Intranasal Vaccination with Attenuated Measles Virus. (28131)

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Successful isolation and adaptation of measles virus in tissue culture systems heralded development of an attenuated measles virus vaccine by Enders and his associates (1-3). Recent studies have demonstrated the efficiency of this attenuated virus by parenteral inoculation in producing modified measles and immunity to natural wild measles (4-8). Attempts to inoculate human volunteers with attenuated virus into conjunctiva, oral cavity or respiratory tract have been investigated by several workers because such routes are more convenient, more natural and presumably safer. However, the results have been inconsistent(7-12). This paper presents successful results of vaccination with tissue culture adapted measles virus by repeated nasal drops in groups of children.

Material and methods. Virus vaccine. Edmonston strain of measles virus was used for vaccination. It had previously been passed 24 times in primary human kidney cells, 28 times in primary amnion cells, 6 times in developing chick embryo and 19 times in chick fibroblast cells before a further 12 passages in primary chick embryo fibroblasts in this laboratory. Tissue culture fluids were used for intranasal vaccination after safety testing on blood agar plates, in suckling mice and in 2 Taiwan monkeys. Infectivity in HeLa cell tubes was 10^4-10^5 per ml after 7 days incubation.

Test subjects. Forty-nine children were selected for vaccination. Forty-three were residents of 2 orphanages and 6 were from families in Taipei City. There were 20 children 7-19 months of age and 29 children 3-8 years old. None of them had a past history of measles.

Vaccine administration. One-tenth ml of vaccine was inoculated into each nasal cavity by dropper and repeated in 10 minutes. After inoculation, subjects were kept in supine position for 15 minutes.

Clinical observation. After vaccination, tested children were checked closely for body temperature twice a day by parents or nurses in the orphanage. After the fourth day of vaccination they were also examined regularly by the author or orphanage doctor.

Antibody determination. Blood specimens were collected from 15 cases before and 3-6 weeks after vaccination. Post-vaccination specimens only were available from 7 other children. Neutralizing antibody was measured in HeLa cell tube cultures with a 0.2 ml equal volume mixture of serum dilution and Edmonston strain virus antigen (100 $TCID_{50}$/0.1 ml) which had been previously incubated for one hour at 4°C. The results were read at 7 days when the virus control tubes showed extensive cytopathogenic effect with destruction of over half the cells. The neutralizing titer was the highest serum dilution completely protecting duplicate tubes from cytopathic effect (syncytia).

Results. There was no immediate reaction to vaccine, either local or systemic, in any inoculated child. The most common symptom suggesting take of vaccination was fever (78%). Frequency, time of onset and duration of fever in subjects divided into 2 age groups are presented in Table I. The highest fever observed in each child is shown in Table II. Incidence of fever was about the same in each age group, while development of extremely high fever (>40°C) was almost limited to infants. Skin eruptions which appeared in 29% of children were more common in infants (45%) than older children (17%). The rashes observed were fine macu-

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TABLE I. Occurrence of Fever and Rash by Age Group in 49 Children Inoculated with Attenuated Measles Vaccine.

Age group	No. of test subjects	No. with fever	Onset of fever* Mean	Onset of fever* Range	Duration of fever in days Mean	Duration of fever in days Range	No. with rash	Onset of rash* Mean	Onset of rash* Range	Duration of rash in days Mean	Duration of rash in days Range
7-19 mo	20	15	7.8	5-13	3.6	1-7	9	12.8	9-14	1.8	1-3
3- 8 yr	29	23	7.9	5-10	3.5	1-7	5	12.7	12-14	1.8	1-2

* Days after inoculation.

lopapular, sparse and discrete, resembling rubella more than classical measles. The rash persisted one day in 4 cases, 2 days in 8, and 3 days in 2. It involved only the face and neck in 4 cases, the face, neck and trunk in 9 and was generalized in one. Pigmentation was observed only in the case with generalized skin eruption and it disappeared completely in 10 days. Enathema was noted only in 2 infants; the one who had generalized skin eruption had typical Koplick's spots. The general condition and activity of the children showed little or no change from normal even in presence of high fever (Table III). Catarrhal symptoms of any type were found in less than half. Five of the diarrhea cases and 2 of vomiting occurred in one orphanage where similar symptoms were occurring in nonvaccinated infants. Pneumonia, central nervous system involvement, or secondary bacterial infections following vaccination were not encountered in any of the children. Cross infection was not found among uninoculated children.

