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Mode of Action of Acid pH Values on the Development of Vesicular Stomatitis Virus

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The effect of low pH's on the development of vesicular stomatitis virus (VSV) was studied. L cells infected with VSV were incubated at pH 6.6. A 99% inhibition in the yield of infectious particles was observed by comparison with the yield at pH 7.4. Such inhibition was not due to inhibition of viral RNA synthesis, since at pH 6.6 all the known species of VSV RNA molecules were synthesized. Furthermore, all the known species of VSV proteins were also synthesized. However, no viral particles nor nucleocapsids were detected. Raising the pH to 6.9 resulted in the appearance of nucleocapsids and viral particles, although the yield of infectious virus was still inhibited by 90%. The lack of infectivity of these pH 6.9 viral particles was correlated with their inability to promote primary transcription. The hypothesis that low pH's alter the correct positioning of the viral proteins into the cell membrane is presented.

It was previously shown that the development of both vesicular stomatitis virus (VSV) and poliovirus can be modified by the pH of the medium in which the infected cells are incubated (6-8).

In poliovirus, low pH's seem to inhibit viral RNA synthesis (6). However, the pH at which this inhibition occurs is the pH at which the uptake of uridine by the cell is greatly altered, so that it is difficult to analyze the system in terms of molecular events, and to determine at which level of replication inhibition takes place.

In VSV, inhibition of virus growth occurs at much less acidic pH than in poliovirus (7). It was considered of interest to study the mode of action of acidic pH's on the replication of VSV. As will be shown, decreased pH alters the assembly of nucleocapsids and formation of virions without affecting the synthesis of viral RNA nor that of viral proteins.

MATERIALS AND METHODS

Cells and virus. The Indiana strain of VSV was used throughout this study.

L cells were propagated in monolayer with minimum Eagle medium (MEM), supplemented with 10% tryptose phosphate and 5% calf serum (MCV_1).

Before infection, antibiotics (100 U of penicillin and 0.1 g of streptomycin per ml) were added to the medium.

Infection and viral RNA synthesis. L cells seeded in 60-mm plastic petri dishes were infected during the exponential phase of their growth with 0.2 ml of VSV at an MOI of 25 to 50 PFU/cell. The virus was adsorbed to the cells for 30 min at 36 C, and then 1 ml of MCV_1 was adjusted to the selected pH with 28 mM HEPES (*N*-2-hydroxyethyl-piperazine *N'*-2-ethanesulfonic acid) buffer (containing 3 μg of actinomycin D). One hour later, 1 ml of buffered medium, containing ^{3}H-uridine (25 Ci/mmol, 1 μCi/ml) was added, and viral RNA synthesis was determined as previously described (3).

Cell fractionation and analysis of viral RNA, nucleocapsids, and virions. When necessary, cells were fractionated by using Nonidet P 40 (NP 40), according to a previously described technique (3).

For analysis of viral RNA, cytoplasmic extracts were treated with 1% SDS and centrifuged by using 15 to 30% sucrose gradients in SDS buffer (0.1 M NaCl, 0.01 M Tris, pH 7.5, 0.02 M EDTA, 0.05% SDS) for 16 h at 22,000 rpm, 20 C in an SW25.1 rotor of a Beckman Spinco centrifuge.

The nucleocapsids produced in the infected cells were studied by analysis of cytoplasmic extracts, treated with 1% deoxycholate (DOC), in a 5 to 20% sucrose gradient in Tris-NaCl buffer (0.15 M NaCl, 001 M Tris, pH 7.5). The preparation was then centrifuged for 50 min at 41,000 rpm at 4 C in an SW41 rotor (14). The population of viral particles liberated in the medium was analyzed, by using a 5 to 20% sucrose gradient in the buffer described above. The preparation was centrifuged for 40 min at 25,000 rpm at 4 C in an SW41 rotor (14).

Analysis of viral proteins. Viral proteins were extracted according to Summers et al., as previously described (17). They were analyzed by electrophoresis in a 7.5% polyacrylamide gel (8 by 1 cm) for 7 h at 10 mA/gel.

In vitro transcriptase activity. The standard reaction mixture described by Baltimore et al. (1) consisted of 12.5 μmol of Tris-hydrochloride, pH 7.9, 22.5 μmol of NaCl, 1.25 μmol of $MgCl_2$, 0.75 μmol of mercaptoethanol, 0.25 mg of Triton N 101, 0.25 μmol of ATP, CTP, and GTP, and 12.5 nmol of ^{3}H-UTP (17.8 Ci/mmol) in a total volume of 0.250 ml.

