The Intracellular Site of RNA Synthesis of Newcastle Disease Virus. (31453)

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It has been shown that RNA of Newcastle disease virus(1) and its coat proteins(2) are produced in cytoplasm. However, the viral RNA synthesis has not been investigated with pulse label. One may suggest that the cytoplasmic localization of labeled RNA is the result of RNA migration from other cellular structures. In the experiments performed the site of RNA synthesis was determined by brief pulses of H^3-uridine as compared to the label of longer duration.

Materials and methods. Virus. K strain of NDV was obtained as allantoic fluid and concentrated by centrifugation at 25,000 *g* for one hour.

Cells. HEp-2 cells were grown on pieces of slides in Parker's medium containing 10% calf serum and used on the second day of incubation when a monolayer had not been fully formed.

Actinomycin D was a gift from Merck, Sharp & Dohme Co., USA. *H^3-uridine* with specific activity 2.44 C/mmole was obtained from the Radiochemical Centre, Amersham.

Assay of H^3-uridine incorporation. Cells were infected with about 1000 EID_{50} p.c. at +4°C for 30 minutes, washed with BSS and put into warm Parker's medium with 2 μg/ml of actinomycin D. This time represented zero time. Control cells were treated similarly. Every 30-60 minutes the samples were put into one of 3 portions of Parker's medium with 2.0 μg/ml of actinomycin D which contained 10, 2 or 1 μC/ml of H^3-uridine and incubated for 10, 30 or 120 minutes, respectively. The cells washed with unlabeled uridine and fixed with Carnoy solution were treated with cold 5% TCA for one hour at +4°C and stored at +4° for autoradiographic exposure. After 3 weeks the slides were developed, washed and dried, and specimens stained with methylene blue.

Results. There was labeling of a nucleoplasm and a nucleolus of uninfected cells exposed to H^3-uridine during first hour of actinomycin treatment. Only a few cells contained the grains in the nucleoplasm but not in the nucleolus when the contact with H^3-uridine was from one to 2 hours of incubation (Fig. 1A). The cells preincubated with actinomycin for 2 hours or more did not incorporate the label (Table I).

When actinomycin-treated infected cells were exposed to one-hour pulses of precursor, the grains were predominantly localized over the cytoplasm; 2 hours after infection some grains were observed also in the nuclei, mainly in the nucleoplasm. Later the number of grains over the cytoplasm increased while labeling of the nuclei and especially of the

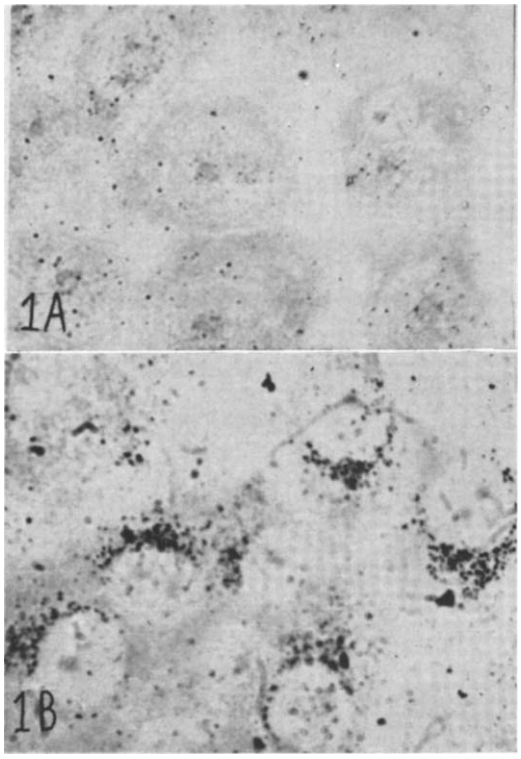

FIG. 1. Radioautographs of actinomycin-treated HEp-2 cells exposed to 1 μC/ml of H^3-uridine from 3 to 4 hr of incubation. A. Uninfected cells. B. NDV-infected cells.

