QUANTITATIVE STUDIES OF THE VIRUS-HOST RELATIONSHIP IN CHIMPANZEES AFTER INAPPARENT INFECTION WITH COXSACKIE VIRUSES*

I. The Virus Carrier State and the Development of Neutralizing Antibodies

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(Received for publication, October 16, 1952)

The experiments to be described deal with a quantitative study of the asymptomatic infection in chimpanzees following the oral administration of different Coxsackie (or C) viruses. These agents lend themselves particularly well to such an investigation because of the relative ease of detecting them in the body. Hence it has been possible to follow the rise and fall of virus in the throat, stools, and blood, and in addition, to measure the immune response resulting from the first experience with the virus and from later homotypic and heterotypic challenges. In this work, the indication of infection has been the production of a virus carrier state, and the measure of immunity has hinged on whether or not the chimpanzee again became a virus carrier when oral challenge of virus was given. Furthermore, the development of neutralizing antibodies has been correlated both with the primary infection and with subsequent experiences with the virus. Studies on the complement-fixing antibodies and their fluctuating titers are reported separately (1).

Because both Coxsackie and poliomyelitis viruses frequent the alimentary tract in chimpanzees (as in man), it was felt that the quantitative studies which could be carried out on inapparent Coxsackie infection might serve as a model for inapparent infection with poliomyelitis virus in which the central nervous system is not involved. In view of the frequency with which both viruses can be isolated from the same patient (2, 3), some experiments were carried out to determine the effect of superimposing an infection with one of these viruses upon an infection with the other.

Materials and Methods

Chimpanzees.—Eight animals, about 2 to 3 years old, were the subjects of this study. They were bled for serum upon arrival in the laboratory and at subsequent intervals as

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

indicated. During the entire experiment, each animal was housed in an individual cage with a false bottom to facilitate the collection of feces, and the daily cleaning and hosing of the cage. Two to four chimpanzees were kept in a room, the general conditions being similar to those used in earlier reports from this laboratory (4, 5).

TABLE I

Viral Strains Used in Study

Strain	Abbreviation	Type	Origin
		I. Coxsackie Viruses	
Conn.-5	Conn.	Conn.-5	Stools of patient with aseptic meningitis, Conn., 1948 (7).
Ohio-1	Ohio	Ohio-1	Stools of patient with non-paralytic poliomyelitis, Ohio, 1947 (8).
Nancy	Nancy	Nancy	Stools of patient with minor, febrile illness, Conn., 1949 (6).
Texas-1	Texas	Texas-1	Flies collected during poliomyelitis epidemic, Texas, 1948 (6).
High Point	Hi. Pt.	Texas-1	Sewage collected during poliomyelitis epidemic, North Carolina, 1948 (6).
Easton-2	E-2	Type 1	Stools of patient with paralytic poliomyelitis, Pennsylvania, 1949 (3).
Easton-10	E-10	Easton-10	Stools of patient with paralytic poliomyelitis, Pennsylvania, 1949 (3).
Easton-14	E-14	Easton-14	Stools of patient with paralytic poliomyelitis, Pennsylvania, 1949 (3).
Fleetwood	D-2	Type 2	Stools of patient with poliomyelitis, Delaware, 1947 (9).
Olson	D-3	Type 3	Stools of patient with poliomyelitis, New York State, 1948 (9).
Alaska-5	Alaska	Alaska-5	Stools of Eskimo patient with minor febrile illness, Northern Alaska, 1950 (10).
		II. Poliomyelitis Viruses	
Y-SK	Y-SK	Type 2 (Lansing)	Stools of patient with non-paralytic poliomyelitis, Conn., 1937 (11).
Texas	Texas	Type 1 (Brunhilde)	Flies collected during poliomyelitis epidemic, Texas, 1948 (12).
Leon	Leon	Type 3 (Leon)	Brain stem and spinal cord of fatal case of poliomyelitis, Los Angeles, 1937 (13).

Virus.—Ten Coxsackie strains and three poliomyelitis strains were used. Each viral strain represented an antigenically distinct type, this being true for both the C viruses and the poliomyelitis viruses (see Table I).

