Radioisotope Precipitation Tests with ^{14}C and ^{51}Cr Labeled Vaccinia Antigens (35849)

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Wulff *et al.* (1) and Downie and Kempe (2) reviewed the significance of the serologic tests for the determination of circulating antibodies after smallpox vaccination. It appears that the neutralization test in tissue culture (NT) yields consistent results after primary vaccination. The outcome of the agar gel diffusion test and the HI, CF, and NT antibody titers against organisms belonging to the human pox group usually fall below demonstrable levels within 18 months after vaccination; whereas the protection induced by immunization lasts longer. We demonstrated in preliminary studies (3, 4) that precipitating antigens labeled with ^{14}C or ^{51}Cr may be helpful in serologic surveys for bacterial, as well as in herpes, rheo, and parainfluenza infections; in the present project, vaccinia antigens feasible for use in the radioimmune precipitation test (RIP) were investigated and compared with the NT in persons immunized against smallpox.

Materials and Methods. Two groups of sera from vaccinated, but not exposed, individuals were used. One was collected from 12 children at the age of 5 to 7 before and 1, 2, 6, 9, and 12 months after primary vaccination; the other from groups of 12 children and adolescents each 2, 2.5, 3, 4, and 5 years after the first immunization. Only sera of young persons with major response ("take") after multiple pressure vaccination with 10^8 to 10^9 $TCID_{50}$/ml were studied.

Protein determinations were carried out by the Lowry *et al.* (5) method.

The radioactive emission of ^{14}C was estimated in the Beckman low-background low-beta counter after drying the radioactive liquid on a filter paper glued into an aluminum planchet. The activity of ^{51}Cr was measured directly in the Chicago Nuclear well-type gamma counter.

The neutralization test (NT) was done in monkey kidney tissue cultures according to standard method (2).

Preliminary tests with sera of convalescents and vaccinated persons and 5 vaccinia strains indicated the feasibility of the Copenhagen strain for these studies. This strain was cultured in monkey kidney tissue cell monolayers. The growth medium for these tissue cells was a mixture of equal volumes of M199 and Eagle's MEM with 0.5% lactalbumin hydrolyzate and 10% agamma calf serum (Grand Island Lab.). After 3-days incubation of the cells in this medium in tissue culture tubes, the growth was washed with PBS. To one-half of the tubes, 2.5 μCi/ml of ^{14}C amino acid mixture (New England Nuclear Co.) were added. The tubes were seeded with 10^3 $TCID_{50}$ vaccinia virus in M199–MEM medium without serum the next day. After 3 more days of culture, or when optimal CPE was observed, the cells were scraped off and disrupted by sonic oscillation for 5 min at 4°. They were mechanically stirred for 4 hr and mixed with 10% (v/v) veronal buffer containing 0.14 *M* NaCl, pH 7.9. After additional stirring for 3 hr, the sonicate was centrifuged at 800*g*. The supernate was clarified through a Gelman GA-4 filter, then dialyzed against distilled water until the dialyzing fluid showed less than 10 counts per minute (cpm) radioactivity. The contents of the dialyzing bags represented the antigen which was lyophilized. This antigen contained 0.5 to 0.8 μCi/mg of protein.

The ^{51}Cr labeled antigen was prepared by the same method. The ^{14}C amino acid mixture was omitted. The lyophilized antigen was dissolved in 0.2 ml of PBS (pH 7.4)/mg. Ten microcuries of $^{51}CrCl_3$ (Inter-

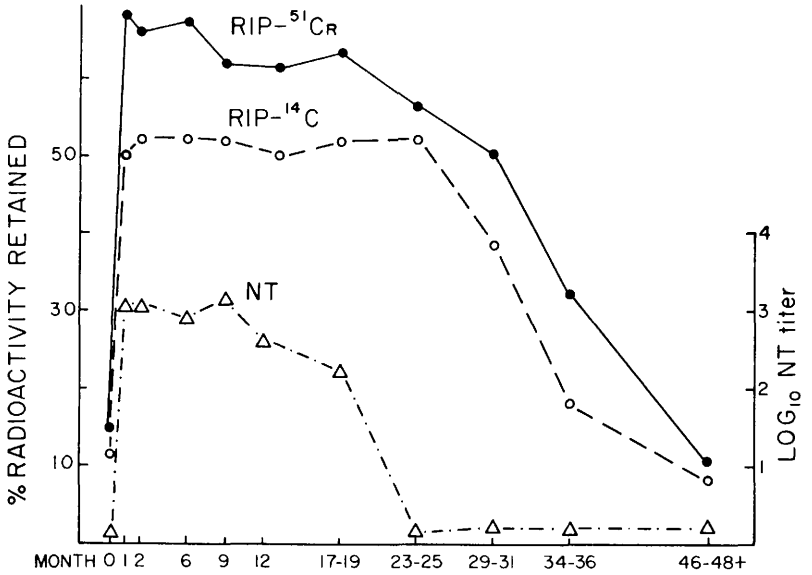

FIG. 1. Geometric means of percentage retention of radioactivity in the RIP test using vaccinia antigen labeled with ^{51}Cr (●-); or ^{14}C (○ - -); and the NT (Δ —•—); in children after primary vaccination; 0 = date of vaccination.

nat. Chem. Nucl. Lab.) per milligram of antigen were added. The mixture was incubated for 24 hr at 4°, then dialyzed until the dialyzing fluid did not contain more than 10 cpm radioactivity. The antigen contained 1 to 2 μCi/mg of protein. It could be used only for 6 to 8 weeks because of the relatively short half-life of ^{51}Cr.

