Epidemiologic Investigations of Rhinovirus Infections. (29611)

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The rhinoviruses(1) are a family of viruses which are grouped together because of common properties of RNA type, ether stability, extreme lability of infectivity at pH 3.0, and small size(1-4). These agents are presently believed to be a principal cause for common cold in adults and a frequent cause for acute upper and lower respiratory tract illnesses in children. The rhinovirus group includes viruses previously designated JH-2060(5), ECHO 28(5), coryzavirus(2,6), rhinovirus (3), murivirus(7), ERC (ECHO 28-rhinovirus-coryzavirus group)(2) and enterovirus-like viruses(8). The majority of viruses of the group which propagate in human cells only are designated H strains while the minority which propagate both in monkey renal and human cells are called M strains (9).

During the past several years, our group has pursued an active program aimed at diagnosis and epidemiology of rhinovirus infections, at definition of clinical syndromes in illnesses caused by these agents, and at classification and serologic typing of viruses of the group(2,6,10-14). In a recent report (14), 53 distinct rhinovirus types were defined and designated. Forty serotypes were recognized among 93 rhinovirus strains isolated in this laboratory and 13 types were established among strains recovered by others (5,8,9,15-17). The present report describes the epidemiology of rhinovirus infections in 7 studies carried out among children, college students and adults during 1959 through 1962. Additionally, data relating to persistence of antibody following rhinovirus infection and to homotypic and heterotypic antibody responses are presented.

Materials and methods. Clinical populations. The clinical populations were of 3 sorts: Children of low socioeconomic background and less than 8 years of age seen in the outpatient clinics of the Children's Hospital of Philadelphia (CHOP studies 1 and 2, 1959-60, 1960-61); students of the University of Pennsylvania who appeared at the student health service for medical care (SHS studies 1 and 2, 1960-61, 1961-62); employees of Merck and Company, Rahway, N. J., who presented themselves at the plant dispensary (Rahway studies 1, 2 and 3, 1959-60, 1960-61, and 1961-62). The patients were selected on the basis of having an acute respiratory illness of 4 days or less duration judged on clinical grounds to be of viral etiology. Persons of comparable background but without evident respiratory illness were included for control purpose. All patients studied were entered in the investigation between Oct. 1959 and April 1962. Further information relating to the populations studied may be found in earlier publications(11, 18-20) and details concerning the time of sampling of cases are presented in Table I in this report. Altogether, 1075 respiratory disease cases and 333 control cases were sampled. *Collection of specimens.* A cotton-swab sample of respiratory secretions was collected from the throat at time of first visit. This was extracted into veal infusion broth and stored frozen in dry ice in flame-sealed glass ampoules until tested for presence of virus up to 2 years later. The virus in such samples was found to be quite stable when stored under these conditions. Blood specimens for serologic study were taken at time of initial visit and again 21 days later, although the latter was sometimes delayed owing to failure of patients to return at the designated time. In a selected group of adult patients, late blood specimens were taken 2 to 4 years after the illness had occurred. *Laboratory studies.* The methods used for

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TABLE I. Ratio of Rhinovirus Isolations to Total Number of Respiratory Disease and Control Cases Studied.

	No. positive/total, according to study among respiratory disease cases and controls											
	1959–1960				1960–1961				1961–1962			
	Rahway #1		CHOP #1		Rahway #2		SHS #1		CHOP #2		Rahway #3		SHS #2	
Month	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Aug.									0/1	0/1				
Sept.									0/22	2/7	0/5		0/13	0/5
Oct.			1/11	0/3			2/14		4/30	0/9	1/16	0/2	2/19	0/7
Nov.	8/40		0/18	0/6	2/15		5/81	0/29	2/29	0/8	0/14	0/5	2/18	0/6
Dec.	7/32	0/10	3/15	0/12	1/16		0/19	0/5	0/19	0/7	0/13	0/3	6/11	0/5
Jan.	1/30	1/21	0/57	0/13	0/15	0/5	1/18	0/2	0/25	0/3	0/3	0/3	2/16	0/5
Feb.	1/21	0/12	1/57	1/22	1/15	0/5	2/19	0/5	3/29	0/7	0/3		1/15	0/5
March	2/10	0/3	3/44	0/13	4/13	0/5	0/16	1/5	1/20	0/7	2/5	0/3	1/8	0/5
April	0/6	0/2	4/41	0/6	2/5	0/6	2/16	0/5	2/28	0/11	0/3		1/3	0/3
May			3/20	0/6		0/1	0/1		1/24	1/9				
June									0/18	0/5				
Totals	19/139	1/48	15/263	1/81	10/79	0/22	12/184	1/51	13/245	3/74	3/62	0/16	15/103	0/41
% Positive	14	2	6	1	13	0	7	2	5	4	5	0	15	0

Rahway (Merck and Co. dispensary); CHOP (Children's Hospital of Philadelphia); SHS (Student Health Service, Univ. of Pennsylvania).

virus isolation, for rhinovirus group designation, for serologic typing and for all other laboratory technology have been described (2,14).

