Fluorescent Antibody and Complement-Fixation Tests of Agents Isolated In Tissue Culture from Measles Patients.* (21957)

SOPHIA M. COHEN, IRVING GORDON,† FRED RAPP,† JOHN C. MACAULAY,‡ AND SONJA M. BUCKLEY.

From the Division of Laboratories and Research, N. Y. State Department of Health, Albany, and Department of Pediatrics, Albany Medical College.

Enders and Peebles(1) observed that human or monkey kidney tissue cultures inoculated with specimens from measles patients undergo, after one or more passages, characteristic nuclear and other cytologic changes. Both infectious virus and specific complement-fixation antigen appeared concurrently in the cultures and the cytopathogenic effect was neutralized by convalescent-phase measles serum. We have applied the fluorescent antibody technic of Coons and his colleagues(2,3) to similar cultures and find that the results parallel those of complement-fixation tests and thus provide immunochemical support for the data of Enders and Peebles and in addition evidence that measles antigen(s) are present in the nuclei as well as in the cytoplasm of infected cells.

Methods. Patients were selected for study during a mild outbreak of typical rubeola. Clinical diagnosis was aided in certain cases by recognition of giant cells of the Warthin-Finkeldey type in stained films of nasal discharge obtained early in illness, as described by Tompkins and Macaulay(4). *Collection of specimens and preparation of tissue cultures.* The procedures outlined by Enders and Peebles(1) were followed. Blood, throat swabs or washings, or nasal discharge were obtained before or within a day after the appearance of the rash. When possible, specimens were inoculated into tissue cultures within a few hours after their collection. Tissue cultures consisted of trypsinized monkey kidney cells grown in sheets(5). Tubes were inoculated with 0.2 or 0.3 ml of the prepared specimens, fed with nutrient fluid to bring the volume to 1.0 ml, and incubated as stationary slants at 36.5°C. Nutrient fluid consisted of beef amniotic fluid containing 5% inactivated horse serum and 5% beef embryo extract. The first passage contained 100 units of penicillin, 100 μg of streptomycin, and 50 μg of nystatin (fungicidin)(6); others, 50 units and 50 and 10 μg, respectively. Transfers to fresh tubes were carried out at 14-day intervals with 0.2-ml amounts of pooled fluids taken 5, 9, and 14 days after inoculation. Controls consisted of uninoculated cultures and of cultures passaged serially with fluids from uninoculated tubes. *Staining with fluorescein-labeled antibody.* The monkey kidney cells were gently scraped from the tube walls, or sedimented by centrifugation of the tissue culture fluid, washed with NaCl buffered at pH 7.6(7), sedimented, and spread on slides. The films were dried in air and fixed with acetone. They were often stored at 4°-6°C for from 1 to 3 days before staining by the indirect method of Weller and Coons(3). The test serum diluted 1:20 was acute- or convalescent-phase measles or heterologous human serum. Fluorescent antibody was prepared from antihuman gamma globulin horse serum generously supplied by Dr. David Gitlin, Children's Medical Center, Boston. The pseudoglobulin fraction was conjugated with fluorescein isocyanate, dialyzed, and stored at −20°C. Before use it was absorbed with mouse liver powder to remove nonspecific staining(2), then diluted 1:10. Prior to staining with the fluorescent antibody solution, films of uninoculated and inoculated preparations were treated, one of each with acute-, the other with convalescent-phase measles serum, to control the staining

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† Present address: Department of Medical Microbiology, University of S. California Medical School, Los Angeles.

‡ Present address: Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass.

technics. The equipment used for fluorescence microscopy has been described by Buckley, Whitney, and Rapp(8). *Complement-fixation antigens* consisted of the fluid phase from tissue cultures in tubes or in 200-ml square bottles. For the latter, 1-ml amounts of inoculum and 7 ml of nutrient fluid were used. Medium 703(9) with or without 5% inactivated horse serum was substituted for Enders' medium(1) in some lots with satisfactory results. The fluids were centrifuged at about 2500 r. p.m. for 15 minutes and inactivated at 56°C for 30 minutes. *Complement-fixation tests* were made with three 50% units of complement. Fixation was at 4°C for 24 hours. For serum titrations, 2-fold dilutions were tested against a previously determined optimum dilution of antigen. Titers were the calculated number of 50% units of complement fixed by undiluted serum.