The RIP test was performed by a modification of the method of Gerloff *et al.* (6) who used polio virus labeled with ^{32}P. Antiviral control serum was prepared in rabbits according to standard method (2). It served to establish the optimal concentration of the antigen in preliminary tests. This appeared to be an amount giving 150 to 200 cpm with ^{14}C labeled, and between 800 to 900 cpm with ^{51}Cr labeled antigens. Sheep antirabbit serum (Behringwerke) was used for the coprecipitation of the rabbit sera.

A pool of human convalescent sera served as the positive control in the tests with the sera of the children. It gave the following titers: NT, 1:2000, HI, 1:640; and CF, 1:160. The Copenhagen strain yielded results with the greatest difference between pooled human negative and positive control sera in the RIP test using the same amounts of antigen as in the preliminary tests with rabbit sera. Commercial (Behringwerke) rabbit antihuman γ-globulin was employed. The amount of the anti-γ-globulin added did not seem to be critical, as shown also in the studies of Gerloff *et al.* (6).

All sera were adsorbed with unlabeled monkey kidney culture cells, 100 mg/0.5 ml of serum, before testing. The cells were centrifuged off at 800*g* after 2-hr incubation at room temperature.

One-tenth milliliter of the radiosotope labeled antigen adjusted to give a cpm as described and 0.25 ml of the serum to be tested were mixed and incubated at 37° for 1 hr. After that 0.25 ml of anti-γ-globulin serum were added, the mixture was incubated at 37° for 2 hr, then at 4° for 1 hr, centrifuged at 1000*g* for 15 min, and the radioactive emission of 0.1 ml of the supernate was determined. The amount of antigen retained in the precipitate was calculated by comparing the cpm of the sample taken from the supernate with that of an equal amount of the supernate from a control tube with negative serum subjected to the same procedure. The results were expressed in percentage of the radioactivity calculated to be retained in the

precipitate.

All tests were carried out in triplicate.

Results and Discussion. Figure 1 shows the geometric means of the results. The SD of the percentage retention in the RIP test was ±9; that of the NT, ±17. It appears that negative sera also retained some radioactivity, perhaps due to some unbound antigenic particles trapped in the bulky precipitate. The percentage of retained radioactivity remained at a high level during the period when NT antibody was elevated. A similar phenomenon has been observed with bacterial extracts (3) and polio virus (6).

It appears that retention of more than 20% of the radioactivity in the precipitate is significant in the RIP test. In the present experiments, the results remained "positive" for approximately 3 years, *i.e.,* for a period during which at least 95% of the successfully vaccinated individuals are immune to smallpox according to epidemiologic evidence (7); whereas the NT became negative between 18 and 23 months.

The RIP method lends itself to large-scale studies. While ^{51}Cr has a relatively short half-life and new batches of the antigen must be prepared frequently, previous tests (4) showed that it gives more easily reproducible results with viruses than ^{125}I, that also increases the radioactive emission. The half-life of ^{131}I is only 8.1 days which makes it less suitable for extensive serologic testing. ^{14}C labeled antigens are stable for a long time but the cpm readings are low, and the radioactive label is expensive.

Summary. The radioisotope precipitation test was performed with ^{14}C and ^{51}Cr labeled vaccinia antigens after primary smallpox vaccination. The test gave positive results with the sera of successfully vaccinated individuals for a longer time than the neutralization test.

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1. Wulff, H., Chin, T. D. Y., and Wenner, H. A., Amer. J. Epidemiol. **90**, 312 (1969).

2. Downie, A. W., and Kempe, C. H., *in* "Diagnostic Procedures for Viral and Rickettsial Diseases" (E. H. Lennette and N. J. Smith, eds.), p. 321. Amer. Pub. Health, New York (1969).

3. Felsenfeld, O., and Parrott, M. W., Health Lab. Sci. **7**, 24 (1970).

4. Felsenfeld, O., Felsenfeld, A. D., and Brannon, R. B., Amer. J. Clin. Pathol. **55**, 124 (1971).

5. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

6. Gerloff, R. K., Hoyer, B. H., and McLaren, L. C., J. Immunol. **89**, 559 (1962).

7. Bedson, H. S., and Dumbell, R. K., Brit. Med. Bull. **23**, 119 (1967).

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