levels of other amino acids would be expected to occur.

Summary. High plasma levels of L-phenylalanine inhibit the entry of 5-hydroxytryptophan-C^{14} into the CSF of dogs. CSF is an accessible body fluid that may be used to study transport of amino acids into the CNS.

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Characterization of Prototype Virus ECHO-32. (29793)

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During studies of infectious agents isolated from Puerto Rican infants with diarrhea(1) several viral agents were recovered which appeared to conform to criteria established for ECHO viruses, but did not belong to any known ECHO group. Two of these agents have been fully characterized and submitted to the Committee on Enteroviruses. One of the viruses, PR17, was reported by the Committee to be related to ECHO virus type 30, the prototype of which is Bastianni(2). The other, PR10, was accepted as the prototype strain of ECHO-32(3). The following report presents characteristics of this virus.

Materials and methods. Source. This agent was isolated from a 20-month-old female admitted with diarrhea, nausea and fever of 3 days' duration. Examination revealed a well-nourished, alert, and well-hydrated infant with moderate upper respiratory distress and a temperature of 101°F. Pertinent laboratory findings included a white count of 21,155 cells/cu mm and numerous *Giardia lamblia* cysts, *Escherichia coli* 019 and Enterococci in her stool. Convalescent phase blood was drawn two weeks later.

Tissue culture. Methods for recovering viruses from fecal sample during this study have been previously described(1). Briefly, the fecal sample was mixed with physiological saline to make approximately a 20% suspension and centrifuged at 3000 rpm for 1 hour at 4°C. One hundred μg of streptomycin and 1000 units of penicillin G were added and this preparation utilized to inoculate rhesus monkey kidney tissue cultures (MKTC). The cultures were maintained in bovine amniotic fluid containing 0.005% phenol red, 100 μg streptomycin and 100 units of penicillin/ml.

Identification. Virus pools were prepared and neutralization tests carried out in cell cultures just described. Antisera for neutralization tests against ECHO viruses types 1

through 28 and 10 candidate enteroviruses were prepared by rabbit immunization. The candidate viral strains Frater, Bastianni, Pett, Vanscoy, 1923/60, Taylor, JV10, JV6, 4707s, and Caldwell were supplied by Dr. H. A. Wenner, Univ. of Kansas. Further neutralization tests were done with pooled antisera of Polioviruses types 1, 2, and 3, with Coxsackie viruses types A9 and B1 through B6. They were also performed with the second candidate virus submitted by the authors, *i.e.*, PR17, as well as with antisera of 2 other unrelated viruses from the same investigation. These neutralization tests were performed using 100 $TCID_{50}$ of virus and 20 antibody units of the enterovirus serotypes. Reciprocal neutralization tests using 100-300 $TCID_{50}$ of the known enteroviruses against dilutions of 1:8, 1:16, and 1:32 of ECHO-32 virus antiserum were carried out. Further identification tests included complement fixation using 2 units of commercial adenovirus CF antigen and a positive human reference serum, as well as hemadsorption and hemagglutination tests using human O, rhesus monkey and rat red blood cells at 4°C, 25°C, and 37°C.

Purification. Plaque studies were performed according to the method of Hsuing and Melnick using rhesus and African green monkey kidney tissue cells with tris buffer overlay as described by these authors(4) and according to the method of Gochenour and Baron(5) adding neutral red after 3 days incubation in the dark. Because these methods were unsuccessful in obtaining plaques, the virus was purified by 3 consecutive terminal dilution passages and pools prepared from the third passage level for rabbit immunization and further studies.

Results. Cytopathogenic effect of ECHO-32 in tissue cultures: Microscopic examination of both living and stained(6) tissue cultures inoculated with ECHO-32 revealed that the cell changes in all stages were comparable with that caused by enteroviruses. Initial cytopathogenic effect (CPE) appeared within 24 hours and destruction of the cell sheet was complete within 4-5 days, achieving a titer ranging from $10^{5.5}$ to $10^{6.5}$. Only MKTC and human embryonic skin and muscle (HES) cells were able to support the growth of ECHO-32. Other tissue cells tested were as follows: human carcinoma lines K. B., HeLa and H.Ep. #2 as well as human sternal marrow (Detroit -6), human liver (Chang) and hamster kidney. No CPE was observed in these cells after 3 consecutive fluid passages at 14-day intervals. As was previously stated, all attempts to produce plaques by the methods utilized(4,5) were unsuccessful. However, it was discovered later that plaques can be produced when 8.8% $NaHCO_3$ is incorporated into the overlay.

