4. Graff, J., Rittenberg, D., *Anal. Chem.*, 1952, v24, 878.

5. Glasscock, R. F., *Isotopic gas analysis for biochemists*, New York, Academic Press, Inc., 1954.

6. Berman, M., Schoenfeld, R., *J. Appl. Physics*, 1956, v27, 1361.

7. Fehling, H., *Arch. f. Gynaek.*, 1879, v14, 221.

8. Runge, H., Baur, M., Hartmann, H., *ibid.*, 1928, v134, 626.

9. Panigel, M., *Physiologie*, 1959, v51, 941.

Received March 20, 1961. P.S.E.B.M., 1961, v107.

Hemagglutination and Hemagglutination-Inhibition with Coxsackie B Viruses. (26708)

LEON ROSEN AND JEROME KERN (Introduced by R. J. Huebner)

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Nat. Inst. Health, Bethesda, Md.

Coxsackie B virus infections are common occurrences in man and are responsible for several distinct syndromes, such as aseptic meningitis and pleurodynia, as well as for febrile illnesses which cannot be readily differentiated from those caused by other infectious agents. Although these viruses can usually be isolated from the throat or anal specimens of patients by the use of primate tissue cultures or suckling mice, these procedures are relatively cumbersome and are still beyond the scope of most clinical laboratories. The report that Coxsackie B type 3 virus agglutinated human erythrocytes(1), and the recent finding in this laboratory that certain strains of Coxsackie B virus types 1 and 5 also possessed this property, suggested that it might be possible to develop a hemagglutination-inhibition (HI) test which would be of use in diagnosis of human infections with these viruses. This report will describe such an HI procedure and the results obtained with it in a study of 25 children infected with Coxsackie B virus types 3, 4, and 5.

Materials and methods. The hemagglutinating strain of Coxsackie B virus type 3 was obtained from Dr. M. Goldfield. The hemagglutinating strains of Coxsackie B virus types 1 and 5 were found in this laboratory among a number of previously unidentified enteroviruses isolated in Toluca, Mexico and kindly made available to us by Dr. A. B. Sabin.

The materials and methods employed in hemagglutination and hemagglutination-inhibition procedures were the same as those used with reoviruses(2) unless otherwise noted. Rabbit antisera were prepared by an immunization schedule described previously (3). Neutralization tests with Coxsackie B virus type 4 were carried out in rhesus kidney cell cultures as described previously(3) using the "Powers" strain of this virus(4).

The human sera employed were from institutionalized nursery children who were under observation in a longitudinal study(5).

Results. The hemagglutinating properties of Coxsackie B virus types 1 and 5 were discovered during an investigation in which unidentified enteroviruses were systematically screened for their ability to agglutinate human erythrocytes. When a hemagglutinating virus could not be typed by HI as a serotype known to possess this property, additional identification technics were used until the agent was identified or was recognized as a new serotype. The identity of the type 1 and 5 strains was established by complement-fixation(6), then confirmed by HI using rabbit antisera. The strain of Coxsackie B type 3 virus described by Goldfield *et al.* is the only hemagglutinating strain of this type which we have encountered. A number of other Coxsackie B type 1 and 5 strains had been tested previously for hemagglutination with negative results.

An investigation of the conditions under which Coxsackie B virus types 1, 3, and 5 hemagglutinated revealed that optimum conditions for each of the 3 types were a sedimentation temperature of 37°C using eryth-

rocytes of newborn infants. Blood from newborn infants was obtained from the umbilical cord when it was severed at time of delivery. If the erythrocytes were to be used in HI tests, type "O" cells were employed.

TABLE I. Hemagglutination-Inhibition Antibody Responses of 10 Children Infected with Coxsackie B Virus Type 3.

Age, mo	H-I titer with indicated antigen B-1	B-3	B-5
12	<10	<10	<10
	<10	≥160	<10
13	<10	<10	<10
	<10	20	<10
15	<10	<10	<10
	<10	40	<10
25	<10	<10	<10
	<10	20	<10
27	<10	<10	20
	<10	20	40
29	<10	<10	<10
	<10	40	<10
33	<10	<10	<10
	<10	20	<10
34	<10	<10	20
	<10	≥160	≥320
34	<10	<10	10
	<10	≥160	10
40	<10	<10	20
	<10	80	40

TABLE II. Hemagglutination-Inhibition Antibody Responses of 8 Children Infected with Coxsackie B Virus Type 4.

Age, mo	H-I titer with indicated antigen B-1	B-3	B-5
13	<10	10	<10
	<10	<10	<10
17	<10	<10	<10
	<10	<10	<10
18	<10	<10	80
	<10	<10	20
19	<10	<10	20
	<10	<10	10
28	<10	40	<10
	<10	640	<10
31	<10	<10	160
	20	<10	160
33	40	<10	640
	320	<10	320
37	<10	<10	<10
	<10	<10	<10

TABLE III. Hemagglutination-Inhibition Antibody Responses of 7 Children Infected with Coxsackie B Virus Type 5.

