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Papers and Originals

Clinical and Laboratory Studies with Rubella Vaccines in Adults*

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Summary: Three live attenuated rubella vaccines were tested in adult volunteers. Clinical reactions were mild, but were more noticeable in vaccinated non-immune subjects than in control subjects. With the exception of two individuals, all of the remaining 54 subjects developed an immune response; the level of antibodies found was somewhat lower than that resulting from natural infection. Though virus could be isolated from some of the sero-negative volunteers after vaccination, no evidence was found of transmission of infection.

Introduction

Rubella is one of the mildest of human virus infections. The mortality is very low and there are few complications. The most serious problem is the effect of the virus on the foetus. Since this was first recognized by Gregg (1941) much new information has been obtained on the whole problem of congenital and other forms of rubella which has important bearings on the problem of prevention. The risk of congenital malformations after maternal rubella is associated with infection occurring in the first 16 weeks of pregnancy, but precise data on the overall effect of maternal rubella are complicated by the fact that foetal damage can result from subclinical infection in the mother. Various estimates have put the risk of foetal damage from death or malformations after rubella in the first 16-week period at between 10 and 30%, but it is very much higher in the first and second than in the third and fourth months (Dudgeon, 1967). Though rubella malformations account for a small percentage of all malformations, they are frequently severe and often multiple. The majority of cases of congenital rubella result from primary infection in the mother. Reinfection with rubella may occur; indeed, recent opinion (McCarthy and Taylor-Robinson, 1967; P.H.L.S., 1968) suggests that it may not be as uncommon as previously thought to be the case, but as yet there is no clear evidence that reinfection has led to foetal damage. In Britain the number of women of child-bearing age without antibody is about 10 to 20%, but the incidence of infection varies greatly from one part of the country to another.

The discovery that rubella virus could be grown in cell cultures opened up the way to the development of vaccines against rubella. Parkman *et al.* (1966) reported progress in the development of a live attenuated rubella vaccine. A strain of virus passaged in African green monkey kidney cells (HPV-77) was found to be of reduced virulence yet immunogenic in monkeys. When tested in susceptible children it was also found to be immunogenic, and very few reactions were observed. Though the majority of those vaccinated excreted virus in the nasopharynx, there was no demonstrable viraemia and no transmission of infection was detected in susceptible contacts (Meyer *et al.*, 1966, 1967).

Recently further attenuated vaccines have been developed in monkey kidney and avian cell cultures (Meyer and Parkman, 1969), in primary rabbit kidney (Huygelen and Peetermans, 1967), in duck embryo (Buynak *et al.*, 1968), and in human diploid fibroblast cells (Plotkin *et al.*, 1967). Because of nasopharyngeal excretion in vaccines and the potential risk to a pregnant woman, clinical trials have for the most part been conducted in enclosed communities, and most of these have been in children confined to institutions. For a number of reasons it was decided that preliminary trials in this country would be carried out in adult volunteers living in enclosed communities. This report presents the preliminary findings of clinical and laboratory studies of three attenuated rubella vaccines in adults.

Materials and Methods

Objects of Trials.—These trials had four objectives. We wished to determine in non-immune and immune subjects (1) the clinical reactions to the vaccines, (2) the immune response, both short-term and long-term, (3) the frequency and duration of virus excretion, and (4) whether transmission of infection occurred.

Study Population.—Volunteers taking part were all members of religious communities. Their ages ranged from 18 to 70 years.

Plan of Trials

The trials were carried out in two parts. The main trials were made in convents and monasteries of enclosed orders where there was no direct contact with the general population, so as to avoid contact of vaccinated individuals with pregnant women. Detailed virological studies in these were undertaken to provide answers to the third and fourth objectives of the trials. Subsidiary trials were carried out in semi-enclosed communities, laboratory studies being limited to serological estimations.

