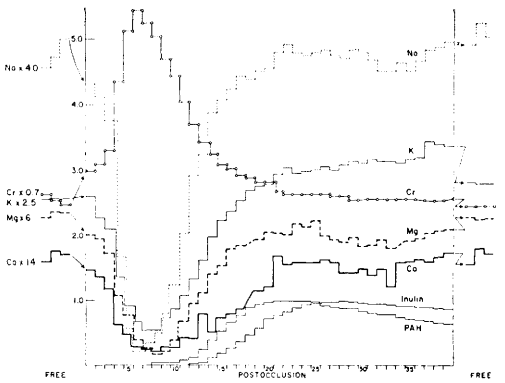

FIG. 1. Electrolyte excretion patterns from a stopped-flow experiment. Creatinine is represented as its U/P ratio, electrolytes (Ca, Mg, Na, K) as clearance ratios to creatinine, and PAH and inulin as fractions of their maximal urinary concentration. U/P and clearance ratios are fitted to arbitrary scale by multiplying by indicated factors. Electrolytes exhibit minima in urine which appear early following release of ureteral occlusion (left) and before appearance of PAH (right), thus relating transport areas to distal segment of nephron. Protocol: Nembutal and chloralose anesthesia; Prime: mannitol 2 g, creatinine 75 mg, KCl 1.5 mM, $CaCl_2$ 0.63 mM, $MgCl_2$ 0.35 mM, dog Ringer-Locke solution 44 ml, all/kilo; equilibration by urine reinfusion 150 min.; 0.6 ml urine samples collected serially following release of 7 min. occlusion; inulin and PAH inj. slowly 2 min. before release.

and Mg minima are "distal" to the region of PAH secretion indicating that these ions are reabsorbed in the "distal" segment of the nephron. The data fail to suggest existence of additional processes of either reabsorption or secretion of Ca or Mg.

A single, distal locus for magnesium transport offers an explanation for the observation of Robinson *et al.*(10) that radioactive Mg appeared only in the "distal" area of a stopped flow graph. Experience with other transport systems, such as sodium and urea in the frog(11,12), indicates that molecules may move opposite to the direction of principal movement while net transport is occurring.

Summary. 1. Stopped flow analysis indicates that calcium and magnesium are reabsorbed strongly in a "distal" region of the nephron approximately coincident with reabsorptive areas for sodium and potassium. 2. The data fail to suggest existence of additional reabsorptive or secretory loci for these ions.

1. Malvin, R., Wilde, W., Sullivan, L., *Am. J. Physiol.,* 1958, v194, 135.

2. Wirz, H., *Helv. Physiol., Pharm. Acta,* 1956, v14, 353.

3. Walker, A. M., Bott, Phyllis, Oliver, J., MacDowell, Muriel C., *Am. J. Physiol.,* 1941, v134, 580.

4. Richards, A. N., Barnwell, J. B. *Proc. Roy. Soc.* (B), 1927, v102, 72.

5. Wesson, L. G., Jr., *Methods in Medical Research,* 1952, v5, 175.

6. Wesson, L. G., Jr., Anslow, W. P., Jr., Raisz, L. G., Bolomey, A. A., Ladd, M., *Am. J. Physiol.,* 1950, v162, 677.

7. Schreiner, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 117.

8. Simonsen, D., Westover, L., Wertman, M., *J. Biol. Chem.,* 1947, v169, 39.

9. Kramer, B., Tisdall, F. F., *ibid.,* 1922, v53, 241.

10. Robinson, R., Murdaugh, H., Peschel, E., *Clin. Res. Proc.,* 1959, v7, 162.

11. Hoshiko, T., Swanson, R. E., Visscher, M. B., *Am. J. Physiol.,* 1956, v184, 542.

12. Love, J. K., Lifson, N., *ibid.,* 1958, v193, 662.

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Homologous and Heterologous Complement Fixing Antibody in Persons Infected with ECHO, Coxsackie and Poliomyelitis Viruses. (24896)

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Although a number of newly recognized viruses recovered from the alimentary tract of man were originally assembled in the ECHO group primarily because they apparently did not belong in already known families of viruses(1), it has become increasingly evident that most of them do share many bio-

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logic properties with each other and with the Coxsackie and poliomyelitis groups of viruses (2). This report will present data on antigenic relationships between these groups of viruses obtained by studying the complement-fixing (CF) antibody responses of persons naturally or experimentally infected with certain ECHO, Coxsackie, and poliomyelitis viruses.

