Attenuation of RA 27/3 Rubella Virus in WI-38 Human Diploid Cells

Stanley A. Plotkin, MD; John D. Farquhar, MD; and Michael Katz, MD, Philadelphia; and Fritz Buser, MD, Bern, Switzerland

THE PROVENANCE of the RA 27/3 attenuated rubella strain has already been described in print.[1,2] Detailed information was given at the London Conference just three months ago.[3] Therefore we shall only outline the history of this strain, before proceeding to examine its in vitro characteristics and its behavior when inoculated in man.

In order to avoid the problem of passenger viruses, the RA 27/3 strain was isolated directly from naturally infected material in WI-38 human diploid fibroblasts.[4]

Explant cultures were made of the dissected organs of a particular fetus aborted because of rubella, the 27th in our series of fetuses aborted during the 1964 epidemic. The third explant, which happened to be from kidney, was selected arbitrarily for further study. Fibroblast cells that grew out from this explant could be subcultivated after several weeks. The presence of rubella virus in the supernatant fluids was confirmed. After four subcultivations of the infected kidney fibroblasts, the supernatant fluid was inoculated directly into a WI-38 culture. Once transferred to WI-38, the RA 27/3 rubella strain was passaged further in the same cell strain.

The RA 27/3 strain was tested again after four and eight passages in WI-38 incubated at 35 C. Subcutaneous inoculation of virus provoked much virus excretion, rash, and spread to contacts.

At this point, two sublines were developed, as illustrated in Table 1, the first by passage in WI-38 cells incubated at 35 C, and the second in the same cultures incubated at 33 C. After reaching the 13th WI-38 passage, the second subline was passaged in cultures incubated at 30 C.

Virus pools at four medium-passage levels were tested in man: the 11th and 14th passage levels of the first subline, and the 15th and 17th passage levels of the second. Although only two subjects were tested for each pool, the results were nevertheless striking (Table 2). The passages of the 35 C subline produced more virus excretion and more clinical reaction than the passages of the 30 C subline. In view of these results, the 35 C subline was

Received for publication March 10, 1969.

From the Wistar Institute of Anatomy and Biology (Drs. Plotkin and Katz), and the Department of Pediatrics, University of Pennsylvania (Drs. Plotkin, Farquhar, and Katz), Philadelphia. Dr. Buser is in private practice in pediatrics, Bern, Switzerland.

Read before the International Conference of Rubella Immunization, Bethesda, Md, Feb 19, 1969. It was also read before the 23rd Symposium on Microbiological Standardization: Rubella Vaccines, London, Nov 19, 1968.

Reprint requests to the Wistar Institute, Philadelphia 19104 (Dr. Plotkin).

Table 1.—Passage History of RA 27/3

Passage No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16									
Dilution passed (C)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35									
Passage No.	...	...	...	...	...	...	...	...	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Dilution passed (C)	...	...	...	...	...	...	...	...	33	33	33	33	33	30	30	30	30	35	30	35	30	30	30	30	35

dropped, and further work was done on the low-temperature subline.

Tests in man were conducted with 25th passage virus, which had been cloned five times by terminal dilutions, and revealed desirable properties of a vaccine strain.

Accordingly, we decided to use the 25th passage-level RA 27/3 virus as seed material for later pools and to expand the trials with virus at about this level. The results will be described below.

Characterization of RA 27/3 Virus

In vitro characteristics of the RA 27/3 virus have been investigated, and several markers have been noted.

Because the vaccine strain is adapted to replication at 30 C, we compared different passage levels with respect to growth at 37 C and 30 C. As illustrated in Table 3, RA 27/3 virus adapted to growth in RK_{13} cells replicated better at 37 C than at 30 C, which was also true for RA 27/3 through the 14th passage in diploid cells. Later passages in WI-38 cells of RA 27/3 vaccine virus showed definitely better replication at 30 C than at 37 C.

