ECHO and Poliomyelitis Virus Antisera Prepared in Guinea Pigs with Fluorocarbon-Treated Cell Culture Antigens. (26004)

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The preparation of virus antigens and antisera by cell culture grown virus has led to difficulties in using these sera in the complement fixation (CF) test, since the fixation between cell culture host antigens and antibodies may interfere with the specific fixation between virus antigen and antibody. The authors(1) and other investigators(2,3,4) have shown that host antigen CF activity can be removed from many viral CF antigens prepared in cell cultures by fluorocarbon treatment, without loss of specific virus CF antigenicity. Although a simple procedure, fluorocarbon treatment would be more convenient for many purposes if it could be used for preparation of the immunization antigens, in which case untreated CF antigens could be used with these antisera.

This report will present data on virus and host CF antibody titers in guinea pigs immunized by fluorocarbon-treated cell culture antigens of ECHO and poliomyelitis viruses.

Material and methods. Virus strains. The following virus strains were used to prepare immunization and CF antigens: prototype strains of ECHO types 1 through 20(5,6), "Mahoney," "MEF-1" and "Saukett" strains of poliomyelitis types 1, 2, and 3, respectively. *Cell cultures.* Monkey kidney cell cultures obtained from Microbiological Associates, Bethesda, Md., were grown in a medium containing 2% calf serum, 0.5% lactalbumin hydrolysate, and 97.5% Earle's solution. At time of inoculation, the cultures in 32 oz prescription bottles were washed twice with Hanks' solution and 40 ml of maintenance medium containing 2% guinea pig serum (for immunization antigens) or 2% calf serum (for CF antigens), 0.5% lactalbumin hydrolysate, and 97.5% Earle's solution was added.

Immunization antigens. Monkey kidney cell cultures were inoculated with 0.3 ml undiluted virus. The bottles were incubated at 37°C. After complete degeneration of cells had taken place the fluids were harvested and treated once or twice with fluorocarbon† by the method reported previously(1). Each fluorocarbon-treated antigen was titrated in the CF test against 4-8 antibody units of anti-monkey kidney guinea pig serum to test host antigen activity. The immunization antigen of normal monkey kidney cells was prepared by mechanically removing cells from monkey kidney cultures. The cells, 3 times centrifuged and washed, were used as a 50% solution for immunization.

The antigens were stored at −20°C. All antigens containing penicillin were treated with penicillinase before being used in immunization of guinea pigs.

Immunization of animals. Groups of 4 guinea pigs were immunized 3 times each with 1.0 ml of each antigen and bled 1 week after last injection. The first and second injections were given subcutaneously, at an interval of 3 weeks. The last injection, 4 weeks after the second, was given intracardially or intraperitoneally.

Complement-fixing antigens. CF antigens were prepared as immunization antigens except that they were not treated with fluorocarbon. The specific CF activities of the antigens of ECHO types 1 through 14 and 3 poliomyelitis types were tested with homologous antisera prepared in monkeys(7) and ECHO type 20 with control positive human serum. A "normal" monkey kidney cell antigen was prepared by inoculating a monkey kidney cell culture with Coxsackie B type 5 virus.

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† Genetron 113, obtained from Allied Chemical & Dye Corp., New York.

TABLE I. Host and Virus CF Reactions of Fluorocarbon-Treated and 2 Untreated Immunization Antigens and Corresponding CF Antibody Titers in Pooled Sera of 2 to 4 Guinea Pigs. Only groups, which, in each of the 3 injections, received an antigen with no host CF reaction, are listed. 4 = no hemolysis; 3, 2, 1 = different degrees of hemolysis; 0 = complete hemolysis.

Immunization antigen	Fluorocarbon treatment	Reaction of immunization antigen with: Anti-monkey kidney G.P. serum, Antigen dilutions 1:1	1:2	1:4	Homologous monkey antiserum, Antigen dilutions 1:1	1:2	1:4	Reciprocal of CF antibody titer in pooled G.P. antisera: Host	Virus
ECHO- 1	+	0	0	0	4	4	1	32	512
2	+	1	0	0	4	4	2	<16	128
6	+	0	0	0	4	2	0	32	1024
6	—	4	4	3		ND		512	2048
8	+	0	0	0	4	2	0	<16	128
13	+	0	0	0	4	1	0	64	2048
14	+	0	0	0	4	1	0	<16	512
14	—	4	4	4		ND		128	512
15	+	0	0	0		ND		<8	512
16	+	0	0	0		ND		<8	256
17	+	0	0	0		ND		<8	512
Polio- 1	+	0	0	0	4	ND	ND	<8	1024
2	+	0	0	0	4	ND	ND	<8	1024
3	+	0	0	0	4	ND	ND	<8	512

ND = not done.

CF test. Complement fixation tests were performed by the standard technic used in this laboratory(1).