Both pH 6.9 and 7.4 virus preparations were purified according to Roy and Bishop (14) by using PEG 6000 precipitation followed by equilibrium centrifugation through 20 to 70% (wt/vol) sucrose gradients in Tris-NaCl buffer, and by velocity centrifugation through 15 to 30% sucrose gradients in Tris-NaCl buffer. After passage through Sephadex G25 to remove sucrose, the following measurements were made on both virus preparations: (i) infective titer, (ii) ^{32}P-radioactivity, (iii) optical density, and (iv) amount of proteins. We checked carefully that the ratios ^{32}P/OD or ^{32}P/mg of protein were the same for both stocks. Under these conditions the ratio pH 7.4 PFU/pH 6.9 PFU was approximately 10.

Virions synthesized at pH 6.9 or pH 7.4 were used at selected concentrations (see Fig. 6 legend). The mixture was incubated at 37 C, and 20-μliter portions were withdrawn at the indicated time. The reaction was stopped by chilling the samples in an ice bath and by adding 20 μliters of 0.1 M EDTA. Thereafter, the RNA synthesized was precipitated with cold 10% trichloroacetic acid in the presence of 0.4 mg of bovine serum albumin. The precipitate was filtered through membrane filters (type HA, Millipore Corp.) and radioactivity was determined in Bray's solution (2).

RESULTS

Cells infected with VSV were incubated at various pH levels. The results presented in Table 1 show that the final viral yield decreased by 90% at pH 6.9 as compared to that at pH 7.4. With progressively lower pH's, inhibition of viral production became more and more pronounced until, at pH 6.5 to 6.6, there was no further production of virus.

Viral RNA synthesis at pH 6.6. We previously reported that poliovirus multiplication was inhibited at low pH (6) and that this inhibition was mainly due to a decrease of viral RNA synthesis. It was, therefore, of interest to investigate whether the same held true for VSV.

TABLE 1. *Viral titers at end of first replicative cycle at different pH levels*[a]

pH	Viral titers (PFU/ml)
7.4	2×10^8
6.9	1.5×10^7
6.8	9×10^6
6.7	9×10^5
6.6	1×10^4
6.5	5×10^3

[a] L cells (10^6 cells/petri dish) were infected 24 h after seeding with 0.2 ml of VSV at an MOI of 50 PFU/cell.

VSV-infected cells were maintained either at pH 7.4 or pH 6.6, and viral RNA synthesis was followed by measuring the incorporation of

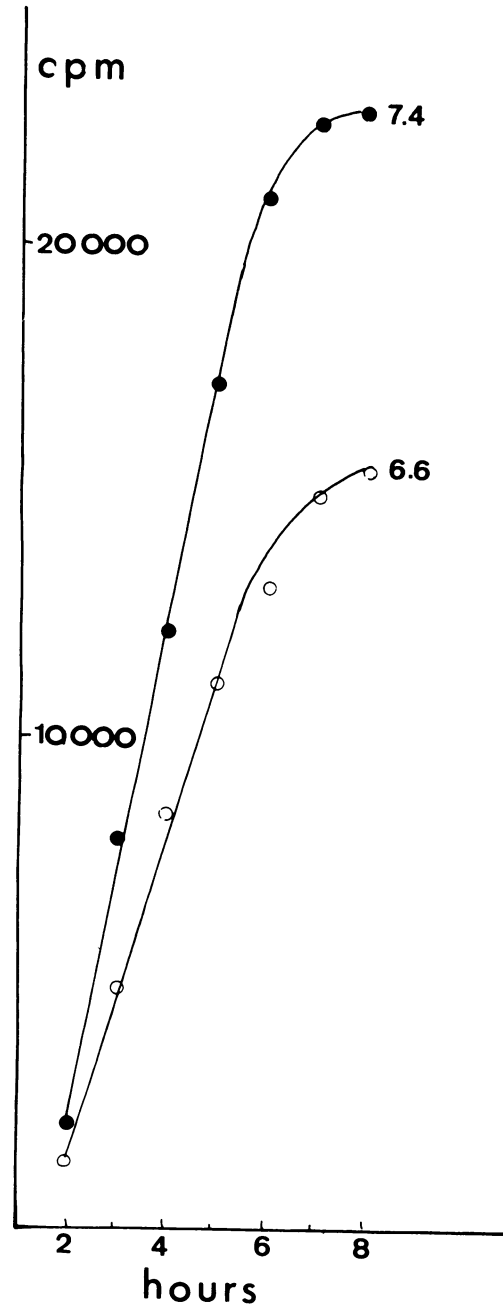

FIG. 1. *Viral RNA synthesis at pH 6.6. L cells were seeded in 6-cm plastic petri dishes and infected 24 h later with 0.2 ml of VSV, and the time course of viral RNA synthesis was determined. Symbols: ●, infected cells incubated at pH 7.4; ○, infected cells incubated at pH 6.6.*

radioactive uridine into acid-precipitable material in the presence of actinomycin D. Incorporation of [^{3}H]uridine into viral RNA was 30% lower at pH 6.6 (Fig. 1).