Initially, blood was taken from the volunteers to determine their immune status to rubella. Vaccination was usually carried out within 7 to 10 days of collection of this blood sample. Of 556 individuals from 17 convents and

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monasteries who volunteered 62 (12·1%) were found to be non-immune (seronegative) and 55 of them were vaccinated. Eighty-seven subjects with pre-existing immunity (seropositive) were also vaccinated.

Depending on the numbers available, and in particular on the numbers of non-immune (seronegative) subjects in each community, the volunteers for the main trials were allocated to three groups:

Group 1. Non-immune (seronegative) group: *vaccinated*.

Group 2. Non-immune (seronegative) group: *initially unvaccinated* in order to determine whether transmission of infection occurred.

Group 3. Immune (seropositive) group: *vaccinated*.

The allocation of seronegative individuals to groups 1 and 2 was made in a proportion of approximately 2:1. Five of the 21 individuals allocated to group 2 were chosen on account of either age (over 56 years) or a medical contraindication to vaccination ; otherwise allocation was random.

The main trials were carried out in two phases. In phase I the seronegative individuals allocated to group 1 were vaccinated and those allocated to group 2 were left unvaccinated. A number of individuals (group 3) with varying levels of rubella antibody (seropositive) were also vaccinated. Nose and throat swabs were collected for virus studies during the 29 days after vaccination from each volunteer in the three groups. Blood samples were collected on day 29 and again during days 41 to 43 after vaccination, when phase II was started with the vaccination of the unvaccinated individuals who had comprised group 2. Subjects who were over 56 years of age or for whom there was a medical contraindication for vaccination remained as unvaccinated control subjects throughout both phases of the trials. In phase II the same procedure was followed as in phase I.

On all matters there was close consultation with the practitioners caring for these communities. They assisted in the initial clinical assessment, in particular to determine whether adenopathy was present before vaccination, and kept observations throughout the trials assisted by the infirmarians staff, who were responsible for ensuring that specimens were collected and observations recorded.

Each individual participating in the trials recorded his or her temperature twice daily on rising and on retiring from the day of vaccination (day 0) to day 29. They also recorded on forms provided any symptoms on the day when these were observed.

Though the daily routine varied from one community to another, the general pattern of communal life was similar. There were frequent opportunities for daily contact in chapel, at refectory, at work, and during study, totalling about 12 hours per day. Volunteers occupied separate cells, with the exception of two convents (Nos. 11 and 12) where they slept in dormitories.

Later, as the results of the main trials became available, subsidiary trials in certain less strictly enclosed religious communities, such as seminaries and training colleges, were carried out. Swabs were not collected in these groups ; serological tests were used to detect the immune response and whether there was any evidence of cross-infection.

Vaccines

Much information has already been obtained with the HPV-77 strain, so it was decided to carry out trials with three other attenuated vaccines which had recently been developed. Details of these are given in Table I. All three vaccines were presented in a lyophilized form and were stored at −20° C. until used. The vaccines were reconstituted in distilled water, and 0.5 ml. was injected intramuscularly into the left deltoid region. The vaccines[1] had been approved by the Medical Research Council's Committee on Immunological Products and by the Dunlop Committee.

TABLE I.—*Attenuated Rubella Vaccines Used in Trials*

Vaccine	Vaccine Strain Designation	Cell Substrate	Passage Details	Developed by
Vaccine A	Cendehill 51/2	Primary rabbit kidney cultures	51st passage	Huygelen and Peetermans (1967)
Vaccine B	HPV-77 DE 5	Duck embryo tissue cultures	HPV-77 duck embryo—5	Buynak *et al.* (1968)
Vaccine C	RA/27/3	Human diploid fibroblast cultures (WI-38)	Seed virus in 25th passage; 2 additional passages in WI-38 in U.K.	Plotkin *et al.* (1967)

Laboratory Studies

Sera.—Before vaccination and on days 29 and 41–43 after vaccination 5 to 10 ml. of blood was obtained by venepuncture. Sera were separated aseptically and stored unheated at −20° C. Further specimens are to be collected at yearly intervals to determine the persistence of antibody.

