JOURNAL OF VIROLOGY, Mar. 1968, p. 248-255
Vol. 2, No. 3


# Isolation and Properties of Newcastle Disease Virus Nucleocapsid

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Received for publication 4 December 1967

Deoxycholate (DOC) disrupted virions of Newcastle disease virus (NDV), releasing viral nucleocapsids. The nucleocapsids sedimented at about 200*S* in sucrose gradients and measured from 1.3 to 1.4 $\mu$ long by electron microscopy. NDV nucleocapsids were resistant to pancreatic ribonuclease. These nucleocapsids contained all the 50*S* ribonucleic acid (RNA) in NDV virions, while virus-associated RNA sedimenting at less than 50*S* was external to the virions.

Rapidly sedimenting (50*S*), apparently homogeneous ribonucleic acid (RNA) has been isolated from Newcastle disease virus (NDV) particles (6, 8, 15; W. R. Adams, Federation Proc. **24**:159, 1965). However, as with other members of myxovirus subgroup II (16), the virions of NDV differ widely in size, and the amounts of RNA-containing helical nucleocapsid per virion appear to vary as well (17).

Recently, Hosaka et al. (7) presented data showing that in HVJ (another member of myxovirus subgroup II) the unit length of viral nucleocapsid, corresponding to one genome, is approximately 1 $\mu$, although some virions contain several such lengths of nucleocapsid. Similarly, Compans and Choppin (3) obtained remarkably homogeneous preparations of nucleocapsid (averaging about 1 $\mu$ in length) from cells infected by another related virus, simian virus 5 (SV5).

This paper reveals that disruption of NDV virions with deoxycholate (DOC) releases viral nucleocapsids that sediment homogeneously in sucrose gradients and are 1.3 to 1.4 $\mu$ in length. In addition to other observations on the morphology of NDV nucleocapsid, we illustrate that the contained RNA is 50*S* RNA and that there are no other RNA species within the virion.

## MATERIALS AND METHODS

Methods for propagating the "C" strain of NDV, for obtaining NDV labeled with $^3$H-uridine, and for purifying unlabeled and labeled virus were described previously (8, 9). Over 99% of the radioactivity associated with purified $^3$H-labeled virus was in RNA (9).

*Density gradient centrifugation.* Virus preparations treated with 0.01 to 0.02 M DOC in 0.01 M $Na_2HPO_4$, 0.14 M NaCl, and 0.003 M KCl, *p*H 7.5 (PBS) were layered on 29-ml linear 5 to 20% sucrose gradients in the same buffer containing 0.01 M DOC. After centrifugation at 22,500 rev/min for 2 hr at 4 C, by use of an SW 25.1 rotor in a Spinco model L centrifuge, absorbance of gradients at 254 m$\mu$ was analyzed with an ISCO model D density gradient fractionator (Instrumentation Specialties Co., Lincoln, Neb.), equipped with a 1-cm flow cell. In addition (or alternatively), 1-ml fractions were taken for radioactivity determinations or for electron microscopy. Acid-insoluble radioactivity was measured by liquid scintillation counting of samples solubilized with Hyamine, as previously described (9). DOC did not affect the count rates.

*Electron microscopy.* Density gradient fractions containing rapidly sedimenting, ultraviolet-absorbing material (such as components, A and B, Fig. 4) were examined. Drops of the sample were placed on Formvar grids that had been lightly coated with carbon. After 2 min, excess fluid was removed with bibulous paper. Negative staining was accomplished by inverting the grids onto either 2% phosphotungstic acid (PTA, *p*H 7.2) or 2% uranyl acetate (UA, *p*H 4.5) for 1 min. After staining, the excess fluid was extracted, and the grids were examined as soon as possible in a Siemens Elmiskop I electron microscope by use of double-condenser illumination. Microscope magnifications were checked with a grating that had 54,864 lines per inch (E. F. Fullem, Inc., Schenectady, N.Y.). Length measurements of nucleocapsids were made from photographic prints by use of a map-measuring device.

Other experimental details are given in the figure legends.