Results. Rhinovirus isolations. Tables I and II summarize the recovery of 93 rhinoviruses from 1075 respiratory disease cases and 333 nonrespiratory control cases entered into the study during 1959-1962. The cases in CHOP study 1 and in Rahway study 1 had all been tested previously for influenza, parainfluenza, reovirus, adenovirus and respiratory syncytial virus infections. The specimens tested for rhinovirus were selected from among those which presented negative evidence for infection with these other agents. The specimens from the other studies were not so selected. Among the total group of specimens tested, 10.4% of cases of respiratory disease in adults and 5.5% of cases in children yielded virus. The occurrence of virus in control cases represented subclinical infection among these persons and amounted to only 2.6% in children and 1.1% in adults. The percentage of cases which yielded virus in any month was quite variable and ranged from 0 to 54%. In the overall, the percentage of specimens which yielded rhinovirus should have been less than was found had the cases for rhinovirus study been chosen entirely at random.

Distribution of serotypes. Fig. 1 and Table III, in composite, show the distribution of rhinovirus serotypes according to time and study group among the persons studied. It is clear that multiple serotypes were circulating simultaneously during the majority of the time periods. However, occurrence of a particular type tended to be limited in time. By way of example, type 11 was common during Nov.-Dec. 1959, type 32 during Feb.-March 1961, type 42 during March-May 1961, and type 12 was recovered every month from Oct. 1960 through Feb. 1961. Types 11, 13-19, 21, 23-26, and 28-30 occurred exclusively during the respiratory season of 1959 and 1960; types 4, 6, 8, 9, 12, 33, 38, 39, 41, 42, 47, 50, and 53 exclusively during 1960-61; and types 34-37, 40, 45, 46, 48, 49, and 51 exclusively, during 1961-62.

TABLE II. Summary, Rhinovirus Isolations Among Children and Adults in Respiratory Disease Cases and Controls.

Group	Respiratory disease cases: Total No. tested	Respiratory disease cases: Positive No.	Respiratory disease cases: Positive %	Controls: Total No. tested	Controls: Positive No.	Controls: Positive %
Child	508	28	5.5	155	4	2.6
Adult	567	59	10.4	178	2	1.1
Totals	1075	87	8.1	333	6	1.8

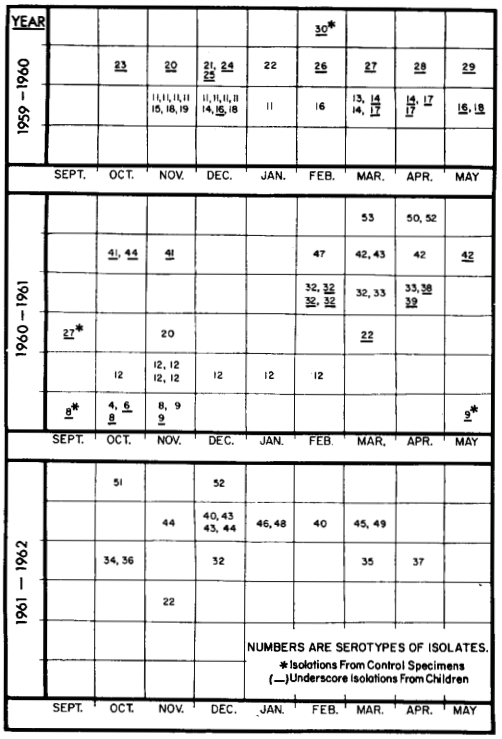

FIG. 1. Distribution, 93 rhinovirus isolates, recovered from October 1959 through April 1962.

Only types 20, 22, 27, 32, 43, 44, and 52 were recovered in more than 1 season. Types 6, 17, 23-30, 38, 39, and 41 were found exclusively in cases in children.