Results. Transmissible agents, presumably viruses, were isolated from both blood and a throat swab of one patient, and from nasal discharge and a throat swab, respectively, of 2 others whose blood was not obtained. The throat swab specimens had been stored in a dry ice chest for 7 days. No agents were detected in specimens from 5 additional cases. Inocula from 3 of the latter had been stored at 4°-6°C for 2 days before culture; tests of inocula from the other 2 were discontinued after 3 apparently negative serial passages. These circumstances may have contributed to our failure to isolate agents.

Enders and Peebles(1) and Rustigian *et al.* (10) encountered latent virus-like agents that induce marked vacuolization and syncytial masses in monkey kidney tissue cultures. The cellular degeneration characteristic of these "monkey-kidney agents" frequently appeared in our cultures, both in those inoculated with specimens from measles patients and in controls; hence cytologic criteria for recognition of measles agents were difficult to apply. In some tissue culture series the "monkey-kidney agents" destroyed the cell sheets in 10 to 14 days. We therefore relied on tests for the presence of measles antigen to identify the 3 agents cultivated from measles inocula.

The reactivity of the measles agents in both fluorescent antibody and complement-fixation tests seemed to be specific. Cells from control monkey kidney cultures, some with extensive "foamy" areas and multinucleated cells, did not exhibit fluorescence nor did control tissue culture fluids fix complement with either acute- or convalescent-phase sera from measles cases. Cells infected with measles agents, however, were brightly fluorescent with the 3 measles convalescent-phase sera tested but not with 3 acute-phase sera or with human antisera to herpes simplex, influenza Types A and B, or mumps viruses. The proportion of brightly and specifically fluorescent cells increased as serial passages continued. After 4 passages, many of the cells apparently contained measles antigen. One of the strains has been carried through 7 passages.

In tests of infected cells, fluorescence was observed in some cultures as early as the 5th day of incubation (Table I). Earlier tests were not made. Between the 5th and 10th day, fluorescence, when present, was usually confined to the cytoplasm. After about 2 weeks, however, bright yellow-green fluorescent intranuclear masses were frequently seen in fluorescent cells, very rarely in non-fluorescent cells (Fig. 1-3). The masses were surrounded by an unstained dark nuclear zone. Attempts to correlate the appearance of specific fluorescent masses in the nuclei with the

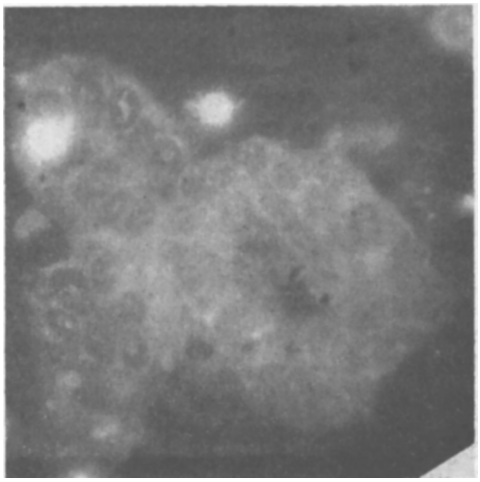

FIG. 1. Monkey kidney cells 14 days after infection with measles agent No. 5584. Indirect fluorescent antibody stain with convalescent-phase measles serum. The lighter areas are those showing specific yellow-green fluorescence. 400×.

