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RESPIRATORY SYNCYTIAL VIRUS DISEASE IN INFANTS DESPITE PRIOR ADMINISTRATION OF ANTIGENIC INACTIVATED VACCINE[1, 2]

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Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen and R. H. Parrott (Children's Hosp. of D.C., Wash., D.C. 20009). Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Amer. J. Epid.*, 1969, *89*: 422–434.—In response to three injections of alum precipitated, 100X concentrated, formalin inactivated RS vaccine (lot 100), 43% of infant vaccinees displayed a 4-fold or greater rise in serum neutralizing antibody and 91% displayed a 4-fold or greater rise in serum CF antibody. When RS virus became prevalent in the community, the rate of RS virus infection in infants who received this vaccine was not remarkably different from that in control infants who received parainfluenza vaccines. However, 80% of RS vaccinees required hospitalization at the time of RS infection whereas only 5% of such infections among parainfluenza vaccinees resulted in admission to the hospital. Illnesses among the RS vaccinees who underwent natural infection included pneumonia, bronchiolitis, and bronchiolitis with pneumonia in a majority and rhinitis, pharyngitis and bronchitis in a few. It seems clear that infants who received this vaccine were not protected against natural infection and also, when they became naturally infected their illness was more severe than that seen in cohorts who received a similar parainfluenza type 1 vaccine. These findings indicate that vaccine-induced RS virus serum antibody alone does not protect against illness and suggest that serum antibody without local respiratory antibody may play a part in the production of disease. We have also observed that the highest incidence of serious RS virus illness occurring naturally is under six months of age when maternally derived serum antibody is present. These findings together suggest that RS virus illness in infants is an immunologic phenomenon wherein the virus and serum antibody interact to produce severe illness.

antibodies; immunity; immunology; pneumonia; respiratory syncytial virus; respiratory tract diseases; vaccines; viral vaccines

There seems little doubt that the respiratory syncytial (RS) virus is the single most important respiratory tract pathogen for infants and children (1–12). Infection with this agent is prevalent for a three to

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four month period every year in virtually all pediatric populations in which it has been studied, and when it is prevalent in the community, hospitalization for bronchiolitis and bronchopneumonia among infants reaches a peak (4). An effective vaccine against early infection with this agent could be expected to produce a significant reduction in lower respiratory tract morbidity and hospitalization among infants and children. Initial efforts at immunoprophylaxis have been concentrated primarily on the development of an inactivated vaccine. Progress in the development of a live attenuated vaccine has proceeded at a slower pace since susceptible laboratory animals do not develop pulmonary pathology nor are genetic markers available which correlate with virulence.

A successful inactivated RS virus vaccine must satisfy certain unusual requirements. Epidemiologic and serologic evidence indicates that moderate levels of serum neutralizing antibody do not provide effective protection against serious RS virus disease during early infancy (13). In addition, the most serious RS virus illnesses occur in early infancy and therefore protection must be provided in the first few months of life at a time when infants possess maternally transmitted serum antibody (13). Thus the goal of an immunoprophylactic program for infants must be either to stimulate serum antibody levels higher than those provided by maternal transfer or alternately to induce the development of local respiratory tract antibody. Attainment of the former goal with inactivated vaccine, however, is made difficult by the poor yield of RS virus in tissue culture and the poor antigenicity of RS viral antigens.

As a part of a collaborative effort in vaccine development we have tested the antigenicity in infants of a variety of inactivated experimental vaccines for RS and parainfluenza viruses. We have limited our studies to infants less than one year of age, since primary infection with RS and type 3 parainfluenza viruses and associated severe illness occur most often during this period. This report will describe the failure of an antigenic inactivated respiratory syncytial virus vaccine to provide protection against infection and illness. In addition, we will describe an altered response of infant vaccinees in whom illness associated with naturally acquired RS infection was of greater than usual severity.

