by a process of selection from strain RM3-56 which requires whole chick embryo extract and horse serum in addition to the defined components of medium 73.‡ This suggests the possibility that additional vitamins are required for the RM3-56 strain of rabbit fibroblasts.

Summary. A strain of rabbit fibroblasts designated strain RM3-73 requires folic acid, nicotinamide, pantothenic acid, pyridoxal, riboflavin, and thiamine for continuous proliferation in a medium containing 2% (v/v) dialyzed horse serum. The concentration of each vitamin which permits optimal proliferation was determined by propagating the cells serially in media containing graded concentrations of the compound. A group of eight amino acids which are not required for serial propagation in the presence of pyridoxal replaced the requirement for this vitamin under experimental conditions employed.

1. Haff, R. F., and Swim, H. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 200.

2. Eagle, H., *J. Exp. Med.*, 1955, v102, 595.

3. Snell, E. E., and Guirard, B. M., *Proc. Nat. Acad. Sci.*, 1943, v29, 66.

4. Stokes, J. L., and Gunness, M., *Science*, 1945, v101, 43.

5. Eagle, H., Oyama, V. I., Levy, M., and Freeman, A., *ibid.*, 1955, v123, 845.

Received February 15, 1957. P.S.E.B.M., 1957, v94.

Viremia in Coxsackie B Meningitis. (23086)

ALEXIS SHELOKOV AND KARL HABEL

U. S. Department of Health, Education and Welfare, Public Health Service, N.I.H., Nat. Inst. of Allergy and Infectious Diseases, Bethesda, Md.*

Viruses of the Coxsackie B group have been frequently isolated from the stools and less frequently from the throat and the spinal fluid of aseptic meningitis patients. However, demonstration of viremia has received only a passing mention in the literature(1,2). The present report describes isolation of a Coxsackie B2 virus from the serum of a patient 3 days after onset of a febrile illness, but 5 days prior to the onset of frank meningitis.

The patient, a 36-year-old male physician engaged in virus research, became ill in November 1955 while investigating an outbreak of a disease characterized by fever, dizziness, chest pain, myalgia and sometimes nuchal discomfort. His studies included examination of patients, collection and testing of blood, pharyngeal and fecal specimens, some of which were found to contain Coxsackie B2 virus.

Methods. Monkey kidney cultures prepared by a modified Youngner technic(3) were used both for virus isolation and neutralization tests. The cells were grown in the lactalbumin hydrolysate-calf serum medium recommended by Melnick(4) and after 4 days of incubation were changed to a maintenance medium in which the concentration of the calf serum was decreased by one half. The human amnion cell cultures were prepared according to a procedure described by Takemoto and Lerner(5). In virus isolation attempts, 0.3 ml of undiluted sera and spinal fluid were added to several tubes each; the throat swabs in Hanks' solution were first treated with 500 u penicillin, 500 μg streptomycin, and 100 u mycostatin per ml; the stools were emulsified to make 10% suspension in Hanks' solution and treated with 1,250 u penicillin and streptomycin both before and after centrifugation at 8,000 rpm for 30 minutes. Serum-virus neutralization tests employed mixtures of equal volumes of appropriate serum and virus dilutions representing 100 tissue culture infectious doses which after 1 hr. at room temperature were inoculated into culture tubes. All sera used in neutralizations, including serial specimens from the patient, were heated at 56°C for 30 minutes.

* Lab. of Infectious Diseases.

TABLE I. Clinical Course and Coxsackie B2 Isolations in Tissue Culture and Suckling Mice.

Date	Clinical course	Specimen	Virus isolations TC	SM
11/12/55	Fever, dizziness, myalgia	None		
13	Same + pleurodynia, nausea	"		
14	Same + headache, anorexia	Throat Blood	Neg. Pos.	Neg. Pos.
15	Some improv'm'nt	None		
16	Same	Stool	"	"
17	Chest pain, backache	None		
18	Same + ? stiff neck	"		
19	Frank clinical meningitis*	Throat CSF	Neg. "	Neg. QNS
22	Some improv'm'nt	Stool	Pos.	ND

* CSF-76 cells (50% PMN), protein 52 mg. Nuchal and spine rigidity, fever, headache and vomiting.

Virus isolations in tissue culture were confirmed by intraperitoneal (0.04 ml) and intracerebral (0.02 ml) inoculation of Swiss white suckling mice of less than 24 hr. of age.

Results. Table I summarizes the major clinical manifestations chronologically with the results of virus isolation attempts. The virus was present in the blood but not the throat secretions on the third day of the initial phase of illness. Its titer was found to be 10^2 per ml of serum at that time. Two days later, during the period of relative improvement, the virus was present in the stool at a titer of 10^5 per g of fecal material. At the time of clinical meningitis, the virus was again not found in the throat and could not be isolated from a sample of spinal fluid. During early convalescence the virus was still present to a titer of 10^4 per g of stool specimen.

