IV. RESPIRATORY SYNCYTIAL VIRUS

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FOR many years pneumonia and bronchiolitis of infants and small children have been an important public health problem which has far exceeded that of poliomyelitis and many other infectious diseases in mortality. Until recently there has been a relative lack of public health interest in lower respiratory tract disease of the young, due in large part to a limited understanding of its etiology. In the past ten years, advances in laboratory methodology have made possible the recovery and classification of a large number of heretofore unrecognized nonbacterial agents from the respiratory tract. Recent controlled epidemiologic studies indicate that members of at least four different groups of these agents are associated with severe respiratory disease of early life. The present report will summarize our knowledge of the respiratory syncytial (RS) virus, which appears to be the most important single cause of such illness.

History and Properties

RS virus was first recovered in 1956 by Dr. J. A. Morris from a chimpanzee during an outbreak of coryza in a colony of these animals.[1] In 1956 we recovered the agent from an infant with pneumonia and from another infant with croup in Baltimore, Md.[2,3] Subsequently the virus has been recovered every year from infants and small children with pneumonia or bronchiolitis in the Maryland-District of Columbia area. Isolation of the agent has been reported from similar illnesses in Illinois during 1958-1959, New York 1959, Pennsylvania 1959-1960, California 1961, England 1961, and Australia 1961.[4-9]

As shown in Table 1 the virus is medium sized and sensitive to inactivation by ether.[2] Infection of human cells in tissue culture results in the formation of syncytia or pseudo giant cells and it is this property for which the agent is named.[2] Little is known regarding the antigens of the virus other than that the antigen reactive in the complement-fixation reaction is smaller than the viral particle and easily separable from it by centrifugation.[2] The virus is not related antigenically to any of the other agents that produce syncytial changes and at the present time it is not possible to classify RS virus in any established viral group. Although the virus has not been shown to grow in eggs or to hemagglutinate, its other properties, plus the development of eosinophilic cytoplasmic inclusions, suggest that RS virus may be an aberrant myxovirus.[10]

The virus as it exists in human respiratory secretions is extremely labile, being easily inactivated by a single cycle of freezing and thawing. Beem and associates showed that throat swab specimens must be tested immediately, without prior freezing, in order to achieve optimum isolation efficiency.[4]

Association of RS Virus with Childhood Respiratory Disease

Although the virus has been known since 1956, conclusive evidence that it causes human respiratory illness has been obtained only recently.[4,11-13] The original Baltimore study suggested that

Table 1—Properties of Respiratory Syncytial Virus

1. Growth in (a) continuous human and (b) primary human and monkey cell cultures. The former more sensitive for virus isolation.
2. Characteristic cytopathic effect in tissue culture is formation of syncytium or "Pseudo Giant Cell" in which eosinophilic cytoplasmic inclusions are prominent.
3. Infectious virus first detected 10 hours after inoculation of HEP-2 tissue culture cells.
4. Specific antigen, first detected by fluorescent antibody technic 10 hours after inoculation of HEP-2 cells, restricted to cytoplasm throughout growth cycle.
5. Virus size of 90-120 mμ.
6. Complement-fixing antigen smaller than virus particle and separable from it by centrifugation.
7. Inactivated by 20% ether.
8. Does not grow in eggs.
9. Hemagglutination not demonstrable.
10. Not pathogenic for mouse, guinea pig, or rabbit.

RS infection was associated with pneumonia in pediatric outpatients, but the association was considered tentative in view of the small sample size.[3]

Evidence for the etiologic association of RS virus and respiratory illness is presented in Table 2, which summarizes the isolation experience at Children's Hospital of D. C. over a two-year period. The virus was recovered significantly more often from infants and children with respiratory illness than from controls free of such disease. The most striking association of RS infection with illness was observed in young infants with bronchiolitis or pneumonia where the virus recovery rates were 39 and 23 per cent, respectively. It appeared that infection in older infants and children tended to result in milder illness than that which occurred in the first half year of life. Upper respiratory tract disease associated with RS infection was not benign, however, since in 95 per cent of instances a temperature elevation above 100° F (rectal) was noted.[11] The over-all virus recovery rate from bronchiolitis suggests that RS virus is one of the major causes of this serious illness. It would appear that the agent is also an important cause of infantile and childhood pneumonia.