Materials and methods

Vaccines

In the present study, three different vaccines were evaluated. 1) *A respiratory syncytial virus vaccine* (lot 100) was prepared from the Bernett strain which was grown in vervet monkey kidney tissue culture. This virus was isolated in primary human embryonic kidney cultures from a throat washing at the National Institutes of Health in May, 1961, and was passaged three additional times in primary human embryonic kidney cultures and 10 times in vervet monkey kidney cultures. A 1:50 dilution of this seed virus was inoculated into vervet monkey kidney bottle cultures which were maintained with Eagle's basal medium in Earle's salt solution without serum. Early harvests were collected after four days incubation at 36 C when 10 to 25 per cent of the cells exhibited a cytopathic effect; late harvests were collected after the cultures were held at 36 C for seven days and had developed 50 to 90 per cent cytopathic effect.

The various sublot tissue culture fluid harvests were clarified individually by filtration through Millipore SM membranes and inactivated with formalin (1:4,000 final) at 36 C for 72 hours. The fluids then were centrifuged at 50,000 rpm in a Sharples rotor and the resultant pellet was resuspended in Eagle's basal medium in Earle's salt solution to give a concentration factor of 25-fold. A further 4-fold concentration was produced by precipitation with alum (4 mg/ml) and

resuspension in one-fourth the original volume of Eagle's medium, thus achieving a final concentration factor of 100-fold. Polymyxin B, neomycin, and streptomycin at 200 units/ml each and benzethonium chloride preservative (1:40,000 final) were added. At the conclusion of potency testing and completion of tests for possible adventitious agents, three sublots from early harvests and 11 sublots from late harvests were pooled to prepare vaccine lot 100. Potency tests were carried out in guinea pigs and cynomologous monkeys, which were tested for complement-fixing (CF) and neutralizing antibody after vaccination. Antigen extinction endpoints were determined in guinea pigs which were injected intraperitoneally with 0.5 ml of a vaccine dilution, given another similar injection seven and 14 days later and bled seven days after the last injection. The 50 per cent antigenic extinction CF antibody endpoint in guinea pigs was greater than 1:40 on one occasion and 1:64 on another.

The safety test protocol was based upon the requirements of the Division of Biologics Standards for an inactivated monkey kidney tissue culture derived measles vaccine (14). Safety tests were conducted in cynomologous monkeys, guinea pigs, rabbits and mice, with satisfactory results. Tests in tissue cultures for adventitious agents, including SV40, were negative. Vaccine lot 100 was packaged in 3.0 ml vaccine vials with 3.4 ml fills.

2) *A parainfluenza type 1 virus vaccine* (lot 23) was prepared from an isolate obtained from an infant with croup by essentially the same procedure as the RS virus lot 100 vaccine. The virus was isolated and propagated in human embryonic kidney culture. Subsequently, it was grown in vervet monkey kidney tissue culture and this system was used for the preparation of vaccine. After millipore filtration and formalin inactivation, tissue culture fluid harvests were concentrated 25-fold by centrifugation and then 4-fold further by alum precipitation. The final concentration was 100-fold.

3) *A trivalent aqueous parainfluenza virus vaccine* (lot 6279) was prepared from amniotic fluids harvested from seven-day-old chick embryos infected with type 1, type 2, or type 3 parainfluenza virus. The amniotic fluids were concentrated 3-fold by centrifugation and inactivated by the addition of formalin to a final concentration of 1:4000. The monovalent amniotic fluid harvests were then pooled to make up the final trivalent vaccine.

All vaccines were safety tested according to standards of the Division of Biologics Standards of the National Institutes of Health and each vaccine was administered to adult volunteers to demonstrate that it did not produce local or systemic reactions prior to its use in infants.

Composition of study group

Infants between two and seven months of age who attended a Child Health Center were selected for administration of vaccine after parental consent was obtained. The infants lived at home and came from a population of relatively low socioeconomic status families, primarily Negro.

Plan of study

The schedule of vaccine administration was as follows: two 0.5 ml intramuscular injections were given one month apart and a third injection was given three months later. Initially the parainfluenza type 1 virus vaccine and the RS virus vaccine were alternated among infants who were otherwise comparable in age, socioeconomic status and location of residence (table 1). Later the type 1 parainfluenza virus vaccine was replaced by the trivalent parainfluenza virus vaccine in the alternation. On December 29, 1966, the injection of RS virus vaccine was stopped for reasons to be recounted later. From December, 1965 to June, 1966, there was at all times a comparable number of infants in the RS and parainfluenza 1 vaccine groups. There-

after infants who received the trivalent parainfluenza vaccine constituted a further control group for those receiving RS virus vaccine.