TABLE I. Number of Grains in Cellular Structures of NDV-Infected Actinomycin-Treated HEp-2 Cells Exposed to 30-Min and 1-Hr Pulses of H^3-Uridine.

Time contact with H^3-uridine	Uninfected cells			Infected cells		
	C*	N	n	C	N	n
0–30 min	0	41.0 ± 1.3	13.2 ± .7	0	69.1 ± 1.5	13.1 ± 1.6
1 –1.5 hr	0	0	0	0	16.3 ± 1.7	4.2 ± .6
1.5–2 "	0	0	0	0	0	0
2 –2.5 "	0	0	0	0	0	0
3 –3.5 "	0	0	0	0	0	0
3.5–4 "	0	0	0	.7 ± .5	7.9 ± 1.7	12.0 ± 1.4
4 –4.5 "	0	0	0	11.0 ± 1.3	33.5 ± 1.6	17.0 ± 1.1
4.5–5 "	0	0	0	31.0 ± 5.0	48.7 ± 2.9	23.5 ± 2.6
1–2 hr	0	0	0	17.8 ± 1.7	15.0 ± 1.7	2.3 ± .6
2–3 "	0	0	0	27.9 ± 1.5	5.4 ± .8	1.6 ± .4
3–4 "	0	0	0	44.4 ± 2.6	1.5 ± .3	.4 ± .2
4–5 "	0	0	0	43.4 ± 2.1	4.3 ± .5	.7 ± .2

* C, cytoplasm; N, nucleoplasm; n, nucleolus.
1.0 and 2.0 μC/ml of H^3-uridine were used for 1-hr and 0.5-hr pulses respectively.

nucleoli disappeared. The grains were distributed either at random over the cytoplasm or over perinuclear zone (Fig. 1B).

When actinomycin-treated infected cells were exposed to 30-minute pulses during the first 1.5 hours after infection, the number of grains seen over the nuclei exceeded the number of grains in uninfected cells. There was no labeling from 1.5 to 3.5 hours, but later the virus-induced incorporation of H^3-uridine into infected cells occurred. In the cells exposed to H^3-uridine from 3.5 to 4 hours after infection the grains were concentrated into nucleoli, and the number of grains in the cytoplasm was not significant (Table I, Fig. 2). When the precursor was added between 4 and 5 hours the incorporated radioactivity was higher than previously, but the number of grains in the cytoplasm was increased more significantly than in the nucleus so that at 5 hours the number of grains in the cytoplasm and in the nucleolus was nearly similar.

When the infected cells were exposed to 30-minute pulses at 4 and 4.5 hours after infection, 4 types of labeled cells were observed: a) with grains over nucleoli (11.8%), b) with grains over nucleoli and nucleoplasm (31.8%), c) with grains over nucleoplasm (10.0%), d) with grains over nuclei which poured into the cytoplasm as a broad flow (46.4%). The flow of grains was usually observed in juxtaposition to the nucleus. In some cells a bipolar position was observed.

In the cells exposed to 10-minute pulses 4 and 5 hours after infection all incorporated radioactivity was concentrated over nucleoli and the nearest regions of the nucleoplasm (Fig. 3).

Discussion. The results obtained indicate that tritiated uridine added for 10 minutes at the period of viral RNA synthesis(3) is incorporated into nucleoli. When the contact with the precursor is prolonged, the label is observed in the cytoplasm. It would appear that the RNA of Newcastle disease virus replicates in nucleoli and migrates into the