Materials and methods. Sera. Five groups of paired human sera were studied. One group consisted of sera from 11 adults experimentally infected with ECHO type 10 virus (3); a second consisted of sera from 7 persons with naturally acquired ECHO type 16 infections; (these sera were kindly furnished by Dr. F. Neva); a third consisted of sera from 3 adults experimentally infected with ECHO type 20 virus(3); a fourth consisted of sera from 5 adults experimentally infected with Coxsackie B3 virus(3); and the fifth consisted of sera from 6 persons with naturally acquired type 1 paralytic poliomyelitis infections (these sera were kindly furnished by Dr. E. H. Lennette). *Virus strains.* The following virus strains were used to prepare CF antigens: prototype strains of ECHO types 1 through 20,(1,2,4), "Connecticut-5" strain of Coxsackie B1,(5), "Texas pleurodynia" strain of Coxsackie B3, (6) "Powers" strain of Coxsackie B4,(7), "Faulkner" strain of Coxsackie B5,(8), "Mahoney" strain(9) of poliomyelitis type 1, "MEF-1" strain(9) of poliomyelitis type 2, and "Saukett" strain(9) of poliomyelitis type 3. *CF antigens.* ECHO and poliomyelitis antigens, with one exception, were prepared as reported previously(10), except that 2% calf serum was used in place of 1%. In brief, the method was as follows: Rhesus kidney monolayer cultures in 32 oz. prescription bottles with 40 ml of medium consisting of 2% calf serum, 0.5% lactalbumin hydrolysate, and 97.5% Earle's solution were inoculated with virus material so diluted that complete degeneration of tissue did not take place in less than 2 days. After complete degeneration had taken place, the bottles were frozen and thawed once, and the fluids then harvested, and were not centrifuged. Fluids were then stored at approximately −20°C until used as antigen. Multiple freezing and thawing of the antigens was avoided, although no significant decrease in antigen CF titers could be demonstrated after 3 to 4 such cycles. Antigens for ECHO types 4, 5, 6, and 14, and all 3 poliomyelitis types were treated with fluorocarbon before use. Antigens for Coxsackie B viruses and ECHO type 20 were prepared in HeLa cell cultures grown in 32 oz prescription bottles with 40 ml of medium consisting of Eagle's basal medium, plus 10% human serum. After about 6 days, when cultures were very heavy, they were washed 3 times with 40 ml of Hanks' solution, and 30 ml of a medium containing 5% chicken serum, 25% tryptose phosphate broth, and 70% medium 199 was added. Cultures were then inoculated and handled as described above for rhesus kidney cultures. None of HeLa antigens were treated with fluorocarbon. *CF test.* Complement fixation tests were performed by standard technic used in this laboratory(10,11). No antigens were heated. Each antigen in each test was titrated against a standard homologous antiserum, monkey serum for ECHO and poliomyelitis antigens, and mouse serum for Coxsackie antigens. All antigens used had titers of 1:2 or 1:4 and at least 2 units of antigen were used in all tests.

Results. ECHO type 10. Homologous and heterologous CF titers of paired sera from 11 adults who were experimentally infected with ECHO type 10 virus and who subsequently showed a rise in homologous CF antibody are shown in Table I. With one exception, no individuals had a rise in CF antibody against any of the heterologous ECHO, Coxsackie, or poliomyelitis antigens used. Volunteer No. 6, who did show a rise in ECHO type 20 CF antibodies, worked in a hospital where he was in contact with volunteers who had been experimentally infected with ECHO type 20 virus. It was demonstrated that this individual also developed a rise in neutralizing antibody against ECHO type 20.

ECHO type 16. CF titers against various antigens of paired sera from 7 persons with naturally acquired ECHO type 16 infections ("Boston exanthem disease") are shown in Table II. Each pair of sera showed a homologous neutralizing antibody rise and ECHO

TABLE I. ECHO, Coxsackie, and Poliomyelitis CF Antibody Titers of Sera Collected from 11 Persons before and 28 Days after Experimental Infection with ECHO Type 10 Virus.

