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Mercaptoethanol-Sensitive Neutralizing-Antibody in Natural Infection With Coxsackievirus B5.*† (31237)

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Many investigations have shown an early appearance of 19 S antibody in animals including man either artificially immunized or naturally infected with viruses(1-7). In most studies the 19 S antibody proved sensitive to treatment with 2-mercaptoethanol (2-ME). The early appearance of 2-ME sensitive antibody seemed to provide a valuable indicator for detecting recent infection with certain viruses. However, there have been only few reports on appearance of 2-ME sensitive antibody during natural virus-infections in man (2,6).

The present communication describes the development of 2-ME sensitive and resistant neutralizing-antibody in sera obtained from children and infants infected with Coxsackievirus B5 and discusses the diagnostic significance of detecting the 2-ME sensitive antibody at an early stage of infection.

Materials and methods. Sera. Serum specimens were obtained from patients of aseptic meningitis caused by Coxsackievirus B5 in the 1961 epidemic in Aomori City(8,9). The patients from whom sera was obtained had been determined as Coxsackievirus B5-infected by virus isolation and/or serodiagnosis. Sera at acute and convalescent phases, together with sera obtained 2 years after the illness(10) were examined. All sera were stored at —20°C and then inactivated at 56°C for 30 minutes on the day of use.

Determination of neutralizing antibody. The virus used was the AM-L-69-63 strain which was a representative strain of Coxsackievirus B5 isolated in the 1961 epidemic (8). Neutralization tests were performed by the conventional tube test method employing HeLa (S_3) cell monolayers. Two-fold serial dilutions of serum or fractionated serum in 0.01 M phosphate buffered saline of pH 7.2 (PBS) were mixed with equal volume of 100 TCD_{50}/0.1 ml of virus and then incubated at 4°C overnight. After incubation, 0.2 ml of the mixture was inoculated into duplicate tubes and incubated at 37°C for 4 days. Titer of neutralizing antibody was expressed as reciprocal of highest serum dilution which protected cells completely.

Treatment with 2-mercaptoethanol (2-ME). One to two dilutions of whole serum in PBS or fractionated serum was mixed with equal volume of 0.2 M 2-ME made in PBS. The mixtures were incubated at 37°C for 2 hours and then dialyzed against PBS at 4°C for 2 days in order to remove 2-ME. Both untreated and treated samples of single serum were tested simultaneously.

Sucrose gradient centrifugation. Zonal density ultracentrifugation was accomplished in a 40P Hitachi Centrifuge employing a swinging bucket rotor and a preformed gradient of sucrose ranging from 10 to 37%. Sera (about 0.5 ml) were layered over the gradient. Centrifugation was carried out for 15 hours at 35,000 r.p.m. About 20 fractions of approximately 0.25 ml were obtained by a puncture

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TABLE I. Titer of Neutralizing Antibody Against Coxsackievirus B5 in Sera of 17 Patients with Aseptic Meningitis Before and After Treatment with 2-ME.

Patient No.	Age at onset of illness (yr-mo)	Period after onset of illness	Antibody titer		Fold reduction
			Untreated	2-ME treated	
58	0-2	16 days	256	32	8
		2 years	1024	1024	0
63	0-8	3 days	64	<4	≧32
		36 "	512	128	4
1314	0-8	34 days	64	32	2
		2 years	32	16	2
84	0-9	40 days	128	64	2
		46 "	64	64	0
		2 years	64	64	0
19	1-3	29 days	64	16	4
		40 "	64	32	2
85	1-3	45 days	512	512	0
		53 "	512	256	2
408	1-4	3 days	8	<4	≧4
		36 "	128	32	4
		2 years	64	64	0
56	1-5	33 days	16	16	0
		2 years	16	16	0
40	2-0	44 days	128	128	0
		2 years	64	64	0
410	3-11	37 days	128	32	4
		2 years	16	8	2
107	4-2	9 days	16	8	2
		2 years	16	16	0
384	5-0	2 days	16	4	4
		16 "	128	128	0
		2 years	128	128	0
544	5-2	2 days	4	<4	≧2
		2 years	32	32	0
553	6-5	6 days	16	<4	≧8
		13 "	16	<4	≧8
1409	6-5	10 days	8	<4	≧4
		17 "	4	<4	≧2
		2 years	512	512	0
4	7-6	20 days	16	<4	≧8
		2 years	128	128	0
55	11-2	25 days	16	<4	≧8
		35 "	≧256	16	≧16