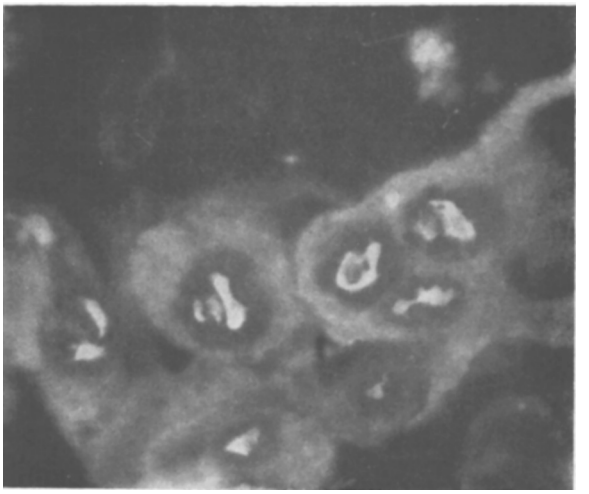

FIG. 2. Monkey kidney cells 13 days after infection with measles agent No. 5585. Stain same as Fig. 1. 700×.

intranuclear inclusions described by Enders and Peebles are in progress.

In direct comparisons between development of specific fluorescence and of complement-fixation antigen, the fluorescent antibody procedure seemed the more sensitive of the two methods, since it detected antigen earlier in the course of incubation than did the complement-fixation test (Table I). This difference may have been due to the fact that fluorescent antibody tests were done on cell spreads, whereas complement-fixation antigens were made from centrifuged fluids. The fluid antigens were relatively weak; they rarely were active when diluted more than twofold. The antigen titer of one fluid taken 25 days after inoculation was considerably increased by addition of an extract of cells from the same cultures. The cells were frozen and thawed for 6 cycles in NaCl solution buffered at pH 7.6; the supernate was the extract.

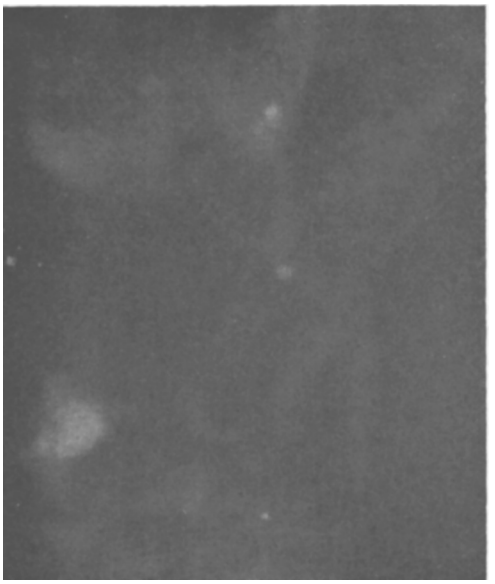

FIG. 3. Control for Fig. 2; same cells stained with acute-phase measles serum. 700×.

Only 3 pairs of acute- and convalescent-phase sera could be obtained from measles patients. The complement-fixation titer of each acute-phase serum was <4; those of the convalescent-phase specimens were 40, 64, and 200. In tests with 21 sera from 13 cases of measles the distribution of titers agrees with that found by Enders(11) (Table II). Among the 26 heterologous sera tested were convalescent-phase sera from cases of virus infection such as herpes simplex, influenza, and mumps, and unclassified sera received for routine tests for evidence of virus or rickettsial infections. Twenty-three gave relatively low complement-fixation titers.

Discussion. Demonstration that tissue cultures inoculated with specimens from measles patients produce antigens that react specifically with convalescent-phase measles sera substantiates the findings of Enders and

TABLE I. Correlation of Results of Fluorescent Antibody Tests with Appearance of Complement-Fixation Antigen in Monkey Kidney Tissue Cultures Infected with Measles Agents.

Strain	Tissue culture: Passage level	Tissue culture: Day post-inoculation	Fluorescent antibody results with: Acute-phase serum	Fluorescent antibody results with: Convalescent-phase serum*	CF titer*
5582, 5583† (blood, throat swab)	3	14	0	+	300
	4	9	0	+	NT
		14	0	+	211
	5	9	0	±	NT
		14	0	+	179
5584 (throat swab)	3	4	NT	NT	<4
		9	NT	NT	>64
		14	0	+	303
	4	10	NT	NT	<8
		14	0	+	<8
		18	NT	NT	<8
		21	0	+	112
	5	14	0	+	<4
	6	5	0	+	<4
		10	0	0	97
		14	0	+	55
		25	0	+	221
5585 (nasal discharge)	1	14	0	±‡	NT
	2	14	0	+	NT
	3	14	0, ±	+	NT
	4	13	±	+	NT
	5	5	0	+	<4
		10	0	0	<4
		14	0	+	<4
		25	0	+	195

CF = complement-fixation. NT = not tested.

