amount of phosphate secreted from interstitial fluid to tubular lumen. This is compatible with the concept requiring that tubular handling of phosphate includes reabsorptive and secretory processes.

Summary. Transient renal responses to close arterial instantaneous injections of NaH_2PO_4 and creatinine were studied in phosphate-loaded dogs. The increment of phosphate excretion per unit injected per one circulation of injected substance was greater than that of creatinine. Such a finding, interpreted as net minimal secretion of phosphate into the renal tubule, was found in 8 consecutive experiments.

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Viremia in ECHO Type 6 Virus Infection.* (24988)

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Extensive use of improved tissue culture procedures in the study of infectious diseases has prompted the recognition of an increasing number of cytopathogenic agents with characteristics of ECHO (Enteric Cytopathogenic Human Orphan) viruses(1). While the importance and etiologic role of these agents in human disease require further clarification, several already have been associated with outbreaks of non-paralytic illnesses, lymphocytic meningitis and infantile diarrhea. Their detection has been accomplished more often in stools than in pharyngeal secretions and cerebrospinal fluid of clinically ill individuals. However, limited data are available(2,3,4) on occurrence of viremia in ECHO virus infections. The present report describes isolation of an agent, antigenically indistinguishable from prototype ECHO 6 virus, from blood of a 7 year old female prior to fatal termination of a paralytic illness not associated with a stiff neck or other signs of meningeal involvement. Results of serologic studies of familial associates also are included.

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Methods. Primary human amnion cell cultures prepared by modified Lahelle procedure (5) were utilized for both virus isolation and neutralization studies. The trypsin dispersed cells were grown in medium consisting of 0.5% lactalbumin enzymatic hydrolysate in Hanks solution and supplemented with 15% heated (56°C for 30 minutes) horse serum. After 5-10 days incubation at 36.5°C the monolayer cultures were washed once with Hanks solution, then 1 ml of lactalbumin maintenance medium containing 2% heated calf serum was added/tube. The latter medium also was used for propagation and maintenance of monkey kidney tube cultures prepared by modified Youngner technic(6). In virus isolation tests, 0.2 ml of each specimen were inoculated into 6 tubes each of human amnion and monkey kidney cell cultures. Certain specimens and tissue culture fluids also were tested by intraperitoneal (0.03 ml) and intracerebral (0.02 ml) inoculation of suckling mice less than 24 hours old. Throat swabs were eluted into 3 ml portions of Hanks solution prepared with 500 *u* penicillin, 250 μg streptomycin and 40 *u* mycostatin/ml;

TABLE I. Summary of Clinical Features and Virus Isolation Attempts.

Day of illness	Clinical features	Specimen	Virus isolation attempts TC	SM
1	Abrupt onset, muscular weakness lower extremities, no fever	None		
2	Fever, flaccid paralysis, abdominal reflexes diminished, deep tendon and gag reflexes absent	Throat swab	Neg.	Neg.
		Stool	"	"
3*	Progressively worse	Throat swab	Neg.	Neg.
		Stool	"	"
		Blood	Pos.	"
		CSF	Neg.	ND
4	Same			
5	" + pneumonic infiltration			
7	Expired	Sigmoid colon and contents	Neg.	Neg.
		Ileum	"	ND
		Mesenteric lymph nodes	"	"
		Cervical and lumbar cord	"	"
		Medulla and pons	"	"
		Cerebral cortex	"	"

TC = tissue culture; SM = suckling mice; ND = not done.
* CSF = 3 cells/cmm (all lymphocytes); total protein 18 mg %.

Hanks solution containing 2000 *u* penicillin and 1000 μg streptomycin/ml was used in preparing 20% suspensions of stool and post-mortem specimens, clarified by centrifugation at 5800 x g for 45 minutes prior to use. Sera employed in neutralization tests were inactivated at 56°C for 30 minutes. Neutralization mixtures prepared with equal volumes of serially diluted serum and constant amounts of virus were incubated at room temperature for 1 hour before inoculation; 0.2 ml of each mixture being added to each of 3 tube cultures.

Results. Correlation of clinical course with time of collection of specimens and with results of virus isolation attempts is shown in Table I. A single whole blood specimen taken 3 days after onset of illness and on 2nd day of paralysis produced specific cytopathogenic changes in both human amnion and monkey kidney cell cultures. The degenerative changes first became evident 8 and 6 days, respectively, after inoculation of the 2 host systems and complete destruction of cellular growth occurred within additional 72 hours in each instance. Subsequent titrations performed in 2 lots of amnion cultures revealed approximately 65 TCD_{50} of virus/ml of whole blood. Presence of virus also was demonstrated in patient's serum obtained on 3rd day of illness and stored at −20°C, for 3 weeks, before inoculation into different lots of amnion and kidney cultures.