Each infant under study was visited by an epidemiological nurse at least once a week and more often if intercurrent illness occurred. Regardless of illness, weekly throat and anal swab specimens were obtained for virus and mycoplasma studies until three weeks after the third injection of vaccine. During the immunization period serum was obtained by antecubital venipuncture from each infant at least five times, generally at monthly or bimonthly intervals. In addition, serums were obtained before and after the periods of community-wide RS virus prevalence. After completion of the series of vaccine injections, the infants were not visited routinely at home, but their parents were asked to report to the vaccine study team whenever respiratory tract symptoms occurred. When ill these infants were examined by an epidemiological nurse or a physician. Throat and anal swabs and paired specimens of blood for serum (acute and convalescent) were obtained for virus and mycoplasma studies.

Beginning in December of 1966 the RS vaccinees were sampled more often for the possible presence of RS virus in the oropharynx than were infants who had received parainfluenza vaccine because at that time we first suspected that exaggerated respiratory tract illness was associated with RS infection in infants who had received RS vaccine. However, serum specimens were obtained from all vaccinees at two to three month intervals. These sera made it possible to compare the occurrence of RS virus infection in the RS and parainfluenza vaccinees in an unbiased manner. Except for the more vigorous attempts to recover RS virus from RS vaccinees after December, 1966, all vaccinees were observed and evaluated in a similar manner. The vaccine study team did not influence the decision to hospitalize an infant with infection or illness; this decision was made by the members of the regular hospital staff.

TABLE 1

Composition of vaccine groups by time

Month/Year	No. of infants receiving indicated no. of injections of designated vaccine (*cumulative*)					
	RS lot 100		Para 1 lot 23		Trivalent parainfluenza lot 6279	
	1 Inj.	3 Inj.	1 Inj.	3 Inj.	1 Inj.	3 Inj.
Dec., 1965	3		2			
Jan., 1966	8		7			
Feb.	11		11			
March	16		16			
April	17	2	18	2		
May	19	5	20	8		
June	21	8	21	11	3	
July	23	13		14	6	
Aug.	27	15		16	7	
Sept.	27	16		18	10	
Oct.	28	17		19	11	3
Nov.	29	21			13	6
Dec.	31	23			15	6
Jan., 1967					17	9
Feb.					20	10
March					21	12
April						13
May						15
June						19
July						20

Laboratory procedures

Virus recovery. Anal and throat swab specimens were collected in veal infusion broth with 0.5 per cent bovine serum albumin and were inoculated into tissue culture either immediately or after two to three hours storage at 4 C. These specimens were then stored at −70 C for reference and for attempts at re-isolation of recovered agents. Two-tenths ml of each specimen was inoculated into at least two roller tubes each of primary rhesus monkey kidney tissue culture and HEp-2 tissue culture. The roller tube cultures were observed for cytopathic effect several times a week and the monkey kidney cultures were tested for hemadsorption at seven-day intervals. Agents producing a cytopathic effect or hemadsorption were further identified by appropriate methods including complement fixation for RS virus, hemad-

sorption neutralization for the parainfluenza viruses and differential hemagglutination-inhibition or neutralization for the adenoviruses.

Neutralization tests. In the early phase of the study, neutralization tests for RS virus were performed in HEp-2 roller tube tissue cultures, utilizing cytopathic effect as the endpoint (15). In later studies, a modification of this technique employing microtiter plates was used. Serum titers were essentially the same when tested by the roller tube or microtiter plate technique. Sixteen to 64 fifty per cent tissue culture infectious doses ($TCID_{50}$) of the Bernett strain of RS virus were used in all tests. Serum was heat inactivated (56 C for 30 minutes) and diluted in 2-fold steps starting with an initial dilution of 1:2. All serum neutralization titers are expressed as the final dilution of serum after addition of an equal volume of RS virus. The serum-virus reaction mixture also contained a final concentration of 5 per cent normal unheated guinea pig serum; this provided heat labile accessory factor which is required to demonstrate RS antibody in certain sera (16).