The C viruses were in the form of 20 per cent suspensions of torsos of infant mice which had become paralyzed following intraperitoneal inoculation. The three poliomyelitis strains were in the form of 20 per cent suspensions of spinal cords of monkeys which had exhibited signs of poliomyelitis following intracerebral inoculation. In the case of Y-SK, half of the suspension was composed of cords from *rhesus* and the other half of those from *cynomolgus*

monkeys. The Texas (Brunhilde) strain was used as infected *cynomolgus cords*, and the Leon strain as *rhesus* cords.

Administration of Virus to Chimpanzees.—5 ml. of the indicated strain was mixed with 50 ml. of milk (or a similar quantity of crushed banana), the mixture was placed in the food bowl, and offered as the first meal of the day. It was taken readily by the chimpanzee. In one experiment with chimpanzees 21 to 24, a mixture of seven C viruses was inoculated, each animal receiving 1 ml. of the mixture intramuscularly and 1 ml. subcutaneously.

Testing Chimpanzee Blood, Throat, and Stools for the Presence of C Viruses.—Blood, withdrawn daily, if possible, from the animals during the 1st week after the administration of virus, was inoculated intraperitoneally or subcutaneously into 16 baby mice (2 litters), each mouse receiving 0.02 ml. Usually the blood was frozen whole and centrifuged after thawing. In some experiments, plasma, washed white cells, and washed red cells were each tested separately.

Throat swabs were obtained, in several instances almost daily, for 2 weeks after virus was fed. Two cotton swabs were used to collect virus from the throat of each chimpanzee. These cotton swabs were then extracted with 0.01 M phosphate buffer, pH 8, in the following manner: The cotton was removed from the applicator sticks and placed in the barrel of a 1 ml. syringe. 1 ml. of the buffer was placed in a small, sterile test tube and the buffer drawn into and forced out from the syringe about 8 to 10 times. The concentration of this extract was considered as being undiluted (10^0) throat swab extract. 2000 units of penicillin and 10 mg. of streptomycin contained in 0.1 ml. were added to the extract before it was inoculated into 16 infant mice.

Stools were collected daily from each animal, from a pan underneath the individual cages of the chimpanzees. Each day this pan was removed, the feces collected, and the pan thoroughly cleaned before being replaced. Stools thus collected were either prepared for inoculation on the same day or frozen at −20°C. for later use. In most instances the stools were tested individually; in a few instances, however, the collections from several days were pooled before testing. Specimens were prepared for inoculation in the following manner: A 33 per cent suspension of stools was made with water; this was then spun in the cold at 18,000 R.P.M. for 20 minutes in the "multispeed attachment" of the International PR-1 refrigerated centrifuge. 2000 units of penicillin and 10 mg. of streptomycin were added to each milliliter of the supernate before inoculation.

Criteria for the presence of a C virus in the blood, throat, or stools of chimpanzees were: (*a*) weakness and paralysis in one or more of the extremities of infant mice after an incubation period of 3 to 14 days; (*b*) fatal termination of the disease in mice within 1 to 2 days from onset; (*c*) reproduction of the disease in other infant mice on passage of bacteriologically sterile, infectious tissue; and (*d*) the demonstration of characteristic myositis in the sick mice when examined histologically. Criteria (*c*) and (*d*) were used at infrequent intervals, for example when there was some question as to specificity of the signs in the mice and when the isolation was made from specimens collected at crucial periods, as from blood within a few days after feeding.

Positive samples were often rethawed and titrated using serial tenfold dilutions.

Testing of Chimpanzee Stools for the Presence of Poliomyelitis Virus.—Stools were collected under the same conditions as they were for C virus. A 33 per cent suspension was made with water and treated as described above. The supernatant fluid, obtained after spinning at 18,000 R.P.M. (and containing the virus if present) was then spun in the Spinco ultracentrifuge, model L, at 40,000 R.P.M. for 60 minutes (average gravitational force 105,000 *g*). The gelatinous pellets obtained as sediment were reconstituted in one-tenth the volume. This concentrate was then clarified by spinning at 18,000 R.P.M. for 20 minutes. The same concentrations of antibiotic as indicated above were added and 1 ml. of the suspension then inoculated intracerebrally into a *rhesus* monkey.

