Structural Phosphoproteins associated with ten Rhabdoviruses

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SUMMARY

The phosphorylation of structural proteins of ten rhabdoviruses has been studied. Each virus preparation has been found to possess at least one phosphoprotein which, in the case of Chandipura, Cocal, Piry viruses or vesicular stomatitis virus (VSV), Indiana and New Jersey serotypes, is the minor NS protein. In rabies virus the sole phosphoprotein appears to be the N protein of the nucleocapsid. For Mokola and Lagos bat viruses, two or three phosphoproteins have been observed, one of which is the N protein. Spring viraemia of carp virus (SVCV) preparations contain two phosphoproteins one of which is, or has a mobility close to, that of the N protein. Kern Canyon virus preparations also possess two phosphoproteins, one with the same electrophoretic mobility as (and possible identity to), the virus glycoprotein while the other has a mobility slightly slower than the N protein. Antigenically the core proteins of rabies, Mokola and Lagos bat viruses have been shown to be related to each other but distinct from those of SVCV or VSV.

INTRODUCTION

The structural proteins of the vesicular stomatitis subgroup of rhabdoviruses have been shown to consist of three major proteins, a glycoprotein (G), a nucleocapsid protein (N) and a membrane protein (M) in addition to two or more minor proteins (L, NS, A and B) (Kang & Prevec, 1969; Wagner, Schaitman & Snyder, 1969; Burge & Huang, 1970; Cartwright, Talbot & Brown, 1970; Mudd & Summers, 1970; Bishop & Roy, 1972; Wagner et al. 1972; Wunner & Pringle, 1972; Obijeski et al. 1974). Recently it has been shown that VSV, Indiana serotype, contains a phosphoprotein (the NS protein) as well as a protein kinase activity which is capable of phosphorylating some of the virus proteins (Strand & August, 1971; Sokol & Clark, 1973; Imblum & Wagner, 1974).

The proteins of rabies virus consist of four major components including a glycoprotein (G), a ribonucleoprotein (N) and two membrane proteins (M1 and M2) (György, Sheehan & Sokol, 1971; Sokol, Stanček & Koprowski, 1971; Wagner et al. 1972; Sokol &

* Dr F. Sokol died suddenly on 26 May 1974.
Clark, 1973). Minor proteins in rabies virus preparations have not yet been characterized. It has been shown that both the intracellular nucleocapsids and mature rabies virus particles possess a phosphorylated N protein as well as a protein kinase activity (Sokol & Clark, 1973). These observations have been extended in the present investigation with regard to rabies defective virus particles and to rabies related viruses; Mokola and Lagos bat virus (Shope et al. 1970) as well as fish rhabdovirus (spring viraemia of carp virus, SVCV, Fijan et al. 1971; Lenoir, 1973). In addition evidence for phosphorylated protein associated with viruses of five members of the VSV subgroup of rhabdoviruses is presented, as well as for defective particles of the unrelated Kern Canyon virus (KCV) (Sokol & Clark, 1973).

METHODS

Reagents. [3H]-labelled individual or mixtures of l-amino acids were purchased from New England Nuclear Corp., Boston, Mass., or Schwarz-Mann, Orangeburg, New York. [32P]-orthophosphate (carrier-free) was obtained from Schwarz-Mann.

Cells and viruses. The W clone of the ERA strain of rabies virus (Kuwert et al. 1968) and plaque-purified strains of KCV and VSV-Indiana (Aaslestad et al. 1971) or Chandipura, Piry, and VS-New Jersey viruses (Obijeski et al. 1974) were used. Cocal virus was obtained from Dr F. A. Murphy, C.D.C., Atlanta, Georgia) and cloned three times by picking a plaque from primary monkey kidney plates which contained less than five plaques per plate. Lagos bat and Mokola viruses (Shope et al. 1970) were cloned in agarose-suspended baby hamster kidney cells (BHK21-S13) as described elsewhere (Sedwick & Wiktor, 1967). SVCV was cloned at 23 °C in BHK21 cell monolayers. Virus stocks were mixed with foetal calf serum (20 %) and stored frozen at −70 or −90 °C.