Plaque formation in RK_{13} cells [5,6] under various overlayers has revealed certain attributes of RA 27/3, as shown in Table 4. Here the RA 27/3 strain is compared to HPV-77 and a low-passage fresh isolate. RA 27/3 produced relatively small turbid plaques, similar in appearance to the low-passage strain. However, in the case of the low-passage strains plaqued under agar overlayers, good numbers of plaques could be produced only with the addition of diethylaminoethyl (DEAE)-dextran to the overlayer, while RA 27/3 produced plaques with or without the presence of DEAE-dextran in the agar.

A system for plaque formation was developed using BHK21 cells which were infected with RA 27/3 and then suspended in agarose.[7] Whereas plaques were produced by the RA 27/3 virus growth in BHK21 or in WI-38, no plaques were produced by other strains, as shown in Table 5. Neither Cendehill nor HPV-77 attenuated strains, nor five unattenuated strains produced plaques. Plaque formation did not occur with the original RA 27/3 virus or with the same strain after eight passages in WI-38 cell cultures. However, by the 15th passage the RA 27/3 virus had acquired the ability to produce plaques in this system. Isolates from the throat of subjects vaccinated with RA 27/3 also formed plaques in BHK21, whereas, in contrast, isolates from Cendehill vaccinees did not.

Clinical Results

Initially, the RA 27/3 was inoculated subcutaneously. Trials in institutionalized children and in normal families yielded no consistent clinical reaction except for an increase in palpable cervical lymph nodes. As shown in Table 6, which illustrates family studies, almost all vaccinees developed antibodies in the range of 1/80 to 1/60, but no spread occurred to seronegative contacts.

Late in 1967 we tried administration of RA 27/3 by a different route—the intranasal one.[8] Attenuated viruses administered parenterally multiply in the nasopharynx. However, HPV-77 [9] and Cendehill [10] attenuated viruses have not regularly induced antibodies when given intranasally. It was therefore interesting

that, in a series of institutional trials, we found evidence for intranasal antigenicity of RA 27/3.

By intranasal administration we mean inoculation by dropper of 0.5 to 1 ml of virus-containing material. In most of these early trials, subcutaneous vaccination was performed in some of the children, so that comparative data could be acquired. Table 7 shows that the serologic response of children to intranasal vaccination was comparable to that after subcutaneous vaccination.

For example, as shown in Table 7, in *trial E,* thirteen seronegative, mentally retarded children were given RA 27/3 vaccine, six subcutaneously (500 plaque forming units [PFU]) and seven intranasally (1,000 PFU). Clinical reactions were absent in these children, except for one child in each group who developed palpable postauricular lymph nodes; six weeks later all those inoculated subcutaneously, and all but one of those inoculated intranasally had developed antibodies.

In *trial F,* seven seronegative, orphaned children were given RA 27/3 vaccine intranasally (1,000 PFU) and placed in contact with another seven unvaccinated seronegative children. A seronegative woman was also vaccinated intranasally. The adult and one of the vaccinated children developed enlarged postauricular nodes during the second week after inoculation, but there were no other clinical reactions to the vaccine. Blood samples were taken nine weeks later; seven of the eight vaccinees but none of the seven contacts had rubella antibodies in their sera.

To determine whether nasopharyngeal virus excretion is similar after subcutaneous and intranasal vaccination, swabs were collected in trials E, H, and I. The results for trials E and H are combined in Table 8. The frequency of recovery was not substantially different in the intranasal vs the subcutaneous groups.