Serological tests. Neutralization tests against enteroviruses and reciprocal neutralization tests with known enteroviruses against ECHO-32 antiserum were negative, as were all hemadsorption, hemagglutination and complement fixation tests. Neutralization tests with patient's sera using 100 $TCID_{50}$ of virus revealed no neutralizing antibodies in either acute or convalescent phase serum at an initial dilution of 1:8. However, tests performed on 2 pools of sera each containing 5 specimens from normal Puerto Rican adults showed one of the pools to have a neutralizing antibody titer of 1:32. Furthermore, tests of 2 similar pools of sera from normal adults living in Washington, D. C., revealed antibody titers of 1:32 and more than 1:256, respectively.

Animal tests. Two duplicate litters of 12-24-hour-old suckling mice were injected intracerebrally (i.c.) with 0.01 ml of virus, while a second litter was given 0.02 ml intraperitoneally (i.p.). At least 2 additional blind passages were made using 20% suspension of mouse brain or muscle. At the same time, these suspensions were titrated in MKTC to determine infectivity. Rabbits, guinea pigs, hamsters and adult mice were given 0.5 ml i.c. and monkeys were inoculated with 0.5 ml i.c. and 0.1 ml intraspinally. All the animals were observed daily for 30 days. Daily rectal temperatures were taken on the monkeys. Complete autopsies were performed on the monkey and microscopic examination of sections of cerebrum, cerebellum, brain stem, and entire spinal cord disclosed no evidence of past or present inflammation. Ten embryonated chicken eggs, 7-10 days old which

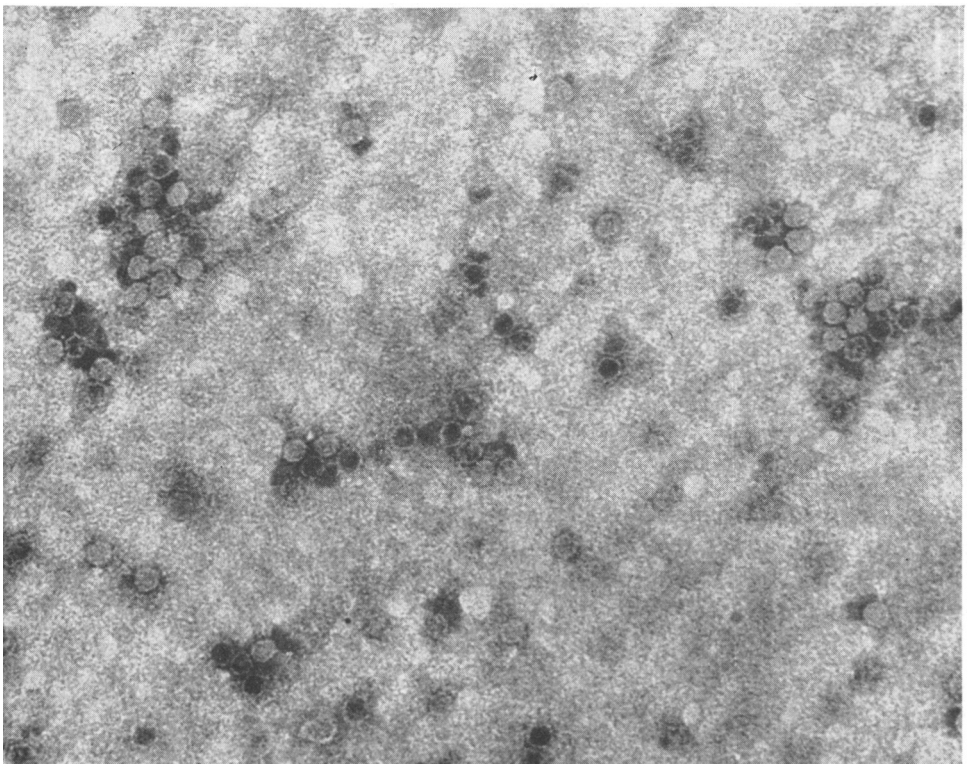

FIG. 1. Electron photomicrograph of ECHO-32-phosphotungstic acid, negative stain. Magnification 133,000 ×.

were inoculated with 0.1 ml of virus by the yolk sac route, yielded negative results.

