and increase the F_t. Second, the removal of proteins from the suspending medium lowers the density of the medium, increasing the density gradient between the cells and the medium and thus favoring cell packing. Although this change in density gradient could play a part in bringing about the difference in plasma trapping between Ringer suspension of cells and whole blood in a given species (goat), it is not the factor causing the difference in plasma trapping of whole blood among the species studied. As shown in Table I, the densities of cells and plasma do not exhibit striking species differences, and the small differences observed cannot be correlated with variations in F_t. The amount of albumin in albumin-I^{131} solution added to the Ringer suspensions was sufficient to avoid adsorption of albumin-I^{131} onto glassware(6). If a significant degree of adsorption had occurred, the Ringer suspensions would have had lower, instead of higher, F_t values than the corresponding whole blood.

For a given species, F_t is greater with the micro-method than with the macro-method. Since a shorter duration of centrifugation was used in the micro-method, the lesser plasma trapping or better cell packing is undoubtedly due to the greater force of centrifugation employed.

Summary. With the use of albumin-I^{131}, the volume of plasma trapped in the packed cell column of hematocrit tubes was determined for blood samples obtained from 5 animal species. The volume of plasma trapped was least in blood samples obtained from elephant, man and dog (MCV 112 to 72 μ^3), larger in sheep blood (MCV 37 μ^3) and largest in goat blood (MCV 18 μ^3). Increasing the force of centrifugation or removal of plasma proteins reduced the volume of fluid trapped in the packed cell column, and the reduction was most pronounced for goat blood.

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Antigenic Relationship Between Strains of Echovirus Types 6 and 30.* (30403)

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Minor antigenic relationship between the prototype strain and one prime variant of echovirus type 6 and 2 strains of echovirus type 30 has been reported from this laboratory(1). This relationship was discussed at a recent meeting of the Panel for Picornaviruses; further study of the above problem was suggested.† This communication reports

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TABLE I. Cross Neutralization Tests with 3 Strains of Echovirus Type 30 and 3 Strains of Echovirus Type 6.

Virus	Antisera					
	E-30 (Bastianni)	E-30 (Frater)	E-30 (Giles)	E-6 (D'Amori)	E-6′ (D-1)	E-6″ (Burgess)
E-30 (Bastianni)	6400*(550)†	4000 (550)	25 (550)	<25 (550)	<25 (550)	<25 (550)
E-30 (Frater)	250 (316)	1000 (316)	<25 (316)	<25 (316)	<25 (316)	<25 (316)
E-30 (Giles)	250 (176)	<25 (176)	6400 (176)	<25 (176)	<25 (176)	<25 (176)
E-6 (D'Amori)	<25 (550)	<25 (550)	<25 (550)	1600 (550)	3200 (550)	4000 (550)
E-6′ (D-1)	<25 (316)	<25 (316)	<25 (316)	3200 (316)	1600 (316)	6400 (316)
E-6″ (Burgess)	<25 (550)	<25 (550)	<25 (550)	6400 (550)	1600 (550)	≥6400 (550)

* Reciprocal of serum dilution. † TCD_{50} in parentheses.

that: (a) major antigenic relationship could not be detected between strains of echovirus types 6 and 30 either by serum neutralization or hemagglutination-inhibition; and (b) one-way crosses were obtained between strains of echovirus 6, and three representative strains of echovirus 30 by complement fixation.

Materials and methods. Viruses. Four strains of echovirus type 30, namely Bastianni (the prototype), Frater, Giles and PR-17(2) were selected for this study. Three strains showed consistent hemagglutination at this laboratory. Echovirus type 6 strains, namely D'Amori (6), D-1 (6′), and Burgess (6″) were from our reference enterovirus stocks.

Antisera. All antisera were prepared in rhesus monkeys according to the method described earlier(3).

Monkey kidney (MK) cell cultures. Kidney cells from rhesus monkeys were grown and maintained, unless otherwise stated, as described by Melnick *et al*(4).

Serum neutralization (SN) test. The neutralization tests were performed in MK tubes with 100-500 TCD_{50} of virus and 4-fold serial dilutions (1:25 to 1:6,400) of antisera (inactivated at 56°C for 30 minutes). Serum endpoints were determined after 7-9 days of incubation by the method of Reed and Muench(5).

Hemagglutination (HA) and hemagglutination-inhibition (HI) tests. HA antigens were prepared in MK cells inoculated with approximately 10 TCD_{50} of virus and maintained on medium 199 without serum(6). As recommended by Kern and Rosen(7), all strains of echovirus types 6 and 30 were tested for HA with human O erythrocytes at pH 5.8, 6.9 and 7.4, and at temperatures 4°C, 25°C and 37°C. HI tests were performed by the microtiter system(8) with 4 units of HA antigen and 0.5% human O erythrocytes. All sera were treated with kaolin and absorbed with erythrocytes prior to testing. Antigen-antiserum mixtures were incubated at 25°C for 1-2 hours before addition of erythrocytes, after which the plates were incubated at 37°C for 90-120 minutes. The HA and HI tests were read by the pattern method.