Table 2.—Results of Clinical Trials of Strain RA 27/3 at Different Passage Levels in WI-38 Tissue Culture

WI-38 Passage	Nasopharyngeal Virus (Mean Duration) (days)	Rash	Neutralizing Antibody Response
11 (35 C)	3.0	2/2	64.16
14 (35 C)	5.5	2/2	16.16
15 (30 C)	0.5	0/2	16.4
17 (30 C)	0	1/2	8.8

Table 3.—Growth at 30 C of RA 27/3 Strain at Different Passage Levels in WI-38 Cell Cultures

Passage	Log_{10} Virus Titer 30 C	37 C	(30 to 37 C)
RK_{13}*	<1.5	3.5	−2.0
8	1.5	5.0	−3.5
14	1.2	3.3	−2.1
15	2.5	2.5	0.0
21	3.2	2.0	1.2
25	3.5	2.5	1.0

* Low-passage RA 27/3 subsequently cultivated in RK_{13}.

Table 4.—Influence of Overlayer on Plaque Number and Morphology of Three Rubella Virus Strains

Virus Strain	Overlayer * Agar 0.9% † No.	D	Char	Agar + 500μg DEAE Dextran per ml No.	D	Char	Carboxymethyl Cellulose No.	D	Char
RA 27/3	60	2	T	81	3	C	90	2	T
HPV-77	62	3	C	68	4	C	60	2	C
H-600	5	1	T	47	2	T±	52	2	T

* PH = 6.9 to 7.0.
† No. indicates number of plaques per two dishes; D, mean diameter (in millimeters); Char, character (C = clear, T = turbid. T± = slightly turbid).

Table 5.—Plaque Formation in BHK 21/13S Cells Suspended in Agarose

Strain Name or Group	Cell Substrate	No. of Strains	Plaque Formation
RA 27/3	BHK	1	+
Cendehill	1°RK	1	0
HPV-77	GMK *	1	0
RA 27/3	Explant	1	0
RA 27/3	WI-38/8	1	0
RA 27/3	WI-38/15	1	+
RA 27/3	WI-38/25	1	+
RA 27/3	WI-38/27	4	+(4/4)
RA 27/3-vaccine isolates	...	5	+(5/5)
Cendehill vaccine isolates	...	3	0(0/3)
Brown	GMK	1	0
Throat isolates	GMK	4	0(0/4)

* GMK indicates African green monkey kidney.

Table 6.—HI Antibody Responses in Family Studies to Inoculation of RA 27/3 (27th Passage) * Vaccine

	Vaccinated Child †				Contacts					
					Child				Mother	
Family	Age (yr)	Sex	Pre-vaccination	Post-vaccination ‡	Age (yr)	Sex	Pre-vaccination	Post-vaccination ‡	Pre-vaccination	Post-vaccination ‡
1	6	M	<10	80	19 mo	M	<10	<10	<10	<10
2	5	F	<10	40	9	M	<10	<10	80	80
3	6	F	<10	80	4	M	<10	<10	80	80
4	2	F	<10	80	4	M	<10	<10	160	160
5	4	F	<10	160	6	M	<10	<10	<10	<10
6	12	F	<10	80	10	F	<10	<10	20	20
7	3	F	<10	40	7 mo	F	<10	<10	160	160
8	5	F	<10	160	1	F	<10	<10	160	80
9	3	F	<10	320	...	...	...	...	<10	<10
10	2	F	<10	80	5 mo	F	<10	<10	80	80
11	5	F	<10	80	3	F	<10	<10	<10	<10
12 §	3	F	<10	160	...	...	...	...	<10	<10
13	4	F	<10	320	18 mo	F	<10	<10	320	320
14	2	F	<10	640	6	M	<10	<10	80	NO ‖
15	28 mo	F	<10	160	4 mo	F	<10	<10	640	640

* In WI-33 cell culture.
† Vaccine given subcutaneously.
‡ Six weeks postinoculation for first seven families, nine weeks for remainder.
§ Second inoculation in the same family.
‖ NO indicates not obtained.