Among 15 paired sera obtained, 2 had antibody before vaccination and showed no rise, while 13 without antibody before vaccination all developed neutralizing antibody. Seven additional sera collected 3-6 weeks after vaccination from children on whom no acute blood was obtained all contained neutralizing antibody. These 20 convalescent sera showed titers of 1:10 to 1:60. The mean titer of those bled 3 weeks after vaccination was 1:28 compared to 1:50 in those bled after 4-6 weeks. Two children found to have antibody in their acute serum failed to develop symptoms of illness.

TABLE II. Height of Fever by Age Group in Children Inoculated with Attenuated Measles Vaccine.

	7-19 mo	3-8 yr	Total
No. with fever	15	23	38
37.5–38°C	2	2	4
38.1–39	1	10	11
39.1–40	7	10	17
40.1–	5	1	6
No. without fever	5	6*	11
Total vaccinated	20	29	49

* Includes at least 2 cases with neutralizing antibody prior to vaccination.

TABLE III. Additional Symptoms and Findings in 49 Children Given Attenuated Measles Vaccine.

	7-19 mo	3-8 yr	Total
Test subjects	20	29	49
Cough	5	5	10
Coryza	8	6	14
Conjunctivitis	3	2	5
Anorexia	7	7	14
Sleeplessness	4	1	5
Crying	9	1	10
Diarrhea	5	2	7
Vomiting	2	0	2
Loss of activity	1	0	1

Discussion. In this study administration of live attenuated measles vaccine by the nasal route produced signs and symptoms of modified measles in a very high percentage of subjects and neutralizing antibody in all tested who did not have antibody in their acute serum. This vaccine is inexpensive and children can be inoculated in large numbers without being frightened by use of syringe and needle. Use of this route does not cause unpleasant local or systemic side effects.

Other studies found the takes by nasal or oral route of vaccination to be irregular. Serological responses by this route were found to be 36% by Black *et al.*(10), 4% by Somorodinstev *et al.*(7), and from 12% to 88% in 4 studies by McCrumb and co-workers(8,9). Okuno(12) reported that nasal spray of around 10 $TCID_{50}$ dose of virus produced immunity in nearly all vaccinated children. Our results with nasal

drops are very similar to Okuno's in that both clinical and immunologic reactions were indistinguishable from those obtained by parenteral route.

Rates of complication and death following natural measles are influenced by age of the child when infected, nutritional and health status, and medical facilities of the population. In most well-developed countries, mortality from measles is so low that acceptability of live attenuated measles vaccination has been questioned because of side reaction of high fever. The situation in Taiwan is different. Data from the Provincial Public Health Administration of Taiwan reveals that about the same number of deaths per year (500) could be attributed to measles in Taiwan as in the entire United States with 20 times the population. Prevention of measles infection as a cause of death is more important in Taiwan than in western countries. Consequently, we do not believe that side reactions of attenuated measles vaccine will discourage use of this vaccine on Taiwan.

Large scale field studies of protection of vaccinated children in measles epidemics are needed to verify advantages of live attenuated vaccine administered by the nasal route.

Summary. Edmonston strain measles virus attenuated by repeated passages in chick fibroblast cells was inoculated by nasal drops into 49 infants and children. No signs of immediate reaction to vaccine were observed. Five to 13 days after vaccination 78% of the tested children developed fever. Mild skin eruptions occurred in 29% with onset 9-14 days after inoculation. Sickness or restricted activity was rare even during periods of fever. Both high fever and skin eruption occurred more frequently in infants under 20 months of age. All 13 children tested who did not have antibody in their acute serum developed neutralizing antibody after vaccine inoculation.

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Antigenic Relationship of 1961-1962 Type B Influenza Viruses to Earlier Type B Strains. (28132)

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The changing antigenic constitution of influenza viruses is under continual surveillance to meet the practical problems which arise when viruses become prevalent which differ from predecessors of the same type. The evolution of a new strain of virus presents prob-

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