Complement fixation tests. Complement fixation tests for RS virus were carried out by a modification of the Bengston method utilizing microtiter plates, 1.7 units of complement, and overnight incubation at 4 C (17). The antigen was prepared from the Long strain grown in HEp-2 culture; 16 units of CF antigen were used in all tests (2).

TABLE 2

Respiratory syncytial virus recovery from infants and children with respiratory tract illness. Per cent recovery by months

Month/Year	No. Tested	No. Pos.	Per cent Pos.
Dec., 1965	99	1	1
Jan., 1966	90	4	4.4
Feb.	91	12	13.2
March	141	16	11.3
April	122	11	9
May	85	3	3.5
June	63	0	—
July	72	1	1.4
Aug.	68	0	—
Sept.	84	2	2.4
Oct.	106	0	—
Nov.	94	2	2.1
Dec.	123	24	19.5
Jan., 1967	136	52	38.2
Feb.	97	34	35
March	105	7	6.7
April	98	7	7.1
May	95	0	—

RESULTS

RS virus prevalence in the community. Patients with various types of acute respiratory tract disease were studied for evidence of RS virus infection as part of an ongoing epidemiologic investigation. Information from this study, which was based upon the recovery of RS virus, provided a basis for estimating the activity of this virus in the community during the vaccine trial (table 2). A period of prevalence of RS virus infection began in January, 1966, and subsided by June, 1966. Only sporadic infections were detected until December, 1966, when another area-wide period of prevalence began; this subsided by May, 1967. Thus, there were two periods of prevalence of RS infection during the interval of this investigation.

Lack of toxicity of vaccine. No unusual pain, tenderness, erythema, fever or other immediate or delayed local or systemic reaction was observed in infants receiving the experimental paramyxovirus vaccines.

Antigenicity of RS vaccine. As might be expected from their young age, all the infant vaccinees possessed some measurable neutralizing antibody prior to the first administration of vaccine. In most instances this was probably maternal antibody. RS vaccinees and a group of infants who had received parainfluenza 1 vaccine during the same time period had comparable geometric mean RS neutralizing antibody titers at entry into the study (table 3). The titer rose gradually in the RS vaccinees, from whom RS virus was not re-

TABLE 3

RS virus serum neutralizing antibody in infants who received RS virus vaccine, parainfluenza 1 virus vaccine or trivalent parainfluenza virus vaccine and from whom RS virus was not recovered during immunization

Vaccine	No. of infants	Geometric mean RS virus neutralizing antibody titer‡ (reciprocal) at indicated time:				
		Pre-inject.	1 mo. after 1st inject.	1 mo. after 2nd inject.	prior to 3rd inject.*	3 weeks after 3rd inject.†
RS virus vaccine (lot 100)	23	24	24	48	48	48
Parainfluenza 1 virus vaccine (lot 23)	17	16	12	12	8	8
Trivalent parainfluenza virus vaccine (lot 6279)	6	24	16	16	8	8

* 3 months after 2nd injection.
† 3 weeks following 3rd injection.
‡ <1:4 considered as 1:2 for purpose of determining geometric titer.

covered during the immunization interval, and fell in comparable parainfluenza vaccinees. All of the RS vaccinees had detectable antibody at the end of the vaccination period, and their geometric mean titer was six times that of the parainfluenza vaccinees. These findings, which are representative of the experience of the entire RS and parainfluenza type 1 vaccine groups, are presented in lieu of the total experience since the serums were tested in one series of tests and thus the comparative results are particularly meaningful.