Nose and Throat Swabs.—Swabs were taken from each volunteer on the day of vaccination (day 0) and on alternate days after vaccination from day 7 through to day 29. Nose and throat swabs were collected separately into 3 ml. of virus transport medium and were stored at −20° C. until tested for virus.

Media.—Virus transport medium consisted of medium 199 with 0·08% sodium bicarbonate, 0·5% bovine plasma albumin, and 400 units of penicillin and streptomycin per ml. The growth and maintenance media for V3A and RK 13 cells were the same as previously described from this laboratory (Plotkin *et al.*, 1963 ; Dudgeon *et al.*, 1964). The maintenance medium for primary patas monkey kidney consisted of Eagle's medium, 1% lactalbumin hydrolysate, 0·5% bovine plasma albumin, and 1% foetal calf serum.

Virus Isolation.—Inocula were prepared from aliquots of nose and throat swabs, which were thawed immediately before inoculation. 0·2 ml. was inoculated into four culture tubes of each of the following cell cultures: (1) V3A (a continuous line of vervet monkey kidney cells) for detection of respiratory and other viruses, and (2) RK 13 and (3) primary patas monkey-cell cultures for detection of rubella virus. Cultures were placed on to maintenance medium before inoculation and were incubated at 35° C. on roller drums.

V3A Cells.—Material from swabs collected on day 0 and day 7 only were inoculated. Cultures were examined on day 5 and again on day 7 for evidence of cytopathic effect and haemadsorption. Cultures were not passaged unless there was evidence of virus growth.

RK 13 Cells.—Cultures were inoculated with material from swabs collected on day 0 and on alternate days from day 7 to day 29. Tubes were examined microscopically on days 5, 7, and 10 for evidence of cytopathic effect, after which the cultures were harvested.

Patas Cells.—The material inoculated was the same as for the RK 13 cells. After 10 days' incubation two tubes from each set of inoculated cultures were tested for interference by challenge with 100 $TCID_{50}$M6 E.C.B.O. virus. Tubes were examined for evidence of cytopathic effect from the M6 virus. After 48 hours' incubation the cultures which had not been inoculated with the challenge virus were harvested.

All specimens in both RK 13 and patas monkey kidney cultures were passaged blindly three times after primary inoculation.

[1] We are indebted to Dr. A. Prinzie, R.I.T., Genval, Belgium, for supplies of vaccine A ; to Dr. Maurice R. Hilleman, Merck Institute for Therapeutic Research, West Point, Penn., U.S.A., for supplies of vaccine B ; and to Dr. A. H. Griffith, Wellcome Research Laboratories, Beckenham, Kent, for supplies of vaccine C.

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Serology

Haemagglutination-inhibition (H.A.I.) Test.—Sera were tested with the micromethod described by Stewart *et al.* (1967), with the modification that the suspension of day-old chick red cells (0·3%) was prepared in dextrose gelatin–veronal buffer containing 0·5% B.P.A. Known positive and negative control sera of human and rabbit origin were included in each test. The end-point was taken as the reciprocal of the highest dilution of serum showing complete inhibition of agglutination. Titres were expressed as the initial serum dilution before addition of the antigen.

Neutralization Tests.—These were carried out in RK 13 cells on the majority of sera by the method previously described from this laboratory (Dudgeon *et al.*, 1964). Sera were tested unheated.

Complement-fixation Tests.—These were carried out by a microtechnique with a cell-associated antigen according to the method described by Sever *et al.* (1966).

Results

The results of the main trials are set out in Tables II–V and are summarized in Table VI together with the results of the subsidiary trials.