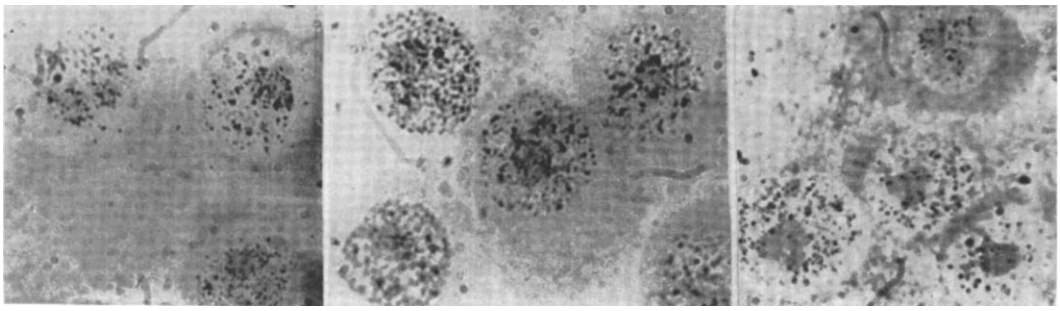

FIG. 2. Radioautographs of actinomycin-treated HEp-2 cells infected with NDV and exposed to 2 μC/ml of H^3-uridine from 3.5 to 4 hr after infection.

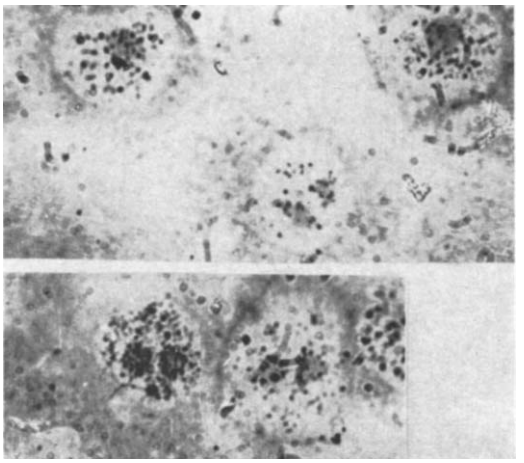

FIG. 3. Radioautographs of actinomycin-treated NDV-infected HEp-2 cells exposed to 10 μC/ml H^3-uridine from 4 to 10 hr after infection. × 600.

cytoplasm when it functions as a template for S- and V-antigen synthesis. This migration occurs more quickly than in the case of Sendai(4) and influenza(5) viruses.

Since the reproduction of Sendai virus occurs also in nucleoli(4), it may be assumed that nucleolar structures play an important role in RNA synthesis of paramyxoviruses.

The label found in nuclei during the first hours after infection seems to migrate also into the cytoplasm. The origin of this label is not clear; it may represent the synthesis of some early RNA. Such RNA was shown to be induced by Sendai and FPV(6).

Summary. The newly synthesized actinomycin-resistant RNA as determined by brief pulses of H^3-uridine at the period of NDV-induced RNA synthesis is detected in the nucleolus. When the contact with H^3-uridine is prolonged the label is found over the cytoplasm. It is suggested that the RNA of Newcastle disease virus replicates in the nucleolus and migrates into the cytoplasm.

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Received June 20, 1966. P.S.E.B.M., 1966, v123.

Growth and Cytopathic Effect of H Type Rhinoviruses in Monkey Kidney Tissue Culture. (31454)

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Rhinoviruses have been divided into two groups based on their capacity to multiply and produce cytopathic effect (CPE) in primary monkey kidney tissue cultures (MKTC)(1). Those virus strains which multiply in MKTC have been designated M strains and those which do not are called H strains. Both H and M type rhinoviruses have the capacity to propagate and produce CPE in tissue cultures of human origin. It is not clear whether this division represents true differences in biologic properties of viruses or is dependent on other factors. In addition, since differences in antigenicity and in the seasonal pattern of infection and illness have been reported between M and H type rhinoviruses (2,3), it was of interest to investigate the stability of the difference in capacity of rhinoviruses to grow in MKTC. In the present study it was possible to demonstrate growth and CPE in MKTC of 5 rhinovirus serotypes previously considered to be H strains.

Materials and methods. Source of virus. The H type rhinoviruses used in this study were prototype strains NIH 1734, 353, 11757, 1059, and 33342(4). Each strain had been