small animals. To make this technique usable for cardiac output determinations (Fick principle), the venous cannula is now permanently implanted in the right ventricle where mixing of the venous blood is adequate. Two hundred forty-one adult male rats and 61 adult ground squirrels of both sexes were cannulated in this way. Postoperative survival was 100%. The animals regained preoperative body weight after 3.9 days and continued afterwards to grow normally. Except for a few animals in which the cannula slipped from the ventricle into the atrium, the patency of the cannulas seemed to be limited only by the life span of the animal.

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Infection of Human Volunteers with Pett Virus.* (28438)

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Although the Pett virus had been recovered from children during an outbreak of respiratory disease(1,2), it could not be etiologically associated with this illness. Moreover, the relationship of this virus to disease in adults also remains undetermined. To gain data on clinical, virologic and serologic responses of Pett virus infection, adult volunteers were inoculated with virus strains immunologically related to the known serotype. This report describes the results of these experiments.

Materials and methods. Clinical procedures. The participants in this study were adult male inmates between the ages of 21 and 35 from various Federal Correctional Institutions. Selection was based on willingness to participate, apparent good health, and lack of detectable neutralizing antibody. Prior to inoculation each volunteer received a complete history and physical examination. Laboratory tests included urine analysis, complete blood count, chemical tests of liver and kidney function, X-ray of chest and bacteriologic examination of nose, throat and rectum. These laboratory studies were repeated at weekly intervals for 3 weeks.

The volunteers were isolated 3 per room. 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. The examining physicians were unaware of the type of inoculum administered the volunteers until termination of the experiment.

Virus inocula. The virus used to prepare inoculum No. 1 was recovered from an anal specimen of a young child residing in an institution in Washington, D. C.† The virus used to prepare inoculum No. 2 was recovered from a throat specimen of a volunteer in this study who had been given inoculum No. 1. Inocula were identified in neutralization tests with prototype hyperimmune rabbit serum. The material used was the second passage in primary human embryonic kidney. The maintenance medium, which consisted of 98 parts Eagle's basal medium(3) and 2 parts chicken serum (inactivated 56°C 30 minutes), also contained 250 units of penicillin and 250 μg of streptomycin per ml. The inocula were prepared and safety-tested as previously described(4).

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Two groups of 3 men each received inoculum No. 1. All men were given 1.0 ml of undiluted inoculum by pipette in the nasal cavity (0.5 ml per nostril); 3 of these subjects were given an additional 1.0 ml of undiluted inoculum in the oropharynx by a DeVilbiss No. 127 atomizer.

Another group of 3 volunteers was inoculated with 1.0 ml of undiluted inoculum No. 2 by pipette in the nasal cavity.

Immediately following administration of the virus a sample of the inoculum was titrated in primary human embryonic kidney cultures. After a 15-day incubation period, the titration endpoint was determined by the method of Reed and Muench(5). (Table I).

Virus isolation and serologic procedures. Throat and anal specimens were collected as noted in Table I. Specimens collected during the 4th and 5th week were obtained at the resident institutions. All specimens were stored at −20°C until tested. Virus isolation attempts were made immediately after specimens were collected in Hep. 2 cultures by a procedure previously described(6). The maintenance medium was the same as used for the preparation of virus inocula.

Virus typings and antibody determinations were done with WI-26 cultures by the neutralization test(1). Two isolates from each man were typed in tests with prototype hyperimmune rabbit serum. Neutralizing antibody titers of sera collected from volunteers prior to and 28 days after inoculation were determined against 50 $TCID_{50}$ of inoculum No. 2 and prototype viruses. The prototype virus used was the 25th Hela and KB tissue culture passage.

Results. All volunteers were afebrile and asymptomatic throughout the 3-week hospitalization period and there was no laboratory evidence of illness. The bacterial flora of the nose and throat remained unchanged after viral inoculation.

The virologic and serologic results in the 9 men given Pett virus are presented in Table I. Infection was demonstrated in all men by virus isolation from either throat or rectal specimens. Virus was recovered at least once from throat specimens of 7 men and from anal specimens of 8 men. None of the specimens from either sampling site was positive earlier than 48 hours after inoculation. With one exception, the last rectal specimen of each volunteer was negative.

No appreciable differences in virus shedding patterns were noted among the men given inoculum No. 1. Except in one instance, virus was never recovered from throat specimens beyond the first week whereas rectal excretion of virus was evident throughout the 5-week period.

The relative frequencies of positive throat cultures among the men given inoculum No. 2 resembled those among men given inoculum No. 1. However, the relative frequencies of positive rectal specimens among men infected with inoculum No. 1 were significantly higher than those seen among men receiving inoculum No. 2 (Wilcoxon 2-sample test, $U=1$, $p=.05$).

High levels of neutralizing antibody did not develop in volunteers following infection with either inoculum. Serologic evidence of infection was demonstrable in all men by neutralization tests with inoculum No. 2. In contrast, neutralizing antibody was detectable in post-inoculation sera of only 3 men by neutralization tests with prototype virus.

In view of the results obtained in neutralization tests with human sera, reciprocal tests were done using rabbit sera prepared against both strains. The results of these tests are shown in Table II. Although an immunologic relationship between the 2 strains was established in tests with prototype rabbit antiserum, antigenic differences between these strains were detected in tests with inoculum No. 2 rabbit antiserum.