Green-yellow fluorescence due to labeled antibody was graded as follows: 0 = none, ± = faint or rare cell bright, + = bright.

* Convalescent-phase serum 55291 used for both procedures.

† Combined blood (5582) and throat swab (5583) isolates from the same patient.

‡ Serum 5586 used in this test.

Peebles(1). We did not repeat their filtration experiments, but were unable to recover fungi, or bacteria including pleuropneumonia-like organisms and leptospira from infected tissue cultures and presume, therefore, that the antigens that produced specific reactions in fluorescent antibody and complement-fixation tests were derived from measles virus. On the whole, antigen in infected tissue cultures was detected earlier by the fluorescent antibody method than by complement-fixation tests. Preliminary data suggest, however, that the latter are of value in differential diagnosis of exanthemata and encephalitides of unknown origin.

The intranuclear fluorescence that appeared after prolonged incubation of infected cultures looks like that observed by Leduc, Coons, and Connolly(12). They made indirect fluorescent antibody stains of sections of lymph nodes from rabbits immunized with diphtheria toxoid and found spots of antibody within nuclear shadows that closely resemble the intranuclear measles antigen we noted (*cf.* their Fig. 4 and 5, our Fig. 1 and 2). Whether the intranuclear measles antigen is associated with inclusion bodies demonstrable by Giemsa or other stains(1) is still to be determined. One of us has recently observed that poliomyelitis virus antigen appears later in the nuclei than in the cytoplasm of monkey kidney cells under circumstances quite similar to those in the present study(13).

Summary. Agents isolated in monkey kidney tissue cultures from 3 cases of measles were identified by immunologic tests with convalescent-phase measles serum. Measles antigen was detected in infected cells by the in-

TABLE II. Complement-Fixation Titers of Sera with Measles Agent Tissue Fluid Antigens.

Titer range*	Sera from cases of measles: Days after onset† <5	Sera from cases of measles: Days after onset† >5	Heterologous sera
<4	7	0	7
5- 25	1	1	11
26- 50	0	1	5
51-100		3	2
101-200	1		1
>200		4‡	

* Titers = Number of 50% units of complement fixed.

† Exact date of onset unknown in some cases; may refer to earliest symptoms or appearance of rash.

‡ Three additional sera from one patient taken 3 to 4½ months after onset fell in this group.

direct fluorescein-labeled antibody technic before specific complement-fixation antigen appeared in the fluid phase. Specific fluorescence due to the presence of measles antigen was noted in the nuclei as well as in the cytoplasm of some cells from infected cultures.

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Chemotherapy of Experimental Schistosomiasis. IV. Oral Activity of Antimony Trichloride Antibiotic Complexes. (21958)

GEORGE W. LUTTERMOSER, HOWARD W. BOND, AND JOHN F. SHERMAN.
(Introduced by Nathan B. Eddy.)

From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda, Md.

1 - (Diethylaminoethylamino) - 4 - methylthiaxanthone, Miracil D, was the first nonantimonial compound found to be of value for the oral treatment of schistosomiasis(1). In later studies, however, Miracil D proved to be toxic to man at therapeutic dosage levels(2,3). Other compounds which have been used with varying degrees of success in the oral treatment of schistosomiasis in man or experimental animals include antimony potassium tartrate, tartar emetic(4); sodium antimony III bis-pyrocatechol-2,4-disulfonate, Fuadin (5); various thioantimonials(6); and some symmetrical diaminodiphenoxyalkanes(7). During the course of screening compounds in this laboratory, it was found that the antimony trichloride complexes of oxytetracycline, Terramycin; of chlortetracycline, Aureomycin; and of tetracycline were schistosomacidal in white mice. The present report includes a comparison of the oral activities of these complexes and of other antimony compounds.

Materials and methods. Samples of the 3 complexes were synthesized by Dr. Philip N. Gordon and were submitted for evaluation by Chas. Pfizer & Co., Inc. The dithiastibiole compound mentioned below was supplied