus was taken directly from sucrose gradients, whereas sucrose gradient purified 6S antigen was further purified by immune precipitation as described for Fig. 3. The dual labeled virus (b) and the 6S immune precipitate (a) were separately denatured and examined by polyacrylamide gel electrophoresis. The ^3H radioactivity (\circ) and ^{14}C radioactivity (\bullet) were determined. In the inset in each figure is plotted the ratio of ^{14}C counts to ^3H counts for that region of the gel which contains ^{14}C -glucosamine radioactivity.

has been reported (9; McClain et al., unpublished data) that antibody to one serotype shows CF activity with antigens of the heterologous serotype. Viral antigens responsible for the cross-reacting CF activity were investigated by use of anti-virus core antibodies and anti-virus coat antibodies. Cells were infected with the New Jersey serotype of VSV. After they were harvested, the virus particles and soluble antigens were concentrated and purified by sucrose gradient centrifugation (by means of the same procedures already described for the Indiana serotype). The optical density (280 nm) along the sucrose gradient was monitored as successive 1-ml fractions were collected. Each fraction of the gradient

was separately tested for CF activity with antibodies to New Jersey virus particles, to Indiana core protein, and to Indiana coat protein.

Figure 8a illustrates the CF activity of B particles of the New Jersey serotype (located in fractions 15 to 20 of the gradient). Reactivity to New Jersey virus antiserum was observed as expected in the region of the virus particles. Antibody to core protein of the Indiana serotype also showed CF activity with New Jersey virus particles. This result suggests that the core proteins of the New Jersey and Indiana serotypes of VSV are antigenically similar. The result, however, raises the problem of how it is possible for an antibody to react with a protein which is

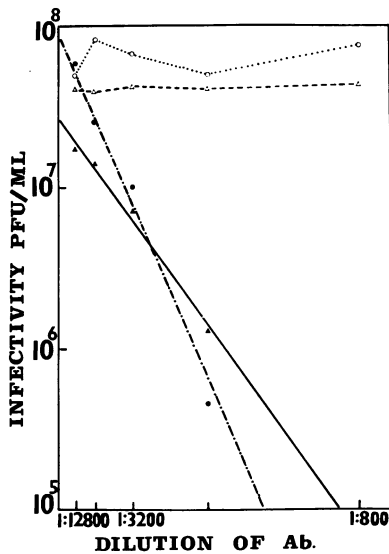


FIG. 7. Absence of cross-neutralization of infections VSV by heterotypic antisera. Antisera to partially purified virus particles of the Indiana serotype and of the New Jersey serotype were prepared in rabbits as previously described (7). To 0.5 ml of appropriately diluted antiserum was added an equal volume of either Indiana or New Jersey infectious virus stock. The mixtures were incubated at 37 C for 30 min; they were then appropriately diluted and the residual plaque-forming units were determined. Antiserum to Indiana virus was tested against infectious Indiana VSV (▲) and New Jersey VSV (○); antiserum to New Jersey virus was also tested against Indiana VSV (Δ) and New Jersey VSV (●).

located in the interior of a virus particle. The reaction of core antibody with whole virus particles from sucrose gradients also occurs with the homologous serotype (*unpublished data*). Since VSV is susceptible to partial degradation during purification procedures, it is possible that the core antibody is reacting with partially disrupted virions. Alternatively, the antibody and components of complement may be capable of attacking the core protein via the central axial hollow of the virion.

Antibody to Indiana coat proteins, on the other hand, failed to show any CF activity with the New Jersey virions. The finding that coat protein preparations contain the proteins VP2 and VP4 (7) suggests that these two proteins are antigenically distinct in the two serotypes. Since virus-neutralizing activity is contained in antibody to coat proteins, this result is consistent with the serotype specificity of neutralizing antibody.

In Fig. 8b, the locations of the 20S and 6S antigens on the gradients are apparent from the CF activity with New Jersey virus antibody. The

Indiana virus-coat antibody failed to show CF activity with either 20S or 6S New Jersey antigens. Thus, the 6S antigen (VP2a) also appears to be distinct in the two serotypes. Antibody to core proteins of the Indiana serotype, however, showed cross-reacting CF activity with the 20S and the leading edge of the 6S New Jersey antigens. This is in agreement with the finding that the core proteins of the two serotypes are antigenically related.

DISCUSSION

The immunological data presented in this paper confirm our hypothesis, based on polyacrylamide gel analysis, that the protein constituent of the 20S antigen is identical to the protein of the virus core (7). We have further demonstrated that another antigenic fragment, the 6S antigen, also contains a protein identical to viral core protein VP3. The molecular weight of VP3 has been estimated to be approximately 60,000 daltons (7, 13). Since neither the composition nor the exact sedimentation coefficients of the soluble antigens are known, no direct conclusions are possible from these data.

It is possible that the 20S antigen may represent fragments of ribonucleoprotein cores, but we have been unable to detect ribonucleic acid in 20S antigen, either by ³H-uridine labeling or by treating the 20S material with ribonuclease prior to analysis on sucrose gradients (*unpublished data*). The structure and functional origin of these core-related antigens still remain to be determined.

On the basis of its strong CF activity and serum blocking power when tested with coat antibody, the 6S antigen appears to be very closely related to the proteins of the viral coat. On the other hand, the glycoprotein VP2a, which constitutes the 6S antigen, shows a mobility on polyacrylamide gels that is slightly different from that of VP2, the glycoprotein-containing fraction from virus particles. There appears to be twice as much glucosamine per unit protein in VP2a as in VP2.

Thus, although VP2 and VP2a are undoubtedly closely related antigens, they do not appear to be physically identical. Whether the differences are due to the carbohydrate moieties or to the peptide portions of these glycoprotein containing fractions remains to be determined.

