Use of Complement Fixation in the Differentiation of Strains of Coxsackie Virus. (17586)

BEATRICE F. HOWITT AND URSULA R. BENEFIELD

From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.*

During the past year a group of viruses has been isolated from the tissues and excretions of individuals suffering from illnesses of undetermined etiology. These agents are lethal for suckling mice and hamsters and are similar to the recently designated Coxsackie viruses isolated and described first by Dalldorf and Sickles, *et al.*(1,2) and later by Melnick, Shaw, and Curnen(3). It had been observed by these workers and also in this study that the Coxsackie virus strains were not all serologically similar. After receiving antisera for viruses designated as Types 1, 2, and 3 from Dr. G. Dalldorf of the New York State Laboratory, it was found by means of the neutralization test that certain of the strains isolated here corresponded to Types 1 and 2, but none so far to Type 3. Two additional distinct types have been differentiated in this laboratory and are called Types 4 and 5. Although the strains obtained could be typed by the neutralization method, it was thought advisable to determine whether the complement fixation test could be used both for this purpose and for determining the presence of antibodies in human sera.

Methods. (a) *Immune Sera.* Ten per cent suspensions of mouse brains or filtered muscles containing active virus for each of Types 1, 2, 4, and 5 were prepared in buffered saline and kept frozen until needed. Six-week-old mice were given biweekly intraperitoneal injections of 0.2 ml of the stock vaccines over a period of 3 weeks. One week after the last inoculation, the animals were bled and the serum frozen. Adult hamsters could be immunized in the same manner with infected hamster tissues.

(b) *Antigens.* Filtered muscle tissue was used for the antigens because the skeletal muscles of suckling mice or hamsters contained high concentrations of virus. The whole mouse could be employed also after removal of viscera, but did not seem quite as potent as the more concentrated muscle virus filtrates. The skeletal muscles were removed from 20 to 30 mice that had shown definite muscle paralysis from 2 to 5 days after intramuscular inoculation of 0.03 ml of virus. The skinned muscles were weighed and ground in a Waring blendor with buffered saline to make a 20% suspension. The material was removed to a flask, left overnight at 4°C, and then centrifugated for 30 minutes at 2500 rpm in the horizontal centrifuge. The supernatant fluid was passed through a Seitz filter. After alternately freezing and thawing the filtrate 5 times in an alcohol-dry ice mixture, it was centrifugated for ninety minutes at 13000 rpm in the angle centrifuge in the cold room. The supernatant fluid was stored in small bottles at 4°C. No preservative was added. The antigens for each type of virus were standardized, and usually 4 units of antigen were used in the test against human sera. However, the antigen unitage was not determined when typing new strains since no homologous antiserum was available; therefore, undiluted antigen for each new virus was used against standard antisera of the known types.

(c) *The Complement Fixation Test.* The complement fixation tests were performed according to a modified Kolmer technic employing one-half the quantities with a total volume of 1.5 ml. Three per cent washed sheep red blood cells and 2 full units of complement were used, the complement having been titrated with the antigens before each test. Serum, antigen, and complement were placed in the

* From the Virus and Rickettsia Branch, Laboratory Division, Montgomery, Ala.

1. Dalldorf, G., and Sickles, G. M., *Science,* 1948, v108, 61.

2. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.,* 1949, v89, 567.

3. Melnick, J. S., Shaw, E. W., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 344.

TABLE I.
Comparison of Complement Fixation and Neutralization Tests in Typing Coxsackie Viruses.

Location of viruses isolated	Total cases typed	No. pos. by C.F. Virus type				No. pos. by neutralization Virus type			
		1	2	4	5	1	2	4	5
Alabama	13	1	2	8	2	1	2	8	2
Delaware	4	1	1	2		1	1	2	
Florida	2				2				2
Georgia	1		1				1		
Louisiana	4		3	1			3	1	
Oklahoma	1		1				1		
Tennessee	10	1	3	5	1	1	3	5	1
Totals	35	3	11	16	5	3	11	16	5

TABLE II.
Comparison of Neutralization and Complement Fixation Tests with Coxsackie Virus Antigens.