The response of vaccinees to RS vaccine as measured by CF and neutralization, is shown in table 4. A 4-fold or greater rise in serum neutralizing antibody developed in 43 per cent of the vaccinees following three injections of RS virus vaccine. Both in response to vaccine and natural infection, the CF technique appeared more sensitive than the neutralization method in detecting a fourfold or greater rise in antibody. Thirteen per cent of infants responded with CF antibody after one injection, 62 per cent after two injections, and 91 per cent after three injections; the mean fold CF antibody rise after three injections was 30-fold. Infants from whom RS virus was recovered prior to completion of vaccine administration were excluded from these tabulations. However, the development of CF antibody by RS vaccinees who later became infected with RS virus under natural circumstances is shown in order to compare their response with that of infants who did not receive the RS vaccine. RS vaccinees appeared to develop a more marked CF antibody response (geometric mean convalescent titer 1:384) following natural RS infection than did parainfluenza vaccinees (geometric mean convalescent titer 1:64) following similar infection. This finding supports the view that the vaccine was antigenic or, alternately, it may be a manifestation of the relatively greater severity of illness in RS vaccinees than in parainfluenza vaccinees.

The failure of RS vaccine to protect against infection and illness. RS virus infection in the RS and parainfluenza vaccine groups could not be compared solely from the virus isolation results since a more intense effort was made to recover virus from RS vaccinees during the winter of 1966–1967. However, such a comparison could be made from the results of serologic studies performed with serial serums obtained before, during, and after the RS virus epidemics of 1966 and 1967. These serums were collected in essentially

TABLE 4

RS complement fixing (CF) and neutralizing antibody (NA) status of infants who received inactivated RS virus vaccine (lot 100) or inactivated parainfluenza virus vaccines (lot 23 and lot 6279)

Vaccine	Time of antibody determination	No. in group	4-fold or greater rise in antibody			
			CF antibody		Neutralising antibody	
			No. infants	Mean fold rise	No. infants	Mean fold rise
Respiratory syncytial vaccine (lot 100)	After 1 injection	31	4 (13%)	2.7	1 (3%)	0.8
	After 2 injections	29	18 (62%)	21	5 (17%)	1.6
	After 3 injections	23	21 (91%)	30	10 (43%)	2.6
	Natural RS virus infection with recovery of virus	16	15 (94%)	165	12 (75%)	21
Parainfluenza 1 vaccine (lot 23) and Trivalent parainfluenza vaccine (lot 6279)	After 1 injection	40	0 (−)	0	N.D.*	
	After 2 injections	40	2 (5%)	0.3	N.D.	
	After 3 injections	39	7 (15%)	2.3	N.D.	
	Natural RS virus infection with recovery of virus	14	13 (93%)	71	N.D.	

* N.D. = Not done.

the same manner from both groups and all serums were tested by a standardized CF antibody technique. Sixty-five per cent of RS vaccinees developed serologic evidence of RS virus infection as compared with 60 per cent of infants who received parainfluenza 1 vaccine, 45 per cent of those who received trivalent parainfluenza vaccine, and 53 per cent of all infants who received one of the parainfluenza vaccines. Thus, the rate of infection in the vaccine groups was not remarkably different.

Although it is clear that RS vaccine did not provide protection against RS virus infection, there is no evidence that it produced an increased susceptibility to infection. However, 80 per cent of the RS vaccinees required hospitalization at the time of infection, whereas only 5 per cent of infections among parainfluenza vaccinees resulted in admission to the hospital (table 5). In addition, infants who had received the RS vaccine became more severely ill when infected with RS virus than did the otherwise comparable infants who received the parainfluenza vaccines.

These findings were amplified when RS and parainfluenza vaccinees were compared using both virus recovery and CF for detection of infection (table 6). Twenty-three of 31 RS vaccinees experienced RS virus infection sometime during or following the vaccination period. Illnesses among these infants included pneumonia in six, bronchiolitis or bronchiolitis with pneumonia in 13, and mild rhinitis, pharyngitis or bronchitis in four. Eighteen required hospitalization. Of 40 infants who had received one of the parainfluenza vaccines, later RS virus infection was detected by virus recovery in 14 or by a fourfold or greater CF antibody rise in 21—a total of 21 infections. Illnesses in these infants included pneumonia in two, bronchiolitis or bronchiolitis with pneumonia in two, relatively severe bronchitis or pharyngitis in two, and mild rhinitis, pharyngitis or bronchitis in 15. Only one of

TABLE 5

RS virus infection and serious illness in comparable groups of infants receiving one or more injections of inactivated RS and parainfluenza vaccines