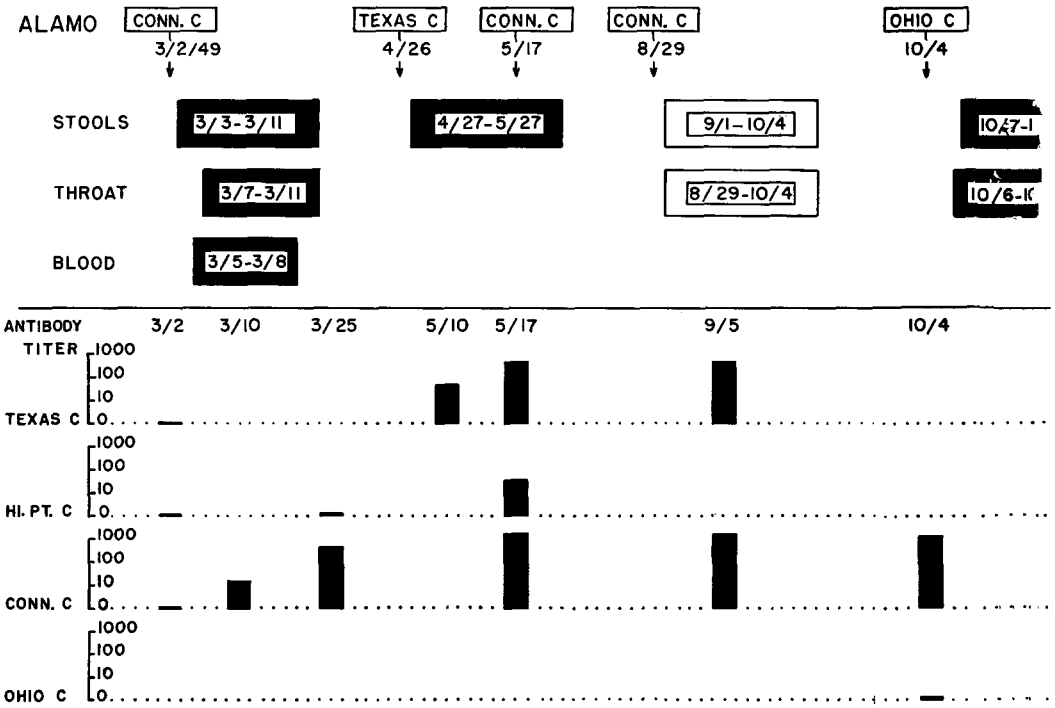


FIG. 1. Isolation of virus from the stools, throat, and blood of chimpanzee Alamo, correlated with th

The arrows indicate the date of feeding the virus designated in the rectangular blocks at the top of the c of virus for the dates shown. The dates on which serum samples were taken are shown above the antib Hi. Pt. C, the High Point strain, and Ohio C, the Ohio-1 strain.

In each case, the period during which virus was detected was followed by a period during which i negative tests are omitted from them. Only those negative results obtained after the chimpanzees had be ever, the positive stool tests, which were limited to a few days after the virus feeding, are omitted fror not of virus multiplication in the host.