All pharyngeal, stool, cerebrospinal fluid and post-mortem specimens failed to yield cytopathogenic agents after 2 consecutive passages in each culture system. Decreasing the concentration of inocula in cultures gave no evidence that "blocking" or "interference" effect was responsible for these negative results. The pharyngeal and stool materials as well as virus containing whole blood produced no demonstrable illness in suckling mice during 2 consecutive passages made at 10-day intervals. Likewise, virus-containing culture fluids from 4th amnion and kidney cell passages ($10^{-6.7}$ and $10^{-6.4}$, TCD_{50}/ml respectively) of blood isolate failed to produce signs of illness in suckling mice. In the latter tests virus could be recovered from pooled brain and muscle of mice 2 but not 12 days after inoculation.

Whole blood and serum isolates were identified as ECHO type 6 virus in neutralization tests performed with 1:100 dilution of first passage culture fluids and 1:10 dilution of prototype ECHO 6 (D'Amori strain) rabbit antiserum. In quantitative homologous and heterologous neutralization tests performed with the prototype antiserum and blood isolate antiserum prepared in rabbits (Table II), no significant antigenic difference was found

TABLE II. Titration of Antisera against ECHO 6 Viruses.

Rabbit antiserum	Blood isolate 32*	Blood isolate 100	Blood isolate 500	Prototype 6 (D'Amori) 32	Prototype 6 (D'Amori) 100	Prototype 6 (D'Amori) 500
Prototype ECHO 6	320†	320	160	320	160	160
Blood isolate	640	640	640	640	640	320

* TCD_{50} of virus used in tests.
† Reciprocal of serum dilution neutralizing indicated amount of virus.

between the 2 virus strains when indicated amounts of virus were employed. Relationship between homologous and heterologous serum titers at any given virus concentration was constant regardless of whether the tests were read on 4th or 8th day, although serum titers usually were 2-fold lower at the latter time. Neutralizing antibodies were not demonstrable in virus-containing patient's serum.

Three months after patient's fatal illness, sera† were obtained from 6 household associates, aged 3 to 34 years, and tested for neutralizing antibodies to the blood isolate and prototype 6 strains. Only one familial contact, aged 14 years, possessed serum antibodies (1:8 titer) to both virus strains. This finding could indicate a present or past subclinical infection with ECHO 6 virus.

Discussion. Information obtained from the study of this case indicates that systemic infection with ECHO type 6 virus may occur without frank meningeal involvement and suggests that viremia may be a significant part of pathogenesis of such infections. Detection of a viremic stage at least 2 days after development of definitive paralysis is of particular interest in view of absence of demonstrable virus in pharyngeal secretions and intestinal tract. This suggests that if the intestinal tract was the locus of initial infection, as currently purported for ECHO viruses, then viral multiplication was minimal at this site and probably occurred relatively early in the incubation period. The possibility also exists that virus multiplication in tissues other than those tested may have contributed to the late viremia.

† We are grateful to Dr. R. E. Harper, Colbert County Health Officer, for procuring these sera for us.

Viremia in pathogenesis of ECHO 6 infection has not been previously recorded. However, an outbreak of exanthem disease in Pittsburgh, Neva and Zuffante(2) isolated virus from blood of one patient on second day of illness. This agent since has been identified as ECHO 16 and is related to if not identical with strains recovered earlier from similar cases in Boston(7). Brief reference was made by Sabin *et al.*(3) to detection of ECHO type 9 viremia prior to onset of clinical manifestations. No details were given but they indicated that viremia rarely was demonstrable within 24 hours after onset of febrile period. Observations by Yoshioka and Horstmann (4) also indicate that type 9 viremia is more likely to precede clinical disease.

It is reasonable to expect that further search will reveal presence of viremia in systemic infections with other ECHO viruses. Clarification of its role in pathogenesis of various clinical manifestations will necessitate more frequent inclusion of multiple blood specimens in virus isolation studies of suspected ECHO infections.

Summary. ECHO type 6 virus was isolated from whole blood and serum obtained 3 days after onset and on 2nd day of paralysis of fatal illness in which there were no meningeal signs. Virus could not be demonstrated in pharyngeal secretions, stools, CSF and postmortem materials. Neutralizing antibodies were not present in the virus-containing serum.

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