Vaccine	Category of infants	No. and age of infants during designated time period of RS virus prevalence				Total No. infants
		1965–1966		1966–1967		
		No. infants	Age§ (mo.)	No. infants	Age§ (mo.)	
RS lot 100	At risk*	20	5.1	25‡	12.7	31
	RS infection†	5		15		20 (65%)‖
	Hospitalized	4		12		16 (80%)¶
Para 1 lot 23	At risk*	20	5.0	17‡	15.8	20
	RS infection†	2		10		12 (60%)‖
	Hospitalized			1		1 (8%)¶
Trivalent parainfluenza lot 6279	At risk*			20	8.4	20
	RS infection†			9		9 (45%)‖
	Hospitalized			0		0
Total parainfluenza	At risk*	20	5.0	37	11.8	40
	RS infection†	2		19		21 (53%)‖
	Hospitalized			1		1 (5%)¶

* No prior natural infection.

† As indicated by 4-fold or greater CF antibody rise only; reinfection counted only once.

‡ One infant was not available to follow up.

§ Mean age at peak of RS prevalence.

‖ Per cent of infants at risk who sustained RS infection.

¶ Per cent of infected individuals who were hospitalized.

these infants required hospitalization at the time of RS infection.

The severity of illness in the RS vaccinees was greater than in unvaccinated infants and children who were admitted to the hospital during the community wide outbreak of RS virus infection. The mean period of hospitalization of the RS vaccinees was 10.5 days, whereas the mean period of 30 age-matched unvaccinated infants with RS bronchiolitis and/or pneumonia was 6.7 days.

The RS vaccinees who became seriously ill during RS infection included both recent vaccinees and individuals whose course of injections was completed as long as 11 months before the onset of illness (table 7). Those who were infected and became ill included two infants who had received only one injection and eight who had received two injections. All of the infants possessed moderate to high levels of serum neutralizing antibody just prior to and also during the acute phase of illness. Approximately a third of those who became infected had RS virus CF antibody in their serum prior to illness or during the acute phase of illness; however, few of these sera had CF antibody levels comparable to those seen during convalescence from RS virus illness. These findings indicate that vaccine-induced serum neutralizing antibody persisted until the time of infection. In addition, the findings clearly indicate that moderate to high levels of serum neutralizing antibody, as such, did not provide effective protection against RS virus lower respiratory tract disease.

Two infants died, one at age 14 months, the other at age 16 months. Each had received three inoculations, one beginning at

TABLE 6

RS virus infection and illness in groups of infants after receiving 1 or more injections of inactivated respiratory syncytial and parainfluenza vaccines

Vaccine received	No. vaccinees	No. vaccinees with later RS infection as indicated by			No. vaccinees with designated illness at time of RS infection				No. requiring hospitalization at time of RS infection
		Virus recovery	CF antibody rise	Virus recovery and/or CF rise	P*	B† or B-P	BR PH‡	URI§	
RS lot 100	31	18	20	23	6	13	0	4	18
Para 1 lot 23	20	7	12	12	2	0	0	10	1
Trivalent parainfluenza lot 6279	20	7	9	9	0	2	2	5	0
Total parainfluensa	40	14	21	21	2	2	2	15	1

* P = Pneumonia.
† B = Bronchiolitis.
‡ BR-PH = Severe bronchitis pharyngitis.
§ URI = Mild rhinitis, pharyngitis and/or bronchitis.

age two months, the other at age five months. The major findings at post mortem examination were extensive bronchopneumonia and patchy atelectasis with emphysema and pneumothorax in one infant. Microscopic histologic findings indicated peribronchiolar monocytic infiltration with some excess in eosinophiles, findings which are consistent with the limited literature on the histologic appearance of bronchiolitis. RS virus was grown readily from the respiratory tract and lung in both infants. At least 10^4 $TCID_{50}$ of RS virus were present per gram of lung of the younger infant.

In addition a pure culture of *E. coli* was recovered from the trachea, blood, spleen and lung of one infant and a pure culture of *Klebsiella* was recovered from the lung, trachea and nose culture of the other.

