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ECHO Virus Type 12, Isolation and Characteristics.*† (24764)

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Although ECHO viruses have received considerable attention within the past 4 years, adequate descriptions of many prototype strains have been lacking. This report concerns isolation and characteristics of prototype ECHO-12 virus. Included herein is a description of its properties in cell cultures, in laboratory animals and in serologic tests.

Materials and methods. Monolayer cell cultures of trypsinized rhesus monkey kidney (MKCC) were used in isolation, neutralizing antibody (NA) tests, preparation of virus pools, and plaque studies. Serologic methods have been described(1). Plaque studies were performed according to method of Hsiung and Melnick(2). Animals were immunized by repeated intramuscular injection of an emulsion containing equal volumes of Arlacel-Bayol F adjuvant and unconcentrated untreated virus-containing MKCC fluids.

Results. Establishment of ECHO-12 prototype virus. Prototype ECHO-12 virus, Travis 2-85-4,§ as first described by ECHO Virus Committee(3), was isolated in 1954(4) from rectal swab taken Aug. 18, 1953 from a healthy 6-year-old son of an American military family (Travis) stationed at Clark Air Force Base (CAFB) in the Philippines. A serum from this child taken 14 days earlier contained no detectable NA to this agent, whereas that collected 14 days after positive rectal swab had a titer of 1:128. Two and 3 months following virus isolation the serum titer was 1:32(1). Prior to acceptance as ECHO-12 prototype, 2-85-4 virus and its antiserum (titer 1:1,000 *vs.* 100 $TCID_{50}$) were tested reciprocally by our laboratory and those of Melnick and Sabin against 12 other original ECHO prototype viruses and their relatively low-titered antisera. These tests revealed no cross relationships. Subsequently, aliquots of ECHO-12, MKCC passage 5, were sent to Dr. Herbert A. Wenner and Microbiological Associates for preparation of hyperimmune monkey and rabbit antiserum respectively. The composite NA tests with monkey antisera (Wenner) obtained in 4 different laboratories have been reported(5). It was found that low dilutions of Wenner ECHO-12 antiserum neutralized significant amounts of ECHO viruses 1 and 8 and the original prototype ECHO-13 virus. In contrast, however, ECHO-12 virus was not neutralized by ECHO 1, 8 and 13 antisera (Wenner).

To investigate these findings the above 4 ECHO prototype strains were purified by plaque isolation or terminal dilution passage and antisera prepared to these purified viruses. The results obtained in our laboratory with terminal dilution purified ECHO-12 antisera (titer 1:2,048) against approximately 100 $TCID_{50}$ of purified ECHO viruses 1, 8, 12 and 13 are shown in Table I. ECHO viruses 1, 8 and 13 were not neutralized by this antiserum. No neutralization occurred when purified ECHO-12 virus was tested against antisera prepared to purified ECHO viruses 1, 8 and

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§ 2-85 is the number given this child and 4 is the number of specimen from which virus was isolated.

TABLE I. Neutralizing Antibody Titers of a Monkey Antiserum Prepared to Terminal Dilution Purified ECHO-12 Virus.

	Purified ECHO virus types			
	1	8	12	13
$TCID_{50}$ employed	50	20	500	20
Type 12 serum titer*	<4	<4	2,048	<4

* Reciprocal of dilution calculated to afford protection to half of inoculated tubes.

13 (not shown in Table 1). ECHO-12 and its antisera were also tested reciprocally in MKCC against the other 23 presently recognized ECHO viruses, the 5 Coxsackie B viruses, Coxsackie A-9 and the 3 polioviruses and their respective antisera; no cross-reactions occurred.

General characteristics of ECHO-12 virus. Prototype ECHO-12 virus, 2-85-4 Travis, was not pathogenic for 1-day-old suckling mice tested by serial blind passage. Similarly it was not pathogenic for 7-day-old chick embryos inoculated by yolk-sac route. Adult guinea pigs and rabbits, given intramuscular injections, were not demonstrably ill. No illness developed in 5 rhesus monkeys following intracerebral and or intramuscular inoculation. No pathology was noted in brain and spinal cord of one monkey given intracerebral injection of virus 21 days previously. Cytopathogenic effect (CPE) in rhesus MKCC cannot be differentiated morphologically from that of most other enteroviruses. Strain 2-85-4 first shows CPE within 24 hours, proceeds to completion within 4 to 5 days at which time it reaches endpoint of approximately 10^{7}/0.1 ml. Replication of ECHO-12 virus with CPE occurred in HEP #2, HeLa, and Detroit 504 Fb-L(6) cell lines and in primary cell cultures of human amnion.‖ No CPE was obtained with monkey heart cells (7) and with primary cell cultures of hamster and gerbil¶ kidney. Large, 10-14 mm, round and clear plaques are produced on rhesus MKCC within 4 to 7 days. Hsiung and Melnick(8) reported that prototype ECHO-12 produces tiny delayed plaques on monolayer cultures of *Erythrocebus patas* MKCC.