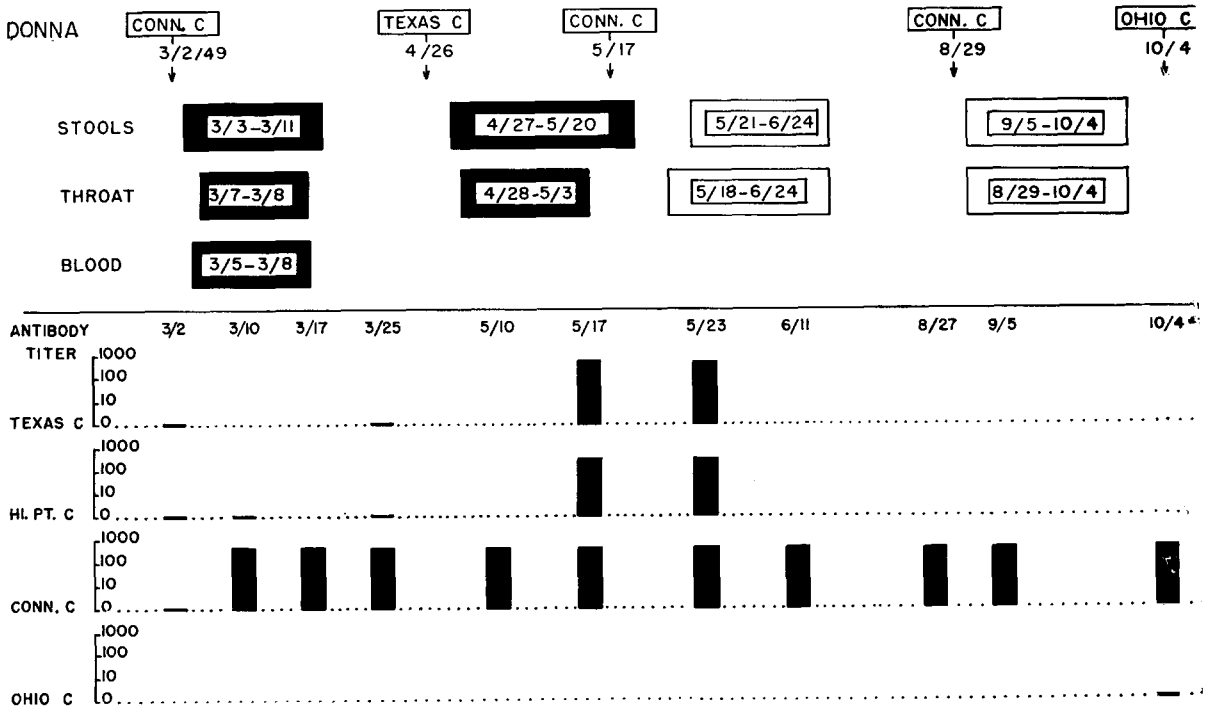
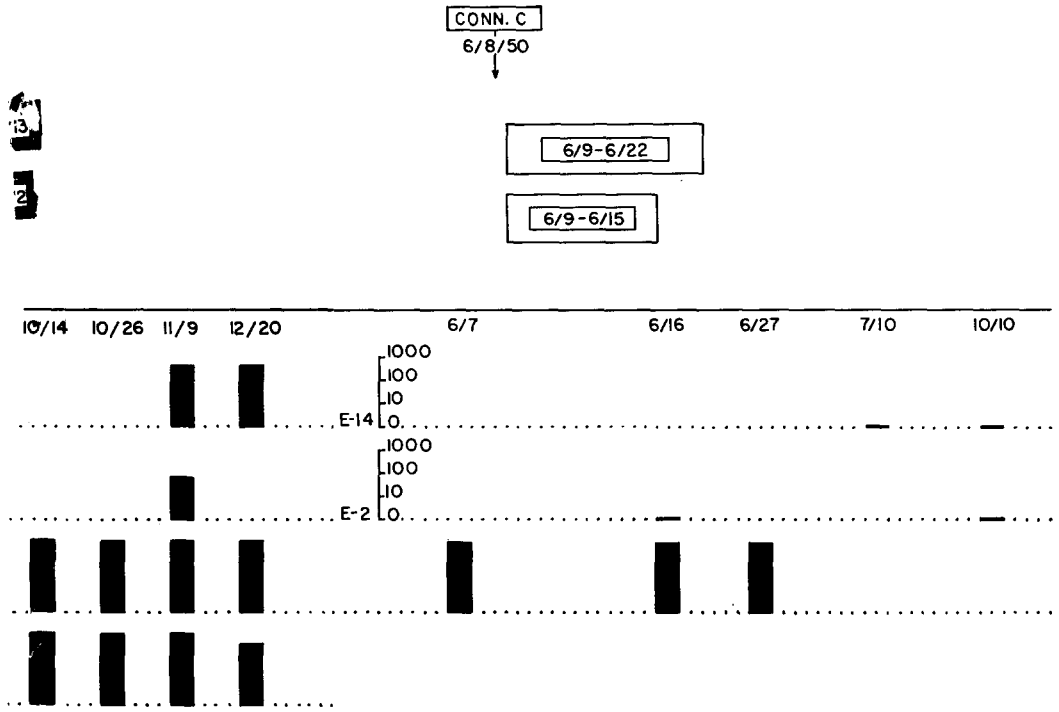