The age at which RS vaccinees developed RS virus lower respiratory tract disease is shown in table 8. This age distribution differs from that of unvaccinated infants who developed RS virus disease during the January, 1966–June, 1966, and December, 1966–April, 1967, epidemics. In the community-wide outbreak the occurrence of severe illness requiring hospitalization was highest during the first two months of life and decreased significantly with increasing age. The RS vaccinees could not be compared, in a strict sense, with the unvaccinated infant population because vaccine was not administered until the vaccinees were two to seven months of age. However, the frequent occurrence of serious RS virus respiratory tract disease in vaccinees who were over six months of age suggested that prior administration of vaccine produced an alteration in host response wherein the older vaccinees developed the type of lower respiratory tract disease most commonly associated with infection of early infancy.

DISCUSSION

From these findings it seems clear that infants who received a monkey kidney tissue culture grown, inactivated, 100X concentrated, alum precipitated respiratory syncytial virus vaccine not only were not protected against infection, but paradoxically they experienced an altered, exaggerated clinical response when natural RS infection occurred. These findings were entirely unexpected. We now feel that in some way administration of the inactivated RS vaccine was responsible for the exaggerated illness caused by RS virus infection. We lack a definite explanation for this phenomenon, except to say that the

TABLE 7

Infants receiving inactivated respiratory syncytial virus vaccine lot 100 who later underwent natural RS virus infection

Vaccinee No.	Diagnosis	Age/Months		Evidence of RS infection		No. of inoc. before illness	Time after last inoc. to illness	Days between last serum & acute illness serum	Reciprocal of RS virus CF antibody titers				Reciprocal of RS virus neutralizing antibody titers			
		First inoc.	Illness	Virus recovered	CF antibody rise				Preinject.	Last serum before illness	Acute illness serum	Convalescent serum	Preinject.	Last serum before illness	Acute illness serum	Convalescent serum
1	B	2	7	+	+	3	28 d	29	8	<4	<4	256	64	48	64	192
2	B-P‡	2	14	+	N.T.	3	8 ½ m	31	<4	<4	<4	—	12	12	4	—
3	URI*	2	11	+	0	3	4 m	70	<4	64	—	32	128	64	—	48
4	P	2	12	+	+	3	6 m	11	<4	4	4	256	16	4	96	N.T.
5	P	2	3½	+	+	1	52 d	28	<4	<4	<4	32	48	24	24	96
6	P	2½	12	+	+	3	5 ½ m	32	<4	<4	128	>512	24	48	128	384
7	P	3	5	0	+	2	23 d	28	<4	<4	32	128	32	32	48	64
8	B*†	3	5	+	+	2	38 d	8	<4	16	—	128	24	12	—	64
9	B	3	5	+	+	2	30 d	30	<4	<4	—	256	12	12	—	48
9‖	B-P	3	15	+	+	2	11 m	32	<4	16	8	512	12	24	24	768 or >
10	B-P	3	4	+	+	2	14 d	15	4	<4	<4	>512	48	32	24	768 or >
11	B	3	9½	+	+	3	2 m	5	<4	8	16	>512	48	24	16	384
12	B	3	4	+	+	1	1 m	6	4	<4	<4	32	256	128	96	768 or >
13	P	3½	13	+	+	3	5 m	13	<4	<4	<4	>512	48	128	96	384
14	B	5	6	+	+	2	17 d	18	<4	<4	32	>512	24	12	64	768 or >
15	B-P‡	5	16	+	N.T.	3	5 ½ m	8	<4	128	64	—	16	48	—	—
16	B-P	5	18	+	+	3	9 m	17	<4	<4	<4	512	6	12	16	128
17	B	6	8	0	+	2	35 d	6	<4	64	—	512	6	32	—	384
18§	B	6	16	N.T.	+	3	6 m	16	<4	16	—	>512	12	96	—	768 or >
19**	URI*	6	17	+	+	3	8 m	51	<4	4	<4	>512	6	16	12	16
20	P	6	11	+	+	3	1 m	8	<4	8	32	>512	96	192	384	384
21	B-P	7	13	+	+	2	4 m	26	<4	16	16	>128	8	32	48	384

* Not admitted to hospital. † Influenza B also recovered. ‡ Deceased. § Hospitalized at other hospital. ‖ Reinfection occurred during December 1966 outbreak. ** Reinfection with interval of 28 days. On both occasions infant had URI with low grade fever.
B = Bronchiolitis. P = Pneumonia. N.T. = Not tested. d = day(s). m = month(s).

