TABLE I.
Cross-Neutralization Tests with Coxsackie Viruses.

Virus strain*	Controls	Immune serum		
		Dallas—M.B.	Conn. No.5	Coxsackie—T.T. (type 1)
Dallas—M.B.	2,2,2,3,3†	S,S,S,S	4,4,4,4	3,3,3,4
Bonham—N.V.	2,2,2,2,2,3,3,3	S,S,S,S,S	3,4,4,4	4,4,4,5,5
Texas 1-2	3,3,3,3,4,4,4	S,S,S,S	—	3,4,4,4,4
Conn. No. 5	5,5,5,7,7,7,7	5,5,5,5	S,S,S,S	3,3,3,5,5,5,5
Coxsackie—T.T. (type 1)	3,3,3,3,3,3,3	3,3,3,3	3,3,3,3	S,S,S,S,S
" —Fl. (type 2)	2,2,2,2,3	3,3,3,3,3	3,3,3,3,3	—
" —J.Ol. (type 3)	2,2,2,2,3	2,2,2,2,3	2,2,2,3,3	—
B.J.	5,5,7,7,7	7,7,7,7	5,5,7,7	—

* 10% infected mouse brain suspension.
† Figures indicate days of death or paralysis.
S—Animal survived.
— Tests not done.

1, 2 and 3 are serologically distinct. Dalldorf(6) has further indicated that the Texas 1-2 strain of Melnick differs serologically from the strains which he has designated types 1, 2 and 3. Melnick and associates(3) have reported that the Connecticut No. 5 and Texas 1-2 strains are unrelated.

Thus it appears that at least 5 serological types of Coxsackie virus have been encountered. Whether the B.J. strain represents another serological type or is a member of the Coxsackie types 1, 2 or 3 has not been determined.

The observation that 2 of the strains (Dallas—M.B. and Bonham—N.V.) isolated in this laboratory are similar to the strain isolated by Melnick from flies collected in the Rio Grande Valley in 1947(3) and the relationship of the latter to a strain isolated from sewage collected in North Carolina(3) may have epidemiological significance. To our knowledge human infection with this type has not been previously reported.

Summary. A serological comparison of 2 virus strains recently isolated from human paralytic infections and other viruses of the Coxsackie family is presented. The results indicate that at least 5 serological types of Coxsackie virus have been encountered. To our knowledge human infection with the type isolated in this laboratory has not been previously reported.

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Complement Fixation with a Coxsackie Virus. (17676)

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The need for a rapid and simple method of diagnosis in Coxsackie virus infections prompted an investigation of the complement-fixing capacity of virus suspensions and specific immune serums. Because of the widespread occurrence of neutralizing antibodies in presumably normal persons, the neutralization test as previously described(1,2) and as done in this laboratory may prove to be of limited value in the diagnosis of Coxsackie virus infections. In addition, the procedure is time-consuming and costly. Immune guinea

1. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, v89, 567.
2. Melnick, J. L., Shaw, E. W., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 344.

TABLE I.
Complement Fixation by Immune Guinea Pig Serum and Coxsackie Virus Infected Tissue Antigens.

Nature of antigen	Dilution of immune guinea pig serum	Antigen dilutions: Virus antigen 8	16	32	64	128	Control antigen 4	8	16	32
Crude suspension	1:4	4	4	4	2	0	4	3	2	1
	1:16	4	4	1	0	0	—*	0	0	0
	1:32	4	4	0	0		—	0	0	
	1:64	4	1	0	0		—	0		
	1:128	2	1	0			—	0		
	1:256	1	0	0			—	0		
Centrifuged 2500 rpm 30 min.	1:4	4	4	4	2	0	3	1	0	0
	1:16	4	4	1	0	0	0	0	0	
	1:32	4	1	0	0		0	0		
	1:64	4	1	0	0		0	0		
	1:128	0	0	0			0	0		
	1:256	0	0				0	0		
Centrifuged 5000 rpm 30 min.	1:4	4	4	2	1	0	2	0	0	0
	1:16	4	1	0	0	0	0	0	0	
	1:32	4	1	0			0	0		
	1:64	0	0				0	0		
	1:128	0	0				0	0		
	1:256	0	0				0	0		

4 = Complete fixation.
3, 2, and 1 = proportionately lesser fixation.
0 = Complete hemolysis.
* Reading of hemolysis not accurate due to turbidity of crude antigen in this dilution.