It was found, however, that penetration of uridine into intracellular acid soluble material was decreased by the same percentage at pH 6.6 whether the cells had been infected or not.

Such alterations of the penetration of uridine could therefore totally account for the inhibition of viral RNA synthesis at pH 6.6.

Since it is known, however, that different species of virus-specific RNAs exist in the infected cell (16), the effect of pH 6.6 on each of these species was investigated. L cells were infected with VSV and incubated at either pH 7.4 or 6.6 in the presence of actinomycin D. One hour after infection, radioactive uridine was added, and 4 h later cytoplasmic extracts were prepared. The extracts were treated with sodium dodecyl sulfate (SDS), and then analyzed by centrifugation by using 15 to 30% sucrose gradient in SDS buffer (Fig. 2).

The sedimentation profile of the RNAs synthesized at pH 7.4 (closed circles) showed three peaks of RNA: 40 to 42*S* virion RNA (fractions 10 to 16); 28*S* viral mRNA (fractions 20 to 26); and 13 to 15*S* viral mRNA (fractions 32 to 40). This profile corresponds to that previously described by others (10, 11, 15, 16, 21).

The sedimentation profile of the viral RNA molecules synthesized at pH 6.6 (open circles) was exactly similar to that at pH 7.4, but the amount of radioactivity in the three species of RNA molecules was reduced by approximately 30%.

A slightly greater inhibition of the labeling of the 40*S* material was noted. This probably reflects the lack of synthesis of virus particles at pH 6.6, since it is known that 40*S* RNA synthesis seems to be inhibited whenever virus formation is prevented. (These results are indeed reminiscent of observations using RNA$^+$ ts mutants grown at nonpermissive temperatures [Printz Ané, personal communication].)

The fact that the synthesis of all these types of viral RNA molecules was inhibited to approximately the same extent at pH 6.6 shows that the 30% inhibition of total viral RNA synthesis was not due to preferential inhibition of that of any particular class of viral RNA molecules, but that it is probably due to inhibition of the penetration of uridine.

Viral protein synthesis at pH 6.6. To check whether all of the viral proteins were synthetized at pH 6.6, VSV-infected L cells were incubated at either pH 7.4 or pH 6.6. Two hours after infection, the cells were trypsinized and resuspended in leucine-free medium and then

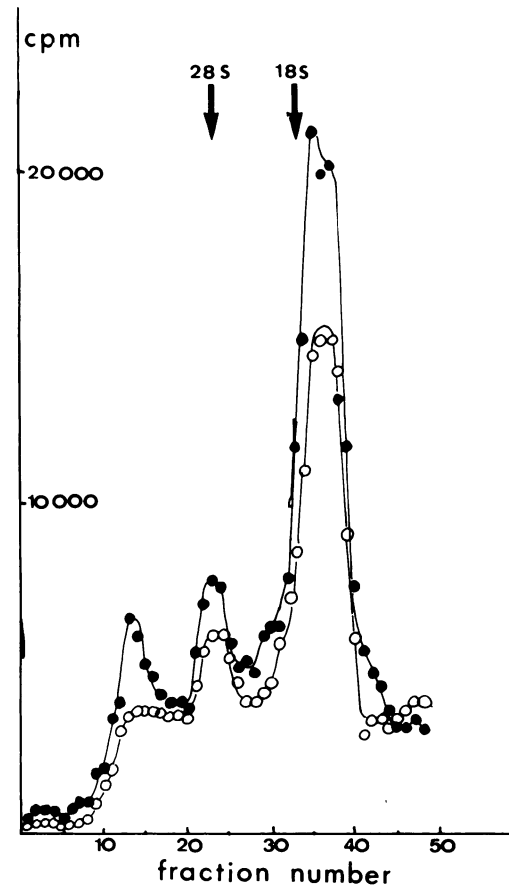

FIG. 2. *Viral RNA molecules synthesized at pH 6.6. L cells were infected as for Fig. 1 except that the labeling was done with 20 μCi of [^{3}H]uridine (25 Ci/mM) per ml. Cytoplasmic extracts were prepared 5 h after infection from the cells of two petri dishes. Part of each extract was made 1% with respect to SDS and analyzed as described in Materials and Methods. The arrows labeled 38S and 18S refer to the position of ribosomal RNAs. Symbols: ●, infected cells incubated at pH 7.4; ○, infected cells incubated at pH 6.6.*

adjusted to the desired pH. The reason for such a treatment has been previously discussed (3).

