strongly positive on 2nd and 3rd egg passages.

In continuing experiments, efforts are being made to develop a variant of mumps virus which will be more uniformly fatal for suckling, and if possible, for adult mice. A development of such virulence, if it could be achieved, should make it possible to perform neutralization tests in mice. As described above such tests are already under study.

Summary. 1. Mumps virus has been carried through 20 passages in mouse embryo tissue culture in the course of which it developed a progressive ability to grow in brains of suckling mice, without apparent illness of the animals. 2. Another strain of mumps, after passage in brains of suckling hamsters, also multiplied readily in the brains of suckling mice, leading to illness and, in a small proportion, to death on continued passage. 3. Practical and theoretical implications of this adaptation of mumps virus to mice have been discussed.

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Occurrence of Coxsackie Virus Complement Fixing Antibodies in Sera of Normal Monkeys.* (19669)

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In the course of investigations regarding Coxsackie virus complement fixing (cif.) antibodies in monkeys, it was found that a homologous hyperimmune Leon type poliomyelitis monkey serum manifested a high titer (1:64) for one of the Coxsackie virus (C virus) prototype antibodies (Easton-10)(1). The possible reasons for its presence in a specific poliomyelitis monkey serum seemed to warrant additional experimentation.

Materials and methods. Sera. Homologous hyperimmune monkey sera were employed as follows: the Petry strain (Leon type poliomyelitis) and the Brunhilde strain were received from Dr. Jonas Salk; Ilda and Stoddard strains (Brunhilde types), as well as MV and MEF1 strains (Lansing types) were received from Dr. Louis Gebhardt; and the Lansing strain from Dr. Robert Ward. Hyperimmune monkey and mouse sera prepared in this laboratory for the FA strain of murine encephalomyelitis virus were also employed, as were sera from monkeys hyperimmunized with the Texas-1 type of Coxsackie virus (Monkey 4197) using infant mouse brain as immunizing agent, and with the Easton-2 (Dalldorf's type 1) Coxsackie virus (Monkey 4766) using muscle-bone suspensions from infected infant mice as antigen. Specific hyperimmune Coxsackie virus mouse sera were prepared for all types of antigens used in the c.f. tests (see below)(2). Sera were obtained from 19 rhesus (*Macaca mulatta*) and 15 cynomolgus (*Macaca irus*) monkeys which had been in the laboratory for periods of time varying from 1 to 43 days. They had been uninoculated prior to bleeding. Ten of the rhesus monkeys were bled at 4- to 7-day

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intervals for 23 days. Also two pools of serum from a number of uninoculated rhesus and cynomolgus monkeys were used.

Complement fixing antigens. Ten Coxsackie virus types were employed in the majority of the complement fixation tests. They were: Easton-10 (E-10), Easton-14 (E-14), Dalldorf's type 2 (D-2), Dalldorf's type 3 (D-3), Alaska-8 (Alaska), Texas-1, Easton-2 (E-2 Dalldorf's type 1), Nancy, Connecticut-5 (Conn-5), Ohio-1. Complement fixation tests with antigens of 5 additional types were performed on the sera of 5 of the normal rhesus monkeys, the latter types including Texas-12, Texas-13, Texas-14, Texas-15, and Israel-7(1). The description of the preparation of the antigens (20% protamine treated muscle-bone suspensions) has been reported(3). Concentrated purified antigens were prepared by ultracentrifugation for the E-10, D-3, and Texas-1 viruses according to the method described previously(4). The antigen of FA murine encephalomyelitis virus was the supernate resulting from the centrifugation at 18,000 r.p.m. for 30 minutes (International PR-1 centrifuge) of a 10% suspension of infected mouse brains. That of the infant mouse-adapted MEF1 strain of poliomyelitis virus of Casals and Olitsky(5) was the supernate of a 50% suspension of infected 3-day-old mouse brains centrifuged at 4°C at 18,000 r.p.m. for 1 hour. Normal antigen was prepared in like manner from uninoculated mice of the same age. *Complement fixation test.* The c.f. titers reported below were obtained by means of the 50% hemolysis method according to Wadsworth(6). Many of the results were checked by the plate c.f. test of Fulton and Dumbell(7,3).† There were no significant differences in the results obtained with the two methods.