Table 7.—Trials of Intranasal and Subcutaneous Vaccination of Seronegative Subjects With RA 27/3 Rubella Vaccine

	Subcutaneous Vaccination			Intranasal Vaccination			Contacts
		Postvaccination * HI Titers			Postvaccination * HI Titers		
Trial	No. Seroconverted */ No. Vaccinated	Median †	Range †	No. Seroconverted */ No. Vaccinated	Median †	Range †	No. Seroconverted */ No. Exposed
E	6/6	160	40-640	6/7	80	80-640	...
F	...	...	...	7/8	80	40-320	0/7
G	3/3	80	80-160	4/4	60	40-160	...
H	5/5	160	40-160	4/6	160	160-320	...
I	10/12	80	20-160	14/14	80	40-160	0/45

* Six weeks postvaccination.
† Among those subjects who did develop antibodies.

Table 8.—Nasopharyngeal Virus Excretion After Intranasal and Subcutaneous Inoculation of RA 27/3 Vaccine, 27th Passage * (Trials E and H)

	No. Vaccinees Excreting Virus								
	Days Postinoculation:								
Type of Vaccination	0	3	7	9	11	13	14	17	21
Subcutaneous (N=11)	0	0	0	1	5	4	2	1	0
Intranasal (N=10) †	0	0	2	3	5	5	3	0	0

* In WI-38 cell culture.
† Excludes three children who did not develop antibodies.

Table 9.—Titrations of RA 27/3 Rubella Vaccine by Intranasal Route

	Study				
Dose Log_{10} TCD_{50}	Philadelphia 1	Philadelphia 2	Lyon, France 1	Lyon, France 2	Total
10,000	...	...	5/5	4/4	9/9
1,000	6/6	5/5	5/5	3/3	19/19
500	5/5	3/3	...	...	8/8
100	4/8	4/6	2/5	2/3	12/22

Table 10.—Family Trials HI Antibody Response Following Intranasal Vaccination With RA 27/3 Vaccine (27th Passage) * †

	Vaccinated Child				Seronegative Contacts			
Family	Age (yr)	Sex	Pre-vaccination	Post-vaccination	Age (yr)	Sex	Pre-vaccination	Post-vaccination
1	6	F	<10	80	7	M	<10	<10
2	1½	F	<10	160	39 ‡	F	<10	<10
3	6	F	<10	40	5	F	<10	<10
	9	M	<10	40	33 ‡	F	<10	<10
4	5	M	<10	80	3	M	<10	<10
5	6	F	<10	320	3	F	<10	<10
6	3	F	<10	320	1	F	<10	<10
	2	F	<10	160	...	...	...	...
7	2½	M	<10	80	1	M	<10	<10
8	5	F	<10	160	3	M	<10	<10
9	5	F	<10	1,280	6	M	<10	<10
10	6	F	<10	320	5	F	<10	<10
	3	M	<10	320	...	...	...	...
11	5	F	<10	320	2	M	<10	<10
12	4	F	<10	160	3	F	<10	<10
13	1	M	<10	320	2	M	<10	<10
14	10	M	<10	320	12	F	<10	<10
15	10	F	<10	640	6	F	<10	<10
16	2½	F	<10	80	3	F	<10	<10
17	6	F	<10	160	2	F	<10	<10
18	10	M	<10	320	6	F	<10	<10
19	10	F	<10	80	3	F	<10	<10
20	9	F	<10	1,280	8	F	<10	<10

* Total = 23; geometric mean = 120 (including two failures), 220 (not including two failures).
† Data from Bern, Switzerland (F.B.).
‡ Mother.

Table 11.—Family Studies of Vaccination With RA 27/3 Rubella Vaccine

	Vaccinees			Contacts		
Route of administration	SQ *	IN *	Both	SQ	IN	Both
No. vaccinated	75	48	123	125	45	170
HI antibodies						
Prevaccination	<10	<10	<10	<10	<10	<10
Postvaccination	20-1,280	40-640	20-1,280	<10-<10	<10-<10	<10-<10
Median	160	160	160			

* SQ indicates subcutaneous; IN, intranasal.