TABLE II.—*Symptoms Observed in Vaccinated Groups and Controls*

Vaccine	No. of Subjects	Temp. > 99°	Temp. > 100°	Rash	URTI	Adeno-pathy	Joint Pains
		Group 1. Seronegative Vaccinated					
A	22	8	1	2	5	8	—
B	18	3	2	—	4	3	7
C	15	4	—	1*	4	—	—
		Group 2. Seronegative Unvaccinated					
A	11	1	—	—	1	1	—
B	10	—	—	—	—	—	—
C	5	1	—	—	—	—	—
		Group 3. Seropositive Vaccinated					
A	26	3	1	1	2	3	—
B	20	—	—	—	3	5	—
C	41	1	—	—	2	—	—

* Herpes zoster.

Clinical Evaluation

The symptoms recorded for the three groups were all mild (Table II). They occurred in all three groups, but were more noticeable and consistent in the seronegative vaccinated group (group 1). A slight fever (⩽100° F. ; 37·8 C.) and sore throat following all three vaccines were noted on or about day 6 or 7 ; again this was more noticeable in group 1. A number of subjects receiving vaccines A and B developed a rubella-like adenopathy around the 9th to 13th post-vaccination day.

A mild arthralgia involving knee, ankle, elbow, or metatarso-phalangeal joints developed in seven female volunteers given vaccine B. The joint pains lasted one to three days and occurred between the 14th and 28th day after vaccination.

With vaccine A two volunteers developed rashes which appeared to be allergic in type (one of these had suffered from the same type of rash previously and they both responded to antihistamines). One individual developed herpes zoster on the skin supplied by the first division of the trigeminal nerve seven days after receiving vaccine C. There were no instances of rashes resembling rubella.

At one monastery (Table IV, No. 6) an outbreak of respiratory infection occurred in the community a few days after vaccination. Parainfluenza viruses were isolated from a number of individuals participating in the trials.

Antibody Responses

Details of the immune responses to the individual vaccines are shown in Tables III, IV, and V together with the results of the virus isolations. With the exception of one individual receiving vaccine B and one receiving vaccine C, all sero-negative vaccinated subjects developed an immune response (see Table VI). The geometric mean antibody titres for the three vaccines were 44, 59, and 61 respectively. No obvious differences in the antibody response could be detected between those from whom virus was recovered and those from whom it was not. None of the unvaccinated seronegative subjects developed antibody following exposure to vaccinated individuals who were shown to be excreting virus. Subsequently when they were vaccinated they developed an immune response. The results of neutralization tests also showed a rise in rubella antibody, but none of the 20 sera tested showed any evidence of complement-fixing antibody 42 days after vaccination.

Twelve out of 87 subjects with pre-existing antibody who were vaccinated showed a fourfold to eightfold increase in haemagglutination-inhibition antibody (Table VI). These increases in relation to prevaccination titres were as follows:

	Prevaccination H.A.I. Titres 4–8	16–32	> 64
No. showing fourfold to eightfold increase/No. vaccinated	5/33	6/30	1/24

TABLE III.—*Cendehill Vaccine (A)*

Community No.	Phase	Vaccinated. No. Antibody (Seronegative): Subject No.	Age	H.A.I. Antibody Levels: Pre-vacc.	D.29	D.42	Virus Isolated Nose/Throat Swab	Unvaccinated Controls. No. Antibody (Seronegative): Subject No.	Age	H.A.I. Antibody Levels: Pre-vacc.	D.29	D.42	Virus Isolated Nose/Throat Swab
Convent 2	I	63	37	<4	64	32	—	61	40	<4	N.T.	<4	—
		73	25	<4	16	16	11,13	80	43	<4	N.T.	<4	—
		82	23	<4	16	32	—	83	34	<4	N.T.	<4	—
		77	42	<4	32	64	11						
	II	80	43	<4	32	32	—	61	40	<4	<4	<4	—
		83	34	<4	64	64	9						
Convent 5	I	174	46	<4	64	64	13, 15	166	71	<4	N.T.	<4	—
		181	24	<4	64	64	—	176	41	<4	N.T.	<4	—
	II	176	41	<4	32	64	—	166	71	<4	<4	<4	—
Convent 12		726	20	<4	64	128	—	753	20	<4	N.T.	<4	—
		736	23	<4	64	64	13, 17	754	19	<4	N.T.	<4	—
		737	18	<4	64	64	—	757	18	<4	N.T.	<4	—
		740	25	<4	64	128	—	768	27	<4	N.T.	<4	—
		747	19	<4	64	128	—						
	II	753	20	<4	32	32	—						
		754	19	<4	16	16	—						
		757	18	<4	128	128	—						
		768	27	<4	32	16	—						
Convent 3	I	160	59	<4	16	16	11, 13, 25						
Convent 4	I	149	36	<4	32	16	11, 13, 15						
Convent 15	I	836	52	<4	8	8	—						
		838	21	<4	64	128	—						