## RESULTS

*Release of a rapidly sedimenting, ribonuclease-resistant component from NDV by DOC.* After sucrose gradient centrifugation of DOC-treated $^3$H-NDV, a radioactive component was observed which sedimented more slowly than intact virus (A, Fig. 1). This component contained about 70%

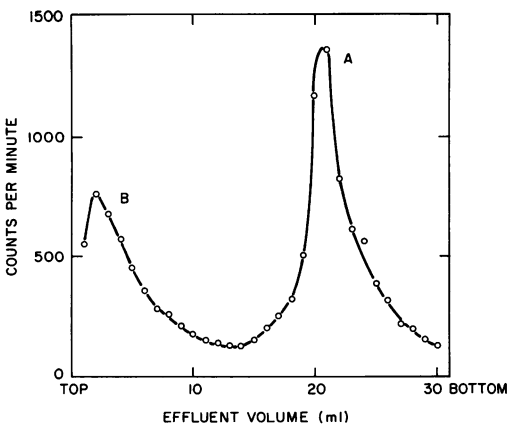


FIG. 1. *Sucrose gradient centrifugation of DOC-treated $^3$H-NDV. Approximately $10^9$ plaque-forming units of $^3$H-NDV in 2 ml PBS were mixed with 0.01 M DOC. After centrifugation on a sucrose gradient as described in Materials and Methods, acid-precipitable radioactivity of 1-ml gradient fractions was determined.*

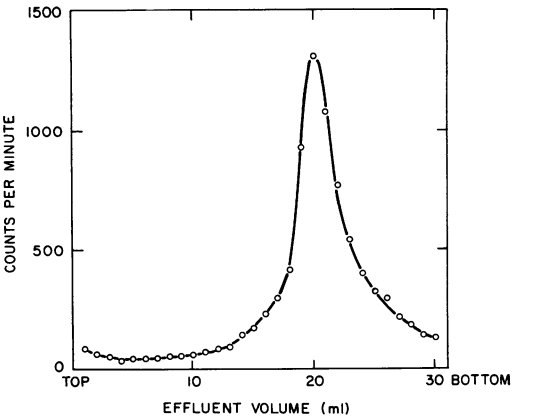


FIG. 2. *Sucrose gradient centrifugation of ribonuclease- and DOC-treated $^3$H-NDV. Ribonuclease (10 μg/ml) and DOC (0.01 M) were added to $10^9$ plaque-forming units of $^3$H-NDV in PBS. Then the preparation was centrifuged on a sucrose gradient as described in Materials and Methods.*

of the labeled RNA associated with the virus. Less than 5% of the label was found in the pellet, while the remainder sedimented slowly and heterogeneously (B, Fig. 1). Compared with chick embryo ribosome monomers centrifuged in the same way, the rapidly sedimenting component of NDV sedimented at approximately 200*S* (11), which indicated that it was a large structure, although smaller than the virus.

Although pancreatic ribonuclease made the slowly sedimenting RNA acid-soluble, the enzyme had no effect on either the sedimentation rate or the radioactivity of the 200*S* component (Fig. 2). Thus, the RNA sedimenting at 200*S* was complexed with, and protected by, another material; as will be shown later, this material is protein.

*Isolation of RNA from the 200S NDV component.* Since RNA isolated from NDV with sodium dodecyl sulfate (SDS) (Fig. 3A) or with phenol sediments predominantly at 50*S* (6, 8, 15; W. R. Adams, Federation Proc. **24**:159, 1965), and since most of the label in NDV was found in the 200*S* component after DOC (Fig. 1), it seemed likely that the RNA associated with the 200*S* component was exclusively 50*S*. This was shown to be the case by isolating the 200*S* component, dissociating it with SDS, and centrifuging the RNA on a sucrose gradient (Fig. 3B).

*Slowly sedimenting RNA associated with NDV virions.* Since the RNA of the 200*S* component was 50*S* RNA (Fig. 3B), it was apparent that the ribonuclease-labile RNA in DOC-treated NDV preparations (Fig. 1, 2) was identical to

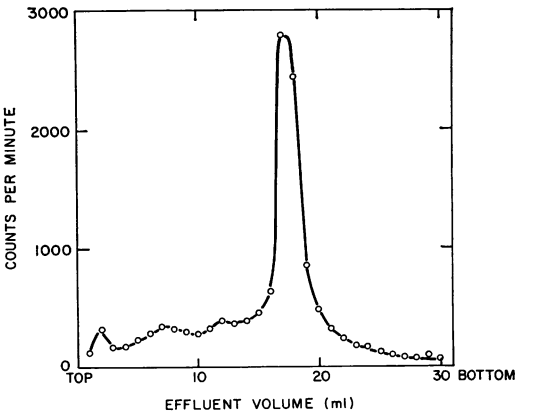


FIG. 3A. *Sucrose gradient centrifugation of SDS-treated $^3$H-NDV. Approximately $10^9$ plaque-forming units of $^3$H-NDV in 2 ml 0.005 M tris(hydroxymethyl)-aminomethane-chloride, 0.001 M ethylenediaminetetraacetate (pH 7.4) received SDS to a final concencentration of 1%. The preparation was layered on a linear 5 to 20% sucrose gradient (29 ml) in the same buffer and centrifuged 17 hr at 20,000 rev/min, 4 C. Acid-insoluble radioactivity was determined on 1-ml fractions.*

