that a number of dipeptides introduced into the intestine of the dog appear in mesenteric plasma almost completely as free amino acids. Their results, then, are in general agreement with our own. Similar experiments done *in vitro* by Newey and Smyth confirm earlier work of Agar, *et al.*(14) which showed that dipeptides introduced on the mucosal side appeared on the serosal side almost entirely as free amino acids. The small and variable though definite passage of some peptide leaves open the possibility that such absorption might occur *in vivo* in cases of surgically altered or diseased gastrointestinal tract.

Summary. Dogs prepared with catheters in the superior mesenteric vein were fed meals of liver and homologous plasma protein. Analysis of the superior mesenteric blood plasma showed marked rises in amino acids up to 6 hours later. Digestion of the homologous plasma protein took essentially the same course as that of liver protein, contrary to results reported by others(7). No peptides were found and both proteins were apparently completely degraded to amino acids. Rises in lymph amino acids were qualitatively similar to those of the mesenteric plasma, though not as marked. An amino acid conjugate was isolated from all plasma samples, and its amino acid composition was determined. Its changing structure, and failure to respond in an obvious way to the meals leads us to believe that it is not of direct dietary origin.

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ECHO-9 Virus Antibody Titrations in a HeLa Cell System. (24874)

MARION K. COONEY, LLOYD E. BOYD AND HENRY BAUER
(Introduced by Anne C. Kimball)
Minn. Dept. of Health, Division of Medical Labs., Minneapolis

The need to perform ECHO-9 virus antibody titrations during an outbreak of this infection in summer of 1957(1,2) prompted us to attempt the use of HeLa cells for these titrations because HeLa cells were more readily available to us than monkey kidney cells. HeLa cell adapted(3) ECHO-9 virus (Hill strain) was used as the antigen.* The results reported here clearly demonstrate that ECHO-9 antibody titrations can be readily executed in HeLa cell tissue culture.

* Seed virus was kindly supplied by Dr. Herbert A. Wenner, Univ. of Kansas School of Med., Kansas City.

Materials and methods. HeLa cells were grown in milk dilution bottles according to methods originally outlined by Syverton and Scherer(4). Lactalbumin hydrolysate-yeast extract medium (LHYE) containing 20% human serum was used as growth medium. After 7 days incubation at 37°C, bottles were scraped, cells dispersed in growth medium, a cell count made, and suspension prepared to dispense 50,000 to 60,000 cells/tube in 0.75 ml of growth medium. Tubes were incubated at 37°C for 3 or 4 days. On day tubes were used, the growth medium was removed, the cell sheet rinsed 3 times with Hanks' balanced salt solution (BSS) following which

0.9 ml of maintenance medium, consisting of a solution of LHYE containing 2% calf serum, was dispensed into each tube. *Virus pools.* One ml of a 10^{-1} dilution of HeLa-adapted ECHO-9 (Hill strain) virus was inoculated into each of several bottles of HeLa cells, in which cell sheets had been rinsed with Hanks' BSS and to which 10 ml of maintenance medium had been added. Bottles were incubated at 37°C, and when cell sheets showed maximum cytopathogenic effect (2 to 3 days after inoculation), they were placed at –20°C for storage. Virus pools were prepared by thawing and pooling stored tissue culture fluids. The fluids were then centrifuged at 1000 rpm for 10 minutes in horizontal centrifuge. The supernatant fluid subsequently used in neutralization tests was ampouled, and stored at –20°C. The virus suspension was titrated in half-log dilutions to determine $TCID_{50}$ of the virus for HeLa cells. Average $TCID_{50}$ for several lots tested was 0.1 ml of a $10^{-3.5}$ dilution. The dose was 100 $TCID_{50}$. *Serum specimens.* Acute and convalescent phase sera submitted in 1957 from persons with symptoms of acute central nervous system (CNS) disease (aseptic meningitis syndrome) were tested. A group of 48 pairs of serum specimens submitted in 1956, when Coxsackie B5 virus(5) infection was prevalent, was also tested. *Technic of test.* Serum specimens were diluted 1:4 with Hanks' BSS and heated at 56°C in waterbath for 30 minutes. Five additional 4-fold dilutions (through 1:4096) of serum specimens were prepared in Hanks' BSS. An equal volume of ECHO-9 virus suspension, adjusted to contain 100 $TCID_{50}$/0.1 ml, was mixed with each serum dilution and allowed to stand for one hour at room temperature. An inoculum of 0.2 ml of each serum-virus mixture was used/HeLa cell culture tube. A positive serum control was included in each series of tests. Virus titration, using 100, 10, and 1 $TCID_{50}$ of virus, in presence of negative human serum was also included to determine precise virus dose present in test. Serum neutralization endpoints were determined when half, or more than half of virus titration tubes inoculated with 1 $TCID_{50}$ of virus showed cytopathogenic effect. Titers were expressed as