N.T. = Not tested.

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TABLE IV.—*Duck Embryo Vaccine (B)*

Community	Phase	Vaccinated. No. Antibody (Seronegative)						Unvaccinated Controls. No. Antibody (Seronegative)					
		Subject No.	Age	H.A.I. Antibody Levels			Virus Isolated Nose/ Throat Swab	Subject No.	Age	H.A.I. Antibody Levels			Virus Isolated Nose/ Throat Swab
				Pre-vacc.	D.29	D.42				Pre-vacc.	D.29	D.42	
Monastery 6	I	89	23	<4	16	32	13, 15, 17, 19	93	25	<4	<4	<4	—
		107	37	<4	32	32	—	100	34	<4	<4	<4	—
		112	40	<4	64	128	*	110	39	<4	<4	<4	—
		119	45	<4	32	32	11	135	75	<4	<4	<4	—
		124	54	<4	32	64	11,13						
	II	93	25	<4	16	16	—	100	34	<4	<4	<4	—
		110	39	<4	64	64	15, 17	135	75	<4	<4	<4	—
Convent 7	I	211	54	<4	32	32	15, 17, 25	209	56	<4	<4	<4	—
		220	39	<4	32	32	—	210	56	<4	<4	<4	—
		223	34	<4	64	64	15, 17, 19						
		227	33	<4	<4	<4	—						
	II	209	56	<4	16	32	—						
		210	56	<4	64	64	—						
Convent 16	I	853	37	<4	128	256	—	872	69	<4	N.T.	<4	—
		854	36	<4	128	128	—	855	67	<4	N.T.	<4	—
		866	36	<4	32	128	—						

* Parainfluenza virus type 3 isolated.

TABLE V.—*Diploid Vaccine (C)*

Community No.	Phase	Vaccinated. No. Antibody (Seronegative)						Unvaccinated Controls. No. Antibody (Seronegaztive)					
		Subject	Age	H.A.I. Antibody Levels			Virus Isolated Nose/ Throat Swab	Subject No.	Age	H.A.I. Antibody Levels			Virus Isolated Nose/ Throat Swab
				Pre-vacc.	D.29	D.42				Pre-vacc.	D.29	D.42	
Convent 11	I	466	20	<4	32	32	—	460	20	<4	<4	<4	—
		462	20	<4	<4	<4	—	536	29	<4	<4	<4	—
	II	460	20	<4	16	32	—	536	29	<4	<4	<4	—
Seminary 10	I	287	28	<4	256	128	17	317	24	<4	<4	<4	—
	II	317	24	<4	64	64	—						
Convent	I	823	32	<4	8	32	—	814	33	<4	<4	<4	—
		818	32	<4	256	256	—						
Seminary 9	I	317	24	<4	64	32	—						
		374	26	<4	32	64	—						
Convent 8		206	20	<4	128	128	—						

TABLE VI.—*Summary of Results in Vaccinated and Unvaccinated Subjects in Main and Subsidiary Trials*