Date serum collected	ECHO antigens														Coxsackie B antigens		Poliomyelitis antigens		
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E14	E20	B1	B3	P1	P2	P3
7/22/57	<8	<8	16	<8	<8	32	<8	<8	<8	<8	32	<8	<8	<8	—*	—	<8	<8	<8
8/19	"	"	<8	"	"	16	"	"	"	128	16	"	"	"	—	—	"	"	"
7/22	"	"	"	"	"	32	"	"	"	<8	64	"	"	32	—	—	"	"	"
8/19	"	"	"	"	"	"	"	"	"	64	"	"	"	"	—	—	"	"	"
7/22	"	"	32	"	"	16	"	"	"	<8	32	"	"	<8	—	—	"	"	"
8/19	"	"	"	"	"	"	"	"	"	64	"	"	"	"	—	—	"	"	"
7/22	"	"	<8	"	"	<8	"	"	"	<8	"	"	"	8	—	—	"	"	"
8/19	"	"	"	"	"	"	"	"	"	64	16	"	"	<8	—	—	"	"	"
7/22	"	"	"	"	"	"	"	"	"	<8	"	"	"	8	—	—	"	"	"
8/19	"	"	"	"	"	"	"	"	"	64	<8	"	"	<8	—	—	"	"	"
7/22	"	"	16	"	"	"	"	"	"	<8	32	"	"	8	—	—	"	"	"
8/19	"	"	"	"	"	"	"	"	"	64	16	"	"	32	—	—	"	"	"
7/22	—	—	<8	—	"	—	—	—	—	<8	<8	—	—	—	—	—	—	—	—
8/19	—	—	"	—	"	<8	—	—	—	32	"	—	—	—	—	—	—	<8	<8
7/22	—	—	"	—	"	—	—	—	—	<8	"	—	—	—	—	—	—	—	—
8/19	—	—	"	—	"	<8	—	—	—	16	"	—	—	<8	<8	<8	—	<8	<8
7/22	—	—	8	—	"	—	—	—	—	<4	—	—	—	—	—	—	—	—	—
8/19	—	—	"	—	"	<8	—	—	—	16	<8	—	—	<8	<8	<8	—	<8	<8
7/22	—	—	"	—	"	—	—	—	—	<4	"	—	—	—	—	—	—	—	—
8/19	—	—	"	—	"	<8	—	—	—	8	"	—	—	<8	<8	<8	—	<8	<8
7/22	—	—	16	—	"	—	—	—	—	<4	8	—	—	8	—	—	—	—	—
8/19	—	—	8	—	"	<8	—	—	—	8	"	—	—	"	<8	—	<8	<8	<8

* — = not tested.

type 16 virus was recovered from 6 patients. Although only 2 individuals (WO & FD) had a CF antibody rise against the prototype strain of ECHO type 16 virus, 3 other persons (KW, RM, and AR) had CF antibody rises against one or more of the heterologous antigens used. Three individuals (WO, FD, and KW) had CF rises against a number of heterologous ECHO and Coxsackie antigens (ECHO types 2, 5, 6, 7, 8, 11, 14, 20 and Coxsackie B3).

ECHO type 20. CF titers for paired sera from 3 adults who were experimentally infected with ECHO type 20 virus and who showed a rise in homologous CF antibody are shown in Table III. Each individual developed heterologous CF antibodies (including ECHO types 5, 6, and 11, and Coxsackie B types 1, 3, 4, and 5).

Coxsackie B3. CF titers of paired sera from 5 adults who were experimentally infected with Coxsackie B3 virus and who showed a rise in homologous CF antigens are shown in Table IV. Three individuals had rises in heterologous CF antibodies (which included ECHO types 4, 6, 7 and Coxsackie B1).

Poliomyelitis type 1. CF titers against various antigens of paired sera from 6 persons with naturally acquired type 1 paralytic poliomyelitis are shown in Table V. Although it was possible to demonstrate a rise in homologous titer for 5 individuals, none developed a rise in heterologous CF antibodies against the antigens used. Moreover, a rise in poliomyelitis CF antibodies was not noted in persons infected with any of the other viruses in this study.

Discussion. Multiple heterologous CF antibody responses observed in persons infected with certain ECHO and Coxsackie viruses and absence of a predictable group response indicate that this type of serologic test with the type of antigens employed would be of very limited usefulness for type-specific diagnosis of infection by these viruses. It is quite possible, of course, that persons (*e.g.*, young children) undergoing their first infection with a

TABLE II. ECHO, Coxsackie, and Poliomyelitis CF Antibody Titers of Sera Collected from Persons in Acute and Convalescent Phase of "Boston Exanthem" Disease (ECHO 16).