ECHO-12 does not hemagglutinate chick red blood cells at 4, 25 and 37°C but does hemagglutinate human group O red blood cells at all of above 3 temperatures(9). Infected MKCC fluids can be used in complement fixation (CF), NA and hemagglutination inhibition (HAI) tests for viral identification with hyperimmune antisera and for detection of antibodies in human serum. Capillary tube precipitin and agar diffusion tests have given equivocal results.

Geographic distribution. A total of 8 isolations of ECHO-12 have been made in this laboratory from material collected in the Philippines, including one from throat swab. Thirty Americans from CAFB were tested for NA, of which 50% were positive, 56% among adults and 20% among children. Antibodies were found in a single lot of Japanese gamma globulin tested. Several other isolations of ECHO-12 virus have been reported from United States and Mexico(5).

Discussion. Absence of NA's for any of the presently recognized MKCC cytopathogenic enteroviruses in high titered ECHO-12 rhesus monkey antiserum prepared with a pool of terminal dilution purified ECHO-12 virus is interpreted to indicate that ECHO-12 virus is essentially antigenically distinct from all these other viruses. There is no obvious explanation for the presence of detectable NA's for ECHO viruses 1 and 8 in the ECHO-12 monkey antiserum prepared by Dr. Wenner(5), which titered 1:5,000 to 1:50,000. However, it is possible that since this antiserum was not prepared with a pool of plaque or terminal dilution purified virus, a small amount of either ECHO-1 or 8 virus was present. It is also feasible that an antigen common to both ECHO-1 or 8 and ECHO-12 actually exists and is demonstrable only when monkeys are highly hyperimmunized with ECHO-12 virus. Although Wenner ECHO-12 antiserum neutralized the original ECHO-13 prototype, 2-188-20 Hamphill(5), subsequent studies have shown that the new prototype strain, 11-4-1 Del Carmen, is not neutralized by this Wenner ECHO-12 antiserum(10). It

‖ Tests with human amnion cells were performed by Gladys Sather of this Department.

¶ Tests with gerbil kidney cells were performed by C. M. Hodges and M. B. Dobkin of Dr. F. S. Cheever's laboratory in this Dept. and results kindly given to us.

was also found that the original ECHO-13 prototype strain was a mixture which contained predominantly ECHO-1 virus in late passages(10).

Although no grouping of the ECHO viruses has been designated by the Enterovirus Committee, ECHO-12 virus, because of its ability to produce plaques in both patas and rhesus MKCC, falls into the proposed group B of Hsiung and Melnick which contains types 7, 8 and 12(8). The ability of ECHO-12 virus to hemagglutinate human type O erythrocytes at 4, 25 and 37°C but not chick erythrocytes is also typical of type 7 ECHO virus but not type 8; the latter virus apparently does not hemagglutinate(9).

ECHO-12 virus was not found capable of producing observable illness in suckling mice, adult guinea pigs, rabbits, monkeys or embryonated eggs.

The only association of this virus with human disease to date is limited to serological rises in 2 patients with the aseptic meningitis syndrome(1,4).

Summary. 1) ECHO-12 prototype virus, 2-85-4 Travis, was isolated from rectal swab of a normal American child in the Philippines, collected in 1953. A rise in NA was demonstrated in serial serum specimens. This virus was antigenically distinct from all other currently recognized enteroviruses which are cytopathogenic for rhesus MKCC, as determined by reciprocal NA tests with all these viruses and their respective monkey or rabbit antisera. 2) Specificity and biologic properties of ECHO-12 virus in certain laboratory animals, chick embryos, and cell cultures are described. The ability of ECHO-12 virus to form plaques in both rhesus and patas MKCC and to hemagglutinate human type O erythrocytes at 4, 25 and 37°C further distinguishes this virus from the first 19 of the 24 recognized ECHO types. Prototype ECHO-12 infected rhesus MKCC fluids can be used in CF, NA and HAI tests. 3) This virus has now been repeatedly isolated from individuals in the Philippines, from United States and Mexico. Antibodies are present in Japanese gamma globulin. One isolation of virus was made from a throat swab.

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Action of Certain Tetrapyrrole Derivatives in Experimental *Trypanosoma congolense* Infections. (24765)

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Since the work of Pfeiffer(1) on *Hemophilus influenzae*, and of Novy and MacNeal (2) on *Trypanosoma brucei*, the nature of factors occurring in blood necessary for growth of certain microorganisms has been progressively elucidated Studies on the influenza organism by Granick and Gilder(3) and on non-pathogenic members of the Trypanosomatidae by Lwoff(4,5) have revealed certain differences between porphyrin re-