tium in cow's milk. Thus, strontium-87m as a dilute aqueous solution of strontium chloride was added to milk from a cow which had received strontium-85 intravenously. Urines were collected from 6 adult volunteers after ingestion of this milk mixture. It is shown that the availability of strontium in the milk is equal to that of strontium as chloride in dilute aqueous solution. The consequences of the result upon the specific activity of strontium-90 from fallout in certain body pools are discussed.

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Measles Vaccination. I. Serologic Responses to Vaccination with Inactivated Vaccine. (27452)

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That measles has created a problem in homes for the mentally retarded may be one reason why programs of prophylaxis and studies of attenuated measles vaccine have been carried out in such populations(1,2). However, the same reasons for desiring to protect an unusually susceptible group of patients with involvement of the central nervous system enjoin caution in employing a living vaccine that may produce fever and some of the symptoms of the natural disease. To protect susceptibles in our vulnerable population and minimize side-effects, our interests seemed better served by use of an inactivated measles virus vaccine than by use of a living vaccine.

The following studies were directed toward determining (a) incidence of serologically-susceptible patients in a closed population, (b) antibody responses of serologically-immune and susceptible individuals to vaccination with an inactivated-measles virus-vaccine, (c) persistence of antibody titers induced by vaccination, and (d) protective value of the serologic evidences of immunity (natural or induced) if challenged by naturally occurring measles.

Materials and methods. Neutralizing antibody (NA) titers for determination of serological susceptibility or immunity were carried out in cultures of transformed human amnion cells with the Philadelphia 6 strain (3) of measles virus. Six-tenths ml of undiluted, heated (56°C, 30 minutes) serum was mixed with 0.3 ml of virus (30-50 TCD_{50} per 0.1 ml) in Hanks' balanced salt solution and incubated for 1 hour at room temperature. Each mixture in an amount of 0.2 ml was then added to each of 5 cell cultures and the stationary cultures incubated for 21 days at 36.5°C, during which time they were frequently examined for presence or absence of measles virus-induced cytologic changes. NA titers following vaccination were similarly determined after a 21-day period except that 0.3 ml of serum and 100 TCD_{50} per 0.1 ml of virus were employed. Sub-passages of cells and fluids from cultures in which the indicator virus was neutralized failed to show pres-

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ence of active virus. NA titers reported below were determined on the same day in a single neutralization test.

In several instances, measles hemadsorption-inhibition tests(4) were performed and results confirmed neutralization test data. After examination for presence or absence of cytopathogenic effects, cultures were washed once with 1.0 ml of chilled phosphate-buffered saline (pH 7.2); 1.0 ml of 0.1% thrice-washed, African Green monkey erythrocytes in PBS were added and the cultures microscopically examined for presence or absence of hemadsorption after incubation for one hour in the refrigerator.

Complement-fixing antibodies (CFA) were assayed with the Philadelphia 6 strain. The antigen was prepared in transformed human amnion cells maintained with serum-free Eagle's basal medium. Uninoculated cultures and cultures of other viruses were similarly prepared for control antigens. All sera were heated (56°C for 30 minutes) immediately prior to test. Veronal buffer was used as diluent throughout. In the performance of the complement-fixation test, 0.1 ml of antigen (2 units) was mixed with 0.1 ml of serum and 0.2 ml of guinea pig complement (2 exact units) added. The complement was previously shown by neutralization tests to be free of measles virus antibodies. Fixation was accomplished overnight in the refrigerator after which 0.2 ml of 2% sensitized sheep erythrocytes was added and the mixtures further incubated at 37°C for 30 minutes. Since the CF test was used for determination of susceptibility and immunity only, results were recorded as to whether the dilution of serum employed (1:4) caused any fixation to occur, in which case the patient was considered to be measles-immune.

The chemically inactivated (ethylene oxide) measles virus vaccine employed did not contain preservative and was prepared from a pool of avianized, attentuated measles virus (Edmonston strain) vaccine that had been used in other studies. The absence of living virus particles and other microbial agents was established in the laboratory of origin* and tests for the detection of viable virus were carried out in our own laboratory by introduction of the vaccine pool into cell cultures of Rhesus and African Green monkey kidney, primary canine kidney, chicken embryo fibroblasts, primary and transformed human amnion, transformed Rhesus monkey heart and HeLa cells. The "safety tests" were carried out by adding 0.5 ml of vaccine to the tissue cultures and incubating the cultures for 1 hour at 37°C prior to addition of supportive medium. The cultures were examined at frequent intervals over 21 days for the presence of measles-virus cytopathogenicity and cells and fluid from all cultures were sub-passed twice into fresh homologous cultures. No evidence of measles or other virus-induced cytologic changes could be detected in stained (hematoxylin and eosin) preparations of these cultures.

The vaccination schedule was established arbitrarily. In the *primary vaccination series,* each patient received a single inoculation of 1 ml of vaccine subcutaneously each month for a total of 3 injections. Some of these patients were later revaccinated with a single dose of vaccine, indicated below (*revaccination series*). Blood samples were drawn immediately before each injection and at intervals thereafter over a period of 12 months. The venous blood samples were allowed to clot and were held in the refrigerator overnight, the serum harvested and stored at –20°C until tested.