Infection of cells, preparation and purification of labelled virus. Confluent monolayers of BHK21 cells were used to prepare sufficient quantities of virus for biochemical analyses. VSV (Indiana or New Jersey serotypes), Chandipura, Piry and Cocal viruses were grown in the presence of 0·5 μCi/ml [3H]-amino acids and 10 μCi/ml [32P]-orthophosphate in Eagle’s medium (MEM) containing one-half the normal concentrations of amino acids, and virus was purified exactly as described by Obijeski et al. (1974). Rabies was grown as described by Sokol & Clark (1973). Lagos bat and Mokola viruses were grown at 33 °C in BHK21 monolayers in the presence of 7 μCi/ml of [32P]-orthophosphate in Eagle’s medium (lacking unlabelled phosphate) and containing one-fifth the normal quantities of amino acids together with 1 μCi/ml of a mixture of [3H]-amino acids (Sokol & Clark, 1973). In some experiments the [3H]-amino acid mixture was replaced by 0·5 μCi/ml of [3H]-serine (final sp. act. 3·4 Ci/mmol) and, or [3H]-threonine (final sp. act. 2·5 mCi/mmol). SVCV was used to infect confluent monolayers of BHK21 cells at an input multiplicity of 0·5 p.f.u./cell and grown at 23 °C for 48 h under conditions identical to those described for rabies virus (Sokol & Clark, 1973). Mokola, Lagos bat, SVC and rabies viruses were all purified as described by Sokol et al. (1968). Defective T particles of rabies virus were separated and collected during the purification procedure (Sokol et al. 1968; Sokol & Clark, 1973).

Isolation of intracellular virus nucleocapsids. Nucleocapsids containing the virus RNA and N protein but lacking the virus membrane or glycoproteins were isolated from infected cells as described previously (Sokol, 1973; Sokol & Clark, 1973).

Protein gel electrophoresis. Virus preparations (1 to 3 mg) were precipitated in 10 % (w/v) trichloroacetic acid and recovered by centrifuging at 10000 g and 4 °C for 30 min. Virus pellets were washed with acetone, recentrifuged as before, then dissociated in 0·5 ml of protein dissociation buffer: 0·01 M-sodium phosphate buffer, pH 7·0, containing 2·5 % (w/v)
Rhabdovirus phosphoproteins

SDS, 5 % (v/v) 2-mercaptoethanol, and 10 % (v/v) glycerol. The mixture was heated at 100 °C for 3 min, bromophenol blue tracking dye added (0.001 %) and a sample loaded on a polyacrylamide gel (Sokol et al. 1971; Obijeski et al. 1974). In order to obtain virus protein preparations free from [32P]-labelled phospholipids, RNA or oligonucleotides, the dissociated polypeptides were precipitated at 4 °C for 30 min by the addition of 6 vol. of ethanol in the presence of 0.2 vol. of saturated sodium acetate, brought to 18 °C for 1 h and then centrifuged at 10000 g and 18 °C for 30 min. The pellet was resuspended in 0.05 to 0.50 ml of 0.01 M-sodium phosphate buffer, pH 7.2, sonicated for 30 s to achieve complete suspension, mixed with pancreatic ribonuclease (200 μg/ml) and incubated at 37 °C for 30 min to digest the [32P]-RNA. After incubation, the polypeptides were reprecipitated by ethanol in the presence of sodium acetate, centrifuged at 10000 g and 4 °C for 30 min and the pellet resuspended in protein dissociation buffer as described above. Alternatively, after ribonuclease treatment the polypeptides were recovered by precipitation by trichloroacetic acid and processed as described for the complete particles.

For polyacrylamide gel electrophoresis of protein samples we employed either 7 %, 8 % or 9 % (w/v) polyacrylamide continuous gel systems formulated essentially as described by Maizel (1971) and modified to minor extents in our different laboratories (Sokol et al. 1971; Obijeski et al. 1974) or discontinuous polyacrylamide gel systems having a 3.6 % (w/v) stacking gel on top of a 10 % or 11 % (w/v) resolving gel (Laemmli, 1970) as described by Obijeski et al. (1974). After electrophoresis each gel was sectioned and the distribution of label determined as described previously (Sokol et al. 1971; Obijeski et al. 1974). In double label experiments the counts were corrected for spill-over of counts from one counting channel into the other.

Antigenic comparison of rhabdovirus nucleocapsids. Rabbits were immunized by the administration of 100 μg of purified virus nucleocapsid in 1.0 ml of NT buffer emulsified with 1.0 ml of complete Freund’s adjuvant and divided into two intramuscular doses. The inoculation was repeated once after seven days; test bleedings were performed 14 days following the second inoculation. Complement-fixation tests were performed by a micro-technique described previously (Sokol et al. 1968).