Age, mo	H-I titer with indicated antigen B-1	B-3	B-5
21	<10	80	<10
	<10	80	40
22	<10	<10	<10
	<10	<10	320
22	<10	80	<10
	20	≥320	80
24	<10	<10	<10
	<10	<10	20
24	<10	80	<10
	<10	80	10
26	<10	<10	<10
	<10	<10	80
32	<10	<10	<10
	<10	<10	40

Hemagglutinin titers of 1:128 or 1:256 per 0.4 ml were obtained with each of the 3 types. Partial agglutination patterns, similar to those which have been reported with certain adenoviruses and rat erythrocytes (7), were usually seen with type 3, and sometimes with types 1 and 5, when erythrocytes from adult donors were employed. Erythrocytes from most infants gave complete patterns. Hemagglutinin titers were almost always higher with cells from infants as compared with those from adults. Some variation from one lot of cord cells to another was observed.

On the basis of the above finding an HI procedure was developed which was identical with that described for reoviruses except that erythrocytes from newborn infants were substituted for those from adults and the erythrocytes were allowed to sediment at 37°C (in a warm air incubator) instead of at room temperature.

The results obtained with this procedure in testing sera of children naturally infected with Coxsackie B virus types 3, 4, and 5 are shown in Tables I, II, and III respectively. In each instance the first serum of each pair was obtained prior to infection and the second from 2 to 15 weeks afterwards. Diagnosis had been established in each child by virus isolation and sera were not selected with respect to their antibody responses. A rise in homologous neutralizing antibody was demonstrated in each pair of sera from the children with type 4 infections.

It will be noted that with one exception a significant homologous antibody response was detected in each of the 18 children with type 3 and 5 infections and that a significant heterologous response occurred in 2 of these children as well as in 3 of the 8 children with type 4 infection. In each case where a heterologous response was observed the child had pre-existing HI antibody to one or more of the 3 test antigens.

Discussion. The demonstration that certain strains of Coxscakie B viruses agglutinate human erythrocytes, while others do not, should encourage the search for hemag-

glutinating strains of enteroviruses for which this useful property has not yet been demonstrated. Thus far, in addition to the 3 Coxsackie B viruses, we have been able to confirm the specificity of the hemagglutination previously reported(1,8,9,10,11) for only the following types of enteroviruses: ECHO types 3, 6, 7, 11, 12, 13, 19 and Coe virus. We have also been able to demonstrate specific hemagglutination by a strain of ECHO type 20 (but not the prototype), ECHO type 21, Coxsackie A types 20 and 24, and several newly recognized enteroviruses which have not been numbered. There is every reason to believe that this list will be extended.

Although HI responses observed in children infected with Coxsackie B viruses were relatively type-specific, the occurrence of heterologous responses in some children with pre-existing HI antibody suggests that this degree of specificity will not hold true with older individuals. Additional data are obviously desirable on the responses of adults, time of first appearance of HI antibodies, and duration of antibody persistence. In view of the simplicity of the HI procedure as compared with other serologic tests available, it would appear that this technic warrants further investigation as to its usefulness for diagnostic and epidemiologic purposes.

Summary. Agglutination of human erythrocytes by Coxsackie B virus types 1 and 5 is reported. Optimum titers were obtained with a sedimentation temperature of 37°C and the erythrocytes of newborn infants. A hemagglutination-inhibition (HI) procedure employing Coxsackie B virus types 1, 3, and 5 was used to test paired sera from 25 children infected with Coxsackie B virus types 3, 4, and 5. A significant homologous response was detected in every case except one. A few heterologous responses occurred in children with pre-existing HI antibody to one or more of the 3 test antigens.

The authors are indebted to Mrs. J. Hovis for valuable technical assistance.

1. Goldfield, M., Srihongse, S., Fox, J. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 788.
2. Rosen, L., *Am. J. Hyg.*, 1960, v71, 242.
3. Rosen, L., Johnson, J. H., Huebner, R. J., Bell, J. A., *Am. J. Hyg.*, 1958, v67, 300.
4. Cheever, F. S., Daniels, J. B., Hersey, E. F., *J. Exp. Med.*, 1950, v92, 153.
5. Bell, J. A., Huebner, R. J., Rosen, L., Rowe, W. P., Cole, R. M., Mastrota, F. M., Floyd, T. M., Chanock, R. M., in preparation.
6. Halonen, P., Rosen, L., Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 105.
7. Rosen, L., *Am. J. Hyg.*, 1960, v71, 120.
8. Dardanoni, L., Zaffiro, P., *Boll. Ist. sieroter. milan.*, 1958, v37, 346.
9. Johnson, K. M., Bloom, H. H., Rosen, L., Mufson, M. A., Chanock, R. M., *Virology*, 1961, v13, 373.
10. Philipson, L., *Arch. fur ges. Virusforsch.*, 1959, v9, 251.
11. Lahelle, O., *Acta Path. Micro. Scand.*, 1958, v44, 413.

Received May 1, 1961. P.S.E.B.M., 1961, v107.

Induced Reactivation of Herpes Simplex Virus in Healed Rabbit Corneal Lesions.* (26709)

WILLIAM A. ANDERSON, BROCK MARGRUDER AND EDWIN D. KILBOURNE
Departments of Public Health and Surgery (Ophthalmology), Cornell University Medical College, New York City

The first report of induced reactivation of herpes simplex infection in an experimental animal was that of Good and Campbell(1) who subsequently reported additional observations of induced herpetic encephalitis in rabbits which included virus isolation(2,3). Recently Schmidt and Rasmussen(4) reported the apparent reactivation of herpes

* This investigation was supported in part by grants from Nat. Inst. Health, Division of Allergy and Infect. Dis., Am. Cancer Soc., and Lillian A. Margolyes League, Inc.