nium characteristically contained 6 and lacked 5 proteins and mucoproteins.

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Alteration of Measles Virus Hemagglutinating Properties by Sonication* (32807)

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Hemagglutination titers of unfractionated measles virus pools may be enhanced up to 32-fold by the addition of salts such as $(NH_4)_2SO_4$ to the assay system. The degree of enhancement varies from one preparation to another and appears to be correlated with the presence of measles hemagglutinating particles with different salt requirements for adsorption and hemagglutination (1). One class of particle is dependent upon high concentrations of polyvalent anions for adsorption, and its hemagglutination titers increase with increasing ion concentration. This class is referred to as the salt-dependent agglutinin (SDA) and its physical and hemagglutinating properties have been described (1).

Measles pools also contain variable amounts of hemagglutinating particles which, unlike SDA, adsorb to red blood cells in isotonic saline (2), and which may or may not show enhanced hemagglutination titers following addition of salt to the diluent (1). Collectively, these particles have been well studied (3), although their differential response to salt concentration was unrecognized at the time.

It was the purpose of this study to distinguish between those particles which show enhanced hemagglutination titers in high salt concentrations (S-HA particles) and those which are not enhanced (HA particles). The results of these comparisons plus the finding that HA particles acquire S-HA properties following sonication suggested an interrelationship between particles with different hemagglutinating properties which is discussed. Application of these findings to the preparation of antigens for detection of measles hemagglutination-inhibiting (HI) antibodies is also discussed.

Methods. Preparation of virus pools, reagents, and experimental procedures have been described in detail (1). The S-HA titrations were done in the same way as SDA titrations, i.e., 0.8 *M* $(NH_4)_2SO_4$ in PBS (pH 7.3) was used for diluent except when specific ionic or pH requirements for enhancement were being determined.

Results and Conclusions. The data in Table I show that S-HA hemagglutination reactions are similar to SDA reactions and most differences are quantitative only. One striking difference was demonstrated, however. The S-HA titers increase with increasing Cl^- ion concentration to an optimum at about 1.0 *M* NaCl. On the other hand, SDA was not detectable by hemagglutination at any NaCl concentration tested (0.15–2.0 *M*).

In size and density, S-HA particles as a

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TABLE I. Summary of Properties of Measles Hemagglutinating Particles.

Property	Class of hemagglutinating particle		
	HA	S-HA	SDA
Size	Heterogeneous	Heterogeneous	Average sedimentation coefficient, 22*S*
Buoyant density in CsCl	≦1.16–1.30	≦1.16–1.30	1.29–1.30
Adsorb to erythrocytes in isotonic saline	+	+	—
Adsorption to erythrocytes reversible	—	±	++
Hemagglutination titer increased by:			
SO_4^{2-} concentration[a]	—	+ (0.1 *M*)	+ (>0.2 *M*)
Cl^- concentration[a]	—	+	—
pH (5.6–7.8)	—	+	+
Temperature (4–37°C)	+	++++	++++

[a] Added to PBS.

group are indistinguishable from HA particles. It appears that the two classes may differ in surface properties only—properties which determine salt-enhanceability. This concept is supported by the fact that HA particles acquired S-HA properties after sonication. In the experiment illustrated in Fig. 1, HA titers were increased 6-fold while S-HA titers increased 16-fold. In a subsequent experiment, particles taken from different density levels (1.16–1.26 gm/ml) of CsCl gradient tubes were sonicated and rebanded in CsCl. It was found that increase in S-HA activity was associated with all density levels tested and sonication was not associated with an appreciable alteration in particle-density distribution. Tween–ether extraction (4), a procedure which alters density as well as particle size, in four of five trials did not convert HA particles into S-HA-like particles. In one instance, however, S-HA activity did increase. It is possible, therefore, that salt-enhanceability may simply be a function of viral membrane integrity. That is, envelope-integrated hemagglutinin may be HA in character, hemagglutinin that is not integrated into viral envelope may have SDA properties, and envelope which has been physically modified may have S-HA properties which seem intermediate to those of SDA and HA.

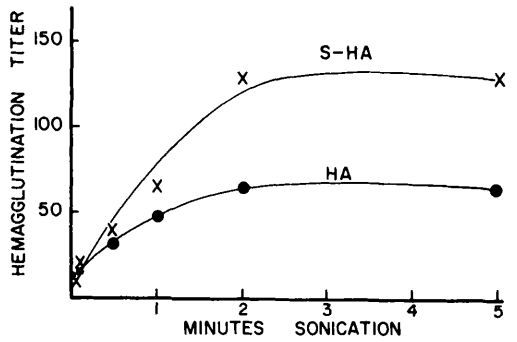

FIG. 1. The HA and S-HA titers of a single pool of measles virus after sonication for various lengths of time. Two-ml virus samples in 13 × 100-mm test tubes immersed in an icebath received 50% of the output intensity from a Bronwill neddle probe.

Salt-enhanceable pools, obtained naturally or following sonication, have been used for detection of measles HI antibodies, incorporating $(NH_4)_2SO_4$ into the system in the same way as for SDA-inhibition tests (1). Although serum titers were from 2- to 4-fold higher than by conventional HI test (5), at no time was a serum negative by HI and positive in $(NH_4)_2SO_4$. Thus, sonication and additional salt offer little advantage in increasing sensitivity of inhibition, but do present advantages in increasing the number of usable antigen pools and in conservation of antigen.

Summary. Significant enhancement of hemagglutination titers of unfractionated measles virus pools by salts such as $(NH_4)_2SO_4$ is reported. Extent of titer increase varies with the ratio of enhanceable hemagglutinins to non-enhanceable hemagglutinins in a preparation. This ratio can be favorably altered by sonication, so that most, if not all, measles pools can be used to advantage in hem-

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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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

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