agglutination-inhibition tests incorporating increased amounts of salts. Properties of measles hemagglutinins classified according to their specific salt requirements are compared and discussed.

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Received Oct. 26, 1967. P.S.E.B.M., 1968, Vol. 127.

Absence of Nucleotide Sequence Complementarity between Sindbis Virus and Newcastle Disease Virus Genomes* (32808)

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The base composition of the total virus-specific RNA in cells infected by Newcastle disease virus (NDV) is complementary to the base composition of the RNA in NDV virions. This indication of nucleotide sequence complementarity between the genomic RNA and most of the virus-induced RNA was borne out by *in vitro* hybridization tests (1,2). Base compositions of RNA's from virions of the arboviruses Sindbis virus (3) and Dengue virus (4) appear complementary to the NDV genome, resembling NDV-induced complementary (—) RNA (Table I). Since Group A arboviruses are the only animal viruses known to have RNA with such a base composition, (7) we thought it of interest to test whether Sindbis virus and NDV genomes are complementary to each other in nucleotide sequences.

TABLE I. Base Compositions of Viral RNA's.

RNA species	(mole percent) A[b]	G	C	U	Ref.
Sindbis (+)[a]	29.6	25.8	24.9	19.7	(3)
NDV (—)	30.4	22.3	25.8	21.5	(1)
NDV (+)	20.1	25.4	23.2	31.2	(1)

[a] (+) = RNA isolated from virions; (—) = RNA isolated from infected, actinomycin D-treated cells (1).

[b] A = adenine, G = guanine, C = cytosine, U = uracil.

Materials and Methods. Chick embryo cell monolayers were infected with Sindbis virus (Strain AR-334) at an input multiplicity of 5 plaque-forming units per cell. Infected cells were incubated 16 hours at 37°C in an atmosphere of 5% CO_2 in air with 25 μC/ml uridine-6-^{3}H (9.3 C/mmole) in Earle's saline containing 0.5% lactalbumin hydrolyzate and 1% gelatin. The ^{3}H-labeled virus was isolated from cell culture fluid by isopycnic centrifugation in potassium tratrate (5). The RNA was extracted from the virus by shaking with phenol at 60°C after addition of 200 μg/ml yeast RNA and sodium deoxycholate to a final concentration of 1%. The RNA was concentrated by precipitation with two vloumes of ethanol, dissolved in 0.005 *M* tris-HCl + *M* EDTA (pH 7.4), and centrifuged into a sucrose gradient made up in the same buffer (Fig. 1). Fractions from the 38 *S* peak of radioactivity and its leading edge were pooled, concentrated by precipitation with ethanol and freed of remaining low molecular weight yeast RNA by passage through a Sephadex G-200 column equilibrated with 0.1 *M* ammonium acetate (pH 5.5).

Results and Discussion. Purified Sindbis-RNA-^{3}H was annealed (1) with various amounts of unlabeled RNA (+) isolated from NDV particles (5). As shown in Fig. 2, NDV

* Supported by USPHS Grant AI 05343 and American Lebanese Syrian Associated Charities (ALSAC).

[1] Holder of USPHS Career Development Award 5-K3-HD 14, 491.

(+) RNA converted no more Sindbis virus RNA to ribonuclease resistance than occurred when Sindbis virus RNA was annealed without additions. The NDV (+) RNA was competent, since it efficiently converted the labeled NDV-induced (—) RNA in extracts from infected cells incubated with actinomycin D (1) to ribonuclease insensitivity on annealing (Fig. 2). Athough NDV-specific RNA represents essentially all of the labeled RNA in such extracts, it represents only about 5% of the total mass (6), hence the differences from Sindbis-RNA in overall specific activity and concentration used for annealing.

We conclude that nucleotide sequences of Sindbis virus and NDV genomes are not complementary, despite apparent complementarity of base compositions. In this case, the complementarity of base compositions must be fortuitous, and not a reflection of an unusual relationship between the genomes and these viruses.

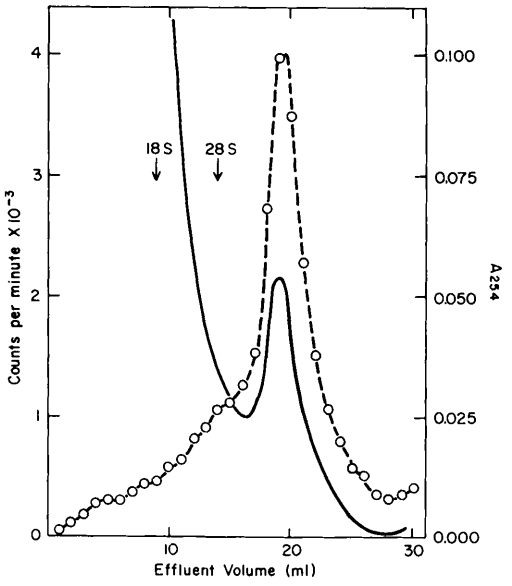

FIG. 1. Isolation of Sindbis-RNA-^{3}H. Viral RNA extracted as described in the text was layered on a 5–20% linear sucrose gradient in 0.005 *M* tris-HCl +0.001 *M* EDTA (pH 7.4) and centrifuged 18 hours at 22,500 rpm at 4° in a Spinco SW 25.1 rotor. The UV absorbance was monitored with a flow-cell and 1-ml fractions were taken. Acid-insoluble radioactivity was determined on 0.03 ml from each fraction. Sedimentation is shown from left to right. Arrows indicate peak positions of ribosomal RNA from chick embryo cells centrifuged at the same time in the same rotor. The large peak of UV absorbance at the top of the tube represents carrier yeast RNA. ——, A_{254}; ○, cpm.

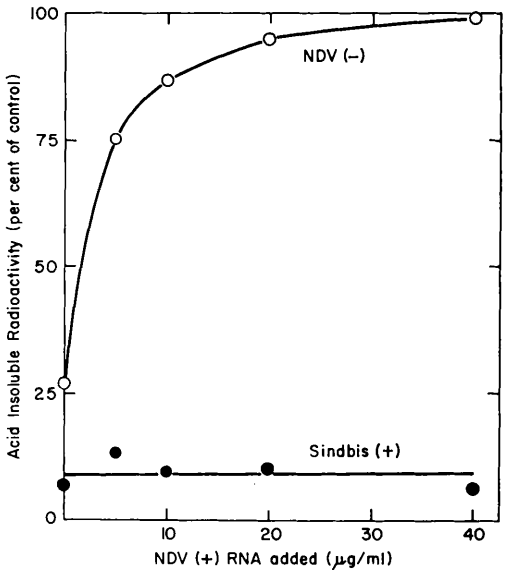

FIG. 2. Annealing of Sindbis (+) RNA-^{3}H and NDV (—) RNA-^{3}H with unlabeled NDV (+) RNA. Various concentrations of NDV (+) RNA, as indicated in the abscissa, were annealed with 200 μg/ml NDV (-) RNA-^{3}H (600 cpm/μg) or 4 μg/ml Sindbis (+) RNA-^{3}H (22,000 cpm/μg). Half of each annealed sample was treated with 10 μg/ml pancreatic ribonuclease "A" for 30 min at 23° and the acid precipitable radioactivity was compared with the other half of the sample, which received no enzyme.

Summary. The RNA isolated from Sindbis virus was annealed with RNA isoated from Newcastle disease virus (NDV). No ribonuclease-resistant hybrid was formed, indicating that these viral RNA's do not have complementary nucleotide sequences, despite complementary base compositions.

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Received October 30, 1967. P.S.E.B.M., 1968, Vol. 127.