Table 12.—Subcutaneous Vaccination of Women With RA 27/3 Rubella Vaccine

		HI Antibodies		Reactions			
No.	Age	Prevaccination	Postvaccination	Fever	Lymphadenopathy	Rash	Joints
HUP 2	25	<10	80	0	0	0	0
HUP 10	20	<10	80	0	0	0	0
HUP 14	21	<10	80	0	+(9) *	0	0
HUP 16	21	<10	320	0	0	0	0
HUP 42	21	<10	320	0	0	0	0
HUP 62	20	<10	160	+(10)	+(9)	+(11)	0
HUP 70	21	<10	80	0	0	0	0
HUP 76	21	<10	80	0	0	0	0
HUP 88	21	<10	160	0	0	±(13)	0
HUP 92	21	<10	160	0	0	0	0

* With coryza and sore throat, nine days.

Table 13.—Intranasal Vaccination of Women With RA 27/3 Rubella Vaccine

No.	Age	HI Antibodies		Reactions			
		Prevaccination	Postvaccination	Fever	Lymphadenopathy	Rash	Joints
HUP 269	19	<10	80	0	0	0	0
HUP 273	20	<10	80	0	0	0	0
HUP 238	20	<10	160	0	0	0	0
HUP 335	20	<10	160	0	0	0	0
HUP 336	20	<10	80	0	0	0	0
HUP 192	19	<10	80	0	0	0	0
HUP 151	20	<10	40	0	0	0	0
HUP 190	20	<10	80	0	0	0	0
HUP 215	21	<10	<10	+(19) *	0	0	0
HUP 101	21	<10	160	+(14) †	0	0	0
HUP 260	25	<10	80	0	+(15)	0	0
HUP 247	19	<10	80	0	0	0	0

* Accompanied by coryza and sore throat.
† Accompanied by diarrhea and vomiting.

To determine the effect of dosage on immunization with RA 27/3, four titrations have been done, with consistent results (Table 9). Two trials were done in Philadelphia and two in Lyon, France, using three different lots of vaccine. All nine vaccinees who received 10,000 TCD_{50}, all 19 who received 1,000 TCD_{50}, and all 8 who received 500 TCD_{50} developed antibodies. However, only 12 of 22 who were given 100 TCD_{50} became immune. Thus, the 50% end-point dose for intranasal vaccination was 100 PFU.

Family Trials for Possible Contagiousness.—Although the institutional trials failed to demonstrate contagiousness of the vaccine given either subcutaneously or intranasally, a more critical test is intrafamilial contact. Families were recruited in which there were at least two seronegative individuals. In each family, one seronegative child was vaccinated, with a seronegative sibling or mother serving as control. We have already shown some results of family studies in which subcutaneous vaccine was used. Similar results were obtained after intranasal vaccine, as shown in Table 10. This table illustrates the results for 23 families. The index child had received 10,000 TCD_{50} intranasally. At the end of six or eight weeks, all the vaccinees had developed antibodies, whereas 21 seronegative siblings and mothers in intimate contact with the vaccinees remained seronegative.

Table 14.—Symptoms Following Vaccination of Nurses in Philadelphia With RA 27/3

Route of Vaccination *	No. Vaccinated †	No. With Symptoms			
		Joints	Rash	Fever	Nodes
SQ	31	0	3	3	7
IN	19	0	1	3	4
Either	50	0	4	6	11

* SQ indicates subcutaneous; IN, intranasal.
† Seroconversion demonstrated in each case.

Tests with 123 families have been completed (Table 11). In 75, the vaccine was administered subcutaneously; in 45, the index child was given vaccine intranasally. Clinical observations of the vaccinees detected no rashes, nor was there any fever that could be attributed to vaccination. Small palpable postauricular lymph nodes developed in about 12% of the vaccinees, but caused no discomfort. At the end of six or nine weeks, blood specimens were collected from all family members. Table 11 summarizes the serologic results. The median hemagglutination-inhibiting (HI) titer in vaccinees was 1:160. None of 170 seronegative contacts developed antibodies.