Additional evidence associating RS virus with lower respiratory tract illness was provided during an outbreak of infection in a Washington, D. C., welfare nursery.[14] Serologic tests indicated that 91 per cent of the 90 infants and children under four years of age experienced RS infection in April and May of 1960. The outbreak was fairly explosive, only 19 days elapsing between the first and last pneumonia illness. Although laboratory studies were not started until the outbreak was half over, 24 strains of RS virus were recovered. A total of 40 per cent of the nursery inhabitants developed a febrile pneumonia and an analyses of the virus recovery data indicated that RS virus was etiologically related to such illness. These findings define, in a dramatic fashion, the risk of lower respiratory tract involvement during infection of the young.

Serologic Response to Infection

The serologic response of infants less than seven months of age is relatively poor as shown in Table 3. Approximately one fifth developed a CF antibody rise and slightly less than one half showed an increment in neutralizing antibody during convalescence.[12] Infants over six months of age responded more often with an antibody rise. Beem and associates reported similar findings for the neutralizing antibody response in infants; these workers, however,

Table 2—Recovery of RS Virus from Infants and Children with Respiratory Disease, June, 1959-July, 1961

Group	Age in Months											
	0–6		7–12		13–36		37–60		>60		Total	
	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.
Bronchiolitis	79	39	36	11	33	12	4	–	2	–	154	25
Pneumonia	74	23	52	4	85	5	35	8	32	3	278	10
Croup	12	8	19	5	62	2	12	–	10	–	115	3
Bronchitis	84	4	50	2	153	1	63	3	78	2	428	2
URI	262	10	254	7	534	5	261	2	145	1	1,456	5
No resp. disease	431	–	215	0.5	411	0.5	221	–	90	1	1,368	0.3

Table 3—Serologic Response of Infants and Children from Whom RS Virus Recovered, March, 1960-July, 1961

Age in Months	Fourfold or Greater Antibody Rise					
	Complement Fixation			Neutralization		
	Number Tested	Positive		Number Tested	Positive	
		No.	%		No.	%
0-6	47	10	21	22	10	45
>6	23	17	74	14	12	86
Total	70	27	38	36	22	61

Adapted with additional data from Parrott, et al.[12]

noted a somewhat greater proportion of CF antibody rises than are shown in Table 3.[4] With the information shown in Table 3 it is then possible to interpret the serologic findings obtained with RS virus over a four-year period in the Washington, D. C., childhood population.

As shown in Table 4 CF antibody rises were most frequently observed in infants or children with bronchiolitis, a finding which is in accord with the virus recovery data. If the known insensitivity of the CF test is taken into account it is probable that the proportion of individuals with bronchiolitis infected with RS virus was closer to 36 per cent. Similarly, the values observed for pneumonia, bronchitis, etc., are probably closer to 22 per cent than to the percentages shown in Table 4. Over-all it can be estimated that 23 per cent of respiratory tract illnesses requiring hospitalization were associated with RS infection over a four-year period.

Proportion of Respiratory Illness Associated with RS Virus in Other Studies

Virus isolation and serologic data from the studies of Beem, et al., and McClelland, et al., are compared with our findings in Table 5.[4,13] It is clear that RS virus was prominently associated with bronchiolitis, pneumonia, and other febrile respiratory illness in Chicago during 1958-1959 and in Philadelphia during 1959-1960.

