necrosis which resemble the local Shwartzman phenomenon. An injection of granules into the skin enhances the reactivity of this area to the reversed passive Arthus phenomenon, and makes it possible to elicit this reaction in leucopenic animals. It is suggested that one or more of the acid hydrolases contained in granules may be implicated in the pathogenesis of vascular damage in the Shwartzman and Arthus reactions.

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Respiratory Syncytial Virus: Observations on Antigenic Heterogeneity.* (28880)

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During the spring of 1962, we recovered 24 strains of RS virus from children admitted to hospital during a respiratory illness (1). Several other strains were recovered later in the same year from children in an orphanage. These strains were so poorly neutralized by prototype (Long) antiserum that they would have been missed had this criterion been the sole means of identification.

All RS strains until recently appeared to be close antigenic relatives. Strains recovered in 1962 suggested that antigenic variants serologically distinguishable from the prototype may be encountered during outbreaks involving human beings. In contrast with the 1962 strains, another isolated in 1963 was serologically closely related to "Long." Recently, Coates *et al*(2) reported on antigenic dissimilarities of 2 RS viruses. We are adding another example of antigenic heterogeneity among these viruses.

Materials and methods. Virus stocks. Strains entered in the study are described in another paper(3).

Immune sera. For the prototype strain "Long." Two ml of undiluted virus stock was inoculated intraperitoneally into each of 3 rabbits, 6 times at intervals of 7 days. Animals were bled on the 49th day. A second series of immunizations identical with the first was started 4 to 5 weeks after the first bleeding. Animals were bled 7 weeks afterwards. After the 2nd bleeding 3 "boosters" were given each a week apart. Rabbits were exsanguinated 5½ months after onset of immunization program. *For strain "87."* This virus, our 1962 prototype, was recovered in Kansas City from an 18-month-old infant with laryngotracheobronchitis. Antiserum was

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TABLE I. Antigenic Heterogeneity of RS Viruses, Strains "Long" and "87" with Several Antisera in Cross-Neutralization Tests.

Antigen	Source of sera	Bleeding	SDE's* "Long"	"87"
RS ("Long")	Rabbit No. 6	6/26/62‡	24	8
		10/29/62‖	192	8
	7	7/ 3/62‡	24	8
		9/20/62§	192	12
	8	6/26/62‡	24	8
		9/20/62§	384	8
		10/29/62‖	192	8
	NIH†		256	16
	Guinea pig	6/26/62	64	16
RS ("87")	Monkey No. 150	8/30/62‖	12	64
		12/ 4/62¶	8	64
	151	7/23/62§	12	64

Pre-inoculation sera of animals failed to neutralize RS virus.

* Expressed as reciprocal of serum dilution.

† Received from Dr. Robert Chanock, Nat. Inst. Health, in 1960.

‡ Sera from 1st scheduled immunization.
§ " " 2nd " " .
‖ " " 3rd " " .
¶ " " 4th " " .

prepared in monkeys by 4 bi-weekly intramuscular inoculations of virus mixed with mineral oil adjuvant (4.0 ml containing equal parts of undiluted virus and adjuvant). Monkeys were bled 2 weeks after the 4th inoculation. Later, monkeys received 3 "boosters" 1 to 3 months apart. Two weeks following each booster blood was taken for antibody studies.

Serological methods. Serum neutralization. Cross-neutralization tests with animal sera (inactivated at 56°C/30′) were made using 2-fold dilution steps, followed by addition of about 100 $TCID_{50}$ of virus. After serum-virus mixtures were incubated at 37°C for 1 hour they were transferred to an ice-water bath. Each was inoculated (0.2 ml per culture) into 4 monkey kidney tissue cultures; cultures were rolled in a mechanical "drum" at 36° to 37°C. *Complement fixation (CF).* The method has been described(4).

Results. Identification of 1962 isolates. As noted earlier the 1962 RS strains could not be identified by "Long" prototype antiserum. Antisera for strain "87," with serum dilution endpoint (SDE) of 1:64 (0.1 ml) enabled us to determine the identity of 23 related viruses.