Our comparison of the antigens of the New Jersey and Indiana serotypes of VSV are of some theoretical as well as practical interest. The core protein VP3, from either nucleoprotein cores or the soluble 20S antigen, is antigenically similar in both serotypes. The coat proteins and the 6S antigen, on the other hand, are antigenically distinct in each serotype. This situation is analogous to that existing within the type A influenza

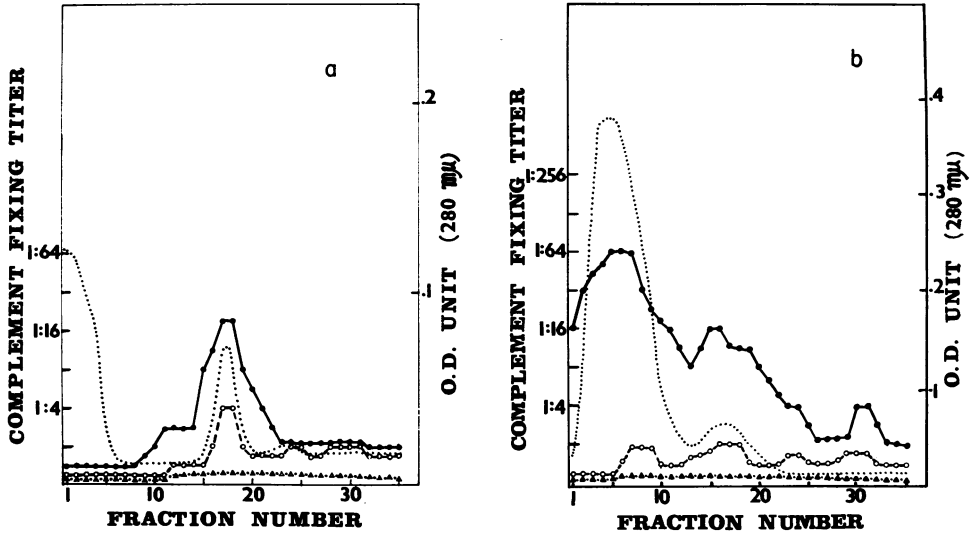


FIG. 8. Complement-fixing (CF) activity of New Jersey serotype B particles and New Jersey 6S and 20S antigens with New Jersey virus antiserum and heterotypic Indiana core and coat antisera. New Jersey virus particles and free antigens were purified from infected lysates by differential centrifugation and ammonium sulfate fractionation as previously described for Indiana serotype antigens (Fig. 2) and reference 7). The virus was examined by rate zonal centrifugation on 5 to 30% sucrose gradients at 4 C and $41,000 \times g$ for 60 min (a); 20S and 6S antigens were separated on 5 to 30% sucrose gradients centrifuged at 4 C and $51,000 \times g$ for 16.5 hr (b). The direction of sedimentation is left to right in the diagrams. The optical density (280 nm) was monitored continuously along the gradients (dotted line) as 1-ml fractions were collected. Each fraction was separately tested for CF activity with antiserum to New Jersey virus (●), antiserum to Indiana core antigens (○), and antiserum to Indiana core antigens (▲).

(1) and avian tumor (8) groups. All these results suggest a greater probability of simultaneous mutational events in the cistrons for coat proteins than in those coding for the core protein.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Cancer Institute of Canada and the National Research Council of Canada.

The excellent technical assistance of D. Takayesu is gratefully acknowledged.

LITERATURE CITED

- Andrewes, C. H., F. B. Bang, and F. M. Burnet. 1955. A short description of the myxovirus group (influenza and related viruses) *Virology* 1:176-184.
- Bradish, C. J., J. B. Brooksby, and J. F. Dillon, Jr. 1956. Biophysical studies of the virus system of vesicular stomatitis. *J. Gen. Microbiol.* 14:290-314.
- Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solution in liquid scintillation counter. *Anal. Biochem.* 1:279-285.
- Brown, F., and B. Cartwright. 1966. The antigens of VSV. II. The presence of two low molecular weight immunogens in virus suspension. *J. Immunol.* 7:612-620.
- Brown, F., B. Cartwright, and J. D. Almeida. 1966. The antigens of VSV. I. Separation and immunogeneity of three complement fixing components. *J. Immunol.* 96:537-545.
- Cotton, W. E. 1927. Vesicular stomatitis. *Vet. Med.* 22:169-175.
- Kang, C. Y., and L. Prevec. 1969. Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. *J. Virol.* 3:404-413.
- Kelloff, G., and P. K. Vogt. 1966. Localization of avian tumour virus group-specific antigen in cell and virus. *Virology* 29:377-384.
- Meyers, W. L., and R. P. Hanson. 1962. Immunodiffusion studies of the antigenic relationships within and between serotypes of vesicular stomatitis virus. *Amer. J. Vet. Res.* 23:896-899.
- Sever, J. L. 1962. Application of a microtechnique to viral serological investigation. *J. Immunol.* 88:320-329.
- Summers, D. F., J. V. Maizel, and J. E. Darnell, Jr. 1965. Evidence for virus specific non-capsid proteins in poliovirus infected HeLa cells. *Proc. Nat. Acad. Sci. U.S.A.* 54:505-513.
- Tishler, P. V., and C. J. Epstein. 1968. A convenient method of preparing polyacrylamide gels for liquid scintillation spectrometry. *Anal. Biochem.* 22:89-98.
- Wagner, R. R., T. A. Schnaitman, and R. M. Snyder. 1969. Structural proteins of vesicular stomatitis viruses. *J. Virol.* 3:395-403.
- Wagner, R. R., T. C. Schnaitman, R. M. Snyder, and C. A. Schnaitman. 1969. Protein composition of structural components of vesicular stomatitis virus. *J. Virol.* 3:611-618.