of bottom of the tube, and then each fraction was diluted in 1:4 with PBS and dialyzed against PBS.

Results. Antibody titers in early and late sera before and after 2-ME treatment. Sera obtained at early and late phases of aseptic meningitis in 17 patients were examined for their titers of neutralizing antibody before and after treatment with 2-ME. The results are shown in Table I. In all but 2 patients, the antibodies in the acute and early convalescent sera were uniformly sensitive to 2-ME, showing 2-fold or greater reduction. However, there was no specific relationship between the 2-ME sensitivity of antibody and the age of the patients.

Results on the 2-ME sensitivity of 56 sera, including the 38 sera listed in Table I, in varied phases of the illness are summarized in Table II. During the first 30 days after the onset of illness, 4-fold or greater reduction in antibody titer could be detected in 75% of the sera tested. However, only 28% of the sera obtained during 31-53 days after

TABLE II. 2-ME Sensitivity of Neutralizing Antibody Against Coxsackievirus B5 in Sera from Patients with Aseptic Meningitis.

Days after onset of illness	No. of sera tested	No. of sera sensitive*(%)	
1–10	9	7 (78)	75%
11–20	10	7 (70)	
21–30	9	7 (78)	
31–40	9	3 (33)	28%
41–50	5	1 (20)	
2 years	14		

* 4-fold or greater reduction.

the onset were sensitive to 2-ME. None of 14 sera obtained 2 years after the onset contained any 2-ME sensitive antibody. A plot of these results against the day after the onset of illness is shown in Fig. 1. The sera sensitive to the action of 2-ME are illustrated as empty circles in the Figure. These circles are predominant before 30 days.

Fractionation of sera by sucrose gradient centrifugation. Several sera obtained at acute or convalescent stages were fractionated by sucrose gradient centrifugation, and each fraction titrated before and after 2-ME treatment. Results of 2 typical examples are shown in Fig. 2. With No. 46 serum obtained 12 days after onset of illness, the antibody activity was distributed over a relatively wide range from fraction No. 5 to No. 16. The highest peak occurred between No. 7 and 10, before 2-ME treatment. However, titers in each fraction dropped to undetectable level after treatment with 2-ME except for a trace left in No. 12. On the other hand, with No. 58 serum which was obtained at 2 years after the illness, the antibody was found in relatively narrow range at slower sedimenting zones than that with the No. 46 serum, showing a peak between No. 12 and 14. This pattern was not significantly altered after 2-ME treatment.

Discussion. Schluederberg(6) reported the development of 2-ME sensitive neutralizing antibody in acute and early convalescent sera of 4 patients infected with Coxsackievirus B5 and one patient with Coxsackievirus B4. Her observation was confirmed by studies in the present paper. Svehag and Mandel(2) described early appearance of 19 S neutralizing antibody in poliovirus type 1 infection of 9 persons. These accumulated data may suggest that an early appearance of 19 S neutralizing antibody as indicated by 2-ME sensitivity with higher titer than that of 2-ME resistant antibody is a common fashion among the enterovirus infections. However, it is not yet certain whether this phenomenon can be generalized to almost all viral infections. Our preliminary studies (unpublished data) on patients infected with respiratory syncytial (R S) virus are not in agreement with results in Coxsackievirus infection. In R S virus infections reduction of antibody titer by 2-ME treatment in early serum was not found in cases so far tested. Schluederberg(6) described only a little reduction of antibody by treatment with 2-ME in the cases of measles and mumps. These data may indicate little or no development of 2-ME sensitive antibody or 19 S antibody in some viral infections other than enterovirus infection. The possibility should also be considered that 19 S antibody is made, but that R S and some other viruses are insensitive to 19 S antibody and thus unable to reveal its presence.