Complement-fixation (CF) test. CF tests were done by the microtiter system using 2 units of antigen and 5 $C'H_{50}$ of complement with overnight fixation at 4°C. CF antigens consisted of centrifuged harvests of infected MK tubes showing 4+ CPE. Antigen unit was determined by box titration and generally 1:8 to 1:16 dilutions of antigen harvests constituted one unit. All sera were inactivated at 56°C for 30 minutes. Veronal buffer saline was used as diluent in all tests.

Results. Cross neutralization tests. Results of cross neutralization tests with Bastianni, Frater, and Giles strains of type 30 and D'Amori (6), D-1 (6′) and Burgess (6″) strains of type 6 and their respective antisera are presented in Table I. In all tests, the presence of a sufficient number of infectious units (TCD_{50}) was established by simultaneous virus titrations. While considerable crossings occurred among the 3 intratypic strains of each type, no crossings were encountered between strains of the 2 distinct serotypes. However, in the course of daily microscopic examinations of the neutralization tubes, it was often observed that some sera delayed the appearance of CPE of heter-

TABLE II. Cross HI Tests with Frater, Giles and PR-17 Strains of Echovirus Type 30 and E-6′ Strain of Echovirus Type 6.

Virus	Antisera			
	Frater	Giles	PR-17	E-6′
Frater	640*	10	160	10
Giles	10	640	≧1280	10
PR-17	<10	80	≧1280	10
E-6′ (D-1)	<10	<10	<10	640

* Reciprocal of serum dilution.

All tests performed by the microtiter system using 2-4 HA units and 0.5% human O erythrocytes at pH 5.8. Antigen-antiserum mixtures were incubated at room temperature for 2 hr prior to addition of erythrocytes.

otypic strains, but break-throughs always occurred and CPE was complete by the seventh day of the observation period.

Hemagglutination-inhibition tests. All 6 strains of echovirus 30 available at this laboratory (Bastianni, Frater, Price, Giles, PR-17, and 1733) and the prototype and 2 prime variants of echovirus 6 were grown and tested for hemagglutination under optimal conditions as described above. Only 3 strains of type 30, namely, Frater, Giles, and PR-17 (the last 2 hitherto reported as non-hemagglutinating viruses), and one strain of type 6, namely D-1, showed significant hemagglutination titers. The highest HA titers for all 4 viruses were obtained at pH 5.8 and 37°C. These 4 viruses and their antisera were then tested by cross HI tests; the results obtained are presented in Table II. No significant heterotypic crossings were detected with the 3 strains of type 30 or the D-1 strain of type 6. With regard to intratypic strains of type 30, the data disclose that while PR-17 antiserum showed significant crossing with Frater and Giles viruses, antisera to Frater and Giles strains showed either considerably less (Giles antiserum against PR-17 virus) or insignificant crossing with other intratypic strains.

Complement-fixation tests. Antigens and antisera encompassed in cross CF tests included three strains of type 30, and the prototype and prime variants of type 6 echoviruses. Uninoculated MK culture fluid overlay served as antigen control in all tests. Results of these tests are recorded in Table III. Type 6 antigens failed to fix complement with the type 30 antisera; conversely, the type 30 antigens fixed complement with type 6 antisera. Similar results, confirming the one-way cross were obtained in two independent tests using either frozen or lyophilized reference antisera.

The titer values obtained with type 6 antisera and type 30 antigens were often equaled, but never exceeded the homotypic titers of type 30 echoviruses, thereby indicating significant relationship between the pairs. The echovirus 6′ (D-1) with generally lower titers in CF tests than the prototype or 6″, also reacted in a corresponding manner in serum neutralization tests.

Discussion. No major antigenic relationship between strains of echovirus types 6 and 30 was detected by SN tests. However, certain sera representing both types delayed the appearance of CPE in heterotypic SN tests, but as mentioned above, break-throughs occurred by the end of the usual observation period. The minor antigenic relationship between certain heterotypic strains of echovirus types 6 and 30, reported earlier from this

TABLE III. Cross CF Tests with 3 Strains of Echovirus Type 30 and 3 Strains of Echovirus Type 6.

Virus	Antisera					
	Bastianni	Frater	Giles	E-6 (D'Amori)	E-6′ (D-1)	E-6″ (Burgess)
Bastianni	≧1024*	512	≧1024	≧1024	64	≧1024
Frater	128	1024	32	128	64	64
Giles	≧1024	256	≧1024	1024	32	≧1024
E-6 (D'Amori)	<8	<8	<8	256	8	64
E-6′ (D-1)	<8	<8	<8	128	64	64
E-6″ (Burgess)	<8	<8	<8	128	64	256

* Reciprocal of serum dilution.