the RNA which sedimented at less than 50*S* when we analyzed isolated viral RNA (Fig. 3A). Such RNA, apparently of low molecular weight and heterodisperse, has been considered a cellular RNA contaminating virus (W. R. Adams, Federation Proc. **25**:422, 1966) or degraded viral RNA (2). This RNA might be not only external to the 200*S* component, but might also be external to the virion. If RNA is outside the virion, it would be digested when intact virions are treated with ribonuclease; if it is inside the

virion, addition of DOC would be necessary to render the RNA accessible to the enzyme.

Ribonuclease solubilized the same amount of radioactivity (Table 1), whether or not DOC was added to the virus, which illustrated that the non-50S RNA was external to the virion. The same conclusion was drawn by W. R. Adams (Federation Proc. **25**:422, 1966), who showed that NDV adsorbed to and eluted from chicken erythrocytes contained only 50S RNA.

*Large-scale isolation of the 200S NDV component.* To obtain sufficient 200S component for electron microscopy, highly concentrated NDV ($10^{11}$ plaque-forming units/ml) was disrupted with DOC and centrifuged on sucrose gradients. An ultraviolet-absorbing component sedimenting at 200S was detected (A, Fig. 4). However, additional, more rapidly sedimenting ultraviolet-absorbing materials were always observed (B, Fig. 4) whenever a highly concentrated virus was examined in this manner. We believe this represents an aggregation of some 200S components, since labeled virus behaved similarly when mixed with concentrated, unlabeled virus. Moreover, we isolated 50S RNA from components sedimenting faster than 200S; ultraviolet-absorbance spectra of 200S and more rapidly sedimenting components (Fig. 5) were identical. Compans and Choppin (3) sometimes observed an aggregation of a fraction of SV5 nucleocapsid isolated by another method.

The presence of DOC in the sucrose gradient appeared to reduce aggregation of 200S components because little or no ultraviolet-absorbing material was found in the expected location when DOC was omitted. In these cases, the ultraviolet-absorbing material appeared in the pellet.

*Chemical properties of the 200S NDV component.* The ultraviolet-absorbance spectrum of the 200S component (Fig. 5) resembled the spectra of tobacco mosaic virus (1) and the

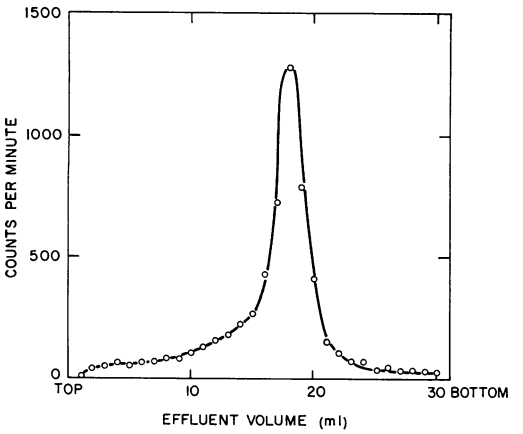


FIG. 3B. *Sucrose gradient centrifugation of SDS-treated 200S component of NDV. $^3$H-labeled 200S component was isolated from a gradient by precipitation of component containing fractions with 2 volumes of ethyl alcohol after addition of 1% SDS and 100 μg of yeast RNA to each fraction. Precipitates were collected by centrifugation, dissolved in 0.005 M tris(hydroxymethyl)aminomethane-chloride, 0.001 M ethylenediaminetetraacetate, 1% SDS (pH 7.4), combined, and analyzed by sucrose gradient centrifugation as described in the legend of Fig. 3A.*

TABLE 1. *Sensitivity to ribonuclease of $^3$H-RNA associated with NDV*[a]

| Sample | Acid insoluble (count/min) | Acid insoluble radioactivity (% of control) |
|---|---|---|
| $^3$H-NDV | 3,306 | — |
| $^3$H-NDV + ribonuclease | 2,435 | 73.5 |
| $^3$H-NDV + DOC + ribonuclease | 2,454 | 74.2 |

[a] A sample of purified $^3$H-NDV, suspended in PBS received 10 μg/ml of pancreatic ribonuclease "A." Another sample received the same amount of ribonuclease and 0.01 M DOC. After 30 min at 23 C, each sample was precipitated with 5% trichloroacetic acid, and the acid-insoluble radioactivity was compared with a sample of virus which did not receive enzyme.