Clinical diagnoses among rhinovirus infections. Table IV shows that 58 (95%) of 61 infections among adults were diagnosed as upper respiratory illness consistent with common cold; one patient exhibited signs of bronchitis, and there was no clinically apparent disease among 2 persons who harbored the virus. Among 32 children, 14 displayed mild upper respiratory illness and in 4 persons there were no symptoms. In contrast to adults, 14 (44%) of the children showed moderately severe disease with lower respiratory tract involvement diagnosed as croup, bronchitis, bronchiolitis, or bronchopneumonia.

Persistence of neutralizing antibody among human beings. Seventeen adult patients with rhinovirus infection who showed antibody increase in convalescence and 5 adults who showed no such response were bled again 2 to 4 years later. The acute, convalescent, and late serum samples were titrated for neutralizing antibody titer employing the homologous virus isolate. None of the 5 persons who failed to respond initially developed antibody during the following 33 to 50 months (Table V). The antibody which developed among the 17 persons who responded initially was remarkably persistent. All persons retained their antibody for 2 to 4 years with only 2- to 16-fold decline in titer. The median decline was 4-fold and the average decline was 5-fold.

Homotypic and heterotypic antibody increases in rhinovirus infection. Nearly all persons with rhinovirus infection respond during convalescence with a neutralizing antibody increase. In a previous report(14) it was recorded that all but one of 51 respiratory disease cases for which paired sera were available showed a significant increase in homologous antibody level during convalescence (3 weeks). Table VI summarizes the homologous and heterologous antibody responses among 18 paired sera from cases tested with the corresponding viruses. Types 8, 12, 22, 32, 33, and 41 were each represented by 2 examples and prototype strain 2060 (type 1) was also included. All paired sera showed a significant increase in antibody against homologous virus and against a second strain of the same homotype, when repre-

sented. Heterotypic antibody responses were rare and occurred only in one patient (case 1665). Antibody against rhinoviruses, in general, was infrequent among the sera from children less than 5 years of age. When present, it was found mainly in the sera of 1.5 and 2 month old infants and this was likely of transplacental origin. By contrast, 9 sera from adults 18 years or older each displayed antibody in the acute specimen against an average of 5 heterologous serotypes. Heterologous antibody was displayed most frequently against types 1, 6, 12, 22, 32 and 46 rhinovirus.

Discussion. The definition of the group characteristics and establishment of serotypes

TABLE III. Distribution, According to Study, of 93 Rhinovirus Strains Representing 46 Serotypes Isolated from October 1959 Through April 1962.

Rhinovirus type	No. of isolations							
	1959–1960		1960–1961			1961–1962		
	Rah. #1	CHOP #1	Rah. #2	SHS #1	CHOP #2	Rah. #3	SHS #2	Totals
4				1				1
6					1			1
8				1	2			3
9				1	2			3
11	9							9
12			2	6				8
13	1							1
14	2	2						4
15	1							1
16	1	2						3
17		3						3
18	2	1						3
19	1							1
20*	1		1					2
21	1							1
22*	1				1		1	3
23		1						1
24		1						1
25		1						1
26		1						1
27*		1			1			2
28		1						1
29		1						1
30		1						1
32*				2	3		1	6
33			1	1				2
34						1		1
35						1		1
36							1	1
37							1	1
38					1			1
39					1			1
40							2	2
41					2			2
42			2		1			3
43*			1				2	3
44*					1		2	3
45						1		1
46							1	1
47			1					1
48							1	1
49							1	1
50			1					1
51							1	1
52*				1			1	2
53			1					1
Total	20	16	10	13	16	3	15	93

* These types were isolated in 2 or 3 successive respiratory disease seasons, all others were recovered in only a single season.

TABLE IV. Clinical Diagnoses Among 93 Rhinovirus Infections.

Diagnosis	No. of cases	
	Adults (61)	Children (32)
	%	%
Upper respiratory illness	58 (95)	14 (44)
Croup	—	1 (3)
Bronchitis	1 (2)	7 (23)
Bronchiolitis	—	3 (9)
Bronchopneumonia	—	3 (9)
No illness	2 (3)	4 (12)

among the rhinoviruses(2,14) made possible meaningful epidemiologic investigations of infections with these agents in child and adult populations. The data obtained in studies of 7 population groups during 3 successive respiratory disease seasons permit certain generalizations concerning the probable epidemiology of these infections.