Temporal Occurrence of Infection and Its Relation to Respiratory Disease Morbidity

RS virus appears to become disseminated extensively in the pediatric population every year. It has been active in the Washington-Baltimore area during each of the past five years, although the months when it was prevalent varied from year to year. The temporal pattern of infection during the past four years in Washington, D. C., is shown in Figure 1. Yearly dissemination of infection was primarily limited to circumscribed intervals of from three to five months. Each of these periods coincided with the peak occurrence of bronchiolitis. Prevalence of pneumonia also correlated with the occurrence of RS infection except during October-November, 1957, when influenza A2 was epidemic. These findings further support the contention that RS virus is a prominent cause of lower respiratory tract illness in the young. It is of interest that the 1958-1959 Chicago outbreak and the 1959-1960 Philadelphia outbreak occurred at the same time that RS virus was prevalent in Washington, D. C.

The circumscribed yearly outbreaks of RS infection differ from the pattern of influenza A or B infection which characteristically recur at two- to three-year intervals. The epidemiology of RS infection also differs from that of parainfluenza 1 and 3 viruses which are easily detectable in the community during almost every month of the year.

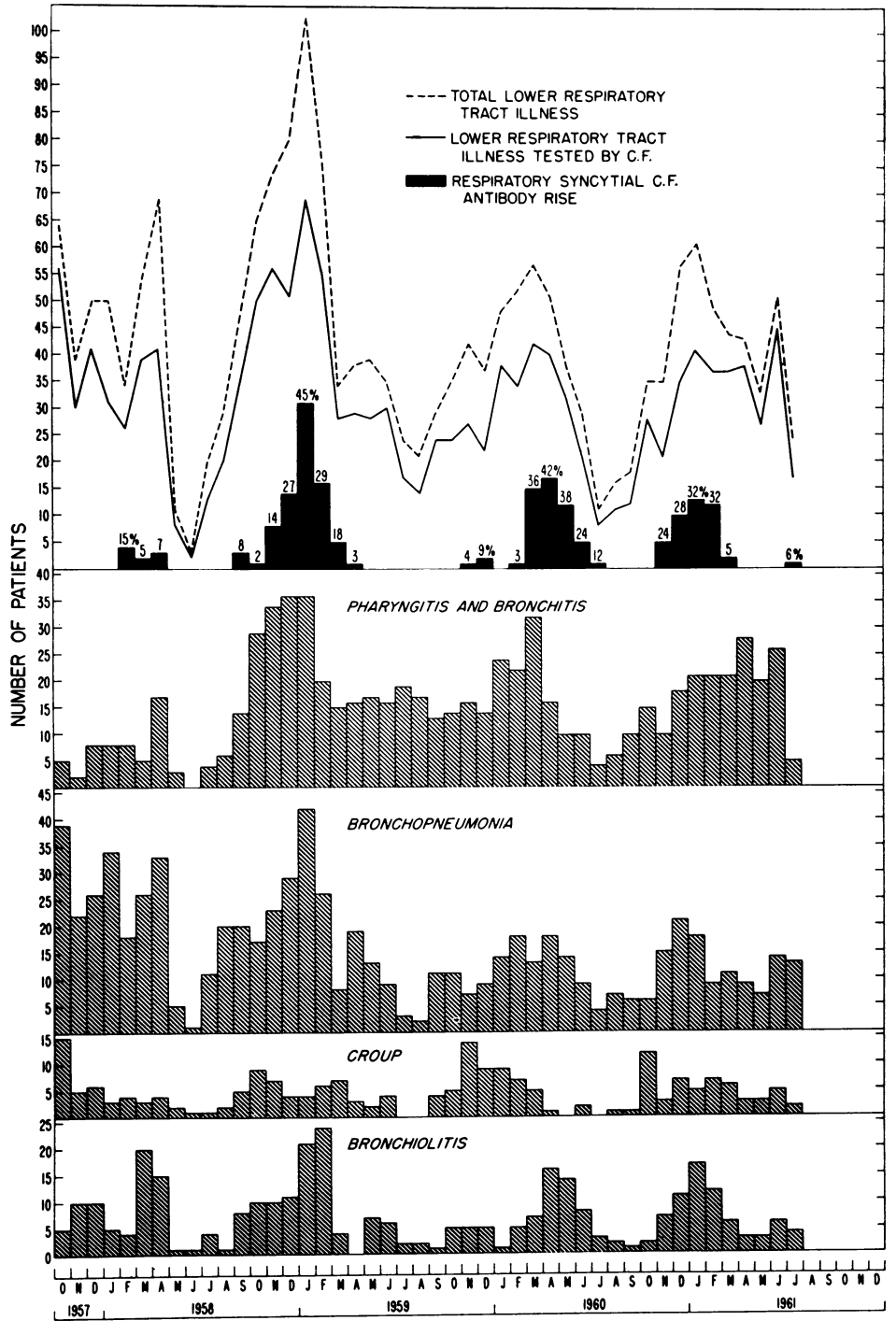
Adult Infection and the Concept of Reinfection