Stability. ECHO-32 undergoes no loss of infectivity titer at 25°C and 37°C for 24 hours but at 56°C it loses 6 logs in one hour and is completely inactivated in 12-16 hours. Full infectivity is maintained by ECHO-32 when heated at 50°C for one hour in the presence of M $MgCl_2$(7). The virus is also stable in a 1:10 dilution of Eagle's Basal Medium at pH 3.0 for 3 hours at 25°C(8). Sodium desoxycholate(9) and ether(10) do not alter the infectivity of ECHO-32.

Nucleic acid determination. Infected cells stained according to the acridine orange procedure of Bertalanftly *et al*(11), gave an intracytoplasmic red staining reaction suggesting that ECHO-32 has a ribonucleic acid core.

Size and shape of virus. Electron photomicrograph studies (Fig. 1) indicate that the virus is isocosohedral-shaped. Size of the virus particles was determined by 2 methods which gave comparable results. The first method is based on the electron photomicrograph (Fig. 1) and the virus particles were determined to be 26 to 27 mμ by comparison to the 88 angstrom polystyrene balls. The second method utilized Swinny adapted millipore filters with 5 ml plastic syringes. To filter the 2 ml of virus during this procedure, 1 ml of air space was maintained between virus and the rubber gasket of the syringe to prevent leakage. Titers of the virus preparations after filtration through Millipore® filters of 3 pore diameters are listed in Table I. These 3 pore sizes are the only ones available in this range. It was decided to apply the principles of Elford's factors as previously used for determination of particle size with gradacol membranes. Since the virus

TABLE I. Titers of Filtered Viral Preparations.

Viral preparation	Millipore filter pore diameter			Unfiltered control
	100 mμ	50 mμ	10 mμ	
1	$10^{6.5}$	$10^{3.0}$	0	$10^{6.5}$
2	$10^{6.5}$	$10^{2.5}$	0	$10^{6.5}$

was partially retained by the 50 mμ filter, Elford's factors(12) were applied as follows:

pore = factor × diameter
p = .33 × 50 mμ = 16.50 mμ lower range
p = .50 × 50 mμ = 25 mμ upper range

In this case the application of the Elford factor gave results similar in range to that of the electron photomicrograph. Attempts to characterize the size of the virus by density gradient ultracentrifugation were unsuccessful.

Discussion. ECHO-32 has satisfied all the criteria required by the Panel on Picornaviruses for a new viral isolate to be designated as an ECHO prototype(13). It possessed antigenic distinctness from all previously described human enterovirus serotypes. Inoculation of laboratory animals, including monkeys, gave no observable effect. The virus was stable to 20% ether and to desoxycholate, indicating a lack of essential lipids and has a ribonucleic acid core typical of enteroviruses.

The two methods utilized to estimate size of the virus particles gave good correlation. While the Swinny Millipore filter has not previously been utilized to determine virus particle size as far as the authors are able to determine, it appears to be a useful, if crude method to arrive at a reasonable estimate.

It is of interest that PR17 (ECHO virus type 30) which was also isolated from a Puerto Rican infant with diarrhea stimulated a neutralizing anibody titer rise from less than 4 to 160 during the infection. PR10 on the other hand gave no such rise. However, tests performed on pooled human sera had varying amounts of neutralizing antibodies present. It must be assumed that more than one of the sera from pooled sera of 5 normal Washington, D. C. adults which gave a neutralizing antibody titer greater than 1:256 were indeed higher than one would expect to encounter in a normal population. There was nothing to indicate that ECHO-32 virus was related etiologically to the gastroenteritis in the infant from whom it was isolated. The infant's stool also contained large numbers of *Giardia lamblia* cysts, as well as a strain of *Escherichia coli* serotype 019 which elicited an hemagglutinating antibody titer rise from 32 to 256 during the attack. Any one of a number of agents may have been responsible for the illness. It can be concluded, however, that human exposure to ECHO-32 is frequent both in Puerto Rico and in the United States, and that this contact can result in a rise in antibody titer.