Presence of the virus in the patient's blood on November 14 was established by repeated isolations from 2 separately frozen vials of the serum. Three of the isolations were accomplished in tubes prepared from different batches of monkey kidney cells. The fourth attempt utilized human amnion cell cultures; the primary tubes did not show cytopathic changes during the 30-day observation period. Aliquots of supernatants harvested at 10, 20, and 30 days and passaged blindly in amnion and monkey kidney tubes generally failed to produce cytopathic effects. The only exception was the 10 day harvest passaged in monkey kidney which after a prolonged incubation period showed passageable characteristic Coxsackie B effect, suggestive of limited survival rather than propagation of the virus in the amnion system. The fifth virus isolation from the serum was directly in 1 day old suckling mice which developed typical experimental Coxsackie B disease. A passageable cytopathogenic agent was re-isolated in monkey kidney tubes from the brain-muscle pool of the 4th suckling mouse passage. Each of the 5 separate tissue culture virus isolates was individually typed as Coxsackie B2.

Table II shows that neutralizing antibodies against Coxsackie B2 prototype virus, as well as the virus strains isolated from the patient's blood and stool, were present in the convalescent serum but not in the "acute" virus-bearing serum or the sera collected 2 and 16 months before the onset of illness.

Discussion. Demonstration of viremia in this patient suggests that it may be a part of pathogenesis of Coxsackie B meningitis. Unfortunately, because serum, pharyngeal, and fecal specimens were not collected daily, it is not possible to correlate the presence of the virus with the development of specific symptoms. However, it appears that viremia either followed or accompanied the onset of fever, myalgia, and pleurodynia, and preceded the development of clinical meningitis. The absence of demonstrable virus in the throat secretions on 2 occasions and its presence in the stool mid-way through the febrile

TABLE II. Titration of Patient's Sera against Coxsackie B2 Viruses.*

Date of serum†	Virus strains: Serum isolate (11/14/55)	Stool isolate (11/16/55)	Prototype B2
7/26/54	<4	ND	<4
9/15/55	"	<4	"
11/14 ‡	"	"	"
12/ 6	64	64	64

* Figures indicate reciprocals of serum dilutions neutralizing 100 $TCID_{50}$.

† All sera were heated at 56°C for 30 min.

‡ "Acute" virus-bearing serum.

illness preceding meningitis suggests that in this patient there was an early locus of viral propagation in the intestinal tract.

Review of the literature disclosed no other findings suggestive of the possible role of Coxsackie B viremia in the pathogenesis of meningitis. Isolation of 4 Coxsackie virus strains from human blood was first reported by Taylor(1) in the course of routine testing of 1,990 specimens from febrile patients in Egypt. Two of these viruses were found to belong to Group A (A3 and A6) and 2 were typed as B2. No definitive data were presented correlating the presence of viremia with the clinical picture(1,6).

Another cursory reference to a single isolation each of Coxsackie A and B viruses from human bloods was made by Bayer and Gear (2). No details were given but apparently all of their patients were admitted with CNS involvement as suspected cases of nonparalytic poliomyelitis. The uncommonness of Coxsackie B viremia after onset of meningitis is attested by the fact that they were able to isolate the virus only once although their group of 200 cases of viral meningoencephalitis included 20 individuals with Coxsackie B viruses in the feces, 9 of whom also had demonstrable virus in the spinal fluid.

It is quite possible that every systemic infection with the Coxsackie B viruses is accompanied by viremia. Elucidation of its role in the pathogenesis of complicating meningitis will require further attempts to isolate the virus from all types of suspected Coxsackie B infections, including those with meningeal involvement at various stages of the disease.

Summary. Coxsackie B2 virus was repeatedly isolated from a serum specimen obtained 5 days prior to the onset of clinical meningitis. The virus was also demonstrated in the stool specimen during the prodromal phase and in convalescence. The convalescent serum contained specific antibodies which were not present either in the virus-bearing serum or in sera collected before the onset of this illness.

The authors are grateful to Mrs. Nancy C. Gregg for valuable assistance.

1. Taylor, R. M., *Atti del VI Congr. Internazionale di Microbiol.,* 1953, v3, 236.

2. Bayer, P., and Gear, J., *S. A. J. Lab. and Clin. Med.,* 1955, v1, 22.

3. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

4. Melnick, J. L., *Ann. N. Y. Acad. Sci.,* 1955, v61, 754.

5. Takemoto, K. K., and Lerner, A. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 179.

6. Taylor, R. M., Rizk, F., and Kader, A., *J. Egypt. Med. Assn.,* 1953, v36, 479.

Received February 18, 1957. P.S.E.B.M., 1957, v94.