It seems likely, based on the information, that a multiplicity of different serotypes may circulate through the population at any one time. Further, these serotypes tend to disappear and not to be represented during successive respiratory disease seasons, although a few exceptions have been noted. The data are presently insufficient to determine whether children are infected principally with a type of virus different from that of adults.

Essentially, every patient responded to rhinovirus infection with the development of homotypic antibody within 3 weeks of onset of illness, often to very high titer. Such antibody, as shown previously(17), appeared to persist for long periods although some of the patients might have been reinfected during the interim period between serum samples. Except for very young infants with maternal antibody, most children were relatively free of rhinovirus antibody. Adults, by contrast, commonly exhibited antibody against several serotypes. The extreme specificity of serologic response indicates that such individuals had prior experience with those serotypes, specifically, which were represented by antibody.

The antibody which appeared during early convalescence seemed sufficient to engender immunity based on analogy with findings in other virus families. Studies of rhinovirus infections in volunteers carried out by other workers(21-23, cf. 24) suggest, however, that additional factors such as interferon or local

TABLE V. Persistence of Neutralizing Antibody in Sera of Human Adults Following Natural Infection.

Rhinovirus type	Case and strain No.	Reciprocal of neutralizing antibody titer: Acute	Convalescent (3 wk)	Late: Time (mo)	Late: Titer	Fold decrease in titer
35	611	<2.5	20	23	10	2×
45	605	"	"	"	"	"
42	474	"	80	33	20	4×
"	457	"	40	34	5	8×
33	465M	"	"	35	"	"
43	463	"	20	"	<2.5*	"
12	MRH	"	"	37	5	4×
20	225	"	"	38	"	"
13	211	"	10	46	"	2×
14	204	"	40	"	20	"
11	68	"	"	49	"	"
"	89	"	20	"	5	4×
12	237	"	"	50	2.5	8×
14	43	"	80	"	20	4×
19	15	"	320	"	"	16×
21	47	"	20	"	5	4×
11	54	"	"	"	"	"
50	477	<2.5	<5*	33	<2.5*	
16	181	"	<5	47	<2.5	
18	93	"	"	49	"	
11	23	"	"	50	"	
"	27	"	"	"	"	

* Partial neutralization.

TABLE VI. Cross-Neutralization Tests with Selected Rhinoviruses and Paired Sera from Children and Adults.

Age of patient	Paired sera: Rhinovirus type	Paired sera: Sample	Reciprocal of neutralizing antibody titer obtained in tests with virus type and (strain): Type 1 (2060)	41 (6360)	44 (6258)	8 (6167)	6 (6211M)	32 (6526M)	41 (6244)	30 (5582M)	8 (6268)	22 (6554)	12 (1444)	32 (1539M)	46 (1979M)	37 (2268)	40 (1963M)	12 (1209)	22 (1924)	33 (1665M)	33 (465M)
1.5 mo	41	Acute	40	20	0	0	40	0	ND	0	0	0	20	0	0	0	0	10	0	0	0
		Conv	40	160	0	0	40	0	20	0	0	0	20	0	0	0	0	10	0	0	0
2 "	44	A	0	0	0	0	0	20	0	0	0	0	80	20	40	10	0	40	0	0	0
		C	0	0	80	0	0	20	0	0	0	0	40	20	40	10	0	40	0	0	0
4 "	8	A	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
	(6167)	C	0	0	0	160	0	0	0	20	40	0	0	0	0	0	0	0	0	0	0
1 yr	6	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		C	0	0	0	0	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 "	32	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(6526M)	C	0	0	0	0	0	160	0	0	0	0	0	160	0	0	0	0	0	0	0
1.5 "	41	A	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0
		C	0	40	0	0	0	0	40	80	0	0	0	0	0	0	0	0	0	0	0
2 "	30	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		C	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
3 "	8	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(6268)	C	0	0	0	20	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0
4 "	22	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		C	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0	20	0	0
18 yr	12	A	40	0	0	0	40	10	0	0	0	0	0	10	80	0	20	0	0	0	0
	(1444)	C	40	0	0	0	40	10	0	0	0	0	160	10	80	0	20	160	0	0	0
18 "	32	A	0	0	0	0	20	0	0	0	0	0	10	0	5	5	0	10	0	0	0
	(1539M)	C	0	0	0	0	40	160	0	0	0	0	5	160	10	10	0	10	0	0	0
19 "	46	A	5	0	0	0	20	20	0	0	0	160	0	10	0	160	0	0	160	0	0
		C	5	0	0	0	20	20	0	0	0	160	0	5	20	160	0	0	160	0	0
19 "	37	A	0	0	0	0	40	0	0	0	0	0	5	0	40	0	5	0	0	0	0
		C	0	0	0	0	80	0	0	0	0	0	10	0	40	640	5	0	0	0	0
20 "	40	A	160	5	0	0	40	20	5	0	0	40	10	10	0	5	0	10	80	80	40
		C	160	5	0	0	40	20	5	0	0	40	20	10	0	5	40	20	160	40	20