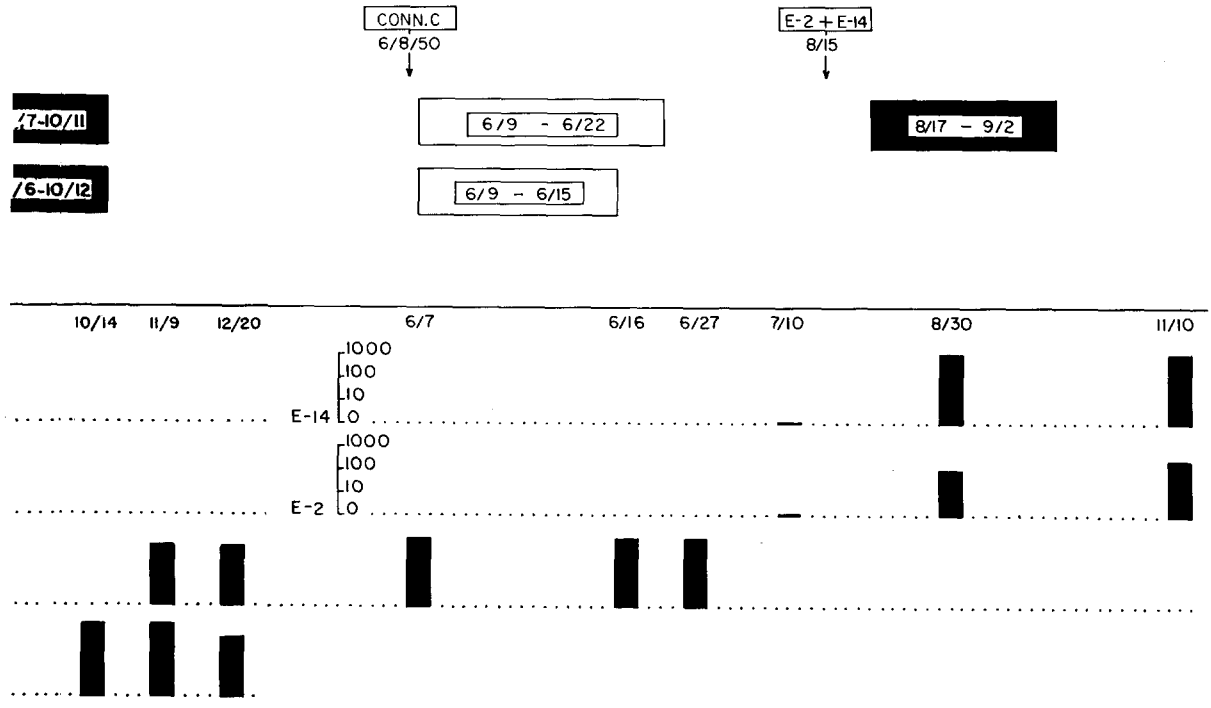