Half an hour later, [^{3}H]leucine was added and the infected cells were collected after 3 h of labeling. Viral proteins were prepared and analyzed by electrophoresis on SDS polyacrylamide gels (17). The results of this experiment are presented in Fig. 3. In the infected cells incubated at pH 7.4 (closed circles), five radioactive proteins were found, corresponding to the L, G, N, NS, and M proteins described elsewhere (18, 19).

In the cells incubated at pH 6.6 (open circles), the same proteins were detected but there was a 50% reduction in the extent of the labeling. This

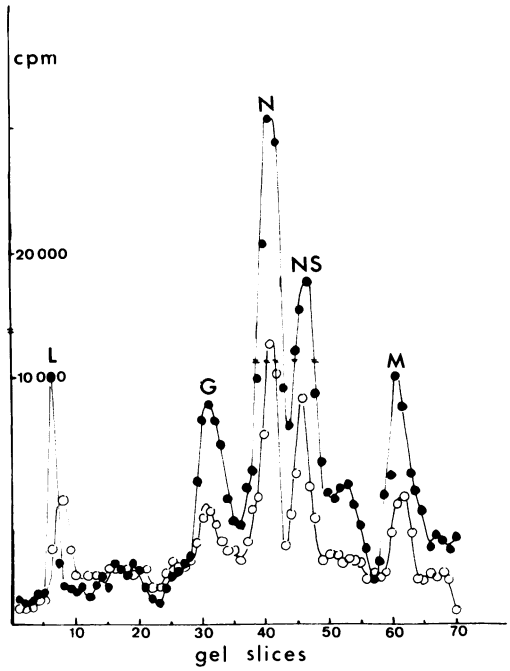

FIG. 3. *Viral proteins synthesized at pH 6.6. L cells infected as previously described were trypsinized 2 h after infection and resuspended at a concentration of 10^6 cells/ml in leucine free medium adjusted to either pH 7.4 (●) or 6.6 (○). The cells were kept in suspension at 36 C, and 30 min later ^{3}H-leucine (10 μCi/ml, 24 Ci/mM) was added. Labeling was for 3 h. Cytoplasmic extracts were prepared and analyzed on 7.5% polyacrylamide gels. The position of the viral proteins are those determined through co-migration of a ^{14}C-labeled purified VSV sample.*

decreased amount of label does not reflect a specific inhibition of viral protein synthesis, but a lower incorporation of amino acids into the acid-soluble pool of the cell and a decrease of cellular protein synthesis (unpublished data). This observation, together with the modification of the penetration of uridine into the cellular acid-soluble pool, seems to indicate an alteration of the cellular membrane at low pH.

Virions and nucleocapsid synthesis at pH 6.6. Since viral RNA and protein synthesis was not affected, it was of interest to investigate whether nucleocapsids and/or virions were assembled at pH 6.6. VSV-infected L cells incubated at either pH 7.4 or at pH 6.6 were labeled with [^{3}H]uridine. Six hours after infection, cytoplasmic extracts and supernatants fluid were analyzed to detect radioactive nucleocapsids and virions by centrifugation using 5–20% sucrose gradients (Fig. 4). The amount of virions (Fig. 4A) and nucleocapsids (Fig. 4B) synthesized at pH 6.6 (open circles), represented only 10 to 15% of the amount synthesized at pH 7.4 (closed circles). Correcting for the 30% decrease in uridine penetration, this amounts to 13 to 20% of the amount found at pH 7.4. Therefore, in spite of the normal or almost normal synthesis of viral RNA molecules at pH 6.6, neither virions nor nucleocapsids were assembled at this pH.

Viral proteins were synthesized normally at pH 6.6; nevertheless, no or very few viral particles were formed. It was questioned, therefore, whether the pattern of insertion of viral proteins into cell membranes was altered at pH 6.6 or not.

A number of experiments have shown that some of the VSV proteins are inserted into the cellular membrane immediately after their synthesis (20). In the case of protein G, Printz and Wagner have shown that in the absence of glycosylation, this protein remains free in the cytoplasm and virions are not assembled (13).