RESULTS

Phosphoproteins associated with rhabdoviruses of the VSV subgroup

Preparations of [3H]-amino acid and [32P]-labelled VSV Indiana, VSV (New Jersey) or Chandipura, Cocal and Piry viruses were dissociated by SDS in phosphate buffer and subjected to polyacrylamide gel electrophoresis in 9 %, pH 7.0, continuous or 11 %, pH 8.9, discontinuous polyacrylamide gels. The results are given in Fig. 1. In each case only one phosphoprotein, labelled with both the [3H]-amino acids and [32P]-phosphate, was identified. As shown previously for VSV (Indiana) (Sokol & Clark, 1973), the phosphoprotein was the minor protein NS which migrated (at pH 7.0) slightly faster than the major ribonucleoprotein (N). However, in the case of Piry virus and VSV (Indiana), the phosphorylated NS protein migrated between the glycoprotein (G) and N protein when the electrophoresis was performed at a higher pH value using the discontinuous gel system (Obijeski et al. 1974). The reason for this change in relative electrophoretic mobility is not known. Note that for this series of experiments all five viruses were grown at the same time and harvested under identical conditions. As can be seen from Fig. 1, the ratio of the two labels in the NS proteins was essentially equivalent for all five viruses, suggesting that in each case the NS protein was phosphorylated to a similar extent.
Fig. 1. Polyacrylamide gel electrophoresis of Cocal, Chandipura, Piry, VS Indiana and VS New Jersey virus proteins. Preparations of $[^3]$H-amino acid and $[^32]$P-orthophosphate labelled viruses of each serotype were dissociated with SDS and 2-mercaptoethanol, the proteins separated from other components as described in Methods, then subjected to electrophoresis for 20 h at 5 mA/gel in 9 % continuous polyacrylamide gels (lower panels) or to electrophoresis for 5 h at 3 mA/gel in 11 % discontinuous polyacrylamide gels (upper panels) as described in Methods. In this and in the other figures, the individual points of the $[^3]$H profiles are omitted for the sake of the clarity of presenting the $[^32]$P data.
**Rhabdovirus phosphoproteins**

![Diagram](image)

Fig. 1(c). For legend see opposite.

**Table 1. The structural proteins of five rhabdoviruses**

<table>
<thead>
<tr>
<th>Virus protein</th>
<th>VSV Indiana</th>
<th>VSV New Jersey</th>
<th>Cocal</th>
<th>Chandipura</th>
<th>Piry</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>2.9 (6)</td>
<td>2.1 (7)</td>
<td>0.4 (5)</td>
<td>0.5 (5)</td>
<td>0.6 (6)</td>
</tr>
<tr>
<td>G</td>
<td>13.9 (30)</td>
<td>7.7 (25)</td>
<td>2.1 (25)</td>
<td>2.3 (24)</td>
<td>2.8 (27)</td>
</tr>
<tr>
<td>N</td>
<td>14.3 (31)</td>
<td>10.4 (34)</td>
<td>3.9 (46)</td>
<td>4.9 (51)</td>
<td>4.7 (45)</td>
</tr>
<tr>
<td>NS</td>
<td>2.2 (5)</td>
<td>1.5 (5)</td>
<td>0.7 (8)</td>
<td>0.6 (6)</td>
<td>0.7 (7)</td>
</tr>
<tr>
<td>M</td>
<td>12.7 (28)</td>
<td>8.5 (28)</td>
<td>1.3 (15)</td>
<td>1.3 (14)</td>
<td>1.7 (16)</td>
</tr>
</tbody>
</table>

| NS            | 1.4         | 0.8            | 0.4     | 0.4     | 0.5     |

*The label contained in each distinguishable protein band (Fig. 1) for each virus was computed (in parenthesis the % this [H] label represents of the total [H] protein for each virus serotype is given).