Results. Twenty-one groups of guinea pigs were immunized by fluorocarbon-treated ECHO and poliomyelitis virus, but only 12 groups (ECHO types 1, 2, 6, 8, 13, 14, 15, 16, 17, and 3 poliomyelitis types) received, in each of the 3 injections, an antigen with no host antigen activity in the CF test. The others were immunized at least once with an antigen from which host CF antigen activity had not been removed. The results of the former group (no host antigen activity) are shown in Table I, where host and virus CF antigen activity of the immunization antigens and corresponding host and virus CF antibody titers in pooled antisera of 2 to 4 guinea pigs are recorded. Nine of the 12 antigens produced no host CF antibodies in guinea pigs at dilutions tested (1:8 or 1:16). However, 3 immunization antigens (ECHO types 1, 6, 13) with no host activity in the CF test produced host antibodies in titers of 1:32 or 1:64. Host antibody titers of guinea pigs immunized with non-treated antigens were 1:128 or 1:256. All 12 fluorocarbon-treated antigens produced specific virus antibodies in titers of 1:128 or more. The difference between virus and host antibody titers was 16-fold or more in antisera prepared with fluorocarbon-treated antigens and 2-fold with non-treated antigens.

The virus and host CF antibody titers of all ECHO and poliomyelitis guinea pig antisera are shown in Table II. As was indicated above, in preparation of many of the ECHO antisera, at least one injection consisted of an antigen with some host CF antigenicity left. Titers of all sera against "normal" monkey kidney antigen were 1:64 or less. Titers against homologous virus CF antigen were between 1:16 and 1:2048. The difference between titers against homologous and "normal" monkey kidney antigen was 16-fold or greater in 15 out of the 20 ECHO types, and in all poliomyelitis types, 8-fold in 2 ECHO types (9 and 10), and 4-fold or less in 3 ECHO types (3, 7, and 19).

No heterotypic CF reactions were found in poliomyelitis antisera. Heterotypic reactions within ECHO types 1, 8, and 13 were the same as reported previously(1,6,7).

Discussion. The results presented indicate that fluorocarbon-treated cell culture grown ECHO and poliomyelitis virus could be used as immunization antigen to prepare antisera in guinea pigs with no host CF antibodies. Although some fluorocarbon-treated antigens with no host CF antigen reaction produced

TABLE II. Reciprocals of Homologous and Host CF Titers, and Some Heterologous Titers of ECHO Type 1-20 and Polio Type 1-3 Guinea Pig Antisera Prepared by Immunizing Animals with Fluorocarbon-Treated Antigens.

	Guinea pig sera																							
CF antigen	ECHO E-1	E-2	E-3	E-4	E-5	E-6	E-7	E-8	E-9	E-10	E-11	E-12	E-13	E-14	E-15	E-16	E-17	E-18	E-19	E-20	Polio P-1	P-2	P-3	Anti-monkey kidney
ECHO- 1	512							0			0	0	512											
2		128																						
3			128																					
4				512																				
5					256																			
6						1024																		
7							64																	
8	512							128					256											
9									512															
10										512														
11	0										512													
12												256												
13	512							0				0	2048											
14														512										
15															512									
16																256								
17																	512							
18																		128						
19																			16					
20																				1024				
Polio-1																					1024	0*	0*	
2																					0*	1024	0*	
3																					0*	0*	512	
"Normal" monkey kidney	16	0	32	0	0	32	32	0	64	64	32	0	64	0	0*	0*	0*	8	16	16	0*	0*	0*	256

0 = <16 0* = <8

host antibodies on repeated immunization of guinea pigs, these were present in such low titers that on simple dilution the antisera would show specific activity only. The practical value of some of the antisera prepared for this study has been proved by using them in CF typing of enterovirus isolates(8).

On the other hand, the data presented show that, besides the host activity, the specific activity of fluorocarbon-treated antigens should be tested before using them for immunization. From ECHO types 15-19 no antisera were available during time of immunization. Hence, the lack of specific virus antibodies in ECHO type 19 antiserum may be caused by lack of specific virus activity in fluorocarbon-treated immunization antigen, which could not be tested.

Since each virus group differs in susceptibility to fluorocarbon treatment(4), the fluorocarbon technic can not be adapted directly to preparation of antisera of other viruses, but if adaptable it might be of value.

Summary. It was found that ECHO and poliomyelitis virus antisera without host CF antibodies could be prepared in guinea pigs by immunizing the animals with fluorocarbon-treated cell culture grown virus. Although in repeated immunization of guinea pigs 3 out of 12 fluorocarbon-treated antigens with no host CF activity produced host antibodies in titers of 1:32 and 1:64, specific virus titers were 16 and 32 times higher. It was also found that, besides the host activity, the specific virus activity of fluorocarbon-treated antigens should be tested before using them for immunization.

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Concentrated Culture of Gonococci in Clear Liquid Medium.* (26005)

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The common practise is to grow *Neisseria gonorrhoeae* on solid media, although the organism can be cultivated successfully on liquid semi-defined(1,2) and undefined media (3). These must usually be enriched with blood, starch, charcoal and similar material, however, and the resulting cloudiness complicates the observation of growth. An important growth-limiting characteristic of the gonococcus is its sensitivity to certain amino acids, fatty acids and possibly other toxic materials that are carried with ordinary medium constituents(4), including agar(5). Adsorptive removal of such inhibitory factors apparently accounts for the necessity of adding various enrichment materials. These might be spatially separated from the actual growth environment and still function, provided that diffusional access to the enrichment material is provided. A simple biphasic flask system (6) was so employed and allowed attainment of gonococcal growth in a water-clear men-

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