Serological heterogeneity with animal sera. Neutralization tests. Neutralizing antibody, expressed as SDE's (Table I) for "Long" virus, reached maximal levels only after stimulation over a 20-week interval by repeated doses of virus. The conditions were essentially similar in monkeys for strain "87," with the exception that SDE's were not enhanced by repeated stimulation. Earlier studies with "Long" produced very low SDE's in monkeys.

The serologic heterogeneity obtained between "Long" and "87" strains of RS virus is summarized in Table I. The patterns of reactivity differ reciprocally illustrating the 2-way serologic differences of these viruses. While restimulation resulted in at least an 8-fold increase against "Long" corresponding rises were not observed for strain "87." *CF tests.* Antigenic similarities between "Long" and "87" viruses were obtained by CF, indicating that each share a common CF antigen. These observations indicate further that strain "87" is RS virus (Table II).

Serologic homogeneity with human sera. Seventy-four paired sera obtained from infants and children between December, 1961 and June, 1962 were tested by serum neutralization and CF against "Long" and "87" viruses. Eighteen positive pairs are listed in Table III. The over-all results indicate that the serological responses were essentially identical.

However, for infants between 3 and 6 months of age, presumably experiencing primary infections SDE's were always greater for "87" than for "Long" virus. Titer differences however are not significant, and not as striking as those obtained with animal sera. On the other hand, among older infants

TABLE II. Cross CF Tests with "Long" and "87" RS Antigens.

Virus strain	Normal rabbit serum	Rabbit anti-"Long" 9/20	Normal monkey serum	Monkey anti-"87" 9/20
"Long"	<8	512	<8	64
"87"	<8	512	<8	64

TABLE III. Serologic Responses to "Long" and "87" Strain of RS Virus of Infants and Children with Respiratory Tract Infections.

Child code No.	Age (mo)	Onset of illness 1961-1962	Virus isolated	Interval between sera (wk)	Serum tested by Neutralization "Long" A Serum	Neutralization "Long" B Serum	Neutralization "87" A Serum	Neutralization "87" B Serum	Complement fixation "Long" A Serum	Complement fixation "Long" B Serum	Complement fixation "87" A Serum	Complement fixation "87" B Serum
1	2	12/4	None	24	≧64	8	≧64	8	<8	<8	<8	<8
148	3	5/3	RS	1	4	8	12	12	<8	<8	<8	<8
69	3	2/26	RS	1	<8	16	<8	32	<8	<8	<8	<8
91	4	3/11	RS	12	<4	8	<8	16	<8	<8	<8	<8
10	6	12/19	Para 2	6	<8	32	<8	≧64	16	16	32	16
17	6	12/31	Para 3	12	<4	24	<8	48	<8	8	<8	16
140	6½	4/27	RS	1	<4	32	ND	48	ND	16	ND	12
130	10	4/10	*	3	<8	256	<8	64	<8	<8	<8	<8
110	15	3/31	None	10	64	64	48	48	<8	<8	<8	<8
121	16	3/10	Adeno 1	1	≧512	≧512	128	64	≧64	≧64	≧64	≧64
98	18	3/5	RS	2	8	32	ND	ND	<8	48	<8	48
11	24	12/2	None	24	<8	32	<8	≧64	<8	<8	<8	<8
49	24	2/1	Infl B	16	≧512	64	128	32	<8	<8	<8	<8
118	36	4/5	None	10	≧512	≧512	≧512	128	≧64	≧64	≧64	64
22	60	12/29	*	12	<8	<8	<8	<8	AC	≧64	AC	≧48
127	60	4/11	RS	3	≧128	≧128	ND	≧64	ND	<8	ND	ND
26	72	1/21	None	20	96	≧128	≧64	≧64	<8	<8	8	8
37	108	1/27	Infl B	20	32	64	≧64	≧64	<8	<8	<8	<8

A, acute phase serum; B, convalescent serum; ND, not done; AC, anticomplementary; RS, respiratory syncytial virus; para, parainfluenza, type 2, etc.; Infl, influenza virus; Adeno, adenovirus.
* Virus not identified.

and children, who earlier in life may have had experiences with RS virus and currently have been "boostered" by a new infection SDE's were in corresponding measure enhanced for "Long."