Trials in Adults.—In view of the arthritic reactions that have been associated with other strains, we searched for similar manifestations in adult women vaccinated with RA 27/3. Initially, small numbers of adult seronegative women, were given

Table 15.—Swiss Trials With RA 27/3 Rubella Vaccine (26th to 27th Passage)*

Group	Vaccinees				Contacts	
	No. Seronegatives	Route of Administration †	Rash or Other Reactions	No. Seroconverted	No. Seronegatives	No. Seroconverted
Institutions	33	SQ	0	30	18	0
Family	24	SQ	0	24	25	0
Single vaccination	21	SQ	0	21	...	0
Family	25	IN	0	23	21	0
Single vaccination	36	IN	0	33	...	0
Totals	78	SQ	0	78 (100%)	43	0
	61	IN	0	56 (92%)	21	0
	139	Either	0	134 (96%)	64	0

* Conducted by F.B.
† SQ indicates subcutaneous; IN, intranasal.

Table 16.—Summary of Vaccinations With RA 27/3 Rubella Vaccine

Country	Route of Administration *	Seronegative Vaccinees			Seronegative Contacts		
		Total No.	No. of Adults	No. With Arthritis	No. Seroconversion	Total No.	No. Seroconversion
United States	SQ	159	32	0	155	108	0
	IN	134	29	0	120	85	0
British Isles	SQ	36	15	0	36	53	0
Switzerland	SQ	78	0	0	78	43	0
	IN	61	0	0	56	21	0
France	SQ	19	0	0	19	20	0
	IN	45	0	0	45	24	0
Israel	SQ	15	15	0	15	0	0
	IN	10	10	0	10	0	0
Iran	SQ	27	0	0	27	14	0
	IN	25	0	0	21	5	0
USSR	SQ	56	0	0	56	6	0
Japan	SQ	112	46	0	112	14	0
Totals	SQ	500	108	0	496	258	0
	IN	275	39	0	232	135	0
	Either	775	147	0	728	393	0

* SQ indicates subcutaneous; IN, intranasal.

vaccine subcutaneously or intranasally without ill effect. For larger trials student nurses were recruited. Reactions were assessed through continuous surveillance by a physician and a project nurse, and by a form listing numerous symptoms, including pains in the joints, which was filled out daily by each subject. As shown in Table 12, ten nurses received vaccine subcutaneously; all seroconverted. One nurse developed lymphadenopathy at nine days, slight fever at ten days, and a rash at 11 days. Another nurse had an evanescent rash at 13 days, and a third nurse had coryza and lymphadenopathy at nine days. No arthritis occurred, and no arthralgia was reported by the nurses.

A second group of 12 nurses was vaccinated intranasally (Table 13). All but one developed antibodies. Fever occurred with coryza at 19 days in the nurse who failed to seroconvert, and with diarrhea and vomiting in one who did convert 14 days after vaccination. Lymphadenopathy was noted in one nurse 15 days postvaccination. There were no rashes, arthritis, or complaints of arthralgia.

More nurses have been vaccinated, and Table 14 summarizes the results to date. Although there have been four instances of rash, six febrile reactions and 11 examples of lymphadenopathy that could be attributed to the vaccine, no joint symptoms or signs have been noted.

Foreign Trials.—The RA 27/3 rubella vaccine has been tested in England, Ireland, France, Israel, Iran, Switzerland, the Soviet Union, and Japan. The results of some of these trials have been published or will be presented later in the meeting. I would like to summarize the Swiss results, however, since Dr. Buser is not at this meeting. Dr. Buser's trials, summarized in Table 15, included both subcutaneous and intranasal vaccination. This trial is especially valuable because the serum specimens were tested blindly by Dr. A. Nicolas in Lyon. A seroconversion rate of 100% was obtained in 78 subjects given vaccine subcutaneously, while 43 contacts remained seronegative. Of 61 subjects vaccinated intranasally, 92% seroconverted, while none of 21 contacts did so.