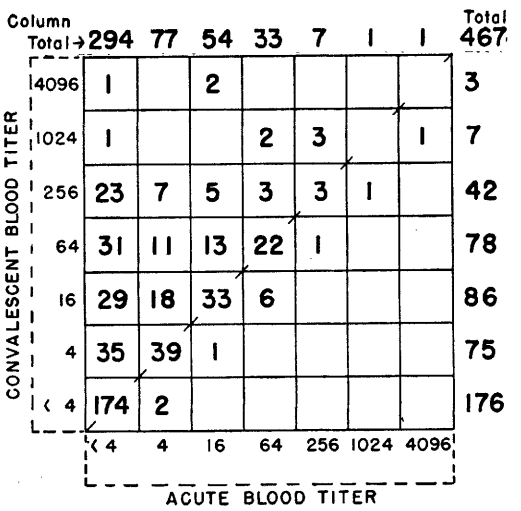

FIG. 1. ECHO-9 antibody results on 467 pairs of acute and convalescent serum specimens. Titers are expressed as reciprocal of highest dilution of serum completely neutralizing 100 $TCID_{50}$ of ECHO-9 virus.

reciprocal of highest serum dilution which completely neutralized 100 $TCID_{50}$ of ECHO-9 virus. *ECHO-9 virus isolation and identification.* Monkey kidney cell tubes were purchased for ECHO-9 virus isolation and typing. Type-specific ECHO-9 antiserum was supplied through courtesy of Nat'l. Fn. for Infantile Paralysis.

Results. ECHO-9 virus neutralizing antibody titrations on paired serum specimens from 467 patients submitted in 1957 during outbreak of aseptic meningitis,† are presented in a correlation square, Fig. 1. The number in each cell represents number of persons who had the initial titer indicated below the square, and the convalescent titer indicated at left of square. Those persons represented in cells on diagonal line showed the same antibody titer in both specimens. The number of persons showing significant increase in titer is represented in cells appearing 2 or more squares above diagonal line. Specimens for virus isolation were available from 281 of 467 patients tested for antibodies and Table I shows relationship between isolation of ECHO-9 virus and antibody results.

† Subsequent studies revealed there were also 26 cases of Coxsackie B5 virus infection, and 64 cases of poliovirus infection to make the total aseptic meningitis experience in Minnesota during 1957.

TABLE I. Association of ECHO-9 Virus Isolation with Antibody Titer.

	No significant rise—Highest titer* observed				Significant (16× or >) rise in titer	Total
	<4	4	16	64 or higher		
Total patients	174	76	52	53	112	467
Patients from whom specimens were submitted for virus isolation	84	37	33	48	79	281
Patients from whom ECHO-9 virus was isolated	2	4	7	27	61	101
ECHO-9 isolation (%)	2.4	10.8	21.2	56.3	77.2	35.5

* Titers expressed as reciprocal of highest serum dilution completely neutralizing 100 $TCID_{50}$ ECHO-9 virus.

A significant rise in antibody was observed more often if first blood specimen was collected during first 6 days of illness. Among 112 patients showing significant rise, the date of onset was known for 106, and 87 of these were collected within first 6 days of illness. In contrast to this, 105 patients showed ECHO-9 antibody but no rise in titer. Date of onset was known for 100 of these, and 51 were collected within first 6 days of illness. Paired serum specimens available from 48 patients ill with CNS disease in 1956 were tested to compare incidence of ECHO-9 neutralizing antibody with the 1957 group. The highest antibody titer in this group was 64 in both specimens on one patient. One patient had a titer of 16 in both specimens, and 5 patients had a titer of 4 in both specimens.

Discussion. Fig. 1 shows that 174 (37%) of patients had no antibody in either serum specimen at dilution 1:4.

The data presented in Table I show that ECHO-9 virus was isolated from only 2, or 2.4% of patients in whom antibody was not detected at serum dilution of 1:4. Virus isolation rates increased with higher titers. The highest isolation rate (77.2%) was in the group in whom a significant (16-fold) rise in antibody titer could be demonstrated.

Monkey kidney cells were used for all ECHO-9 virus isolation and identification. All attempts to isolate ECHO-9 virus in HeLa cells were unsuccessful.

Titrations on paired sera from 48 patients with CNS disease in 1956 indicated that ECHO-9 antibodies were rarely demonstrable in that year since 41 (85%) of paired sera tested had no demonstrable antibody at serum dilution of 1:4.

Significance of the presence of antibody in instances in which a significant rise could not be demonstrated is not clear. In Fig. 1, convalescent titers are clustered at 4, 16, and 64. Fewer titers of 256 were demonstrated, while only a few persons showed titers of 1024 and 4096. In 29 patients showing a significant rise in antibody titer, the peak of antibody titer attained was 16. We interpreted this to mean that titers as low as 4 and 16 may be taken as supportive evidence for diagnosis of ECHO-9 infection under epidemic conditions. The higher virus isolation rates obtained in association with these antibody levels (Table I) support this view.

Summary and conclusions. 1) Use of a HeLa cell system, using HeLa cell-adapted ECHO-9 virus, has definite value in studying neutralizing antibody titers during an ECHO-9 epidemic. 2) 228 cases of CNS disease reported to our Department during summer and fall of 1957 were listed as ECHO-9 virus infections on the basis of clinical, epidemiological and/or laboratory findings. Laboratory evidence for support of the diagnosis was available in 214 cases.

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