Vaccine	Seronegative					Seropositive		
	No. Vaccinated	No. (and %) Showing Seroconversion	Geom. Mean Antibody Titre	Proportion of Virus Isolation	No. of Seronegative Contacts Showing Seroconversion	No. Vaccinated	No. (and %) Antibody Response‡	Proportion of Virus Isolation
A	22	22* (100)	44	7/22†	0/11	26	4 (15·3)	0/26
B	18	17* (94·4)	59	6/16†	0/10	20	4 (20)	0/14§
C	15	14* (93·3)	61	1/10†	0/6	41	4 (9·7)	0/17

* Includes results of main and subsidiary trials. † Results of main trials only. ‡ Fourfold or greater increase in antibody titre. § Parainfluenza virus type 3 isolated from 3/14.

Virus Isolation

Virus was isolated from a proportion of seronegative vaccinees varying from 9 to 50% with all three vaccines. In the case of vaccine A (Table III) virus was recovered between the 9th and 25th days, with maximum isolations from 23% of the vaccinees on the 11th and 13th days. After vaccine B (Table IV) virus was recovered between the 11th and 25th days but maximum isolations (37% of vaccinees) were on the 15th and 17th days. In some subjects virus excretion appeared to be intermittent. Virus was isolated from only one subject receiving vaccine C on day 17, but so far this vaccine has been used less extensively.

Most of the isolations were made on the first passage, a few on the second, and none thereafter. Both cell culture systems were equally sensitive in our hands, but the RK13 cells were easier to read owing to the marked cytopathic effect of the vaccine strains. Preliminary observations indicate that the average titre of virus in the swabs was $10^{0.5}$ to $10^{0.6}$ per ml., which is considerably less than in natural or congenital infections.

None of the unvaccinated seronegative or vaccinated seropositive individuals were found to excrete virus.

In one community (Table IV, No. 6) parainfluenza type 3 virus was isolated in V_3A cultures from the swabs collected on day 7 from one seronegative vaccinee and from three seropositive vaccinees (Table VI).

Transmission of Infection

No evidence was obtained from these studies that infection had been transmitted from vaccinees to susceptible individuals despite the fact that they had been in contact with the vaccinees who were found to be excreting virus. In assessing the significance of these findings it must be emphasized that each community represented a separate epidemiological unit, and, though the numbers in each were small, there were with vaccines A and B about equal numbers of individuals shown to be excreting virus and susceptible contacts. Only one contact with a known virus excreter has been identified with vaccine C. Furthermore, it can be seen from Tables III–V that none of the unvaccinated control subjects showed evidence of sero-conversion and some were in contact for two periods during which time they were exposed to vaccinated individuals excreting virus.

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Discussion

We set out to answer a number of questions concerned primarily with clinical reactions, immune response, infectiousness, and transmissibility of attenuated rubella vaccines. The answers to these are intimately concerned with the safety and acceptability of any rubella vaccine. Within the limitations of these trials, to some extent imposed by the numbers of susceptible subjects and also by the environmental conditions, most of the questions have been answered, some more satisfactorily than others.

The clinical reactions were all mild. They were not confined to the seronegative vaccinated group, but were most frequent in them. The only unusual reaction was arthralgia, which was noted in seven out of nine seronegative females after receiving vaccine B. No such reactions were noted in seven seronegative male volunteers or in any subjects with previous immunity given the same vaccine. None of the symptoms, either arthralgia or adenopathy, were severe enough to interfere with the daily activity of those participating, and it was the general consensus of opinion of all who took part in these trials that these vaccines were certainly acceptable. These reactions, mild though they were, were slightly more noticeable than in previous reports (Lepow *et al.*, 1968 ; Du Pan *et al.*, 1968a, 1968b). This could be related to age, as all the volunteers were adults and clinical manifestations of natural rubella are generally more noticeable in adults than in children.