Although the role of RS virus in childhood respiratory disease is fairly

Table 4—Serologic Evidence of RS Infection in Infants and Children Admitted to Hospital with Respiratory Disease, Oct., 1957-July, 1961

	Age in Months											
	0–6		7–12		13–24		25–48		>48		Total	
Group	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.
Bronchiolitis	112	19	52	23	45	16	18	6	3	–	230	18
Pneumonia	114	12	102	18	129	13	78	18	99	10	522	14
Croup	17	–	31	3	52	8	32	3	24	12	156	6
Bronchitis	87	10	61	16	118	10	125	17	131	8	522	12
Total resp.	330	13	246	17	344	12	253	15	257	9	1,430	13
No resp. disease	78	1	48	2	129	3	196	4	237	3	688	3

Figure 1—Temporal Relationship of Respiratory Syncytial Virus Infection to Total Respiratory Tract Illness



Reprinted from Parrott, et al.,[12] with additional data for October, 1960-July, 1961.

well understood, little is known regarding the consequences of infection in the adult. Experimentally it has been possible to infect male volunteers with second monkey kidney passage RS virus.[15,16] Of 41 men given 160 to 640 TCD_{50}, 33 were infected and 20 developed an afebrile upper respiratory illness which lasted an average of five and a half days. An association between virus challenge and subsequent illness was supported by the finding that illness never preceded the time of initial virus isolation. Also, as shown in Table 6, the occurrence of illness correlated well with the extent of infection; volunteers who became ill generally shed virus for a longer period and were more likely to develop a rise in CF or neutralizing antibody than individuals who did not become ill.

RS virus infection in the volunteers represented reinfection as all the men had moderate to high levels of neutralizing antibody prior to challenge. It is probable that such antibody was responsible for the mild nature of the observed illnesses. Neutralizing antibody, however, may be only one of several factors which determine the nature of illness following RS infection. Thus, a total of 14 of the 21 infants and children over six months of age with bronchiolitis or pneumonia possessed neutralizing activity in their acute phase serum in the Chicago and Washington studies.[4,11] Similarly, 20 of the 36 children who developed pneumonia in the

Table 5—Proportion of Respiratory Disease of Infancy and Childhood Associated with RS Infection in Various Studies

		Virus Recovery			Serologic Evidence of Infection		
Diagnosis	Location	Time	No. Tested	% Positive	Time	No. Tested	% CF Antibody Rise
(1) Bronchiolitis	Wash.,[1] D.C.	Jun., '59-Jul., '61	154	25	Oct., '57-Jul., '61	230	18
	Chicago,[2] Ill.	Nov., '58-Jun., '59	34	50	—*	—	—
	Phila.,[3] Pa.	—*	—	—	Oct., '59-Jun., '60	26	38
(2) Pneumonia	Wash.,[1] D.C.	Jun., '59-Jul., '61	278	10	Oct., '57-Jul., '61	522	14
	Chicago,[2] Ill.	Nov., '58-Jun., '59	22	36	—*	—	—
	Phila.,[3] Pa.	—*	—	—	Oct., '59-Jun., '60	56	39
(3) Other acute respiratory disease—predominantly febrile	Wash.,[1] D.C.	Jun., '59-Jul., '61	1,999	4.4	Oct., '57-Jul., '61	678	10
	Chicago,[2] Ill.	Nov., '58-Jun., '59	101	15	—*	—	—
	Phila.,[3] Pa.	—*	—	—	Oct., '59-Jun., '60	481	16

National Institutes of Health—Children's Hospital, D.C., Collaborative Study, 1957-1961[1]; Beem, et al., 1960[2]; McClelland, et al., 1961.[3]

* Only selected patients tested; therefore proportion cannot be calculated.