Differences in the relative electrophoretic mobilities of the virus G, N or M proteins of VSV Indiana, VSV New Jersey, Cocal, Chandipura or Piry viruses have been demonstrated previously (Wunner & Pringle, 1972; Obijeski et al. 1974). Such differences are also apparent in Fig. 1. In addition it is evident from Fig. 1 that the relative labelling of the three major protein species varied with the virus serotype (Table 1). The structural proteins of VSV (Indiana) have been characterized in several laboratories (Kang & Prevec, 1969;
Fig. 2. Polyacrylamide gel electrophoresis of the proteins obtained from defective particles of rabies (ERA strain) virus. A preparation of [3H]-serine, [3H]-threonine and [32P]-orthophosphate labelled defective virus was dissociated with SDS and 2-mercaptoethanol and the proteins separated from other components as described in Methods. The proteins were then subjected to electrophoresis in an 8% continuous polyacrylamide (lower panel) or a 10% discontinuous polyacrylamide gel (upper panel) as described in Fig. 1.

Fig. 3. Polyacrylamide gel electrophoresis of the structural proteins of Mokola and Lagos bat viruses. Preparations of [3H]-amino acid and [32P]-orthophosphate labelled Mokola and Lagos bat viruses were dissociated by SDS, purified from other components (Methods) and subjected to electrophoresis in 7% continuous polyacrylamide gels as described by Sokol, Stanček & Koprowski (1971).

Wagner et al. 1969; Burge & Huang, 1970; Cartwright et al. 1970; Mudd & Summers, 1970; Bishop & Roy, 1972), and similar variations in the amounts of the VSV (Indiana), G, N and M proteins can be seen from those publications. Although for VSV (Indiana) those results may be explained by the different media, host or strains employed by the various investigators, in the present investigation each virus was grown in BHK21 cells using samples of the same batch of media and all five viruses were harvested and purified by an identical procedure.

The percentage of [3H] label in each identifiable band for each virus is given in Table 1. The resolution was not sufficient to quantitate the label in the minor proteins A and B described for VSV Indiana (Bishop & Roy, 1972). However, it can be seen that the relative...
quantities of $[^{3}H]$ in proteins G, N and M were almost equal for VSV (Indiana), whereas for Chandipura virus there was a 3·6-fold difference in the amount of label in the M and N proteins. Again the reasons for these differences between the various virus species are not known although they could be due to a combination of genetic and host cell physiological causes.

**Phosphoproteins associated with rhabdoviruses of the rabies subgroup**

Preparations of $[^{3}H]$-amino acid and $[^{32}P]$-labelled Mokola and Lagos bat viruses and defective particles of rabies (ERA strain) virus were dissociated by SDS in phosphate buffer and analysed by polyacrylamide gel electrophoresis. The rabies defective virus particles were found to contain 18S RNA species. Proteins obtained from the defective particles of rabies virus were subjected to two different forms of electrophoresis, either employing an 8% continuous or a 10% discontinuous gel system, in order to determine if the proteins could be better resolved by one or the other technique (Fig. 2). Only one phosphoprotein was observed by either analysis, the $[^{32}P]$ peak migrating precisely with the $[^{3}H]$-labelled N protein, indicating that the nucleocapsid protein or a protein of similar mobility is the only phosphoprotein in defective rabies virus. This result is similar to previously published observations for the B particles of rabies (ERA strain) virus (Sokol & Clark, 1973). It should be noted that the $[^{3}H]$-labelled protein band observed between the N and M1 proteins in the 10% discontinuous gel corresponds in mobility to the non-phosphorylated large fragment of N obtained by trypsin treatment of rabies intracellular nucleocapsids, although it was unresolved in this instance from the other proteins in the 8% continuous gel electrophoresis (Fig. 2). It should also be noted (Fig. 2) that all four major proteins of defective rabies virus contain threonine and, or serine. In separate experiments using either $[^{3}H]$-serine or $[^{3}H]$-threonine we have found that both serine and threonine (potential substrates for phosphorylation) are present in each of the four major proteins of rabies (ERA strain) virus (data not shown).

Preparations of $[^{3}H]$-amino acid and $[^{32}P]$-labelled Mokola and Lagos bat viruses were dissociated by SDS in sodium phosphate buffer and the proteins resolved by electrophoresis in 7% (w/v) polyacrylamide gels (Fig. 3). The glycoprotein (G) of Mokola virus was identified in a separate experiment by $[^{3}H]$-glucosamine labelling (data not shown) as the band migrating slightly slower than the N protein. Similarly for Lagos bat virus the glycoprotein was identified as a protein species whose mobility overlapped that of the N protein. Neither virus glycoproteins were phosphorylated. The N proteins for each virus were identified by preparing intracellular nucleocapsids (see Fig. 5). Other proteins in the two virus preparations have not been characterized further. There were some heterogeneous protein species at the top of the gels possibly representing aggregates or cellular contaminants. On the basis of the mobilities of the $[^{3}H]$- and $[^{32}P]$-labelled proteins, it appears that, as for rabies virus, the N proteins of these two viruses are also phosphoproteins. In addition other proteins which migrated faster than the N protein were also phosphorylated. The $[^{3}H]$ to $[^{32}P]$ count ratios in these other proteins were essentially similar to each other and to that of the respective N protein of the virus particle (i.e. 1 to 4·7 for Mokola and 1 to 3·6 for Lagos bat virus). Neither the function nor topology of these other phosphoproteins has been determined.