Age of patient	Paired sera: Rhinovirus type	Paired sera: Sample	Reciprocal of neutralizing antibody titer obtained in tests with virus type and (strain): Type 1 (2060)	41 (6360)	44 (6258)	8 (6167)	6 (6211M)	32 (6526M)	41 (6244)	30 (5582M)	8 (6268)	22 (6554)	12 (1444)	32 (1539M)	46 (1979M)	37 (2268)	40 (1963M)	12 (1209)	22 (1924)	33 (1665M)	33 (465M)
21 "	12	A	0	10	0	0	5	0	10	0	0	5	0	0	80	0	0	0	5	20	20
	(1209)	C	0	10	0	0	10	0	10	0	0	5	160	0	80	0	0	160	5	20	20
21 "	22	A	5	0	0	0	0	80	0	0	0	0	20	40	5	0	0	40	0	0	0
		C	5	0	0	0	0	80	0	0	0	20	40	40	5	0	0	40	40	0	0
22 "	33	A	40	0	0	0	320	40*	0	0	0	20	0	40*	40*	0	80	0	40	0	0
	(1665M)	C	40	0	0	0	320	160	0	0	0	40	0	160	320	0	80	0	40	20	20
34 "	33	A	10	0	0	40	40	80	0	0	40	20	0	40	20	0	0	10	40	0	0
	(465M)	C	20	0	0	40	80	40	0	0	80	40	0	40	40	0	0	10	40	40	40

Least dilution of acute specimens was 1:5 and of convalescent, 1:10 unless otherwise indicated. Single underscore points up homologous virus and paired sera. Double underscore indicates results of tests of paired sera using a heterologous virus strain of identical serologic type. * Asterisk highlights heterotypic antibody increases of case 1665 to heterologous types 32 and 46 as well as to homologous type 33. Note: Types 8, 12, 22, 32, 33 and 41 are each represented twice.

tissue resistance may play a role in resistance to reinfection with the same virus in the early convalescent period.

The extreme multiplicity and constant changing of serotypes in the human population suffices to explain the enigma of the recurrent common cold without need to refer to defective immunologic mechanism in protection against reinfection with the same virus. The diversity of antigenic types among the relatively small number of virus isolates to date suggests that the total number of types may number into the hundreds or even thousands. The possibility of continuing development of new serotypes must also be considered. Little hope is held for the development of a practicable vaccine containing all the types(25,26). It is clear that vaccine development must await further clarification of the epidemiology and must rest on future guidelines.

The proportion of cases of common cold which yield a rhinovirus are generally proportionally small as shown in the present study and in other investigations(11,14,27-29). This suggests that the virus isolation method used for diagnosis might be very insensitive. Alternatively, the majority of cases of such illness may be caused by viruses of an as yet undisclosed biological nature, possibly embracing a family or families of viruses still to be discovered.

As shown in our previous studies(2,10,14) and confirmed by others(30), rhinoviruses may be associated with moderately severe lower respiratory tract infection as well as mild upper respiratory illness. The greater severity of respiratory illness in children compared with adults is not unique to rhinovirus infections. About half of the infections observed in children in the present study showed lower respiratory tract involvement and included the syndromes of croup, bronchitis, bronchiolitis and bronchopneumonia.

Summary. The epidemiology in 93 proved rhinovirus infections caused by 46 different serotypes among 7 different child or adult population groups studied during 3 successive respiratory disease seasons is described. Multiple serotypes were found to circulate

simultaneously in the populations and serotypes tended to disappear and not to be represented in successive seasons. Essentially all patients with rhinovirus infection responded serologically. This response was extremely homotypic and appeared to persist, though in diminished amount, for at least 4 years. The low proportion of cases of common cold yielding a rhinovirus suggests either that the diagnostic efficiency of the virus isolation method is poor or that as yet undiscovered viruses may be responsible for the marjority of such illnesses or perhaps both situations prevail. About ½ of children with rhinovirus infection in the present study displayed lower respiratory tract involvement and their illness was diagnosed as croup, bronchitis, bronchiolitis or bronchopneumonia. Little hope is held for development of rhinovirus vaccines containing a sufficient spectrum of serotypes; vaccine development must await clarification of epidemiology and must rest on future guidelines.