FIG. 2. Isolation of virus from the stools, throat, and blood of chimpanzee Donn



development of neutralizing antibodies.
t. Blocks with heavy margins indicate the presence of virus, and those with light margins the absence
ly titers. Conn. C, indicates the Connecticut-5 strain of Coxsackie virus, Texas C, the Texas-1 strain,

irus was found (as illustrated in Table III). In order not to encumber Figs. 1 to 4, the recordings of these
hallenged with virus to which they had been previously exposed are entered. In the latter instances, how-
the charts. Such transient excretion is indicative of passive transfer of virus through the alimentary tract,



correlated with the development of neutralizing antibodies. Legends as in Fig. 1.

Criteria for the presence of poliomyelitis virus in the stools of the chimpanzees were the appearance in the monkey of the experimental disease with characteristic histological lesions in the upper and lower levels of the spinal cord. A test was considered negative if the animal survived without signs of poliomyelitis for 28 days following inoculation, and when sacrificed, it failed to exhibit histological lesions in the medulla and spinal cord.

Neutralizing Antibody Tests.—Coxsackie Viruses: The tests were based on previously described experiments (6) and were carried out in the following manner: Varying dilutions of sera (undiluted to 1:500) were distributed in 0.2 ml. amounts to small test tubes. An equal volume of virus (200 ID_{50} doses 0.02 ml.) was then mixed with each serum. The mixtures were allowed to stand at room temperature for 1 hour, at which time the tubes were transferred to an ice water bath. Eight or sixteen infant mice were then each inoculated subcutaneously with 0.02 ml. of a virus-serum mixture. Mice were observed daily for 2 weeks for paralysis or death. Those mice which succumbed within 2 days of the inoculation and those which disappeared (eaten) were not considered in the calculations. Antibody titers were calculated by the method of Reed and Muench, the titer being considered that dilution of serum at which 50 per cent of the mice were protected.

The same procedure, as outlined above, was used in an effort to detect neutralizing antibodies in stools and extracts of the throat swabs of the chimpanzees. In all instances, a 33 per cent suspension of stools and undiluted throat swab extracts were employed, mixing them respectively with 100 ID_{50} doses of virus.

Poliomyelitis Virus: Since the Y-SK strain of poliomyelitis virus is a Lansing type, and since the latter, in our hands, has a shorter and more uniform incubation period in mice, neutralizing tests to determine the presence of poliomyelitis virus antibodies following the administration of Y-SK, were carried out with Lansing strain. The neutralization test with Lansing was carried out in the same fashion as in the case of C viruses. In this instance, however, 3 week old mice were employed and 0.03 ml. of the virus-serum mixture was inoculated intracerebrally. Mice were observed daily for 28 days for paralysis or death, and those mice which died within 2 days after inoculation were not considered in the final tabulations. The neutralizing antibody titer was calculated as indicated above.

Neutralization tests were carried out in monkeys in the following manner: 0.2 ml. of undiluted sera was mixed with 0.2 ml. of Leon virus (about 100 PD_{50} doses). The mixture was allowed to stand at room temperature for 1 hour. The entire amount was then inoculated intracerebrally into one *rhesus* monkey.

RESULTS

Experiments with Chimpanzees Alamo, Donna, Becky, and Beti.[1]—The data obtained on these four animals are summarized in Figs. 1 to 4, in which the appearance of virus in the blood, throat, and stool following the feeding of virus has been correlated with the appearance of neutralizing antibodies. The times at which virus was fed to the animals are listed in Table II, and the results with one of the chimpanzees, Becky, are shown in detail in Table III. The protocol of the neutralization tests carried out with the sera of chimpanzee Beti against one virus is shown in Table IV. The results for neutralizing antibodies to other viruses in the serum samples of this chimpanzee (and on the other animals) are not shown in detail but are summarized in the accompanying figures (Figs. 1 to 4).

[1] We wish to acknowledge the assistance of Dr. Ernest W. Shaw in carrying out certain phases of these experiments in 1949.

TABLE II

*Coxsackie and Poliomyelitis Viruses Fed to Chimpanzees Alamo, Donna, Becky, and Beti**