As with the VSV subgroup of rhabdoviruses all three viruses in the rabies subgroup possessed distinctive and distinguishable structural proteins. Mol. wt. estimates using procedures described previously (Sokol et al. 1971) gave values of 80,000, 73,000 and 65,000 for the G glycoproteins of rabies, Mokola and Lagos bat viruses, respectively; while values of 62,000, 64,000 and 61,000 were estimated for the N proteins of the same three viruses,
Spring viraemia of carp

Preparations of \(^{3}H\)-amino acid and \(^{32}P\)-orthophosphate labelled T-particles of KCV, \(^{32}P\)-orthophosphate labelled B particles of SVCV, were dissociated by SDS and 2-mercaptoethanol, the proteins freed other virus components (Methods), and subjected to electrophoresis in 7% continuous polyacrylamide gels.

respectively. The mol. wt. estimates of the M1 and M2 proteins of rabies virus were estimated as 40,000 and 25,000, respectively. The three distinguishable proteins of Mokola virus which moved faster than the phosphorylated N protein possessed mol. wt. of 46,000 (phosphoprotein), 40,000 (phosphoprotein) and 25,000 mol. wt. (not phosphorylated). Similarly the three distinguishable proteins of Lagos bat virus which also migrated faster than the phosphorylated N protein possessed mol. wt. estimated at 45,000 (phosphoprotein), 40,000 (phosphoprotein) and 25,000 (not phosphorylated).

**Phosphoproteins associated with other rhabdoviruses**

Two other rhabdoviruses, Kern Canyon and spring viraemia of carp, have been examined for possession of phosphorylated proteins. As shown previously for the complete particles of KCV (Sokol & Clark, 1973), two phosphoproteins were identified in KCV defective T particles (Fig. 4). One possessed an electrophoretic mobility identical to that of the virus glycoprotein while the other migrated slightly slower than the N protein. In comparison to all other rhabdoviruses examined, the \(^{32}P\)-phosphate to \(^{3}H\)-amino acid ratios in these proteins was markedly low (see Fig. 4 ordinates). For SVCV, two major phosphoproteins
Fig. 5. Phosphoproteins associated with the intracellular nucleocapsids derived from Mokola (a), Lagos bat (b) and SVC (c) virus-infected cells. Nucleocapsids were obtained for Mokola (a) and Lagos bat (b) viruses from the cells in which the virus particles analysed in Fig. 3 were grown. The nucleocapsids were extracted for proteins (Methods) and subjected to electrophoresis in 7% continuous polyacrylamide gels. The SVCV nucleocapsid electropherogram (c) was stained with Coomassie blue and scanned as described previously (Sokol et al., 1971). SVCV nucleocapsids purified following trypsin treatment (d) were similarly analysed.

were identified, one migrated with an electrophoretic mobility close to that of the virus N protein while the other moved faster than the N protein. The mol. wt. of the two phosphoproteins of SVC were estimated as 52,000 (N) and 43,000. The SVCV glycoprotein (G, mol. wt. 88,000) and membrane protein (M, mol. wt. 23,000) were not phosphorylated.

Phosphoproteins associated with intracellular nucleocapsids derived from rhabdoviruses

In a previous publication it has been shown that the free intracellular nucleocapsids of VSV or KCV, isolated by isopycnic sedimentation in caesium chloride gradients, lack phosphorylated proteins although they contain virus RNA and N protein (Sokol & Clark, 1973). However, free intracellular nucleocapsids of rabies virus contain both RNA and a phosphorylated N protein. When the latter nucleocapsids were isolated using trypsin treatment to disperse the infected cells prior to cell lysis, part of the N protein was cleaved to give a small phosphorylated peptide (mol. wt. 7,000) and a large non-phosphorylated
Table 2. Cross complement fixation reactions between the nucleocapsids of five rhabdoviruses and their antibodies*