Temperature of inactivation. For routine use in the c.f. tests the sera were inactivated at 60°C for 20 minutes. A number of sera (see below) were also heated for 20 minutes at the following temperatures in degrees C: 63, 65, 68, 70, 73, 75. All sera were diluted 1:4 before heating. The deviation of the water bath at each temperature was ±0.1°C. *Neutralization tests.* These were carried out according to the method used in this laboratory(2) employing 100 ID_{50} of virus against constant or varying dilutions of the sera. In addition tests employing varying dilutions of the viruses with constant serum dilutions were also performed. For the neutralization tests all sera were inactivated at 56°C for 30 minutes, specific neutralizing antibodies being stable at this temperature(2). *Serum absorptions.* Serum from monkeys 4197, 4766, and 5782 (a normal rhesus monkey) was subjected to the following absorption procedures: 1) Sheep cell absorptions were performed by adding to 0.8 ml of undiluted serum 3.2 ml of centrifugally packed sheep cells which had been washed three times with physiological saline. The mixture was allowed to remain at room temperature with occasional shaking for one hour after which it was centrifuged and the supernatant fluid used for the c.f. antibody determinations. 2. Absorption by boiled guinea pig kidney and boiled beef cells were done according to the methods described by Kolmer and Boerner(8). In each case the serum was heated at 60°C for 20 minutes at the end of the absorption period.

Lyophilization and chloroform extraction. Lyophilization of sera from immunized monkeys 4197 and 4766, and from normal monkey 5782, as well as extraction of the lyophilized samples with chloroform, was carried out. Three volumes of chloroform were added to each sample and removed after standing 15 minutes at room temperature with occasional shaking. This was repeated 3 times making a total extraction volume of 9 times. Reconstitution in distilled water of lyophilized and chloroform extracted sera took place at room temperature for ½ hour after which they were heated at 60°C for 20 minutes. *Dialysis.* One serum (normal rhesus 5789) was dialyzed overnight at room temperature in 1000 volumes of distilled water before being used in the c.f. test.

Results. The c.f. titers obtained with Coxsackie, FA, and MEF1 antigens in various homologous hyperimmune monkey sera are presented in Table I. The reactions between

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TABLE I. Coxsackie Virus c.f. Antibody Titers of Some Specific Homologous Hyperimmune Monkeys and Mouse Sera.*

Sera from monkeys immunized with:	Source	Reciprocals of c.f. titers with following antigens† E-10	E-14	D-2	D-3	Alaska	Texas-1	E-2	Nancy	Conn-5	Ohio-1	MEF1	Normal	FA
Brunhilde	Pittsburgh	64	48	64	48	64	16	0	0	0	0	0	0	0
Ilda (Brun.)	Utah	32	48	24	16	48	0	0	0	0	0	0	0	0
Stoddard (Brun.)	"	6	0	8	0	0	0	0	0	0	0			0
Leon	Pittsburgh	96	32	96	32	4	6	0	0	0	0	0	0	0
MEF1 (Lansing)	Utah	4	0	0	0	0	0	0	0	0	0	128	0	0
MV (")	"	12	12	24	6	12	6	0	0	0	0	64	0	0
Lansing	New York	64	12	48	0	64	48	0	0	0	0	256	0	0
FA (monkey)	Yale	12	12	24	0	24	24	0	0	0	0	0	0	48
FA (mouse)	"	0	0	0	0	0	0	0	0	0	0	0	0	96

0 = titer <1:4.
* Sera obtained from 4 different laboratories.
† See text for explanation of antigens.

