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Persistence of Antibodies to ECHO-16 Viruses Following Boston Exanthem Disease.* (25203)

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Of the diseases now considered to be of ECHO virus etiology, Boston Exanthem disease(1,2) was the first to be recognized as a clinical and epidemiologic entity and associated with its causative agent. Immunological relationship of Boston Exanthem viruses to ECHO-16 virus has since been demonstrated, although their antigenic patterns are not identical(3,4). Reports on the ECHO viruses have dealt with clinical associations, epidemiological studies, or antigenic relationships. Information on the immunological status of man several years after infection with ECHO viruses has not been available. Serum samples were obtained† from patients of the 1951 Boston Exanthem disease epidemic, and also from cases of the Pittsburgh outbreak of 1954. This report provides results of studies on complement fixing and neutralizing antibody levels 3 to 6 years after original infection.

Materials and methods. Neutralizing Antibody (NA) Tests. Explant type roller cultures of embryonic human skin-muscle tissue‡, grown with medium containing bovine embryonic fluids as described elsewhere(4), were used. Prior to inoculation the nutrient was changed to 90% bovine embryonic fluids and the Hanks component omitted. Seven days after inoculation one additional fluid change of the same medium was made. Pools of virus in the 4th to 7th *in vitro* passage were used—2 of the *Floyd* strain which originated from a Boston patient, and 2 of the Pittsburgh *McGrew* strain. Serum samples were stored at −15°C, thawed on the day of use, and inactivated at 56°C for 30 minutes. Except as noted in Table I, all serum specimens from a patient were tested simultaneously. Two- or 4-fold serum dilutions were mixed with virus, held at 5°C for 1½ hours, and 3 cultures each were then inoculated with 0.1 ml of the mixture. In each test 3 or more control cultures were inoculated with an equivalent virus dose. Serum NA titers were determined 3 days after the virus controls showed unequivocal and generalized cellular degeneration. Titration of the virus inoculum was performed concurrently with each test. NA titers are expressed as that dilution of serum, before addition of virus, which effected neutralization of 2 or more of the 3 cultures inoculated. If all 3 tubes at one dilution were neutralized and 2 or all 3 tubes of the next highest dilution failed to be neutralized, an arbitrary intermediate dilution was taken as the titer. *Complement-fixation (CF) tests.* Methods of antigen preparation and the micro-method CF test procedure are reported elsewhere(5). The antigens were concentrated from infected culture fluids and heated at 56°C before use. Three antigens, from the same strain (McGrew) of exanthem virus, were used; a few

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TABLE I. CF and Neutralizing Antibody Titers in Boston Exanthem Disease Patients at Various Intervals after Illness.

Patient, age at onset	Time after onset	CF titer*	Neutralizing antibody titer† vs Floyd‡ (Boston) strain	vs McGrew§ (Pittsburgh) strain
Mr. W.	20 days	128	64	32
26 yr	2 yr, 2 mo	64	"	16
	4 yr	64	>32	nt
	6 yr, 3 mo	64	nt	128 or >
Mrs. W.	20 days	64	16	2
26 yr	2 yr, 2 mo	32	16	8
	4 yr	16	16	nt
	6 yr, 3 mo	16	nt	8
Lel. W.	23 days	64	32	16
3 yr	2 yr, 2 mo	32	>128	128
	4 yr	16	128 or >	64
	6 yr, 3 mo	16	nt	128
Lin. W.	4 yr	32		128
2 yr	6 yr, 3 mo	32		"
M. F.	11 days	128	64	64
2 yr	2 yr	16	nt	>128
	3 yr, 11 mo	128	>128	256
	6 yr, 3 mo	32	nt	128
V. F.	2 yr, 1 mo	64		128 or >
6 yr	4 yr	128		>128
	6 yr, 3 mo	64		128 or >
C. F.	2 yr, 1 mo	8		128
4 mo	4 yr	16		"
	6 yr, 3 mo	<16		"
Mrs. D.	23-26 days	anticomp.		32 or >
24 yr	6 yr, 3 mo	16		32
L. D.	23-26 days	64		16
2 yr	6 yr, 3 mo	16		32
Mr. McG.	23 days	128	16	32
29 yr	11 mo	64	"	"
	2 yr, 10 mo	32	"	"
D. McG.	27 days	128	64	128
4 yr	11 mo	32	>64	256
	2 yr, 10 mo	16	"	128
R. We.	20 days	128		32
5 yr	11 mo	32		64
	2 yr, 10 mo	16		>32
K. We.	20 days	64		32 or >
6 yr	11 mo	16		64
	2 yr, 10 mo	16		32 or >
Mrs. We.	17 days	64		128
30 yr	11 mo	16		"
	2 yr, 10 mo	8		16
Mr. Du.	18 days	256		64
35 yr	11 mo	64		<32
	2 yr, 10 mo	32		32

* All titers expressed as reciprocal of serum dilution before virus or antigen added.