Patients studied. As a representative sample of the total of approximately 2600 patients, 302 patients were studied to determine the presence or absence of measles virus antibodies. Analysis of the sera by complement-fixing and neutralizing technics showed that 82 (27%) had no complement-fixing antibodies, and 20 (7%) of the 302 had no neutralizing antibodies. Serum dilutions of 1:4 were employed for complement-fixing and undiluted sera were used for neutralizing antibody detection. These findings confirm the experience of others(5,6,7) that the absence of neutralizing antibodies under *these test*

* The authors are indebted to Dr. Herald Cox and Dr. Floyd Markham of Lederle Laboratories, Pearl River, N. Y. for preparing this lot of material and to Dr. James M. Ruegsegger for making this vaccine available to us.

TABLE I. Neutralizing Antibody Titers of Serologically-Immune Patients After Vaccination with Inactivated-Measles Virus-Vaccine.

Patient	Pre-vaccination*	Antibody titers (reciprocal) wks post-vaccination 4*	8*	12	20	24
A	6	32	96	96	48	4
B	8	>512	>512	>512	>512	64
C	8	128	128	128	32	8
D	16	>512	>512	>512	>512	128
E	8	64	64	128	48	32

* 1.0 ml vaccine subcutaneously immediately following blood collection.

conditions more truly reflects lack of previous experience with the measles virus than does the absence of complement-fixing antibodies. A group of 24 patients showing serologic evidence of previous experience with the measles virus and 12 who showed absence (NA and CFA) of such experience were selected for the study. Signed parental permission for inclusion of all patients in the study was obtained prior to injection of vaccine.

Results. (A) *Primary vaccination series.* The sera from 5 of the 24 patients, in whom neutralizing antibodies to measles virus *had been detected prior* to vaccination, were selected at random for further study. The results presented in Table I show that all of the children showed significant elevation of neutralizing antibody titer following the first injection of vaccine. Furthermore, the 2 successive vaccinations did not appear significantly to elevate these "boosted" titers. It is of interest that 12 weeks following the last of the 3 injections, there was a significant decrease in antibody titer, particularly in the sera of patients C and E. This decline was emphasized when titers of the serum samples taken 16 weeks after the last injection were determined. The titers of patients A and C were identical with those of pre-vaccination samples, whereas the titers of patients B, D, and E appeared to be declining steadily. These results were so uniform that it was regarded as unnecessary to carry out similar studies on the remaining 24 patients.

Twelve patients, in whom measles virus antibodies *could not* be detected in undiluted *pre-vaccination sera,* were vaccinated with the inactivated-measles virus-vaccine preparation. The results of subsequent antibody determinations are presented in Table II. Ten of the 12 children were converted (serologic conversion) during the course of the primary immunization schedule: 2 after the first injection, 5 after the second injection, and 3 after the third injection. The range of conversion titers was from 1:2 to 1:256. In all instances a sharp decline in neutralizing antibody titer occurred at 8 weeks following the last injection. Only 2 children of 9 converted still had antibodies after a 16-week period following vaccination, and antibody titers of these 2 children were barely detectable (1:2 and 1:4).

(B) *Revaccination series.* It was of interest to determine the effects of revaccination after antibody titers could no longer be detected or had declined to relatively low levels. Twenty-two weeks following the last injection of the primary vaccination series, a single subcutaneous injection of 1.0 ml of vaccine was administered to 11 of the originally serologically-susceptible children. Antibody titers resulting from revaccination are presented in Table II. Prior to revaccination, antibodies could no longer be detected in the sera of 7 children (Patients 1, 3, 4, 7, 8, 10, 11) who had been converted during the primary vaccination series, but were demonstrated in all cases 1-2 weeks following revaccination. Increases in antibody titers occurred in 2 additional patients (patients 5,6) whose "conversion" antibody titers had declined to relatively low levels. An additional patient (Patient 9), though not originally converted, produced antibodies when revaccinated. One patient (Patient 12) was not converted following revaccination. Thus, as a result of both vaccination series, 11 of 12 children were serologically converted. Following revaccination, maximal antibody titers were attained 1-3 weeks later and these titers appeared to decline shortly thereafter, though at a slower rate than following the primary vaccination series. Sixteen weeks after the last injection of the primary vaccination series, antibodies could be detected in the sera of only 2 of 11 children (titers 1:2 to 1:4); whereas, 22 weeks after revaccination, antibody titers were still demonstrable in the sera

TABLE II. Neutralizing Antibody Titers of Serologically-Susceptible Patients after Vaccination and Revaccination with Inactivated-Measles Virus-Vaccine.