<table>
<thead>
<tr>
<th>Immune serum against nucleocapsids of</th>
<th>Antibody titres against nucleocapsids of</th>
<th>Rabies virus</th>
<th>Lagos bat virus</th>
<th>Mokola virus</th>
<th>SVCV</th>
<th>VSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies virus</td>
<td></td>
<td>1024</td>
<td>512</td>
<td>1024</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lagos bat virus</td>
<td></td>
<td>128</td>
<td>1024</td>
<td>1024</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mokola virus</td>
<td></td>
<td>32</td>
<td>1024</td>
<td>1024</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SVCV</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>512</td>
<td>0</td>
</tr>
<tr>
<td>VSV</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1024</td>
</tr>
</tbody>
</table>

* Titres are the reciprocal value of the highest serum dilution giving partial haemolysis in the presence of four antigenic units of nucleocapsid and two units of complement. Titres equal or lower than 4 are considered to be nonspecific and presented as 0.

Fragment (mol. wt. 55000) which migrated on electrophoresis faster than the uncleaved N phosphoprotein (Sokol & Clark, 1973). In Fig. 5 we present the electrophoretic patterns of proteins recovered from free intracellular nucleocapsids of Mokola, Lagos bat and SVC viruses prepared from mechanically harvested infected cells labelled by both $^{32}\text{P}$-orthophosphate and (except for SVCV) $^{3}\text{H}$-amino acids. The nucleocapsids of Chandipura, Cocal, VSV (New Jersey) and Pity viruses were similar to those of VSV (Indiana) and are not shown. Mokola nucleocapsids possessed one principal phosphorylated protein, the N protein (possessing a $[^{32}\text{P}]/[^{3}\text{H}]$ count ratio of 5:4), with trace amounts of other phosphoproteins although whether they were of cellular or virus origin could not be determined. An essentially identical result was obtained for Lagos bat virus although there was a significant minor peak of $^{32}\text{P}$ coincident with a $[^{3}\text{H}]$-labelled band which migrated faster than the phosphorylated N protein. The N protein of the nucleocapsids of Lagos bat virus possessed a $[^{32}\text{P}]/[^{3}\text{H}]$ count ratio of 4:0, i.e. essentially equivalent to that of the Lagos bat virus particles. The amount of $[^{3}\text{H}]$ or $[^{32}\text{P}]$ label in the faster moving phosphoprotein of Lagos bat virus nucleocapsids was, however, markedly less than in the Lagos bat virus particles.

Nucleocapsids isolated from SVCV infected cells gave a major peak of $[^{32}\text{P}]$ label which migrated with the $[^{3}\text{H}]$-N protein as well as a second additional peak like the one found in SVCV particles.

When the $[^{3}\text{H}]$-amino acid and $[^{32}\text{P}]$-labelled nucleocapsids of SVC virus (226 µg) were incubated for 15 min at 20°C with (125 µg) trypsin in 0·13 M-NaCl, 0·05 M-tris, pH 7·8, rebanded by isopycnic centrifuging in caesium chloride solution and the ribonucleoproteins recovered ($\rho = 1·32$ g/ml), the protein moiety was not only smaller than the phosphorylated progenitor N protein but also apparently contained much fewer phosphate residues. A similar result was found with Lagos bat nucleocapsids (data not shown). The mol. wt. was estimated for either preparation as 43000 (see Fig. 5). As found for rabies virus, when suitably labelled Mokola or Lagos bat or SVC virus infected cells were harvested by trypsin treatment, similar dephosphorylated polypeptides were identified in the nucleocapsid preparations. In addition a smaller highly phosphorylated peptide (8000 to 10000 mol. wt.) was also found (data not shown).

Lack of antigenic relationship between the N protein of SVCV and the nucleocapsid proteins of rhabdoviruses from the rabies subgroup

It has been shown by Shope et al. (1970) by virus neutralization test and complement fixation that Mokola, Lagos bat and rabies viruses are antigenically related to each other.
This observation has been extended by Schneider et al. (1973), who demonstrated that these viruses possess common group specific antigens localized in the nucleocapsid. In view of the foregoing results which indicate that SVCV possesses a phosphorylated (N) protein, we decided to determine whether the nucleocapsids of SVCV were antigenically related to those of viruses of the rabies subgroup (Table 2). The results of such cross complement fixation reactions indicate that the nucleocapsid protein of SVCV is not related to the nucleocapsid proteins of Lagos bat, Mokola, rabies or VS (Indiana) viruses. These results are in agreement with the previously reported antigenic cross-reactivity of the core proteins of rabies and rabies-related viruses (Schneider et al. 1973).