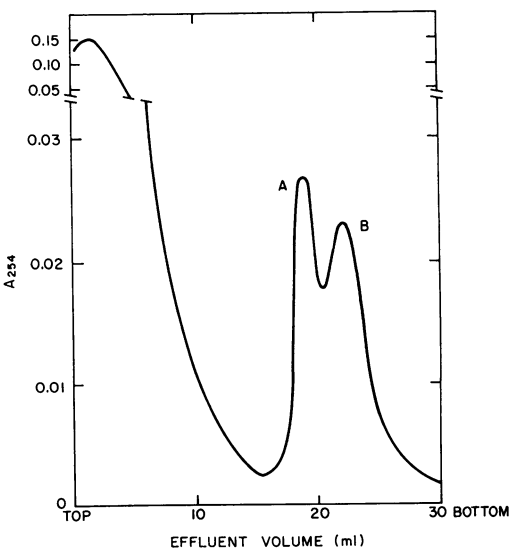


FIG. 4. *Sucrose gradient centrifugation of DOC-treated unlabeled NDV. Approximately $10^{11}$ plaque-forming units of NDV received DOC to 0.1 M in 2 ml of PBS. The preparation was analyzed by sucrose gradient centrifugation as described in Materials and Methods. The large peak of absorbance at the top of the tube represents chiefly slowly sedimenting viral proteins.*

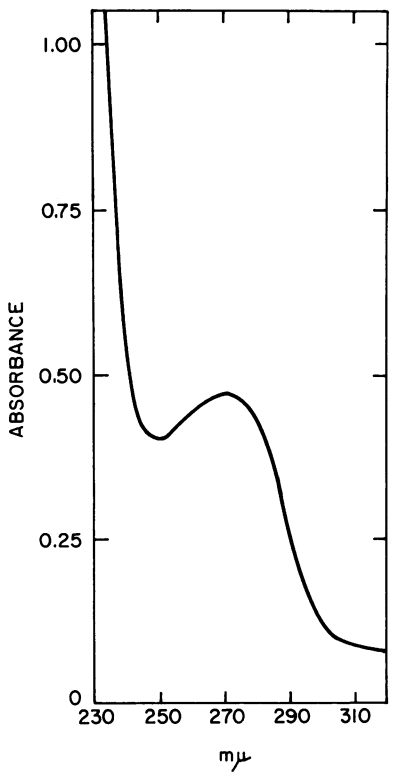


FIG. 5. *Ultraviolet-absorbance spectrum of NDV 200S component. The 200S material collected from several sucrose gradients was combined, concentrated by centrifugation, and dissolved in PBS containing 1% SDS. Absorbance measurements were made at 23 C in 1-cm cells in a Zeiss PMQ II spectrophotometer.*

nucleocapsid of SV5 (3). This evidence that the 200S component was nucleoprotein was supported by colorimetric tests (5, 10) which showed 4.5% RNA by weight relative to protein (average of two determinations on a sample containing 73 μg of RNA).

*Morphology of 200S NDV component.* Well-dispersed preparations of the 200S NDV component isolated from sucrose gradients revealed particles having the appearance of helical nucleocapsid (Fig. 6–8). Measurements were made of 28 UA- and 29 PTA-stained particles (Fig. 9). Particles shorter than 0.5 μ and tangled particles were disregarded. The UA-stained particles had a mean length of 1.33 μ (standard deviation, ±0.12 μ) and the PTA-stained ones had a mean length of 1.44 μ (standard deviation, ±0.20 μ). This difference in length was significant to the 2.5% confidence level.

The average outside diameter of the nucleocapsid was 185 A (±25 A). This measurement was variable, even on the same strand, and appeared to depend on the depth of penetration of the stain in and around the structure units.