Chimpanzee	Date	Strain of virus fed	Titer ID_{50}
Alamo	Mar. 2, 1949	Conn.-5	
	Apr. 26	Texas-1	$10^{-7.4}$
	May 17	Conn.-5	$10^{-6.1}$
	26	Y-SK polio‡	Not done
	Aug. 29	Conn.-5	$10^{-6.6}$
	Oct. 4	Ohio-1	$10^{-5.7}$
	June 8, 1950	Conn.-5	$10^{-6.7}$
	Aug. 15	Leon polio	Not done
Donna	Mar. 2, 1949	Conn.-5	
	Apr. 26	Texas-1	$10^{-7.4}$
	May 17	Conn.-5	$10^{-6.1}$
	26	Y-SK polio	Not done
	Aug. 29	Conn.-5	$10^{-6.6}$
	Oct. 4	Ohio-1	$10^{-5.7}$
	June 8, 1950	Conn.-5	$10^{-6.7}$
	Aug. 15	Leon polio	Not done
	15–16	Easton-2§	10^{-6}
	15–16	Easton-14§	$10^{-6.5}$
Becky	Apr. 26, 1949	Texas-1	$10^{-7.4}$
	May 17	"	$10^{-7.8}$
	26	Texas 1948 polio‡	Not done
	Aug. 29	Texas-1	$10^{-8.0}$
	Oct. 4	Ohio-1	$10^{-5.7}$
	June 8, 1950	Texas-1	$10^{-7.1}$
	Aug. 15	Leon polio	Not done
Beti	May 17, 1949	Texas-1	$10^{-7.4}$
	26	Texas 1948 polio‡	
	Aug. 29	Texas-1	$10^{-8.0}$
	Oct. 4	Ohio-1	$10^{-5.7}$
	June 8, 1950	Texas-1	$10^{-7.1}$
	Aug. 15	Leon polio	Not done
	15–16	Easton-2§	10^{-6}
	15–16	Easton-14§	$10^{-6.5}$

* All animals received about 5 ml. of 20 per cent suspension of infected tissue at one feeding, except when indicated. Aliquots of the samples fed to the chimpanzees were titrated in mice on the day of feeding.

‡ Half the amount was fed in the morning and half in the afternoon.

§ The Easton-2 and Easton-14 strains were fed together, for 2 consecutive days.

The data for only one chimpanzee, Donna, will be considered in detail, for it can be seen that similar patterns were obtained with all four animals.

TABLE III

Isolation of Coxsackie Virus from Stools and Throat of Chimpanzee Becky

Virus fed	Date	Results with infant mice inoculated with stools*	Titer of virus in stools Neg. log of LD_{50}	Results with infant mice inoculated with throat swabbings*	Titer of virus in throat swabbings Neg. log of LD_{50}
	1949				
Texas C	Apr. 26				
	27	10/10 (0)	4.0	8/8 (4)	2.2
	28	6/6 (4)	4.0	6/6 (4)	3.1
	29			7/7 (0)	4.0
	30	6/6 (0)	3.1	8/8 (2)	4.0
	May 2			8/8 (6)	2.3
	3	4/4 (1)	3.5	8/8 (7)	1.5
	4	10/10 (1)	3.1	0/14	0
	5	14/14 (7)	3.5	0/20	0
	7	15/15 (11)	2.2	0/18	0
	10	10/10 (1)	1.9		
	12	11/11 (1)	2.5	0/7	0
	14	18/18 (8)	2.5		
	15	15/15 (11)	2.9		
Texas C	May 17				
	18	7/7 (3)	2.8	0/7	0
	19	18/18 (6)	3.6	0/8	0
	20	14/14 (1)	3.1	0/9	0
	21	15/15 (10)	2.1		
	23	19/19 (12)	1.0	0/9	0
	24	13/13 (6)	1.5		
	25			0/8	0
Texas polio	26				
	27	10/15 (9)	0.5		
	28			0/15	0
	30	7/14 (5)	0.5	0/16	0
	31	2/18 (2)	0.5	0/14	0
	June 1	0/18	0		
	2	0/20	0		
	3	0/12	0		
	6–10	0/16	0		
	20–24	0/17	0		
	27–29	0/16	0		
Texas C	Aug. 29				
	31			0/18	0
	Sept. 1	18/18 (7)	1.0		
	2			0/18	0
	5	15/18 (13)	1.0		
	6			0/18	0
	8	3/18 (0)	0		
	10	0/14	0	0/9	0