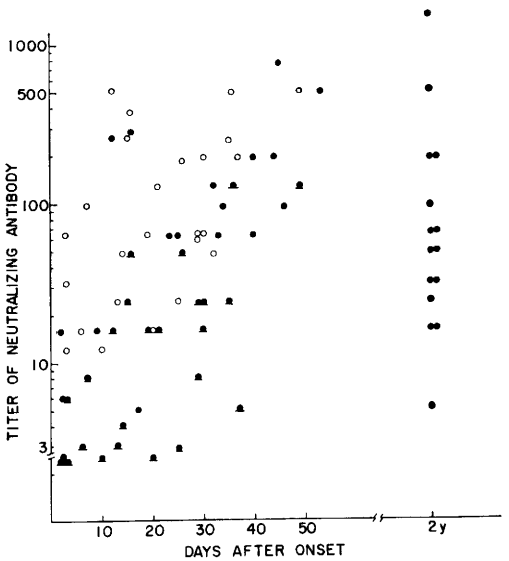

FIG. 1. Neutralizing antibody titers of 56 sera from 35 Coxsackievirus B5 infections, 0-12 years old. ○: Original titer of sera sensitive to 2-ME treatment. ●: Titer of sera after 2-ME treatment.

●: Titer of sera completely resistant to 2-ME treatment.

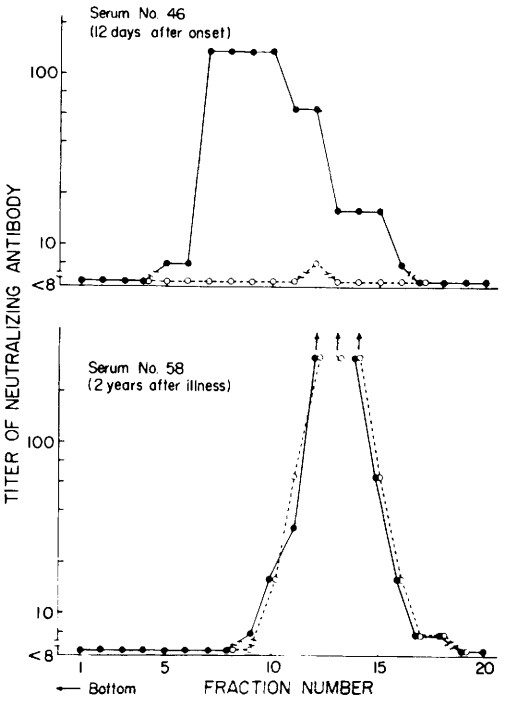

FIG. 2. Sedimentation pattern of neutralizing antibody in sucrose gradient centrifugation. ●—●: Titer of antibody before 2-ME treatment. ○ - - ○: Titer of antibody after 2-ME treatment.

Sera examined in this study were obtained from children and infants who suffered from aseptic meningitis in 1961(8). Development of antibody in those children and infants probably caused by a primary but not recurrent infection with Coxsackievirus B5 because little or no seeding of Coxsackievirus B5 was detected in Aomori City before 1960 and after 1962. It was found only in 1961, as described previously(10). Therefore, production of antibody either sensitive or resistant to 2-ME in this report may be evaluated as a response to the primary infection with Coxsackievirus B5. It is clearly demonstrated that the titer of the 2-ME sensitive antibody is significantly higher than that of the 2-ME resistant antibody in sera obtained within 30 days after onset of illness in 70% or more of the patients tested. This fact may provide an advantage for the serodiagnostic procedures of the viral infection. Usually, the serodiagnosis is carried out by employing paired sera obtained at acute and convalescent stage of the disease and the definite diagnosis is judged by a significant rise of antibody between paired sera. However, it is actually not rare that only single acute or early convalescent serum but not paired sera can be obtained from a patient from whom a definite diagnosis is desired. For such cases, the procedure of 2-ME sensitivity test with single serum may be valid. Even in a case in which a significant rise of antibody titer between the paired sera was not obtained, the diagnosis might be possible if a significant reduction of antibody titer after the treatment with 2-ME in an acute phase serum would be found contrary to the resistance in a convalescent serum.