Patient	Date serum collected	ECHO antigens E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E14	E16	E20	Coxsackie B antigens B1	B3	Poliomyelitis antigens P2	P3
WO	6/22/54	—*	64	—	—	—	64	<8	16	—	—	32	—	8	<8	64	—	16	—	—
	7/15	<8	128	<8	<8	<8	128	16	64	<8	<8	128	<8	32	64	128	—	64	—	—
FD	6/28	—	8	—	—	<8	8	<8	<8	—	—	8	—	<8	<8	8	—	<8	—	—
	7/15	<8	128	<8	<8	32	128	64	16	<8	<8	256	<8	16	32	256	—	64	—	—
KW	6/26	—	8	—	—	—	8	—	16	—	—	32	—	8	<8	16	—	8	—	—
	7/13	<8	32	<8	<8	<8	64	<8	64	<8	<8	128	<8	32	"	128	—	32	—	—
RM	6/22	—	—	—	—	—	—	—	—	—	—	—	—	—	"	<8	—	<8	—	—
	7/13	<8	<8	<8	<8	<8	8	<8	<8	<8	<8	<8	<8	<8	"	"	—	16	—	—
AR	9/13	—	—	—	—	—	—	—	—	—	—	—	—	—	"	—	—	8	—	—
	10/ 1	—	<16	<16	—	<16	<16	<16	<16	—	<16	<16	<16	<16	"	<16	<16	32	<16	<16
WE	6/28	—	—	—	—	—	—	—	—	—	—	<8	—	—	"	—	—	—	—	—
	7/13	—	<16	<16	—	<16	<16	<16	<16	—	<16	"	<16	<16	"	<16	<16	<16	<16	<16
TF	6/22	—	—	—	—	—	—	—	—	—	—	—	—	—	"	—	—	—	—	—
	7/13	—	<16	<16	—	<16	<16	<16	<16	—	<16	<16	<16	<16	"	<16	<16	<16	<16	<16

* — = not tested.

TABLE III. ECHO, Coxsackie, and Poliomyelitis CF Antibody Titers of Sera Collected from 3 Persons before and 28 Days after Experimental Infection with ECHO Type 20 Virus.

Date serum collected	ECHO antigens E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E14	E20	Coxsackie B antigens B1	B3	B4	B5	Poliomyelitis antigens P1	P2	P3
7/22/57	<8	<8	64	<8	<8	<8	<8	<8	<8	<8	16	<8	<8	<8	<8	8	8	<8	<8	<8	<8
8/19	"	"	128	"	"	32	"	"	"	"	64	"	"	64	32	32	32	32	"	"	"
7/22	"	"	16	"	<8	"	"	"	"	"	16	"	"	16	<8	16	<8	<8	8	"	"
8/19	"	"	"	"	16	"	"	"	"	"	64	"	"	64	32	64	"	16	<8	"	"
7/22	—*	—	<8	—	—	—	—	—	—	—	—	—	—	<8	<8	<8	"	<8	—	"	"
8/19	—	—	"	—	<8	<8	—	—	—	—	<8	—	—	32	16	32	32	16	—	"	"

* — = not tested.

TABLE IV. ECHO, Coxsackie, and Poliomyelitis CF Antibody Titers of Sera Collected from 5 Persons before and after Experimental Infection with Coxsackie B3 Virus.

Date serum collected	ECHO antigens												Coxsackie B antigens		Poliomyelitis antigens	
	E1	E2	E3	E4	E5	E6	E7	E8	E10	E11	E12	E14	B1	B3	P2	P3
10/ 1/56	—*	—	—	—	—	<8	<8	—	—	—	—	—	<8	<8	—	—
12	<16	<16	<16	<16	<16	64	32	<16	<16	<16	<16	<16	64	256	<16	<16
1	—	—	—	<8	—	—	—	—	—	—	—	—	—	<8	—	—
16	<16	<16	<16	32	<16	<16	<16	<16	<16	<16	<16	<16	<16	128	<16	<16
1	—	—	—	—	—	—	<8	—	—	—	—	—	<8	<8	—	—
20	<16	<16	<16	<16	<16	<16	16	<16	<16	<16	<16	<16	64	128	<16	<16
1	—	—	—	—	—	—	—	—	—	—	—	—	<8	<8	—	—
22	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	?	32	<16	<16
1	—	—	—	—	—	—	—	—	—	—	—	—	—	<8	—	—
12	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	16	<16	<16

* — = not tested.

virus of the enterovirus family might respond only with type-specific CF antibody and that heterologous responses observed in adults were the result of previous enterovirus infections.

Although CF antibody response is of limited usefulness for diagnostic purposes, it does furnish additional evidence of relationships between various members of the enterovirus group. In this study ECHO virus types 2, 4, 5, 6, 7, 8, 11, 14, 16, and 20, and Coxsackie B virus types 1, 3, 4, and 5 were linked to each other by significant heterologous antibody responses. It is theoretically possible that some apparently heterologous responses were due to actual infection with the heterologous virus. However, the short period of time between the 2 serum specimens and age and environment of persons studied make this possibility unlikely.