Measurements of antibody rises by CF were demonstrable less often than by serum neutralization. Infants less than 4 months of age failed to develop CF antibodies. In others, (Child 1 and 121, Table III) antibodies demonstrated by neutralization but not by CF were very likely derived from the mother, or when demonstrable in high titer by both serologic tests represent recurrent infection with striking "booster" effect.

Discussion. In this report we have presented another example of antigenic heterogeneity among RS viruses. Not all RS viruses will be revealed readily by serum neutralization with prototypic (Long) antisera. Since all strains, including "87" thus far studied have common CF antigens, group relationships can be established.

The data assembled here suggest that subtypes of RS virus are accountable for human infections. A clear declaration, however, cannot yet be made for the existence of different antigenic races of RS virus. The answers obtained with animal antisera suggested existence of antigenic variation, but contrastingly, human sera failed to reveal similar disparity. Human sera may not readily differentiate subgroups. Since from available information infection with RS virus commonly involves young infants, recurrent attacks may except in very young infants obscure any except very major antigenic differences between strains.

We do not have a satisfactory explanation, at least none better than offered by others (2,5), why some strains produce in animals antisera demonstrating antigenic heterogeneity. Tissue cultures may select viruses which in propagation are antigenically different from the viruses propagating in the human host. Coates *et al*(2) described antigenic differences between "Long" and "CH 18537" strains, the latter isolated in Washington, D. C. (1962). In their study ferrets inoculated intranasally were bled for antisera. A common complement-fixing antigen was found between the 2 RS virus strains; but results of their cross neutralization tests differ from ours. Anti-"Long" serum after reinfection of

ferrets not only inhibited strain "Long," but also strain "CH 18537" to about the same titer (1:32 to 1:256). In contrast, we never achieved an increase of the low titer (1:8 to 1:12) to the heterologous virus strain, even after 3 reimmunization cycles.

The antigenic behavior of the RS viruses is in some respects similar to those of the parainfluenza viruses(4). Both parainfluenza type 1, Sendai and HA-2 viruses were substantially neutralized in tissue cultures only by homologous antiserum. Heterologous strains were only slightly neutralized, or not at all. In complement-fixation test, however, Sendai proved to have a broader spectrum than HA-2.

An explanation for this variance has been proposed. Cook *et al*(4) believe that each member of the group possesses a complex of antigens sharing similar chemical configuration, but yet differing in each in a way imparting antigenic specificity. They develop the hypothesis that antigenic fractions shared between virus strains are not equally distributed quantitatively. We can only propose that from time to time a slight change in chemical configuration occurs among RS viruses contributing to different antigenic responses. Further evidence is required to substantiate this proposition.

Summary. Evidence is presented of antigenic heterogeneity between prototype "Long" and "87" strains of RS virus. In cross neutralization tests with animal antisera only the homologous virus strain was inhibited to high titer, while the SDE for the heterologous remained very low, even after 3 or 4 immunization cycles. Human sera failed to reveal disparity. Both strains shared a common CF antigen.

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Comparative Resistance of Vagotomized Monkeys to Intravenous vs. Intragastric Staphylococcal Enterotoxin Challenges.* (28881)

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Vomiting induced in monkeys and cats is the pharmacological response employed in detection of staphylococcal enterotoxin. Parenteral administration of enterotoxin is required with the cat since vomiting is not provoked by feeding of relatively large amounts of enterotoxin. Monkeys, however, are sensitive to intragastrically (i.g.) administered enterotoxin(1).

Studies on the emetic receptor sites for staphylococcal enterotoxin have utilized both animal species. The incidence of vomiting was greatly reduced in the acute, bilaterally vagotomized cats injected intraperitoneally with heated, crude, enterotoxic filtrates, suggesting the existence of receptors in the viscera innervated by the vagus(2). Transthoracic vagotomy of cats did not confer complete protection against intravenous (i.v.) challenge of a highly purified enterotoxic preparation but resulted in a significant elevation of the threshold dose for emesis(3). The data presented suggest that the ED_{50} (50% emetic dose) may have been increased about 5 times.

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