† Neutralized for 3 days after virus control cultures showed degeneration.

‡ Virus dose varied from 20 to 350 ID_{50}.

§ *Idem* 25 to 250 " .

nt = not tested.

tests were done with an antigen derived from another strain (Clauss). Two units of antigen and 2½ to 3½ units of complement were employed. A positive control serum was included in each test and reacted to comparable degree with each antigen used(5). Available serum samples from any one patient were tested simultaneously at least once. Duplicate tests showed good agreement in titers.

Results. The level of CF antibody usually showed a 2- to 4-fold decline in titer within one year after infection, but little change thereafter for as long as 6 years. One Boston patient (M. Flo) exhibited a distinct secondary rise in CF antibody titer between the second and fourth years. However, no significant increase in level of NA was evident over this same interval. CF and NA values for the 15 patients studied are summarized in Table I.

With one possible exception, no decline in NA level was found 3 to 6 years after the original infection. However, unexplained fluctuations in titer of NA were recorded for sera obtained from one family (Will.). This variation was not associated with corresponding changes in CF titer, and in only one patient (Lel. Will.) was the fluctuation in NA titer demonstrable with both test strains of virus.

Discussion. The results indicate that in man after infection with ECHO 16-like viruses, CF antibody titers fall moderately within 1 to 2 years, and then persist at detectable levels for periods of at least 3 to 6 years. This contrasts with the more rapid drop in CF antibody levels following infection with polio viruses(6). Evidence was also obtained that a secondary rise in CF antibody may occur, presumably because of reinfection with the same or an antigenically related agent.

Levels of NA showed no significant decline 3 to 6 years after the exanthem infection; in fact, apparent increases in titer were registered in several cases. However, the significance of these changes in late convalescence is difficult to evaluate. The individuals with unstable antibody levels were all members of the same family. In 2 instances the rise in titer may have represented a gradual increase in antibody after the original infection, since

no samples were tested between early convalescence and 2 years. The absence of a concomitant rise in CF antibody would support this hypothesis. If the changes in antibody levels were the result of infection with antigenically related viruses, a discrepancy in booster antibody response between neutralizing and CF antibodies would have to be postulated.

It is possible that the levels of antibody in the patients studied were maintained by inapparent infections with antigenically related enteroviruses. Detection of changes in antibody titer resulting from such a mechanism would require more frequent sampling of sera. Further investigation will be necessary to determine whether the pattern of immunologic response found in Boston Exanthem disease is also applicable to other ECHO virus infections.

Summary. Persistence of complement fixing and neutralizing antibodies to ECHO 16-like viruses was studied in 15 patients convalescent from Boston Exanthem disease. CF titers fell moderately within 1 or 2 years after infection, but remained demonstrable for 3 to 6 years. Levels of neutralizing antibody persisted without significant decline for a similar period of time.

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Nucleic Acid Content of Various Areas of the Rat Brain.* (25204)

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The present investigation was initiated to provide control values of NA concentrations in local areas of rat brain. Previous reports have, for the most part, concerned themselves with NA content of the whole brain ignoring the differences between the various areas of the brain. Since these areas differ from each other functionally, morphologically, metabolically, and in chemical composition, and since physiological stresses or agents are known to act differently on these various cell groups, it becomes necessary to establish regional control values.

Methods. Adult, white, male rats (300-450 g) were used. The rats were decapitated after receiving a stunning blow on the head and the brain dissected on a cold surface. The tissue was washed with ice-cold distilled water to remove surface blood and blotted on filter paper. More reproducible results were obtained if the tissue was blotted to remove surface water (Table I). The excised tissue was then placed in 10% trichloracetic acid (TCA) or stored in the deep-freeze. A maximum period of 15 minutes elapsed between sacrificing of the animal and the storage or treatment of the tissue with TCA. When tissue weight was 100-200 mg, the homogenates were prepared with a close fitting stainless steel pestle in 16 × 150 mm test tubes. When the hypothalamic and thalamic tissues (20-50 mg) were analyzed for total nucleic acid (TNA), the tissue was homogenized in a 12 ml centrifuge tube using a plastic pestle prepared according to the method of Darbé(1). It was necessary to pool these tissues from 5 animals to obtain enough material for analysis of deoxyribonucleic acid (DNA) using the standard procedure. Enzymatic assays were made following the procedure of McDonald(2) except that it was run at 37°C and different

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