TABLE 8

Age distribution of RS virus illness in vaccine group compared with that of community

Age at which RS bronchiolitis or pneumonia occurred	Patients from community		RS vaccinees No.
	No.	Per cent of total	
1–2	36	31	
3–4	21	18 (65%)	3
5 6	18	16	4
7–8	13	11	2
9–10	5	4	1
11–12	4	3	3 (63%)
13–14	4	3	3
15–16	5	4	2
17–18	2	2	1
19–20	3	3	
21–22	4	3	
23–24	1	1	
Total	116		19

vaccine in some way altered the host response to natural RS infection. This phenomenon is presumably similar to the "paradoxical" vaccine effect which has been reported with parenterally administered, rickettsial vaccines, trachoma vaccine, *Mycoplasma pneumoniae* vaccine and more recently with inactivated measles vaccine (18–22).

In retrospect the above findings recall experience with a group of 54 infants who received a 4X concentrated, formalin-inactivated, alum precipitated RS virus vaccine in 1962 and 1963. In those infants we concluded that the vaccine was not sufficiently antigenic to be considered for further trials. In addition we concluded that the test vaccine was completely ineffective in preventing illness or infection because 21 of the 54 infants who received two or three injections experienced natural RS virus infection during the subsequent period of high prevalence. Ten of these infections were associated with severe lower respiratory tract illness requiring hospitalization. Three of these hospitalized infants had serum neutralizing antibody levels at the time of illness which may have reflected either persistence of maternal antibody or an immunogenic effect of the poorly antigenic RS virus vaccine. At the time we felt that the high incidence of infection and illness in vaccinees was possibly outside the range of normal; however, there was no concurrent control group with which to compare the unusual incidence of illness in the vaccinees. In view of the recent findings, it is likely that the high incidence of serious illness in the infants who received the earlier poorly immunogenic RS virus vaccine was associated with administration of the vaccine.

The parainfluenza type 1 vaccine, which was given to alternate vaccinees during the first half of the investigation, was prepared in a manner virtually identical to that of the RS vaccine. Both vaccines were prepared from virus grown in vervet monkey kidney tissue cultures. Both viruses were concentrated 100-fold and in both instances alum was used as an adjuvant. Since the parainfluenza type 1 vaccinees did not exhibit an apparent altered response to RS virus infection, it is unlikely that monkey kidney antigens, either in sedimentable form unassociated with virus or incorporated into the virion envelope, were responsible for the exaggerated clinical response of RS vaccinees. Thus, a component of RS virus itself, and not a tissue culture host cell antigen, probably was responsible for inducing an altered state of reactivity to RS infection in RS vaccinees.

In reviewing the incidence of RS virus lower respiratory tract illness at Children's Hospital, Washington, D. C. from 1959 to 1966, we observed that the highest rate of such serious disease occurred in the first four months of life, at a time when maternally transmitted antibody was present at a moderate to high level (13). This pattern was repeated during the 1966–1967 RS virus epidemic in Washington. These findings, in addition to indicating that serum neutralizing antibody by itself does

not provide effective protection, suggest to us that passively acquired antibody may play a role in the pathogenesis of RS virus lower respiratory tract disease of early life (23). Possibly antigen-antibody complexes at the respiratory epithelial surface initiate a sequence of events involving complement fixation, chemotaxis and leukocyte damage which leads to the bronchiolar pathology seen in serious RS virus disease (24, 25).

Recent studies with another paramyxovirus, type 1 parainfluenza virus, indicated that local respiratory tract secretory antibody was more important in resistance to infection than was serum antibody (26). If a similar situation obtains for RS virus and if serum antibody plays a role in RS virus pathogenesis, one can then postulate that parenterally administered inactivated RS vaccine produced its alteration of host response by increasing both the level and persistance of serum antibody in a host who lacked respiratory tract secretory antibody. In this sense inactivated vaccine may have acted to extend the period of vulnerability of vaccinees beyond the first few months of life.

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