Although ECHO virus types 1, 3, 9, and 12 were not linked to previously mentioned types by unequivocal CF antibody responses, antigenic relationships between ECHO type 9 and ECHO types 4 and 6, Coxsackie A types 5 and 7, Coxsackie B types 3 and 4, and poliovirus types 1, 2, and 3 have been demonstrated in another investigation(12). Antigenic relationships between poliovirus types and certain other enteroviruses have also been reported in 2 additional studies(13,14). Absence of such reactions in our study may have been the result of small number of sera tested, type of antigen used, technic of the test, dilutions of sera tested, or other unknown factors.

Absence of antigenic relationships between ECHO type 10 virus and the others included in this study is in accord with other data on characteristics of ECHO type 10 virus, which suggest that it is not related to other ECHO viruses(2,15).

Summary. A study of heterologous CF antibody responses in persons naturally or experimentally infected with ECHO virus types 10, 16, and 20, Coxsackie B virus type 3, or poliovirus type 1, indicated an antigenic relationship between the following viruses: ECHO types 2, 4, 5, 6, 7, 8, 11, 14, 16, and 20, Coxsackie B virus types 1, 3, 4, and 5. No antigenic relationship was noted between ECHO

TABLE V. ECHO, Coxsackie, and Poliomyelitis CF Antibody Titers of Sera Collected from Persons in Acute and Convalescent Phase of Type 1 Paralytic Poliomyelitis.

Patient	Date serum collected	ECHO antigens E2	E3	E4	E6	E7	E11	Coxsackie B antigens B1	B3	B5	Poliomyelitis antigens P1	P2	P3
DF	8/ 2/56	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8
	27	"	"	"	"	"	"	"	"	"	32	"	"
RC	9/10	8	8	"	32	16	"	"	"	"	<8	"	"
	10/17	8	8	"	"	"	"	"	"	"	32	"	"
ED	7/25	8	<8	"	<8	<8	"	"	"	"	<8	"	"
	8/13	8	"	"	"	"	"	"	"	"	32	"	"
TG	8/17	8	"	"	16	32	"	"	"	"	<8	"	"
	9/ 7	<8	"	"	8	"	"	"	"	"	16	"	"
DG	8/ 3	"	"	"	<8	<8	"	"	"	"	<8	"	"
	25	"	"	"	"	"	"	"	"	"	16	"	"
RP	9/25	16	8	"	"	16	"	32	32	32	<8	"	"
	10/10	"	8	"	"	"	"	"	"	"	"	"	"

virus type 10 and any of the other enteroviruses.

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1. Committee on the ECHO viruses, Nat. Fn. for Infantile Paral., *Science,* 1955, v122, 1187.

2. Committee on the enteroviruses, Nat. Fn. for Infantile Paral., *Am. J. Public Health,* 1957, v47, 1556.

3. Huebner, R. J., Ward, T. G., Paffenbarger, R. S., Suskind, R. G., unpublished data.

4. Rosen, L., Johnson, J. H., Huebner, R. J., Bell, J. A., *Am. Jour. Hyg.,* 1958, v67, 300.

5. Curnen, E. C., Shaw, E. W., Melnick, J. L., *J. Am. Med. Assn.,* 1949, v141, 894.

6. Huebner, R. J., Risser, J. A., Bell, J. A., Beeman, E. A., Beigelman, P. M., Strong, J. C., *New Eng. J. Med.,* 1953, v248, 267.

7. Cheever, F. S., Daniels, J. B., Hersey, E. F., *J. Exp. Med.,* 1950, v92, 153.

8. Dalldorf, G., Sickles, B. A., in Diagnostic Procedures for Virus and Rickettsial Diseases, *Am. Public Health Assn.,* New York, 1956, 153.

9. Salk, J. E., *J. Am. Med. Assn.,* 1953, v151, 1081.

10. Halonen, P., Huebner, R. J., Turner, H. C., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 530.

11. Bengtson, I. A., *Pub. Health Rep.,* 1944, v59, 402.

12. Johnsson, T., Böttiger, M., Löfdahl, A., *Arch. f. Virusforsch.,* 1958, v8, 306.

13. Zeipel, G. von, Svedmyr, A., *ibid.,* 1957, v7, 355.

14. Hammon, W. McD., Yohn, D. S., Ludwig, E. H., Pavia, R. A., Sather, G. E., McCloskey, L. W., *J. Am. Med. Assn.,* 1958, v167, 727.

15. Hsiung, G. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 387.

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Formalin Treated Chicken Erythrocytes as Indicators of Influenza A Virus (Asian) and Its Antibody.* (24897)

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Formalin treated human erythrocytes have been used to measure the hemagglutinating capacity of influenza virus(1,2), detect antibodies against coupled protein(3,4), haptens (4,5) and enterobacterial antigens(6). While such erythrocytes were reported(1) as early as 1948 to be comparable to normal human and

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