Patient's sera	No. days after onset	A. Neutralization* Coxsackie viruses		B. Complement fixation† Type 1 antigen Serum dilutions				Type 2 antigen Serum dilutions			
		Type 1	Type 2	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
B.	2	+	+	2	0	0	0	4	4	3	0
	35	+	+	4	2	1	0	4	2	1	0
C.	5	0	+	0	0	0	0	4	4	4	1
	34	0	+	0	0	0	0	4	4	1	0
D.C.	4	0	0	0	0	0	0	4	4	4	4
	28	0	+	0	0	0	0	4	4	4	4
L.C.	1	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0
J.	10	0	0	3	0	0	0	4	1	0	0
	42	0	+	4	0	0	0	4	4	4	1
H.	13	+	+	4	4	0	0	0	0	0	0
	44	+	+	4	3	0	0	0	0	0	0
K.	4	+	+	3	1	0	0	4	2	0	0
	26	+	+	4	4	2	0	4	4	3	0
P.	1	0	0	0	0	0	0	0	0	0	0
	26	+	0	4	4	4	—	0	0	0	0

* + = Sera neutralized by at least 1000 LD50 doses of virus. All sera inactivated at 58°C for ½ hr.
0 = Sera did not neutralize 1000 LD50 doses of virus.
† Figures refer to degree of hemolysis: 0 = complete hemolysis; 1, 2, 3 = intermediate hemolysis; 4 = no hemolysis.

refrigerator overnight at 4°C before adding the sensitized red cells. After incubation for thirty minutes at 37°C in the water bath, followed by one hour in the refrigerator, the results were recorded at 4+ (no hemolysis), 3+, 2+, 1+ (intermediate hemolysis), and 0 (complete hemolysis). Controls included those for the sera, antigens, hemolytic system, and known positive sera from each type. Three and four plus reactions were considered of significance in serum dilutions above 1-2, but equivocal if in the latter dilution.

Results of the Tests. The strains of Coxsackie virus could be separated easily into different types by means of the complement fixation method, and in each case there was agreement with the results of the neutralization tests (Table I). A total of 35 strains has

been tested with the majority falling into Types 2 and 4. None so far has corresponded to Type 3 of Dalldorf.(2) A later report will be made when the tests for all of the strains on hand have been completed.(4) The results with the human sera were not generally so clear cut. Twenty-two pairs of acute and convalescent sera were tested against antigens of Types 1 and 2. Typical results for 8 pairs are given in Table II. An occasional rise in titer for the same strain was noted in both tests. Often the antibodies were present soon after the onset. In general, there was agreement between the two methods as to the antibody types found. If the neutralization test was positive for Type 1, for instance, the complement fixation test was positive for the same type. Occasionally antibodies were found for both types in each test.

Summary. The complement fixation test using filtered muscle antigen can be employed for differentiation of the Coxsackie virus into serologically distinct types and for determining the presence of antibodies in human sera.†

4. Hewitt, B. F., in manuscript.

† Since submitting this paper for publication, Casals, Olitsky and Murphy also have reported on the use of the complement fixation test for differentiating the Coxsackie viruses. PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 636.

Received December 6, 1949. P.S.E.B.M., 1950, v73.

A Short Column Procedure for Separating Cytoplasmic Components from Normal and Tumor Tissue. (17587)

VERNON RILEY AND M. W. WOODS* (Introduced by Dean Burk).

From National Cancer Institute, National Institutes of Health, Bethesda, Md.

Columnar chromatography has been shown to be applicable to purification and characterization studies of certain subcellular particulates of wide size and physiological variation, namely the virus-like agent of chicken tumor I(1) and the microscopically visible granules of the Harding-Passey and the S91 mouse melanomas.(2) Most of these studies were made with material that had already been partially purified by differential centrifugation. It has since been found, in working with the latter tissues, that the preliminary purification can be done rapidly without recourse to the centrifuge by employing a procedure involving grinding of the tissue with diatomaceous silica (Celite No. 503) and submitting this slurry to a short column or pad of the same adsorbent. The resulting percolate consists essentially of the subcellular granules and most of the soluble components of the tissue extract. It is devoid of nuclei and intact cells as indicated by microscopic examination. This tissue percolate is suitable for certain enzyme investigations or further purification studies by chromatography or differential centrifugation.

Methods and results. A typical procedure is outlined in the following experiment indicating the relative results obtained with equal quantities of tumor tissue prepared by the short column technic as compared with standard grinding followed by centrifugation. Several Harding-Passey tumors were pooled and fragmented by forcing through a 5 ml syringe without needle, and divided into 2 equal lots of 5 g each termed A and B. Lot A was ground in an iced mortar without sand and with the gradual addition of 5 times its weight of cold distilled water. This suspension was then cleared of tissue debris, unbroken cells, and nuclei by two successive centrifugations† of 5 minutes each at approxi-

* With the aid of John Chambers.

1. Riley, Vernon T., *Science*, 1948, v107, 573.

2. Riley, Vernon T., Hesselbach, M. L., Fiala, S., Woods, M. W., and Burk, D., *Science*, 1949, v109, 361.

† Type SS-1 Serval centrifuge with 9″ angle rotor, run at approximately 3700 rpm in a walk-in refrigerator at 5°C.