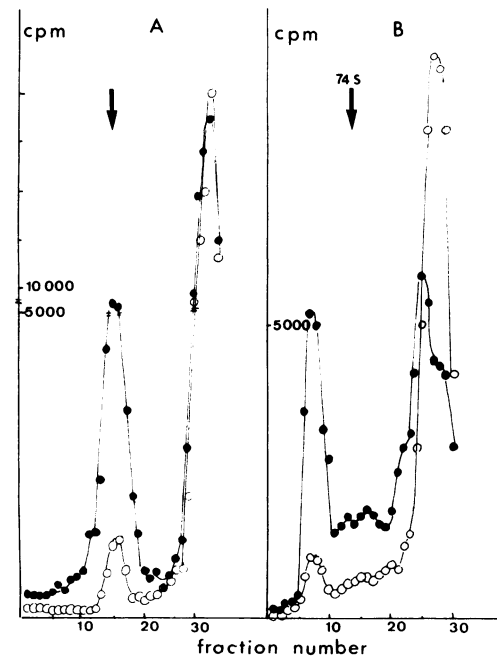

FIG. 4. *Virions and nucleocapsids synthesized at pH 6.6. Panel A, virions. The supernatant of the infected cells used for Fig. 2 was centrifuged for 40 min at 25,000 rpm, 4 C, using a 5 to 20% sucrose gradient in Tris-NaCl buffer. The arrow refers to the position of purified VSV virions. Panel B, nucleocapsids. Part of the cytoplasmic extracts used for Fig. 2 was made 1% with respect both to DOC and Brij and centrifuged for 70 min at 41,000 rpm, 4 C, in the SW 41 rotor of the Spinco, using 5 to 20% sucrose gradients in Tris-NaCl buffer. The arrow labeled 74S refers to the position of ribosomes as determined by optical density. Symbols: ●, infected cells incubated at pH 7.4; ○, infected cells incubated at pH 6.6.*

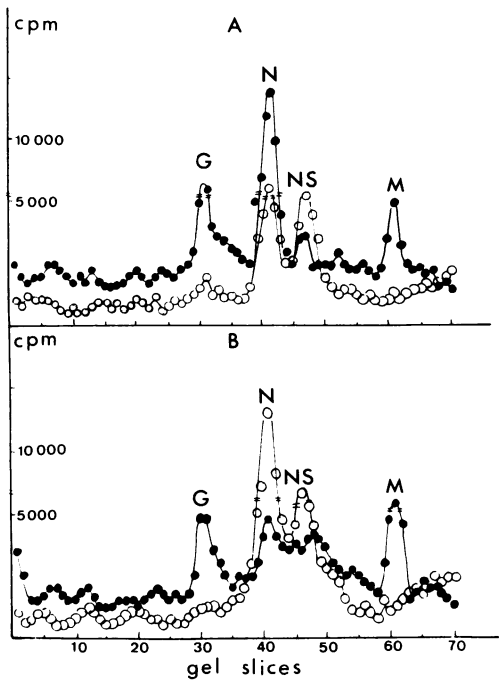

FIG. 5. *Cellular distribution of viral proteins at pH 6.6. L cells were infected and labeled as for Fig. 4. Cytoplasmic extracts, prepared without detergent, were centrifuged for 2 h at 100,000 × g, and viral proteins were prepared from the resulting supernatant and pellet and analyzed as for Fig. 4. Panel A, pH 7.4, sedimentable material, ●; soluble material, ○. Panel B, pH 6.6, sedimentable material, ●; soluble material, ○.*

Thus, the problem was investigated in further experiments. L cells were infected as previously described; thereafter, cytoplasmic extracts were prepared without any detergent. The extracts were centrifuged for 2 h at 100,000 × *g* and the viral proteins in the pellet (membrane-bound proteins) and the supernatant fluid (free proteins) were separately analyzed by electrophoresis in SDS polyacrylamide gels (Fig. 5). At pH 7.4 (panel A) the overwhelming majority of proteins G and M, and approximately two thirds of protein N, were recovered in the pellet (closed circles); whereas most of protein NS, and one third of protein N, were recovered in the soluble fraction (open circles). These results agree with those previously described (13, 20). It should be noted that in this experiment the protein L was hardly detectable, if at all.

The distribution of the proteins synthesized at pH 6.6 (panel B) was, however, quite different. Proteins G and M were correctly inserted into membranes and normally recovered in the sedimentable fraction (closed circles); whereas, most of protein N was not: 80% of it was found in free form with the protein NS (open circles).