Discussion. The results of the volunteer experiments described here indicate that adult males became infected following inoculation with strains of Pett virus. However, the data on virus excretion were not entirely consistent with findings observed in children (2). In both studies virus was more easily recovered from anal specimens whereas virus excretion from the throat was not detectable in children. The latter observation is not necessarily at variance with the data reported here, since the children were experiencing a simultaneous myxovirus infection and the

TABLE I. Virus Excretion and Antibody Responses of Volunteers Following Inoculation with Pett Virus.

Volunteer and inoculum No.	Administered dose ($TCID_{50}$)	Virus isolation (weeks post-inoculation) — Throat						Rectal						Neutralizing antibody titer (*vs.* 50 $TCID_{50}$)	
		1	2	3	4	5	Totals	1	2	3	4	5	Totals	Inoculum #2	Prototype
NI. 1	$1 \times 10^{4.5}$	3/7*	0/2	0/3	0/3	N.T.†	3/15	1/7	1/2	0/3	0/3	N.T.	2/15	<2‡ 16	<2 <2
BO. 1	$1 \times 10^{4.5}$	0/7	0/2	0/3	0/3	0/2	0/17	0/7	0/2	3/3	2/2	1/2	6/16	<2 8	<2 <2
ME. 1	$1 \times 10^{4.5}$	1/7	0/2	0/3	0/3	0/2	1/17	4/7	2/2	3/3	1/2	0/2	10/16	<2 8	<2 8
LE. 1	$2 \times 10^{4.5}$	2/7	0/3	0/3	0/3	0/2	2/18	3/7	3/3	3/3	2/3	0/2	11/18	<2 16	<2 <2
NE. 1	$2 \times 10^{4.5}$	2/7	1/3	0/3	0/3	0/2	3/18	5/7	3/3	2/3	3/3	1/2	14/18	<2 12	<2 <2
BA. 1	$2 \times 10^{4.5}$	2/7	0/3	0/3	0/3	0/2	2/18	5/7	3/3	2/3	1/3	0/2	11/18	<2 8	<2 6
	Totals	10/42	1/15	0/18	0/18	0/10		17/42	12/15	13/18	9/16	2/10		6/6	2/6
BR. 2	$1 \times 10^{5.5}$	1/7	4/7	0/7	0/6	N.T.	5/27	0/7	0/7	0/7	0/6	0/2	0/29	<2 16	<2 <2
MC. 2	$1 \times 10^{5.5}$	2/7	2/7	0/7	0/6	N.T.	4/27	0/7	0/7	1/7	0/7	1/2	2/30	<2 12	<2 6
SM. 2	$1 \times 10^{5.5}$	0/7	0/7	0/7	0/6	N.T.	0/27	3/7	5/7	0/7	0/7	N.T.	8/28	<2 8	<2 <2
	Totals	3/21	6/21	0/21	0/18			3/21	5/21	1/21	0/20	1/4		3/3	1/3

* Numerator indicates number of positive specimens and denominator indicates number of specimens tested.
† N.T. = not tested.
‡ Reciprocal of dilution; first line indicates titer of pre-inoculation serum and second line indicates titer of post-inoculation serum.

TABLE II. Results of Neutralization Tests with Pett Prototype and Inoculum Strain.

Virus strain	Virus $TCID_{50}$	Rabbit antiserum	
		Prototype	Inoculum #2
Prototype	50	80*	<10
	16	80	<10†
Inoculum #2	50	80	40
	16	160	80

* Reciprocal of neutralizing antibody titer.
† Delay in appearance of cytopathic effects at 1:10 dilution.

specific onset of virus infection was unknown.

Inoculation of volunteers with material prepared from a throat isolate resulted in reduced rectal shedding when compared to that from men given inoculum prepared from a rectal isolate. Since it was not due to a lower dose of virus, it suggested that a qualitative change may have occurred in the composition of the virus population. One obvious possibility was that propagation in the throat led to a selection of viral particles less well adapted to growth in the gastrointestinal tract.

The failure to cause illness in these volunteers is difficult to interpret. Whether this indicates a low degree of pathogenicity of the Pett virus for man cannot yet be determined. It is possible that non-immunologic host factors, lack of strain virulence or experimental procedure may have been responsible for these results.

The findings that human neutralizing responses were less frequently demonstrated with prototype virus and the lack of complete reciprocal cross neutralization between the prototype and inoculum strains in tests with rabbit sera require further definition. Whether these differences in antigenic composition reflected stable strain characteristics or were due to emergence of a serologic variant as a consequence of repeated tissue culture passage of the prototype strain cannot be determined from the present data. It is possible that by use of the plaque neutralization procedure an immunologic relationship may be established.

Summary. Inoculation of 9 adult volunteers with a strain of Pett virus produced virus infection but no clinical disease. Although virus was recovered from throat specimens, isolations were more frequently obtained from anal specimens. Serologic rises developed in all men tested against a strain of virus isolated from a volunteer in this study whereas only 3 of 9 exhibited significant rises when tested against the prototype virus. The relative frequencies of positive rectal specimens among men infected with an inoculum prepared from a rectal isolate were significantly higher than those which occurred among men given an inoculum prepared from a throat isolate.

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