Present Status of RA 27/3 Vaccine

Table 16 summarizes the data available several weeks ago on vaccination in man of RA 27/3 at the 25th to 29th passage levels.

With regard to clinical reaction, over 775 seronegative persons have received RA 27/3, 500 subcutaneously and the remainder intranasally. No serious reactions have been noted in these subjects, or in the 300 children vaccinated in Taiwan, or the more than 250 known seropositives that have been vaccinated—a total of more than 1,300 people.

In particular we note that nearly 150 seronegative women have received RA 27/3, of whom more than 100 were subcutaneously inoculated, without joint symptoms. Later in the conference, I understand that other studies will be reported in which there was a low percentage of joint symptoms. None of these, however, was incapacitating.

Virtually 100% of subcutaneous vaccinations, and more than 90% of intranasal vaccinations, have evoked antibodies.

Nearly 400 controls have remained seronegative despite contact with vaccinees.

Summary

In summary, the RA 27/3 strain gives a good antibody response in vaccinees, without significant reaction and without spread to contacts. Its advantages are that it is attenuated and produced in WI-38, it can be given to adult women, and it can be administered intranasally.

This investigation was supported in part by Pennsylvania Department of Health contract 68-593-1, ME-561 from the Commonwealth of Pennsylvania; and Public Health Service grant SO1-FR 05540 from the General Research Support Branch.

Dr. Plotkin is a recipient of an award from Smith, Kline and French, Inc., Philadelphia, for research on rubella vaccine.

References

1. Plotkin, S.A.; Cornfeld, D.; and Ingalls, T.H.: Studies of Immunization With Living Rubella Virus: Trials in Children With a Strain Cultured From an Aborted Fetus, *Amer J Dis Child* 110: 381-389 (Oct) 1965.

2. Plotkin, S.A., et al: A New Attenuated Rubella Virus Grown in Human Fibroblasts: Evidence for Reduced Nasopharyngeal Excretion, *Amer J Epidem* 86:468-477 (Sept) 1967.

3. Plotkin, S.A.: "Development of RA 27/3 Attenuated Rubella Virus Grown in WI-38 Cells," in *Proceedings of the 23rd Symposium on Microbiological Standardization: Rubella Vaccines*, London, November 1968, Basel, Switzerland: S. Karger, 1969.

4. Hayflick, L., and Moorhead, P.S.: The Serial Cultivation of Human Diploid Cell Strains, *Exp Cell Res* 26:585-621 (Dec) 1961.

5. Plotkin, S.A.: Plaquing of Rubella Virus in RK_{13} Cells, *Arch Ges Virusforsch* 16:423-425, 1965.

6. Fogel, A., and Plotkin, S.A.: Markers of Rubella Virus Strains in RK_{13} Cell Culture, *J Virol* 3:157-163, 1969.

7. Sedwick, W.D., and Wiktor, T.J.: Reproducible Plaquing System for Rabies, Lymphocytic Choriomeningitis and Other Ribonucleic Acid Viruses in BHK-31/13S Agarose Suspension, *J Virol* 6:1224-1226 (Dec) 1967.

8. Plotkin, S.A., et al: Intranasally Administered Rubella Vaccine, *Lancet* 2:934 (Nov 2) 1968.

9. Meyer, H.M., Jr., et al: Clinical Studies With A Attenuated Rubella Virus, *Pan American Health Organization Scientific Publication* 147:390-398, 1967.

10. Du Pan, M.R., et al: Clinical Trials With a Live Attenuated Rubella Virus Vaccine Cendehill 51 Strain, *Amer J Dis Child* 115:658-662 (June) 1968.