Since the sedimentable material examined in this experiment not only includes membrane-bound material, but nucleocapsids as well, the observed difference in the distribution of protein N could be due either to the fact that nucleocapsids were not assembled at pH 6.6, or that there was a defect in the insertion of this protein into the cell membrane.

This latter hypothesis was excluded by showing that treatment of the sedimentable material with DOC did not alter the sedimentable (particulate) nature of protein N, although rendering soluble the majority of proteins G and M (20). Thus, during infection at pH 6.6, both proteins G and M are normally inserted into cell membranes, but protein N remains in a free form, due to lack of assembly into nucleocapsids.

Lack of infectivity of viral particles produced at pH 6.9. As shown above (see Fig. 4) a small amount of nucleocapsids could be formed at pH 6.6, although the yield of infectious virus was less than 0.1% of control (Tables 1 and 2). These results prompted up to investigate the amount of infectious particles, virions, and nucleocapsids produced at different pH's between 6.6 and 7.4 (Table 2). With increasing pH levels, the amount of nucleocapsids and viral particles increased. At pH 6.8 this amount reached the level obtained at pH 7.4, although at this pH the production of infectious particles was still inhibited by 90%. These results suggest that at this pH, nucleocapsids and viral particles could be formed, but lacked infectivity.

Using ^{32}P-labeled virions produced at pH 6.9 it was first shown that adsorption and penetration of these labeled noninfectious virions into L cells were the same as those of infectious virions produced at pH 7.4 (unpublished data). However, when primary transcription of the two

TABLE 2. *Relationship between infectious virus total virion and nucleocapsid production at different pH levels*[a]

pH	Viral titers PFU/ml	Virions (%)	Nucleocapsids (%)
7.4	1×10^8	100	100
6.9	2×10^7	100	100
6.8	9×10^6	100	100
6.7	1×10^6	50	50
6.6	1×10^4	10	20

[a] Viral titers at the end of the first replicative cycle as in Table 1. The amount of virions and nucleocapsids was based on the amount of radioactivity under the peaks of virions and nucleocapsids in gradients similar to those in Fig. 4A and B.

types of virions was measured (5) (9), differences were detected between the two preparations: L cells were infected either with ^{32}P-labeled pH 6.9 virions or with ^{32}P-labeled pH 7.4 virions, and the incorporation in the acid-insoluble fraction of [^{3}H]uridine was measured in the presence of actinomycin D (3 μg/ml) and cycloheximide (150 μg/ml) (Fig. 6, panel A).

When the cells were infected with the same amount of ^{32}P virions from both stocks, as measured by optical density and protein content (10 times more infectious virions produced at pH 7.4 than those produced at pH 6.9), in normal virions, transcriptase activity was about 10 times higher when compared to that of virions produced at pH 6.9 (compare open squares and open circles). On the other hand, when cells were infected with the same amount of infectious virions to both stocks (10 times more virions produced at pH 6.9 than those produced at pH 7.4), the yield of viral RNA produced by primary transcription was the same for both samples (compare closed circles and open squares). Identical results were obtained when incubating the infected cells at pH 6.9 instead of pH 7.4 (unpublished data). These data show that the level of primary transcription was directly proportional to the number of infectious virions employed and not to the number of physical particles. In addition, transcription was independent of the pH at which it was performed. This suggests that the lesser infectivity of the pH 6.9 particles might be related to a defect or absence of transcriptase in these particles (1). In fact, absence of transcriptase is very unlikely since the proteins of pH 6.9 particles showed the same electrophoretic pattern as those of pH 7.4 particles when analyzed by polyacrylamide gel electrophoresis (data not shown).

This hypothesis was further strengthened by measuring the in vitro transcriptase activity of both viral preparations (Fig. 6, panel B). When the same amount of ^{32}P virions were assayed in a cell-free system, the amount of [^{3}H]UTP incorporated in the viral RNA by the virions produced at pH 6.9 was 10 times less than those produced with pH 7.4 (compare open circles and open squares). Here again, when the same amount of infectious virions was used, both in vitro activities were identical (open squares and closed circles).

DISCUSSION

The present report shows that the development of VSV is quite sensitive to the acidity of the medium since incubation of VSV-infected cells at pH 6.9 resulted in a 90% inhibition of the final virus yield. At pH 6.6, development of the virus was completely abolished.

At variance with what has been described for poliovirus (6), this inhibition was not due to that of viral RNA synthesis, since at pH 6.6 all the known species of VSV RNA molecules (16) were synthesized at a normal rate and to a normal extent.

