ADDENDUM. Results of experiments now in progress indicate that the method used to test the heat stability of scrapie virus is critical. For example, illness typical of scrapie has appeared in mice inoculated with 10^{-1} and 10^{-2} dilutions of an aliquot of scrapie virus suspension, prepared in 25% meat infusion broth and 5% normal rabbit serum in saline, that was passed serially through millipore membranes of 1200, 800, and 650 mμ average pore diameter and then exposed to 95.5°C for 30 minutes. Yet, mice inoculated with an unfiltered aliquot of this suspension that was similarly exposed to 95.5°C are still healthy, though disease has appeared in mice inoculated with dilutions through 10^{-5} of portions exposed to lower temperatures.

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Infection of Human Volunteers with a Reovirus of Bovine Origin. (28226)

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The reoviruses which have been recovered from lower animals are, with certain possible exceptions, indistinguishable by *in vitro* tests from those which have been recovered from man(1). Moreover, it has been demonstrated experimentally that strains of human origin are able to infect a variety of animals including cattle(2). This report describes the experimental infection of human volunteers with a strain of reovirus type 1 recovered from a naturally infected calf.

Materials and methods. Clinical Procedures. The 3 volunteers used in this study were adult male negro inmates from a federal correctional institution. Selection was based on willingness to participate and apparent good health. Prior to administration of the inoculum, each volunteer received a complete history and physical examination. Laboratory tests included urine analysis, routine blood count, chemical tests of liver function, chest x-ray, an electrocardiogram, and bacteriologic examination of nose, throat and rectum. These laboratory studies were repeated at weekly intervals after inoculation for 2 or 3 weeks.

The volunteers were isolated in a single room for approximately 2 weeks. Oral temperature, pulse and respiratory rates were determined 4 times daily. Once daily physical examination was performed by a team of 2 physicians and this, with a record of any complaints of illness, was recorded before leaving the room. No antibiotics or aspirin were given to the volunteers.

Inoculum. The inoculum was obtained from a bovine anal specimen containing reovirus type 1. The material used was the second passage in primary human embryonic kidney. Maintenance medium consisted of Eagle's basal medium(3) which contained 250 units of penicillin and 250 μg of streptomycin per ml. Following a single cycle of freezing and thawing, fluid from cultures was pooled, centrifuged at 3000 rpm for 15 minutes, filtered through a millipore filter of an 800 mμ pore diameter and frozen at –60°C until used. Aliquots of the inoculum were safety-tested as previously described(4).

The inoculum was instilled by pipette into the nasal cavity (0.5 ml into each nostril). Immediately following administration of the virus a sample of the inoculum was titered in primary human embryonic kidney cultures. The end-point which was determined after a 19-day incubation period by the method of Reed and Muench(5) was $10^{-8.5}$ $TCID_{50}$ per ml.

Virus isolation. Throat and anal specimens were collected daily for a 3-week period and kept frozen at −20°C until tested. Two-tenths ml of the undiluted specimens were inoculated into 2 primary rhesus monkey kidney cultures containing 1.0 ml of Eagle's basal medium. After 15 days incubation at 36.5°C in stationary racks, cultures were frozen and thawed one time and 0.5 ml of the undiluted harvest fluid was inoculated into each of two tubes and observed for 7 days. Virus isolations were identified in the hemagglutination-inhibition test against reovirus type 1, 2 and 3 rabbit antisera.

Hemagglutination-inhibition test. The hemagglutination-inhibition (HI) test was done as previously described(6). For determination of geometric mean titers, a less than 1:10 response was represented as 10×2^{-1}.

Results. The volunteers remained afebrile throughout the 3-week hospitalization period. Bowel movements were normal except in one volunteer who had a history of constipation. Two of the volunteers had emesis on day 9 and one of the volunteers experienced a slight nasal discharge days 3 to 8. Liver function tests, x-rays, total blood and differential white counts, erythrocyte sedimentation rates and urine analyses remained within normal limits.

Despite nasopharyngeal administration of the inoculum, virus was not recovered from the throat. In contrast, virus was readily isolated from rectal specimens in a frequency which indicated the occurrence of an enteric infection. The results of rectal virus shedding during the 3-week post-inoculation period are shown in Table I. Virologic evidence of infection was demonstrated in each of the 3 volunteers. Although the incidence of virus isolation was higher in the first week (90%), a significant number of specimens were virus-positive in the second week (43%). Several isolation attempts with the specimens collected during the third week were repeatedly negative. In most instances, a second tissue culture passage of the rectal specimens was required before characteristic cytopathic effects were evident.

TABLE I. Rectal Virus Isolation from Adult Volunteers Following Intranasal Inoculation with a Bovine Strain of Reovirus Type 1.