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1. Tyrrell, D. A., Chanock, R. M., *Science,* 1963, v141, 152.

2. Ketler, A., Hamparian, V. V., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 821.

3. Andrewes, C. H., *Yale J. Biol. and Med.,* 1961-62, v34, 200.

4. Dimmock, N. J., Tyrrell, D. A. J., *Lancet,* 1962, v2, 536.

5. Pelon, W., *Am. J. Hyg.,* 1961, v73, 36.

6. Hamparian, V. V., Ketler, A., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 444.

7. Mogabgab, W. J., *Am. Rev. Resp. Dis.,* 1963, v88, 246.

8. Johnson, K. M., Rosen, L., *Am. J. Hyg.,* 1963, v77, 15.

9. Taylor-Robinson, D., Tyrrell, D. A. J., *Lancet,* 1962, v1, 452.

10. Reilly, C. M., Hoch, S. M., Stokes, J., Jr., McClelland, L., Hamparian, V. V., Ketler, A., Hilleman, M. R., *Ann. Int. Med.,* 1962, v57, 515.

11. Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., McClelland, L., Cornfeld, D., Stokes, J., Jr., *J. Am. Med. Assn.,* 1962, v180, 445.

12. Hamparian, V. V., Ketler, A., Hilleman, M. R., *Am. Rev. Resp. Dis.,* 1963, v88, 269.

13. Hilleman, M. R., Reilly, C. M., Stokes, J., Jr., Hamparian, V. V., *ibid.,* 1963, v88, 274.

14. Hamparian, V. V., Leagus, M. B., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1964, v116, 976.

15. Mogabgab, W. J., *Am. J. Hyg.,* 1962, v76, 15.

16. Price, W. H., Emerson, H., Ibler, I., Lachaine, R., Terrell, A., *ibid.,* 1959, v69, 224.

17. Connelly, A. P., Jr., Hamre, D., *J. Lab. and Clin. Med.,* 1964, v63, 30.

18. Hamparian, V. V., Ketler, A., Hilleman, M. R., Reilly, C. M., McClelland, L., Cornfeld, D., Stokes, J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 717.

19. McClelland, L., Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., Cornfeld, D., Stokes, J., Jr., *New Engl. J. Med.,* 1961, v264, 1169.

20. Reilly, C. M., Stokes, J., Jr., McClelland, L., Cornfeld, D., Hamparian, V. V., Ketler, A., Hilleman, M. R., *ibid.,* 1961, 264, 1176.

21. Anderson, T. O., Riff, L. J. M., Jackson, G. G., *J. Immunol.,* 1962, v89, 691.

22. Jackson, G. G., Dowling, H. F., Akers, L. W., Muldoon, R. L., Van Dyke, A., Johnson, G. C., *New Engl. J. Med.,* 1962, v266, 791.

23. Cate, T. R., Couch, R. B., Johnson, K. M., *J. Clin. Invest.,* 1964, v43, 56.

24. Mufson, M. A., Ludwig, W. M., James, H. D., Jr., Gauld, L. W., Rourke, J. A., Holper, J. C., Chanock, R. M., *J. Am. Med. Assn.,* 1963, v186, 578.

25. Hilleman, M. R., *Am. Rev. Resp. Dis.,* 1963, v87, 165.

26. ———, *Industry and Tropical Health: V,* Industrial Council for Tropical Health, Harvard School of Public Health, Boston, Mass., 1964, 81.

27. Kendall, E. J. C., Bynoe, M. L., Tyrrell, D. A. J., *Brit. Med. J.,* 1962, v2, 82.

28. Bloom, H. H., Forsyth, B. R., Johnson, K. M., Chanock, R. M., *J. Am. Med. Assn.,* 1963, v186, 38.

29. Hamre, D., Procknow, J. J., *Brit. Med. J.,* 1961, v2, 1382.

30. Taylor-Robinson, D., Johnson, K. M., Bloom, H. H., Parrott, R. H., Mufson, M. A., Chanock, R. M., *Am. J. Hyg.,* 1963, v78, 285.

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