TABLE II. Numbers of Normal Monkeys with c.f. Reactions at 1:4 or Higher Serum Dilution to Indicated Virus Types.†

No. of monkeys	Days after arrival in laboratory	No. of monkeys with c.f. reactions to: E-10	E-14	D-2	D-3	Alaska	Texas-1	E-2, Nancy, Conn-5, Ohio-1 types*	No. of monkeys with no c.f. reactions
6 R*	1	3	5	1	0	0	0	0	1
6 C	7	4	3	3	2	3	1	0	1
3 R	12	2	1	1	0	0	0	0	1
10 R	21	5	6	4	5	1	1	0	4
9 C	43	8	6	6	6	8	1	0	1
Total 34		22	21	15	13	12	3	0	8

* R = rhesus. C = cynomolgus. 0 = titer <1:4.
† Pooled rhesus and cynomolgus sera from other monkeys had c.f. reactions to all 6 types. None of the normal sera reacted with E-2, Nancy, Conn-5, Ohio-1, c.f. antigens.

the Coxsackie virus antigens and mouse hyperimmune sera were specific(1,3), no cross reactions being encountered. Omitted from Table I are data indicating that none of the Coxsackie virus mouse antisera reacted with the MEF1 or FA antigens.

The titers obtained with normal rhesus and cynomolgus monkey sera are similar in every respect to those found in the sera of Table I. Considering titers of 1:4 or higher as positive reactions, Table II indicates the number of normal monkeys, as well as the 2 pools of normal sera, manifesting the Coxsackie virus c.f. antibodies.

Both tables show that when reactions between monkey sera and antigens were seen, they were confined for non-Coxsackie antigens to the homologous reaction (FA and MEF1 in Table I) and to only 6 of the 10 Coxsackie types tested, *viz.* E-10, E-14, D-2, D-3, Alaska, Texas-1. All 6, however, were not present at the same time in every animal. Thus 8 of

TABLE III. Effect of Heat and Dialysis on c.f. Titers in Normal Monkey Serum.

Monkey	Serum treatment	Reciprocals of c.f. titers:† E-10	E-14	D-2	D-3
5789	Dialysis* and heat	60	12	112	24	48
		65	0	24	6	4
		68	0	20	0	0
		70	0	6	0	0
5782	Heat	60	32	32	16	32
		63	24	16	16	24
		65	8	12	8	16
		68	0	0	0	8
		70	0	0	0	0

* See text. Results were similar before dialysis.
† Neither monkey had c.f. antibodies to Alaska, Texas-1, E-2, Nancy, Conn-5, Ohio-1.
0 = titer <1:4.

TABLE IV. Effect of Heat on c.f. Titers in Monkeys Hyperimmunized with Coxsackie Viruses.*

Monkey and immune status	°C	Reciprocals of c.f. titers: E-10	E-14	D-2	D-3	Alaska	Texas-1	E-2	Nancy	Conn-5	Ohio-1
4766											
Pre-immunization	60	8	6	8	6	0	0	0	0	0	0
Immunized with E-2	60	48	64	48	80	56	32	160	48	0	0
	63	16	48	16	64	32	12	96	14		
	65	8	24	8	32	24	8	24	10		
	68	4	12	4	16	8	0	16	4		
	70	0	8	0	12	6	0	12	0		
	73	0	0	0	4	0	0	4	0		
4197											
Pre-immunization	60	0	64	0	48	48	0	0	0	0	0
Immunized with Texas-1	60	64	160	80	64	80	160	0	0	0	0
	63	32	96	20	32	32	128				
	65	24	32	8	12	20	80				
	68	16	8	0	0	6	32				
	70	6	6	0	0	0	16				
	73	0	0	0	0	0	6				
	75	0	0	0	0	0	0				

* c.f. titers of the immune sera after various additional treatments (see text) were not significantly different from those before treatment.
0 = titer <1:4.

34 monkeys had none of the 10 antibodies (Table II), whereas 26 had some combination of the 6 previously mentioned types. When titrated, the serum values ranged from 1:4 to 1:128; most were in the neighborhood of 1:32. Table I indicates that 6 of 8 hyperimmunized monkeys (poliomyelitis and FA immune) also manifested them in high titers. Sera from the latter had been prepared in 4 separate laboratories, although this appears to be of little consequence in view of the reaction taking place in normal monkey sera.