The method of staining had considerable influence on the appearance of the nucleocapsid and particularly on the repeat distance of the structure. In PTA (*p*H 7.2)-stained preparations (Fig. 7), the structure units were more widely separated. Measurements of these particles yielded a repeat distance of 63 A ± 10 A. The UA (*p*H 4.5)-stained preparations seemed more compact (Fig. 8) and had a repeat distance of 60 A ± 10 A. This different appearance cannot be explained on the basis of the *p*H of the stain because PTA of *p*H 5.0 or lower still revealed a loose coil similar to that depicted in Fig. 7.

PTA-stained preparations of material sedimenting faster than 200S (B, Fig. 4) had the same appearance as the 200S material.

## DISCUSSION

Nucleocapsids have been released from virions of NDV and other myxoviruses by shaking with ether (13, 14), but this method has not yielded uniform long nucleocapsids such as those we have obtained from NDV by use of DOC. NDV nucleocapsids released by DOC may be isolated easily for further study because they sediment much more rapidly than other viral components (Fig. 4). Their uniformity and large size indicate that these nucleocapsids represent complete genetic units of the virus; they contain the 50S RNA which presumably represents intact viral genomes (6, 8, 15); W. R. Adams, Federation Proc. **24**:159, 1965).

There may be variation in the number of nucleocapsid units incorporated into an NDV virion (17), as with the related HVJ (7). Nevertheless, the only RNA in NDV virions is associated with nucleocapsids (Table 1), which shows that qualitatively the incorporation of RNA into NDV virions is rigidly controlled. Robinson and co-workers (2, 6, 12) described a "4s" component that contains viral genetic material in their preparations of NDV RNA. Our data however, show that all RNA sedimenting at less than 50S is external to the virion, since it is digested by ribonuclease when intact virus is treated with the enzyme. Ribonuclease-treated virus being fully infectious (D. W. Kingsbury, *unpublished observations*), RNA sedimenting at less than 50S can have no function in NDV replication—whether it arises from breakdown of 50S RNA (2, 6, 12) or from another source (W. R. Adams, Federation Proc. **25**:422, 1966).

Recently, Compans and Choppin (4), by use of osmotic shock, obtained 1.0 to 1.1 μ long