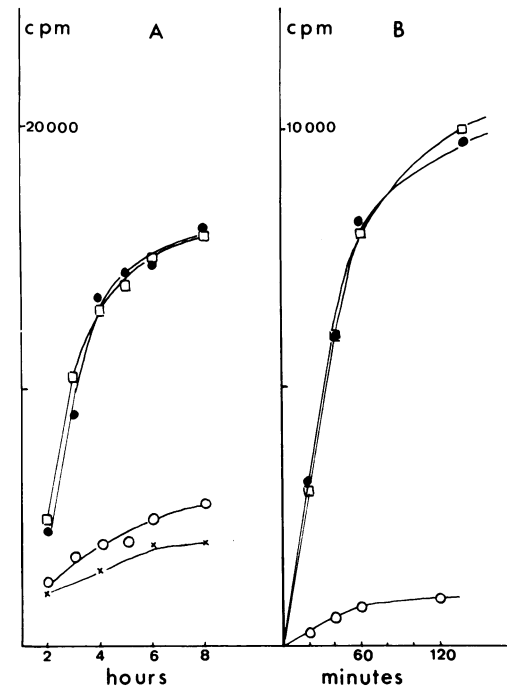

FIG. 6. *Transcriptase activity of the pH 6.9 viral stock. Panel A, Primary transcription in vivo. L cells (10^{6} per petri dish) were infected with either pH 7.4 virions or pH 6.9 virions both labeled with ^{32}P (specific activities were 10^{3} counts per min per 10^{9} PFU for pH 7.4 virions and 10^{3} counts per min per 10^{8} PFU for pH 6.9 virions). After 30 min of adsorption, 2 ml of medium containing 1.5 μg of actinomycin D per ml and 150 μg of cycloheximide per ml were added. After 60 min of incubation, [^{3}H]uridine (10 μCi/ml, 25 Ci/mM) was added and, at hourly intervals, the trichloroacetic acid-precipitable radioactivity was determined as previously described. Symbols: □, cells infected with 50 PFU/cell, pH 7.4 virions; ●, cells infected with 500 PFU/cell, pH 6.9 virions; ○, cells infected with 50 PFU/cell, pH 6.9 virions, ×, uninfected control. Panel B, in vitro transcription. Part of the virus preparations used in the experiment of panel A were assayed for in vitro transcriptase activity. Each point represents the average of duplicate samples. Time zero radioactivity was substracted from the experimental data. Symbols: □, 2 × 10^{9} pH 7.4 PFU/ml of reaction mixture (5 μg of viral protein); ●, 2 × 10^{9} pH 6.9 PFU/ml reaction mixture (50 μg of viral protein); ○, 2 × 10^{8} pH 6.9 PFU/ml reaction mixture (5 μg of viral protein).*

A complete or almost complete absence of VSV particles and nucleocapsids was noted in cells incubated at pH 6.6. Since 42*S* viral RNA and viral proteins were nevertheless synthesized in normal amounts at that pH, it follows that the inhibition probably bears on the assembly of the N protein with mature viral RNA to form nucleocapsids. This was also demonstrated by showing that at pH 6.6, although proteins G and M were normally recovered in association with membranes, little N protein was found in the 100,000 $\times$ *g* pellet of the cytoplasmic extract.

From the results obtained with the ts 52 thermosensitive mutant of VSV (4), it has been shown that the lack of glycosylation of protein G not only prevents the association of this protein with membranes, but also that of protein M, and completely precludes the formation of nucleocapsids (13). This suggests that there might be some relationship between the processing of membrane proteins and the formation of nucleocapsids. Our results indicate, however, that both processes can be uncoupled, since in conditions where formation of nucleocapsids was prevented through acidity of the medium, both the G and M proteins were normally recovered in association with the cell membrane. It could be noted, however, that we have no proof that the G and M proteins were inserted at their correct site in the membrane. Were they not, this could be in favor of the hypothesis that acidic pH's are inhibitory for VSV multiplication because they modify the cell membrane in such a way as to prevent the correct positioning of the viral proteins in the membranes.

According to such an hypothesis, the lack of infectivity of the VSV particles produced at pH 6.9 would be due to improper assembly of the viral proteins, resulting in the inability of the transcriptase to function correctly. At very low pH's (pH 6.6), the modification would further bear on the insertion of the G and M proteins, the incorrect positioning of which would prevent all formation of nucleocapsids. Although there is yet no direct proof that this explanation is correct, the fact that the cell membranes are modified at low pH's can be easily inferred from the altered penetration of uridine and amino acids into the acid-soluble pool of the cell at these pH's.