Table 6—Infection and Upper Respiratory Illness in Adult Volunteers Given RS Virus

Respir. Illness	No. in Group	Time Virus Recoverable from Pharynx—Days		Antibody Rise			
				CF		Neut.	
		0–2	3–6	No	Yes	No	Yes
Yes	20	4	16	5	15	6	14
No	21	16	5	19	2	17	4

NOTE: All volunteers had neutralizing antibody prior to administration of virus.
Adapted from Kravetz (Kravetz, et al.,[15] and Johnson, et al.[16])

Washington nursery outbreak had neutralizing activity in a serum specimen which antedated the outbreak period.[14] It would appear that this neutralizing substance did not afford complete protection against lower respiratory tract illness. The specificity of the neutralizing material under discussion and the nature of the other factors which influence the outcome of RS infection remain challenging problems for future research.

Recently, reinfection of adults with RS virus under natural conditions was observed among marines at Camp Lejeune, North Carolina.[17] During an eight-week period (January-March, 1961) virus was recovered from four persons and 19 additional men developed a serologic response. Neutralizing antibody at a level of 1:16 to 1:256 was present in the preinfection serum specimen of 11 of the 12 men from whom such serum was available. Similar data supporting the occurrence of natural reinfection with RS virus were obtained by Hamre and Procknow in a longitudinal study of respiratory disease in college students in Chicago; in this instance 15 infections were documented and neutralizing antibody was detected in serum specimens which antedated infection.[18]

In the Camp Lejeune study RS infection was detected in 15 of 243 men with respiratory illness and only half as often in the control group, i.e., 8 of 247. Eleven strains of RS virus were recovered from 33 college students with colds in Chicago; whereas 31 persons convalescent from such illness during the same interval failed to yield the agent. These findings suggest that naturally occurring adult reinfection may be associated with mild respiratory illness but this correlation must be considered tentative at the present time.

Summary and Prospects

RS appears to be responsible for a considerable proportion of the severe respiratory illness which afflicts infants and small children. The virus has sharply limited periods of widespread dissemination in the community every year and represents a recurring threat to the pediatric population. Serologic studies indicate that most children become infected by age four.[3,14] Reinfection can occur later in life and is probably associated in a proportion of instances with a mild respiratory illness.

It is clear that an RS virus vaccine, whether live attenuated or inactivated, should receive a very high priority in any future plans for immunoprophylaxis of pediatric respiratory disease.

REFERENCES

1. Morris, J. A.; Blount, R. E., Jr.; and Savage, R. E. Recovery of Cytopathogenic Agent from Chimpanzees with Coryza. Proc. Soc. Exper. Biol. & Med. 92:544–549 (June), 1956.
2. Chanock, R. M.; Roizman, B.; and Myers, R. Recovery from Infants with Respiratory Illness of