In a series of 10 monkeys bled at 4 to 7 day intervals for a period of 23 days, none showed any significant change in Coxsackie virus c.f. titers for that time. Four of these animals never had any of the 10 antibodies even though 2 of them were cage-mates of 2 others which did have some of them. Five of these 10 monkeys were tested with 5 "new" Coxsackie virus types, Texas-12, Texas-13, Texas-14, Texas-15, and Israel-7(1). The antibodies were distributed as follows:

No. of animals	No. of 10 "old" type antibodies present	No. of 5 "new" type antibodies present
3	0	0
1	4	2
1	6	5

Tables III and IV present the c.f. titers resulting when sera from uninoculated and Coxsackie virus hyperimmune monkeys respectively, were subjected to the indicated treatments. Increasing heat of inactivation was the only agent which affected the titers significantly. In hyperimmune monkey 4197 (Table IV), although the only virus given was Texas-1, the E-10, D-2, and Texas-1 c.f. antibodies rose significantly as a result of hyperimmunization whereas in monkey 4766 (Table IV), hyperimmunized with E-2 virus, all antibodies except Ohio-1 and Conn-5 rose significantly or appeared as the result of hyperimmunization.

Tests for *neutralizing* antibodies in Monkeys 4197 and 4766 indicated that only those for Texas-1 in the former, and those for E-2 in the latter, were present after hyperimmunization. They had been absent prior to immunization. *Neutralizing* antibodies for Coxsackie viruses were not detected in any of the normal monkeys nor in the monkeys hyperimmunized with FA virus or the poliomyelitis viruses.

Discussion. From the results presented it seems clear that both rhesus and cynomolgus monkeys, as they arrive in the laboratory, may

possess substances in their sera which are capable of reacting like complement fixing antibodies to certain of the Coxsackie viruses, and that, as far as has been determined, they are not accompanied by heat stable neutralizing antibodies. The striking pattern of the reactions, always being confined to antibodies of from one to 6 virus types of 10 types tested is noteworthy. (Five additional types were tested and antibodies to all were found.) The occurrence of these in normal monkeys explains their presence in the sera of monkeys immunized with poliomyelitis or other heterologous viruses.

Regardless of whether they were found naturally or produced by hyperimmunization of monkeys, these antibodies could not be differentiated by any of the treatments employed, *viz.* absorption by sheep cells, boiled guinea pig kidney, boiled beef cells; lyophilization with or without chloroform extraction; dialysis; the use of ultracentrifugally concentrated, purified antigens in the c.f. test; or increasing heat of inactivation. Only the latter treatment affected them significantly, but it affected both the natural and specifically induced antibodies in identical fashion.

Absorption of specific c.f. antibodies from monkey hyperimmune sera has not yet been accomplished although it has been repeatedly attempted. Be that as it may, the presence of these substances in monkey sera could conceivably reflect possible subclinical infection in them with agents (including additional Coxsackie virus types) which have antigenic relationships with the 6 (or 11) "reacting" types concerned in this report. It may also be that in the pathological processes resulting in the infected mouse tissues, certain Coxsackie viruses cause substances to be formed which are capable of reacting in the complement fixation test with antibody-like material in the serum of some monkeys. Even so, the question of the relationship between these serum substances normally present in monkeys and specifically induced antibodies remains open, since hyperimmunization with specific Coxsackie virus causes the appearance of, or significant rises in, heterologous Coxsackie virus c.f. antibodies, just as infection with a specific Coxsackie virus does in man(9).

Summary. In the sera of normal rhesus and cynomolgus monkeys substances have been encountered which are capable of fixing complement in the presence of as many as 6 (or 11) antigenically distinct Coxsackie, or C, virus antigens. "Antibodies" to 4 C virus types were consistently absent. The behavior of these antibodies did not differ significantly from Coxsackie virus complement fixing antibodies produced by specific immunization. Accompanying heat stable neutralizing antibodies have not been demonstrated in normal monkeys but only in those specifically immunized.

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