Regarding the virus particles produced at pH 6.9, they were at the most 10% as infectious as those produced at pH 7.4. Their lack of infectivity correlated with their inability to promote the primary transcription which is required for the synthesis of the first viral mRNA molecules (5, 9). Since all viral proteins were normally synthesized in the infected cell incubated even at pH 6.6, and also since the pH 6.9 virions show a normal content of proteins when analyzed by polyacrylamide gel electrophoresis (data not shown), it is rather unlikely that such inability was due to lack of virion-associated transcriptase.

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LITERATURE CITED

1. Baltimore, D., A. S. Huang, and M. Stampfer. 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Nat. Acad. Sci. U.S.A. **66**:572-576.
2. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1**:279.
3. Fiszman, M., M. Reynier, D. Bucchini, and M. Girard. 1972. Thermosensitive block of the Sabin strain of poliovirus type I. J. Virol. **10**:1143-1151.
4. Flamand, A. 1970. Etude génétique du virus de la stomatite vésiculaire: classement en groupes de complémentation. J. Gen. Virol. **8**:187-195.
5. Huang, A. S., and E. K. Manders. 1972. Ribonucleic acid synthesis of vesicular stomatitis virus. IV. Transcription by standard virus in the presence of defective interfering particles. J. Virol. **9**:909-916.
6. Léauté, J.-B., M. Fiszman, C. Chany, and M. Girard. 1971. Effet de l'influence d'un abaissement de pH sur le métabolisme cellulaire et sur le développement du virus de la poliomyélite. C. R. Acad. Sci. (Paris) **273**:2024-2047.
7. Léauté, J.-B., M. Fiszman, C. Chany, and M. Girard. 1971. Effet d'un abaissement de pH sur le développement du virus de la stomatite vésiculaire. C. R. Acad. Sci. (Paris) **273**:2176-2179.
8. Lwoff, A., and M. Lwoff. 1960. Sur les facteurs du développement viral et leur rôle dans l'évolution de l'infection. Ann. Inst. Pasteur **98**:173-203.
9. Marcus, P. I., D. L. Engelhardt, J. M. Hunt, and M. J. Sekellick. 1971. Interferon action: inhibition of vesicular stomatitis virus RNA synthesis induced by virion-bound polymerase. Science **174**:593-598.
10. Mudd, J. A., and D. F. Summers. 1970. Polysomal ribonucleic acid of vesicular stomatitis virus infected HeLa cells. Virology **42**:958-968.
11. Newman, J. F. E., and F. Brown. 1969. Induced ribonucleic acids in cells infected with vesicular stomatitis virus. J. Gen. Virol. **5**:305-313.
12. Perlin, M., and J. Hallum. 1971. Effect of acid pH on macromolecular synthesis in L cells. J. Cell Biol. **49**:66-74.
13. Printz, P., and R. R. Wagner. 1971. Temperature sensitive mutants of vesicular stomatitis virus: synthesis of virus-specific proteins. J. Virol. **7**:651-662.
14. Roy, P., and D. H. L. Bishop. 1972. 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.
15. Schincariol, A. L., and A. F. Howatson. 1970. Replication of vesicular stomatitis virus. I. Viral specific RNA and nucleoprotein in infected L cells. Virology **42**:732-743.
16. Stampfer, M., D. Baltimore, and A. S. Huang. 1969. Ribonucleic acid synthesis of vesicular stomatitis virus.

I. Species of ribonucleic acid found in Chinese hamster ovary cells infected with plaque-forming and defective particles. J. Virol. **4**:154-161.
17. Summers, D. F., J. V. Maizel, and J. E. Darnell. 1965. Evidence for virus specific noncapsid proteins in poliovirus infected HeLa cells. Proc. Nat. Acad. Sci. U.S.A. **54**:505-513.
18. Wagner, R. R., T. C. Schnaitman, and R. M. Snyder. 1961. Structural proteins of vesicular stomatitis virus. J. Virol. **3**:395-403.
19. Wagner, R. R., T. C. Schnaitman, R. M. Snyder, and C. A. Schnaitman. 1969. Protein composition of the structural components of vesicular stomatitis virus. J. Virol. **3**:611-618.
20. Wagner, R. R., R. M. Snyder, and S. Yamazaki. 1970. Proteins of vesicular stomatitis virus: kinetics and cellular sites os synthesis. J. Virol. **5**:548-558.
21. Wild, T. F. 1971. Replication of vesicular stomatitis virus: characterization of the virus induced RNA. J. Gen. Virol. **13**:295-310.