a Virus Related to Chimpanzee Coryza Agent (CCA). I. Isolation, Properties and Characterization. Am. J. Hyg. 66:281–290 (July), 1957.
3. Chanock, R. M., and Finberg, L. Recovery from Infants with Respiratory Illness of a Virus Related to Chimpanzee Coryza Agent (CCA). II. Epidemiologic Aspects of Infection in Infants and Young Children. Ibid. 66:291–300 (Nov.), 1957.
4. Beem, M.; Wright, F. H.; Hamre, D.; Egerer, R.; and Oehme, M. Association of the Chimpanzee Coryza Agent with Acute Respiratory Disease in Children. New England J. Med. 263:523–530 (Sept.), 1960.
5. Rowe, D. S., and Michaels, R. H. Isolation of the Respiratory Syncytial Virus from a Patient with Pneumonia. Pediatrics 623–629 (Oct.), 1960.
6. Hamparian, V. V.; Ketler, A.; Hilleman, M. R.; Reilly, C. M.; McClelland, L.; Cornfeld, D.; and Stokes, J., Jr. Studies of Acute Respiratory Illnesses Caused by Respiratory Syncytial Virus. I. Laboratory Findings in 109 Cases. Proc. Soc. Exper. Biol. & Med. 106:717–722 (Jan.), 1961.
7. Adams, J. M.; Imagawa, D. T.; and Zike, K. Epidemic Bronchiolitis and Pneumonitis Related to Respiratory Syncytial Virus. J.A.M.A. 176:1037–1039 (June), 1961.
8. Peacock, D. B., and Clarke, S. K. R. Respiratory Syncytial Virus in Britain. Lancet 2:466 (Aug. 26), 1961.
9. Lewis, F. A.; Roe, M. L.; Lehmann, N. I.; and Ferris, A. A. A Syncytial Virus Associated with Epidemic Disease of the Lower Respiratory Tract in Infants and Young Children. Australian M. J. (in press).
10. Kisch, A. L.; Johnson, K. M.; and Chanock, R. M. Immunofluorescence with Respiratory Syncytial Virus. Virology 16:177–189 (Feb.), 1962.
11. Chanock, R. M.; Kim, H. W.; Vargosko, A. J.; Deleva, A.; Johnson, K. M.; Cumming, C.; and Parrott, R. H. Respiratory Syncytial Virus. I. Virus Recovery and Other Observations During 1960 Outbreak of Bronchiolitis, Pneumonia, and Minor Respiratory Diseases in Children. J.A.M.A. 176:647–653 (May), 1961.
12. Parrott, R. H.; Vargosko, A. J.; Kim, H. W.; Cumming, C.; Turner, H.; Huebner, R. J.; and Chanock, R. M. Respiratory Syncytial Virus. II. Serologic Studies Over a 34-Month Period of Children with Bronchiolitis, Pneumonia, and Minor Respiratory Diseases. Ibid. 176:653–657 (May), 1961.
13. McClelland, L.; Hilleman, M. R.; Hamparian, V. V.; Ketler, A.; Reilly, C. M.; Cornfeld, D.; and Stokes, J., Jr. Studies of Acute Respiratory Illnesses Caused by Respiratory Syncytial Virus. 2. Epidemiology and Assessment of Importance. New England J. Med. 264:1169–1175 (June), 1961.
14. Kapikian, A. Z.; Bell, J. A.; Mastrota, F. M.; Johnson, K. M.; Huebner, R. J.; and Chanock, R. M. An Outbreak of Febrile Illness and Pneumonia Associated with Respiratory Syncytial Virus Infection. Am. J. Hyg. 74:234–248 (Nov.), 1961.
15. Kravetz, H. M.; Knight, V.; Chanock, R. M.; Morris, A. J.; Johnson, K. M.; Rifkind, D.; and Utz, J. P. Respiratory Syncytial Virus Infection in Adult Volunteers. I. Production of Illness and Clinical Observations. J.A.M.A. 176:657–663 (May), 1961.
16. Johnson, K. M.; Chanock, R. M.; Rifkind, D.; Kravetz, H. M.; and Knight, V. Respiratory Syncytial Virus Infection in Adult Volunteers. II. Correlation of Virus Shedding, Serologic Response and Illness. Ibid. 176:663–667 (May), 1961.
17. Johnson, K. M.; Bloom, H. H.; and Chanock, R. M. (To be published.)
18. Hamre, D., and Procknow, J. J. Viruses Isolated from Natural Colds in the U. S. A. Brit. M. J. 1382–1385 (Nov.), 1961.

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V. EATON AGENT: A REVIEW

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IN THE early 1940's the term primary atypical pneumonia (PAP) was first suggested to describe a broad group of nonbacterial pneumonias.[1] A description of PAP as a clinical syndrome was necessary at that time because of a lack of understanding of its etiology. Although specific agents were not associated with this complex syndrome, it was frequently noted that cold agglutinins developed during convalescence, and this test was employed as a nonspecific