pig serum for the Dallas—M.B. strain(3) was used for the initial experiments. Complement fixation tests with two types of antigens have been done. In the first attempts, unsuccessful results were obtained when crude suspensions of brain tissue from infant mice infected with the Dallas—M.B. strain was used as antigen. Following the report of Gifford and Dalldorf(4) that the virus content of paralyzed muscle of infected mice is many times greater than brain material, the legs of mice infected with this strain were harvested and ground with alundum as a 33% suspension in saline. The mixture was centrifuged at 2500 r.p.m. for 2 minutes and the supernatant fluid was tested for its suitability as antigen in the complement fixation test. Positive results were obtained with this preparation, but non-specific fixation occurred as shown by tests with tissue extracts prepared from normal infant mice using the same procedure as for virus antigen. The results led to a third series of experiments with centrifuged virus and control antigens treated in an identical manner. These antigens were prepared from crude muscle tissue suspensions as described above and further treated as follows: 1. Supernatant fluid from ground muscle tissue centrifuged at 2500 r.p.m. for 2 minutes. 2. Supernatant fluid from the same material centrifuged at 2500 r.p.m. for 30 minutes. 3. Supernatant fluid from same material centrifuged at 5000 r.p.m. for 30 minutes.

In the test, the immune guinea pig serum was inactivated at 56°C for 30 minutes and diluted 1:4, 1:16, 1:32, 1:64, 1:128 and 1:256. Virus antigen dilutions of 1:8, 1:16, 1:32, 1:64 and 1:128, and normal tissue extracts (control antigen) dilutions of 1:4, 1:8, 1:16 and 1:32 were prepared. Each dilution of immune serum was tested with each of the dilutions of both virus and control antigens. The immune serum (0.2 ml) was mixed with an equal amount of the appropriate dilution of the antigen and 0.2 ml of complement con-

3. Sulkin, S. E., Manire, G. P., and Farmer, T. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 340.

4. Gifford, R., and Dalldorf, G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 589.

taining 2 exact units. The tubes were incubated in a water bath at 37°C for 2 hours and 0.4 ml of a mixture containing equal parts of 2% washed sheep erythrocytes and hemolysin (2 units per 0.2 ml) was added to each of the tubes. The tests were read after further incubation for 30 minutes. Complete fixation was recorded as 4+ and complete hemolysis as 0. The usual controls were included. The results of these tests are shown in the accompanying table.

An analysis of the results would indicate that antigens prepared from infected mouse muscle tissue give specific fixation of complement in the presence of homologus guinea pig immune serum. These sera failed to fix complement in the presence of comparable dilutions of antigens prepared from normal mouse muscle tissues. Centrifugation of crude antigens further increased the specificity of the test as shown by the comparable titers obtained with the virus antigen after centrifugation at 2500 r.p.m., whereas nonspecific fixation was virtually eliminated. Further centrifugation at 5000 r.p.m. did not improve the antigen. Additional studies on the use of other purification procedures and on the reactivity of antigens prepared with other serologic types are in progress.

Preliminary observations on the use of this procedure in analyzing the sera from patients from whom the virus has been isolated, patients suspected of infection with this virus, and from presumably normal individuals, indicate that the complement fixation test may be of value in the study of Coxsackie infection in the human.

Summary. The technique of a complement fixation test which may prove of value in the study of Coxsackie virus infection in man is described.

Addendum. Since this manuscript was submitted for publication, Casals *et al.*, Howitt and Benefield, and Melnick *et al.* have reported complement fixation with strains of Coxsackie virus.

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Homologous Transplantation of Fetal Endocrine Tissue to Adult Non-Related Host.* (17677)

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This report concerns itself with the surgical transplantation of endocrine tissues to normal animals. Previous investigators have made either autographs [Halstead(1), Ingle (2)] of the parathyroids and the adrenals, or syngenesiographs [Pomerat(3), Robertson(4) and Turner(5)] of the adrenal and ovary. Review of the literature reveals no reports of successful transplantation of endocrine tissue to animals of the same species which are not closely related—a homologous graft. Greene(6) has demonstrated that embryonic tissues, like cancer tissue, possess autonomy and may be transplanted to the anterior

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2. Ingle, D. J., and Higgins, G. M., *Endocrinology*, 1938, v22, 458.

3. Pomerat, C. M., Breckenridge, C. G., and Gordon, L., *Endocrinology*, 1944, v34, 60.

4. Robertson, G. C., PROC. SOC. EXP. BIOL. AND MED., 1945, v59, 30.

5. Turner, C. D., *Anat. Rec.*, 1939, v73, 145.

6. Greene, H. S. N., *Cancer Research*, 1943, v3, 809.