The immune responses to the vaccines are in keeping with the results published by other investigators. At first sight these titres, which are about four times lower than those usually found in patients convalescent from rubella, are somewhat lower than those reported by Du Pan *et al.* (1968a) and Meyer *et al.* (1968), but it does not seem profitable at this stage to compare titres obtained in different laboratories. The technique for measuring haemagglutination-inhibition antibody varies from one laboratory to another as does the interpretation of the titres, and more especially because there is as yet no international reference serum.

As with other investigators, we encountered the occasional case in which an immune response could not be shown after vaccination. One female subject given vaccine B had failed to develop haemagglutination-inhibition antibody by 84 days. She was revaccinated and developed an immune response. One female subject given vaccine C had no demonstrable antibody on day 42 and unfortunately left the country before a further blood sample could be collected. This could have been an instance of delay in antibody development, a feature reported by Cooper *et al.* (1968), or to a failure due to an insufficient antigenic stimulus. The failure to detect complement-fixing antibody after vaccination is in contrast to the response usually found after natural infection. Meyer *et al.* (1968) reported similar results. This could be due to a delay in antibody production or to the cell-associated antigen used in the test.

Meyer *et al.* (1968) and Lepow *et al.* (1968) reported that the antibody level after vaccination was higher in those excreting virus. We could find no such difference. The number of vaccinated subjects found to be excreting virus, which was higher with the rabbit kidney (A) and duck embryo (B) vaccine than with the diploid (C), was lower than in other reported series. This could be due to technical considerations or to a difference perhaps dependent on the age of our volunteer subjects. However, the immunogenic potency of rubella vaccine strains does appear to be related to the factor of virus excretion, as some strains which have been overattenuated and produce little virus excretion show seroconversion rates of 60% or less (P. D. Parkman and H. M. Meyer, personal communication, 1968). But aside from this the important question is in relation to safety. Does virus excretion matter? Can attenuated vaccine strains spread from person to person, and, if so, could they cause damage to a foetus? These are two separate questions, but they are closely linked.

To date, several hundred susceptible subjects have been vaccinated with various rubella vaccines and no cases of contact infection have been reported. The reasons for the lack of transmission are not fully understood. It could be due to quantitative factors, as it is clear that the amounts of virus excreted in vaccinated persons are much less than in natural infections and in congenital rubella infants. Lack of transmission could be simply due to the process of attenuation. There is evidence that some attenuated strains show a lack of infectivity for the nasal mucosa (Du Pan *et al.*, 1968a), and earlier observations by Meyer *et al.* (1967) showed that the attenuated HPV-77 strain was of reduced virulence in pregnant monkeys and did not cross the placenta. This is the crux of the question so far as human beings are concerned, as the ultimate test of safety of a vaccine strain is that, even if the virus does cross the placenta, it should be non-pathogenic for the foetus. Despite the fact that the susceptible unvaccinated subjects were in contact with individuals shown to be excreting virus, in some cases for prolonged periods, no evidence of transmissions was obtained.

It can be argued that the environmental conditions in these communities compared with those, for example, in a household, were not conducive to the study of the spread of rubella, despite the fact that in these communities more than half the day was spent in fairly close contact. This aspect of the trials is probably the least satisfactory; it is one which is being studied in current investigations.

These results, taken in conjunction with others already reported, are encouraging, but inevitably at this stage in the development of a new prophylactic a number of questions arise. The object in developing a rubella vaccine is to prevent foetal damage by active immunization of susceptible individuals before pregnancy. The method must be safe and effective. The evidence obtained so far on the immune responses to the rubella vaccines is encouraging, but evidence on the protection afforded by vaccine-induced immunity compared with that of natural infection is limited (Parkman *et al.*, 1966). Not only will it be important to study the persistence of immunity over a period of years and in this we are in a position to observe the long-term response to vaccination in enclosed communities—but if reinfection occurs it will also be important to determine whether virus multiplication is limited to the nasopharyngeal mucosa. Freedom from reactions is clearly desirable with any vaccine, and in this respect these rubella vaccines earn good marks. All these vaccines have been prepared in different cell substrates, a primary mammalian cell (rabbit), a primary avian cell (duck), and in human diploid fibroblast culture. Which of these will prove to be the most acceptable in the long run it is too early to say, but these are some of the problems that can only be answered by continued observation.