**DISCUSSION**

Evidence has been presented that all of the ten rhabdoviruses examined possess phosphorylated proteins. Viruses classified in the VSV subgroup have only one phosphoprotein, the minor protein NS. Rabies, Lagos bat and Mokola viruses (and their respective cellular nucleocapsids) all possess a phosphorylated nucleocapsid (N) protein, representing a characteristic distinguishing them from the VSV subgroup of viruses.

Of the five viruses belonging to the VSV subgroup, all have distinct and distinguishable proteins (Obijeski et al. 1974; see below) and are only distantly related by RNA sequence homology studies (Repik et al. 1974). It has, however, been shown by serological analyses that certain antigenic sites are common to this subgroup suggesting that they once had a common ancestor (Cartwright & Brown, 1972). Amongst the rabies subgroup viruses, relatedness has been found between Mokola, Lagos bat and rabies viruses by virus naturalization and complement fixation (Schneider et al. 1973; Shope et al. 1970, see below) although as a subgroup they bear no detectable relationship to the VSV subgroup or to SVCV. RNA sequence homology studies (D. H. L. Bishop, M. S. Smith, P. Repik & H. F. Clark, unpublished observations) indicate that the rabies strains HEP, LEP, PM, CVS and ERA are very closely related to each other but essentially unrelated to the viruses of the VSV subgroup or to SVC. Mokola and Lagos bat virus genome homologies have not yet been determined.

Of the two other rhabdoviruses examined, KCV and SVCV, the former appears to possess small amounts of a phosphorylated glycoprotein and another phosphoprotein with an electrophoretic mobility close to that of the N protein (Sokol & Clark, 1973; see below). On the other hand SVCV possesses a phosphoprotein in its nucleocapsid which may or may not be the N protein since the [32P] does not comigrate exactly with the [3H] label. Evidence will be presented in the second communication in this series (in preparation) that during in vitro phosphorylation by a protein kinase activity resident in SVCV, the phosphorylated product also does not comigrate with the N protein of the virus.

Of the ten viruses examined, more than one virus or nucleocapsid phosphoprotein has been observed for KC, SVC, Mokola and Lagos bat viruses or their nucleocapsids. However, difficulties in producing sufficient quantities of the latter three viruses for biochemical analyses may have resulted in contaminant cellular phosphoproteins in our preparations, such as those at the top of the gels.

It is noteworthy that for nine out of ten rhabdoviruses so far examined, phosphoproteins are found either in association with the nucleocapsids (rabies, Mokola, Lagos bat, SVC viruses) or are present with the transcriptase active RNA-ribonucleoprotein complexes derivable from VSV and related viruses by detergent and salt treatment followed by phase separation to remove the solubilized G and M proteins (Bishop & Roy, 1972). KCV
phosphoproteins have not been studied by phase separation yet. Such complexes of VSV possess N, L, NS, A and B proteins as well as the virus RNA. Of these proteins only NS is phosphorylated, the minor proteins A and B are not phosphoproteins nor do they become phosphorylated \textit{in vitro} by the associated protein kinase (unpublished observations). In parenthesis it should be mentioned that centrifuging in caesium chloride solution results in the removal of NS from the RNA-N protein nucleocapsid of VSV and related viruses (see Sokol & Clark, 1973). It should also be noted that six of the ten rhabdoviruses have been shown to possess virus RNA-dependent RNA polymerases (transcriptases) (Aaslestad \textit{et al.} 1971; Chang \textit{et al.} 1974). Although no polymerase activity has so far been detected in rabies virus (rabies related viruses and SVCV have not yet been studied), it has been found that in the presence of cycloheximide rabies virus genomes are completely transcribed in infected BHK cells (D. H. L. Bishop, M. S. Smith, P. Repik & H. F. Clark, unpublished observations). Whether the intracellular transcription requires activation of a latent virus-bound transcriptase remains to be investigated. The role of phosphorylated structural proteins in rhabdovirus infections has yet to be elucidated although the possibility that they may be related to the interrelations of RNA transcription and RNA replication is a hypothesis which is being investigated.

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