Summary. A viral agent, PR10, isolated from a Puerto Rican infant with gastroenteritis has been designated as the prototype strain of ECHO-32 by the Panel for Picornaviruses(3). ECHO-32 (1) produced enterovirus-like CPE in rhesus monkey kidney tissue cultures, (2) bore no antigenic relationship to known enteroviruses, (3) produced no disease in the common laboratory animals or embryonated eggs, (4) was insensitive to desoxycholate or ether, (5) was stabilized by M $MgCl_2$ to heating at 50°C for one hour, (6) demonstrated a red staining reaction with acridine orange indicating an RNA core, and (7) was estimated to be 26-27 mμ in size.

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Production of Interferon by Human Mononuclear Leucocytes.* (29794)

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The production of interferon by suspensions of human leucocytes has been previously demonstrated(1). The present series of experiments was performed to determine whether (a) mononuclear cells or (b) polymorphonuclear leucocytes or (c) both cell types were responsible for the interferon produced under these conditions.

Materials and methods. Viruses. Sendai virus (F. Davenport) obtained from Dr. J. F. Enders was passed many times in allantois of embryonated egg. Stock Sendai was grown in 9-11-day-old embryonated eggs in the usual manner. To obtain high concentrations of virus to the order of 10^{8-9} EID_{50}, the harvested virus in allantoic fluid was ultracentrifuged at 40,000 rpm in the Spinco model L ultracentrifuge for 2½ hours. The virus was suspended in M199 with 50% skimmed milk and stored at −20°C. Infectivity titer was calculated by the method of Reed and Muench. The virus was titrated in embryonated eggs (EID_{50}) using the appearance of haemagglutinin as criterion of infection.

Sindbis virus (59G) obtained from Dr. R. Taylor was propagated in established human amnion cells (HA-FL) and titrated. For purposes of interferon assay, approximately 500 $TCID_{50}$ of the virus was used.

Antiserum. Sendai antiserum (HAI-titer = 1:1024) was prepared in rabbits inoculated 5 times intravenously at 3-day intervals with 0.5 ml of Sendai virus in allantoic fluid. The animal was bled 10 days after the last injection. The serum was inactivated at 56°C for 30 minutes and titrated by haemagglutination inhibition.

Tissue cultures. HA-FL cells (Dr. K. Rozee, Univ. of Toronto) were grown in either milk dilution bottles or screw-capped tubes in Hanks' Balanced Salt Solution containing 0.5% lactalbumin hydrolysate, 0.1% glucose, penicillin and streptomycin, and supplemented with 10% calf serum. Upon virus inoculation, no serum was included in the medium.

Iron. The iron powder used was obtained from Fisher Scientific Co. (Cat. # 1-62). Sterility was ensured by autoclaving.

Stains. Orcein stain was obtained from The British Drug House Ltd., Poole, England. A 2% solution was prepared(2) for use.

Dextran. 6% W/V dextran with 0.9% NaCl, Pharmachen Corp., Bethlehem, Pa.

Preparation of leucocyte suspensions. Fifty ml of heparinized blood was taken from healthy male or female donors in sterile flasks. To this sample, dextran (6%) was added to a final concentration of 1:6 in the blood sample. The blood was then incubated in 10 ml portions in sterile screw-capped tubes inclined at a 45° angle for 30 minutes or longer at 37°C.

After incubation, the plasma containing the suspension of leucocytes was removed and pooled. This sample was then divided into 2 portions.

From one portion, the plasma was removed by centrifugation at 2500 rpm for 15 minutes, and the sediment containing leucocytes was washed 3 times, and resuspended in 5 ml of medium 199 containing 10% calf se-

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