All tests performed by the microtiter system using 2 units of antigen and 5 $C'H_{50}$ of complement with overnight fixation at 4°C.

laboratory(1), may be attributable to such delays in CPE in neutralization tests. On the other hand, results of HI tests performed with 3 strains of type 30, two of them (Giles and PR-17) hitherto reported as non-hemagglutinating viruses, and our only hemagglutinating strain of type 6, namely D-1 (6'), showed a complete lack of cross reactivity.

The CF tests, however, revealed significant crossings between antisera to the 3 strains of echovirus type 6 and antigens derived from Bastianni, Frater, and Giles strains of echovirus type 30. Furthermore, with D'Amori and Burgess antisera, the heterologous titers were at least 4-fold greater than the homologous titers. No crossing was observed between antisera to strains of type 30 and antigens of type 6. Heterotypic CF crossings among enteroviruses using sera from patients infected with these viruses have been reported by many workers(9,10). Such findings, usually without corresponding heterologous neutralizing antibodies generally have been considered as anamnestic responses to prior infection with the heterotypes. With specific monkey or mice antisera, however, little heterologous CF cross reactivity has been observed(11,12,13).

The prototype (D'Amori) and the 2 prime strains (D-1 and Burgess) of echovirus type 6 were isolated from cases of aseptic meningitis and diarrhea(14,15). The prime strains were originally defined as viruses producing antisera which neutralized the prototype strain at high dilutions but themselves were poorly neutralized by antiserum prepared against the prototype virus(16). Later studies, however, showed that considerable crossings, as detected by neutralization test, existed among these strains(3). Preliminary results of work now under way at this laboratory have shown a similar heterogeneity among various strains of echovirus type 30 (17). In the type 30 group, strains Frater (18), 1733(19), Bastianni(20), Giles(21), and Price(22) were all isolated from patients with CNS disease and strain PR-17 (23) from a Puerto Rican infant with diarrhea.

Types 6 and 30 echoviruses, among all other echovirus serotypes, show the widest ranges in antigenic heterogeneity. This is possibly another resemblance in their propensities as human pathogens, but heretofore these characteristics have not suggested antigenic relationship. Only now, with the demonstrable one-way serologic relationship do these properties seem to emerge as of some importance not only in immunology, but also pathogenesis and epidemiology.

The significant one-way CF crosses between type 6 antisera (prepared several years before strains of echovirus type 30 were recognized) and echovirus 30 antigens require further study. At this time type 30 antigens appear to share common components (perhaps soluble antigens) with echovirus 6 strains; it is not clear why echovirus 6 strains do not reciprocate. Possibly 2-way crosses may be demonstrable with recent human isolates of type 6, for it is possible that during many serial passages in tissue culture, they have lost the reacting antigen. A corollary proposition exists: antisera produced against strains of echovirus type 6 not only identify intratypic strains by CF test, but strains of echovirus type 30 as well.

Summary. The minor antigenic relationship between the prototype and one prime variant strain of echovirus type 6 and 2 strains of echovirus type 30, reported earlier from this laboratory, was further investigated. It was found that whereas no significant antigenic relationship between the various heterotypic strains of these two types is detected by serum neutralization and hemagglutination-inhibition tests, significant one-way crosses occur in complement fixation tests with antisera to the 3 strains of echovirus 6 and antigens derived from 3 representative strains of echovirus 30. The significance of this relationship and its application in determinative virology is discussed.

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An Immunologic Classification of Heterotypic Antibody Responses to Adenoviruses in Man. (30404)

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When human beings are infected or immunized with an adenovirus, neutralizing and hemagglutination-inhibition (HI) antibody responses to other adenovirus serotypes frequently occur(1-12). Antigenic relationships between several serotypes have been inferred from the fact that previous exposure with one type is followed by a significantly increased frequency of responses to other types; namely, between types 3, 7, 11 and 14(8,9), and between types 26 and 27(11). More commonly, however, the responses are distributed in an apparently random fashion among the various serotypes. The term "recall" has been used to indicate that such heterotypic responses may represent a non-specific evocation of antibody to serotypes with which the host has had prior contact(9).

Recently, studies in volunteers in this laboratory have suggested an explanation for heterotypic antibody responses to adenovirus type 26 and type 27 infections in man (13). The results presented in that study and the findings reported herein indicate that these responses are specific and reflect the hitherto unrecognized existence of 3 major immunologic groups among the adenoviruses. These groups were delineated when it was noted that heterotypic antibody responses in man separate into 3 groups which coincide with an earlier classification of human adenoviruses by Rosen which is based on the agglutination of rat and rhesus monkey erythrocytes(14).

The present report will describe the studies which relate the proposed immunologic classification of human adenoviruses to the hemagglutination (HA) groups and will assess the

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