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Pregnancy and the Nephrotic Syndrome

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Summary: Nineteen patients with nephrotic syndrome, 13 with histological diagnosis, were studied throughout 31 pregnancies. Eight were diagnosed for the first time during pregnancy.

Antenatal problems due to severe oedema, urinary tract infection, and refractory orthochromic anaemia were encountered. Eight patients were hypertensive at booking, and in two of these pregnancy was terminated; three others had a significant increase in blood pressure. In 12 of the remaining pregnancies a rise in blood pressure of 20 mm. Hg or more occurred towards term.

There were 29 live births (including one set of twins), one stillbirth due to a cord accident, and one neonatal death. The infant birth weight, apart from being affected by hypertension, was related to the maternal serum albumin level.

The patients have been under observation for up to 20 years. Fifteen have not shown any deterioration of renal function during the prolonged period of observation. One developed oliguric renal failure immediately post partum and three others died, two, four, and 12 years after their pregnancies.

Introduction

The relationship between pre-eclampsia and the nephrotic syndrome has remained confused and poorly understood, largely owing to the varied morbidity and clinical course of both conditions. Without renal biopsy or long-term clinical studies the distinction may be extremely difficult. This similarity has led to the misdiagnosis and underreporting of the coexistence of nephrotic syndrome with pregnancy, estimated by Wegner (1937) as occurring in 0·028% of pregnancies. The nephrotic syndrome has been claimed to result from severe pre-eclampsia (Hopper *et al.*, 1961; Sarles *et al.*, 1964), and cyclical nephrotic syndrome has been reported as occurring only during pregnancy (Schreiner, 1963).

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The established view of Dieckmann (1936) and Wegner (1937) that pregnancy had a deleterious effect on chronic renal disease has been challenged by Seftel and Schewitz (1957), Silberman and Adams (1962), Marcus (1963), and Johnston *et al.* (1963), who have stressed the good maternal prognosis during pregnancy and the low rate of foetal loss. This communication is a study of 31 pregnancies occurring in 19 patients with the nephrotic syndrome. Many of these patients have been under careful long-term surveillance for more than 10 years. These pregnancies can therefore be viewed as isolated occurrences against the natural history and pathology of the renal disease, thus correcting the lack of long-term follow-up available in previous publications.

The cases have been selected by rigid biochemical criteria (Squire *et al.*, 1957), all having had at some time a serum albumin of less than 2 g./100 ml. and a proteinuria of more than 5 g./day. In eight patients (Table I) the nephrotic syndrome was first diagnosed during pregnancy, though in two of these there was a past history of previous acute renal disease. In the remaining 11 patients (Table II) renal disease had been present from 1 to 15 years before the pregnancies studied. Five patients in this group were receiving steroid therapy throughout eight pregnancies and two others had already completed successful courses of steroids before the onset of their three pregnancies. Two of these patients (Cases 10 and 11) and one patient (Case 18) who exhibited a progressive spontaneous recovery after the first pregnancy were the only patients who did not show severe clinical and biochemical evidence of the nephrotic syndrome at the time of the pregnancies. Two pregnancies were terminated at 16 weeks by hysterotomy on account of increasing oedema and hypertension. Apart from the 31 pregnancies in the present study, there were two spontaneous abortions without biochemical data.

Histological Diagnosis

Renal biopsy was performed before or after pregnancy in 13 cases. Case 5 had a second biopsy one year after the pregnancy and Case 2 had two renal biopsies following the initial biopsy taken at the time of caesarean section. The staining techniques and criteria of histological diagnosis were those used by Brewer (1964). The clinical history and progress, bio-

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