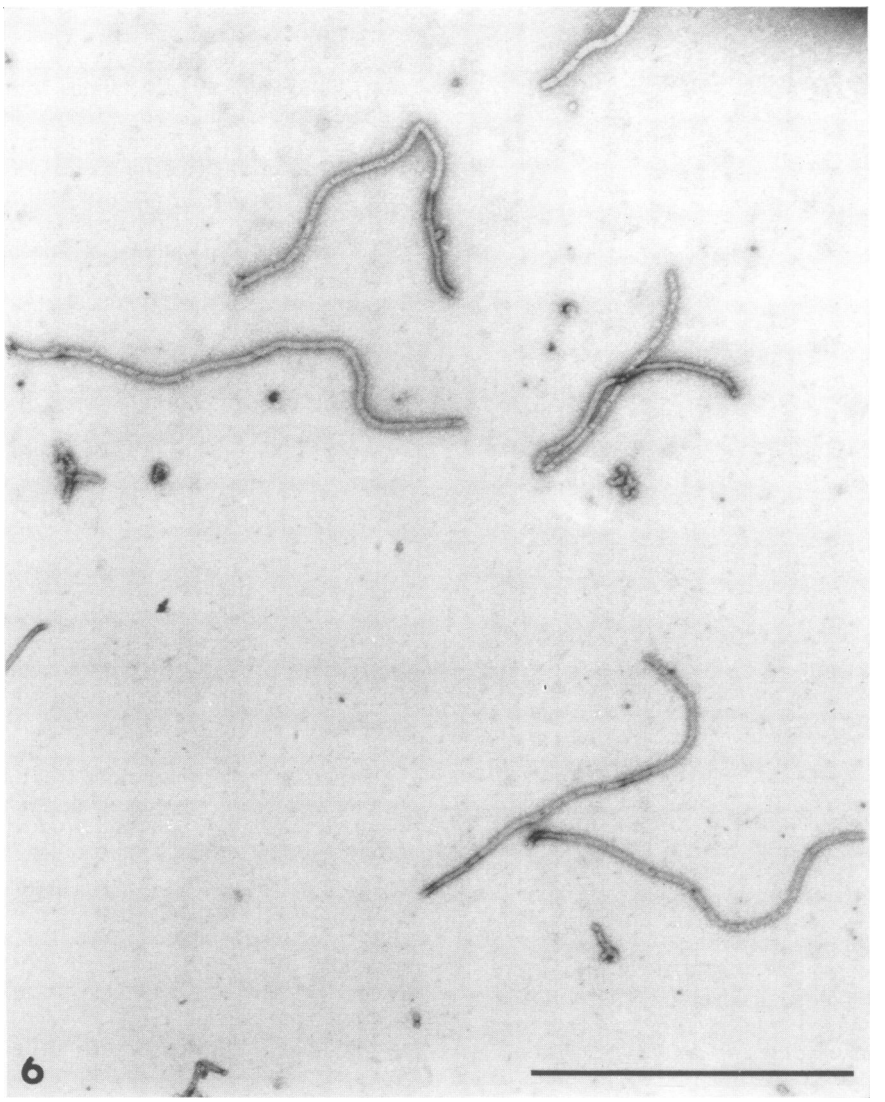


FIG. 6. *Electron micrograph of NDV nucleoprotein stained with uranyl acetate. Bar indicates 1 μ. × 43,000.*

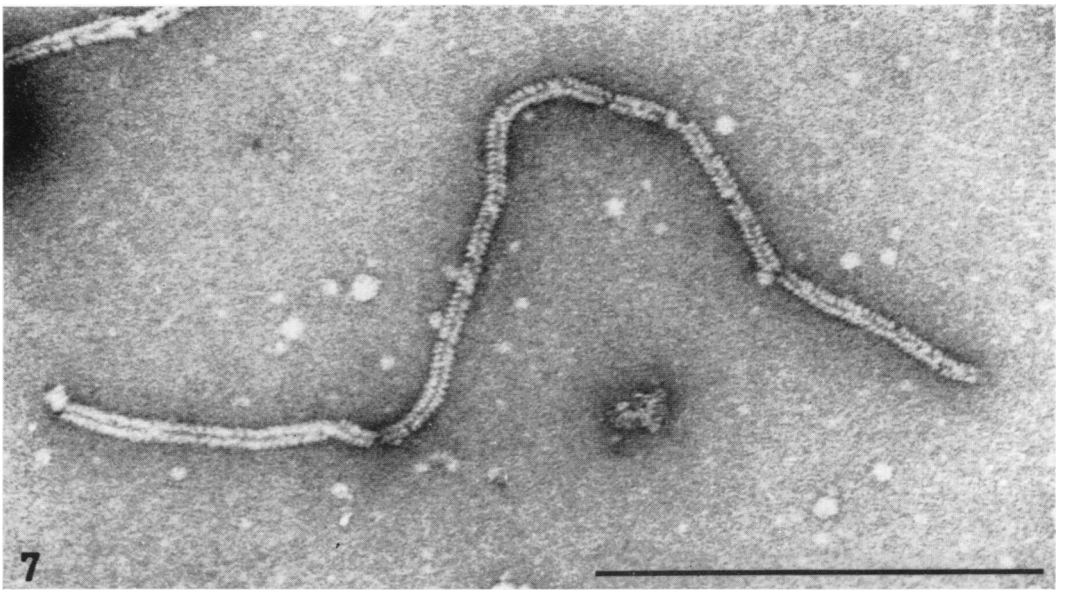


FIG. 7. *A single NDV nucleoprotein particle stained with phosphotungstic acid. Bar indicates 0.5 μ. × 122,000.*