	Antibody titer (reciprocal), weeks post-vaccination													
	Pre-vaccination	Primary vaccination series						Revaccination series						
Patient	0*	4*	8*	11	16	24	30*	30*	30 +4 days	31	32	33	34	52
1	0	2	2	8	2	0	0	0	0	0	32	32	16	4
2	0	0	0	16										
3	0	0	24	24	2	0	0	0	0	6	8	16	8	8
4	0	0	0	4	0	0	0	0	0	0	16	32	4	4
5	0	0	8	128	4	2	2	2	4		128	256	128	8
6	0	2	32	256	8	4	4	4	4	96	192	128	32	16
7	0	0	2	8	0	0	0	0	0	0	2	4	4	0
8	0	0	24	16	4	0	0	0	0		8	16	4	4
9	0	0	0	0	0	0	0	0	0		4	4	8	2
10	0	0	16	64	8	0	0	0	0	16	16	8	8	8
11	0	0	0	16	0	0	0	0	0	4	4	16	4	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* 1.0 ml vaccine, subcut., immediately following blood collection.

of 8 of 11 children (titers 1:2 to 1:16).

Although the likelihood of sensitivity to residual small quantities of egg protein in the avianized vaccine seemed remote, all patient records were carefully examined for evidence of allergic manifestations, either dietary or seasonal. All patients were able to tolerate eggs in the diet. No difficulties from vaccination were observed. Recording of temperature immediately after injection and during the subsequent 24-hour period failed to reveal any evidences of elevation as a result of vaccination. There was no irritation at sites of injection, nor were any systemic symptoms observed.

Discussion. With the general acceptance of the concept that prevention of virus infection is a desirable objective and that living attenuated vaccines are more likely of success than inactivated vaccines, there has been greater interest in the development of attenuated living vaccines and there have been few reports on inactivated-measles virus-vaccine (8,9,10,11).

Our results show rather striking "booster" effects in serologically-immune patients as determined by neutralizing antibody studies, following injection of a single subcutaneous dose of the inactivated-vaccine. These responses clearly indicate that when detectable antibody titers antedate a booster injection, significant antibody rises follow the use of killed vaccine, and that, after withdrawal of the stimulus, antibody titers decline rather promptly.

The use of the inactivated vaccine in serologically-susceptible individuals resulted in less striking elevation of antibody titers and the appearance of antibodies was stimulated in some of the patients only after 3 subcutaneous injections of 1 ml each. The vaccination schedule was completely arbitrary and may not have been optimal. Again when the antigenic stimulus was removed, the titers declined to the previous undetectable level in all but 2 of the patients. In each instance, however, antibodies reappeared following revaccination with a single dose of vaccine 6 weeks later. Though this small group of patients was defined as serologically-susceptible on the basis of undetectable antibody titers, it is possible that they did have previous measles experience but with a decline of antibodies to an undetectable level so that, even in this group, the antibody titers resulted from a "booster phenomenon" and not an actual primary immunization. This possibility cannot be excluded but seems rather remote since the number of children serologically converted following the first injection of the primary vaccination series was considerably less and the antibody titers were significantly lower than after a single revaccination.

Although our study was carried out by design at such a time as to anticipate the season during which measles normally occur (April

and May), no natural challenge of disease occurred. This was unfortunate since one group of serologically-susceptible patients had been prepared for this natural challenge by stimulation of demonstrable antibodies. Furthermore, it is possible that the group of serologically-converted patients in whom antibodies could no longer be detected might not have exhibited clinical signs of illness when exposed to virulent measles virus, but may have reacted with the production of antibodies. In contrast to the experience in the community at large, the institution where this study was carried out was free of measles and no cases were clinically recognized. It is possible that the non-occurrence of measles actually reflects the immunity of the population as suggested by the survey and by the program of vaccination.

Conclusions. A survey of 302 patients from a total population of approximately 2600 patients indicated serologic susceptibility of 20 (7%) as defined by an absence of measles virus neutralizing antibodies. A chemically inactivated (ethylene oxide) measles virus vaccine was administered according to an arbitrary vaccination schedule (1.0 ml subcutaneously at intervals of 4 weeks for a total of 3 injections) and, in 10 of 12 serologically-susceptible patients the formation of measles antibodies was stimulated (serological conversion was accomplished). Patients who had detectable antibodies prior to vaccination showed sharp elevation of titers in response to a single injection of vaccine. In all patients tested, antibody titers promptly declined when the antigenic stimulus was not repeated. However, revaccination of the originally serologically-susceptible individuals resulted in significant increases in antibody titers. Since no natural challenge in the form of measles occurred in the population during the period of study, there was no opportunity to evaluate the *protective* value of these antibody demonstrations.

Addendum. Since this manuscript was prepared, studies concerning successful vaccination with another inactivated-measles virus-vaccine preparation have been reported. (Feldman, H. A., *et al.*, and Winkelstein, W., *et al.*, *J.A.M.A.*, Feb. 10, 1962, v179).

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Comparative Thymolytic Activities of 11β-Hydroxy and 11-Keto Adrenocortical Steroids. (27453)

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Although cortisone and hydrocortisone exhibit similar anti-inflammatory activity, the former has never been shown to be effective *per se*. There is evidence suggesting cortisone is biologically inert and that transformation to the 11β-hydroxy analogue is obligatory for