As previously reported(10), neutralizing antibody against Coxsackievirus B5 persists for at least 2 years after the infection. This long life-antibody was determined to be 2-ME resistant antibody by the studies in the present report. Thus, qualitative and quantitative determinations of both the 2-ME sensitive and resistant antibodies may facilitate seroepidemiological studies on enterovirus infections.

In the experiments reported here, 2 peaks of antibody in a single serum were not clearly separated in the sucrose gradient centrifugation. However, the two kinds of antibody which might be the same or very close to 19 S and 7 S immunoglobulins, respectively, were detected in early and late convalescent sera, respectively (Fig. 2). The rapidly sedimenting antibody was almost completely inactivated by 2-ME but the slowly sedimenting antibody was resistant to it, in the same experimental conditions.

Summary. Serum samples from patients of aseptic meningitis associated with Coxsackievirus B5 were examined to detect both 2-mercaptoethanol (2-ME) sensitive and resistant neutralizing antibodies. In 75% of the sera obtained at an early stage after the onset of illness, the titer of 2-ME sensitive antibody was significantly higher than that of the 2-ME resistant antibody. During the late stage of infection the level of 2-ME resistant antibody exceeded the 2-ME sensitive titer; this was especially true of sera obtained 2 years after onset of illness. Fractionation of sera by a procedure of sucrose gradient cen-

trifugation revealed that the rapidly sedimenting peak of antibody corresponded to the 2-ME resistant antibody. The nature of the 2-ME sensitive antibody and the significance of its detection in diagnosis of the viral infection were discussed.

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Immunophoretic Evaluation of Blood Serum Proteins in Chickens. I. Changing Protein Patterns in Chickens According to Age.* (31238)

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The identification of some protein fractions in immunophoretic preparations of chicken blood serum can be readily made by analogy from similar preparations of human blood serum. Other fractions may be tentatively identified pending verification by ultracentrifugation methods. We have used this principle in analyzing serum protein profiles in normal and experimental chicks subjected to vit E-deficient diets which produce selectively nutritional encephalomalacia or myopathy, and in chickens with genetic muscular dystrophy. Since variations in the pattern were observed in chickens of varying ages, a systematic examination of patterns was made in a series of normal chickens, and the data are presented here.

Methods and materials. Three sets of chickens were studied in 2 separate laboratories: (1) 25 chickens, received at one day of age, of the Arbor Acre strain. (2) 4 chickens of the New Hampshire strain, also used as controls for the chickens with genetic muscular dystrophy. (3) 13 chickens obtained from Cuxhaven, Germany consisting mostly of White Leghorn chicks. These chicks were fed for the first 3 weeks on a commercial preparation for newly hatched chicks.† Afterwards, the New Hampshire chicks were fed on a balanced commercial feed‡ while the Cuxhaven chickens were fed a stock feed suitable for adequate growth and development, and containing a mixture of fish and cereal meal. All chickens were kept in cages which did not permit free mobility to the older chickens. The sex of the older chickens was recorded, and of the younger ones, only those of the Arbor Acre strain were known.

Blood samples were obtained either by ventricular puncture under sodium pentathol anesthesia (100 mg/kg I.P.) from cervical

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† Starteena—Ralston Purina Co., St. Louis, Mo. For the Cuxhaven chicks, a preparation called "Volikrafthorn K Kuken Alleinfutter," by Bergesche Kraftfutterwerke, H. Schmidt, K. G., 4 Dusseldorf 1.

‡ Groweena—Ralston Purina Co., St. Louis, Mo.