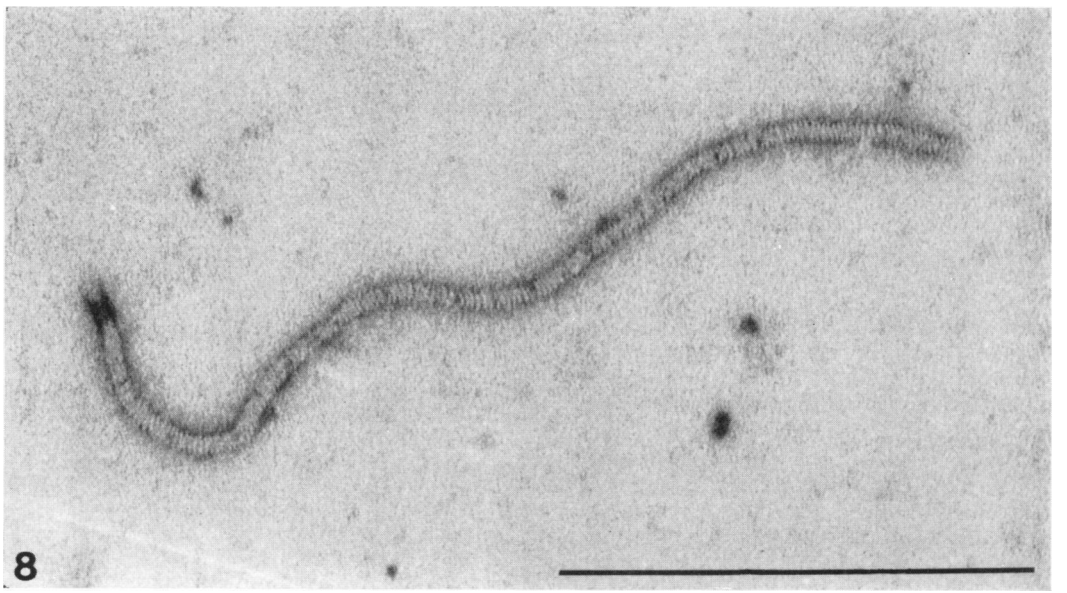


FIG. 8. *A single NDV nucleoprotein particle stained with uranyl acetate. Bar indicates 0.5 μ. × 130,000.*

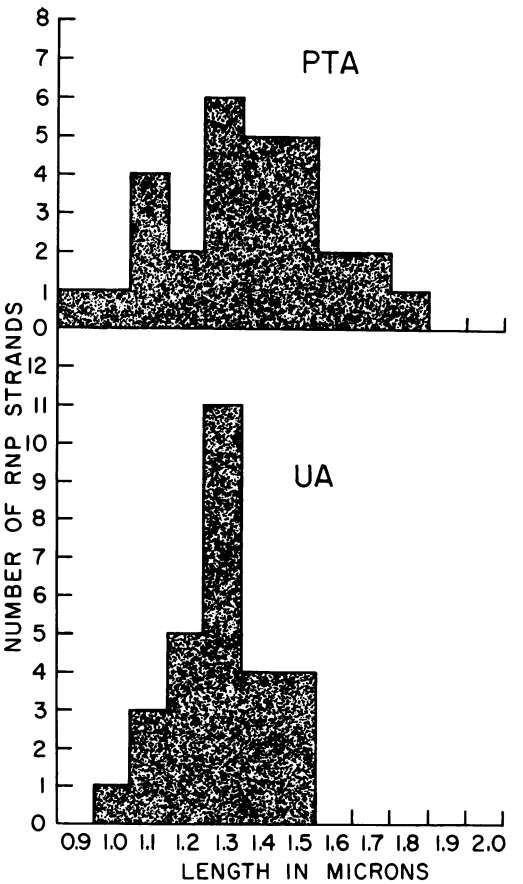


FIG. 9. *Histograms of NDV nucleocapsid (RNP) lengths measured on preparations stained with phosphotungstic acid (PTA) or uranyl acetate (UA).*

nucleocapsids from cells infected by NDV, which indicates that NDV nucleocapsid is morphologically similar to SV5 (3) and HVJ (7) nucleocapsids. However, our measurements on nucleocapsids from NDV virions gave lengths of 1.3 to 1.4 $\mu$. We do not consider our findings in conflict with those of Compans and Choppin (4) because their nucleocapsids seemed more tightly coiled than ours. DOC-released NDV nucleocapsids had apparent periodicities of 50 to 73A compared to 45 to 55A for NDV nucleocapsid from infected cells (R. W. Compans and P. W. Choppin, *personal communication*). These differences in periodicity reflect relative looseness in the DOC-released nucleocapsid coil, and are consistent with the differences in lengths observed; they may have their origin in the different sources of nucleocapsids or the varied methods used to extract and examine them. Inasmuch as NDV nucleocapsids obtained with DOC were resistant to ribonuclease, the looser coiling in our preparations might have occurred during preparation for electron microscopy.

Recently, W. R. Adams (*personal communication*) informed us that he obtained nucleocapsids similar in appearance and dimensions to those described in this paper by treating NDV virions with DOC.

## ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI-05343 and AI-05765, and by American Lebanese Syrian Associated Charities (ALSAC). One of us (D. W. K.) was the recipient of Public Health Service Career Development Award HD 14,491.

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