	Virus recovery (days after inoculation)					
	1 – 7		8 – 14		15 – 21*	
Volunteer	No.	%	No.	%	No.	%
T.M.	6/7†	86	2/7	29	0/4	0
C.D.	7/7	100	3/7	43	0/4	0
C.B.	6/7	86	4/7	57	0/4	0
Totals	19/21	90	9/21	43	0/12	0

* Specimens were not tested on days 18, 19 and 20.

† Numerator indicates No. of virus-positive specimens; denominator, No. of specimens tested.

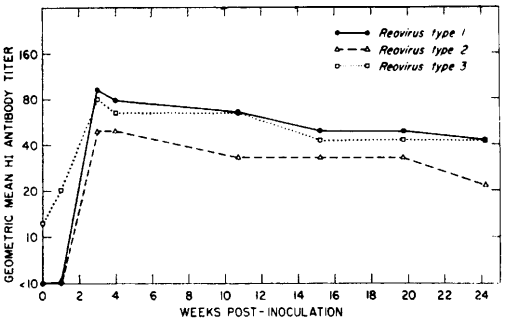

FIG. 1. Hemagglutination-inhibition responses of 3 volunteers inoculated with a bovine strain of reovirus type 1.

Prior to virus challenge, the volunteers had no measurable HI antibody to reovirus types 1 and 2; however, 2 men possessed pre-existing antibody titers of 1:20 to reovirus type 3. With the exception of no rise to reovirus type 2 in one volunteer, significant homotypic and heterotypic HI antibody rises were observed in the post-challenge sera (day 21). The homologous and heterologous HI antibody responses are represented in Fig. 1. Despite the presence of pre-existing HI antibody in 2 volunteers to reovirus type 3, the titers did not exceed those developed to the infecting type. The antibody titers to the 3 serotypes which tended to decrease after 3 weeks were still detectable at approximately 24 weeks after inoculation.

Discussion. These data indicate quite clearly that the inoculated human volunteers became infected with the reovirus type 1 strain of bovine origin. Moreover, the data obtained on virus excretion, serologic response, and the lack of significant clinical manifestations were remarkably similar to those obtained previously from volunteers experimentally infected with a reovirus type 1 strain of human origin(7).

Although it was known that reoviruses of human origin could infect cattle, this is the first demonstration that a reovirus of animal origin can infect man. It has not yet been demonstrated that reoviruses are naturally transmitted from lower animals to man, or vice versa; however, the sum of the presently available evidence suggests that such an event is not unlikely.

Summary. Inoculation of volunteers with a bovine strain of reovirus type 1 produced infection in adult human volunteers. Following intranasal administration of the agent, virus excretion, serologic response and lack of clinical manifestations were similar to those observed in volunteers experimentally infected with a reovirus type 1 strain of human origin.

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Virus Isolation Studies of Stool Specimens Obtained from Patients With Cholera.* (28227)

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The 1958-1959 cholera outbreak in Bangkok, Thailand, was studied by several groups of investigators from the U.S.A.(1,2,3,4) and an account of the viral isolation studies was given by Johnston *et al.*(4). Enteroviruses of recognized serotypes were occasionally isolated. No etiological relationship was found between the enteroviruses isolated and the clinical disease. We also had the opportunity of studying the problem of the association of enteroviruses with cholera(5) and our results are in full agreement with those of Johnston *et al.* Although 4 enteroviruses were isolated, we found no significant association between them and the clinical disease. Besides known enteroviruses, we have isolated a virus (Thai C-18) similar in behavior to the Coxsackie A group of viruses, but not identical with any of the recognized human enterovirus serotypes. The properties of Thai C-18 virus are given in this report.

Materials and general methods. Source of materials. 40 Fecal specimens were secured through the kind cooperation of Drs. K. Goodner, R. Freter and Harry Smythe, Jefferson Medical College, Philadelphia. We received also 17 additional stool specimens through the kindness of Col. Felsenfeld and his associates at SEATO cholera laboratory. The specimens obtained by the Jefferson team were collected at a time when the epidemic was on the wane, and some patients were no longer in the acute stage of the clinical disease. The SEATO laboratory specimens were secured some months later during an apparent recrudescence of the same outbreak. All specimens were frozen promptly and sent, packed in dry ice, to Pittsburgh. Paired sera for antibody studies were obtained from as many patients as possible. Bacteriological studies were carried out in Bangkok and the results made available to us. Although a clinical diagnosis of cholera was made in each instance, some of the cases were regarded as atypical. The *Vibrio comma* isolation rate was approximately 63%.

Source of viruses and immune sera. Poliomyelitis Type 1-3, Coxsackie A Type 7, 9, B Type 1-6, ECHO Type 1-27 viruses were

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