TABLE III—*Concluded*

Virus fed	Date	Results with infant mice inoculated with stools*	Titer of virus in stools Neg. log of LD_{50}	Results with infant mice inoculated with throat swabbings*	Titer of virus in throat swabbings Neg. log of LD_{50}
	1949				
Ohio C	Oct. 4				
	5			1/7 (1)	0 5
	6			6/6 (0)	2.8
	7	5/5 (5)	3.5	5/5 (2)	3.3
	8	9/9 (1)	3.2		
	10			8/9 (8)	3.6
	11	9/9 (6)	1.0		
	12			4/7 (1)	2.4
	13	6/7 (3)	2.2		
	14			2/7 (0)	0
	17	0/9	0		
	Nov. 9	0/10	0	0/9	0
	1950				
Texas C	June 8				
	9–10	1/15 (0)	0	0/20	0
	12–13	14/14 (9)	+	0/25	0
	14–15	0/16	0	0/22	0
	16–17	0/8	0		
	19–20	0/15			
	21–22	0/16			
(Easton-2 and-14)‡	Aug. 21–26	7/7 (3)	2.8		
	Aug. 28–Sept. 2	7/8 (6)	1.9		
	Sept. 4–9	6/8 (5)	1.0		
	12–16	8/8 (5)	1.7		

+ Indicates virus was detected but was not titrated.

* Results in these columns are with the original extracts. If virus was found, the extract was then titered for its virus content. 10/15 (9) indicated that of 15 satisfactory mice in the test group, 10 died, 9 with observable paralysis before death.

‡ The roommate of Becky was fed these two strains on August 15 and the infection spread to Becky.

Donna was fed the Conn. strain on March 2, 1949. Virus was recovered in the stools daily for the next 9 days and then excretion of virus stopped. In view of the fact that virus may be recovered for a few days following its feeding, even in an immune animal, virus excretion in the feces must be detected beyond the first few days after its ingestion to indicate that multiplication has taken place. For the first 4 days after the feeding, virus could not be detected in the throat; it was found on the 5th and 6th days but not thereafter. As virus has never been recovered by us from the throat of an immune animal, its detection there at any time is considered to be evidence of infection (virus multiplication). Virus was not detected in the blood for the first 2 days after feeding; viremia was present on the 3rd,

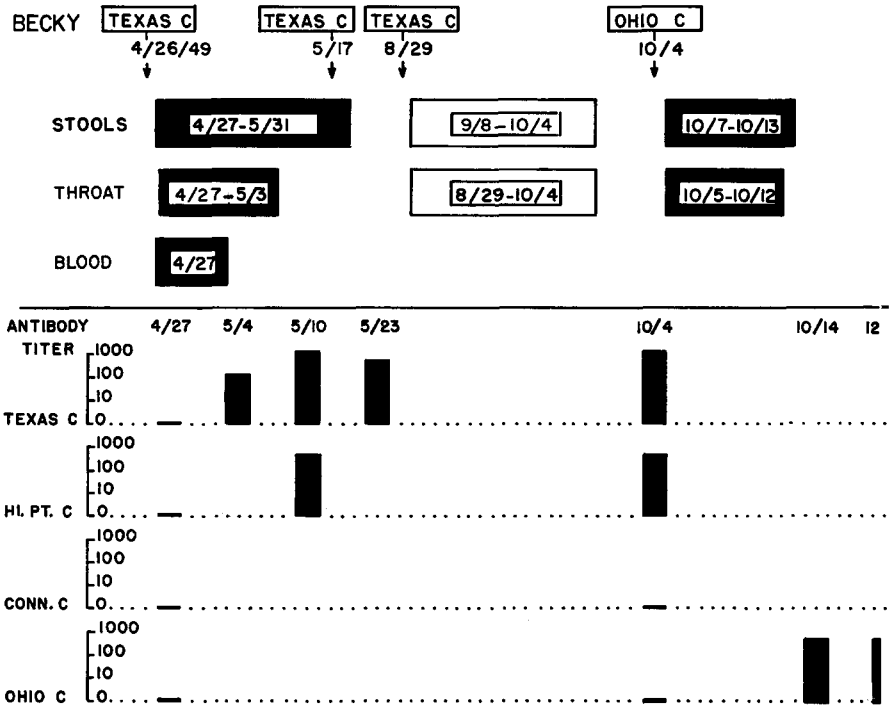


FIG. 3. Isolation of virus from the stools, throat, and blood of chimpanzee Becl

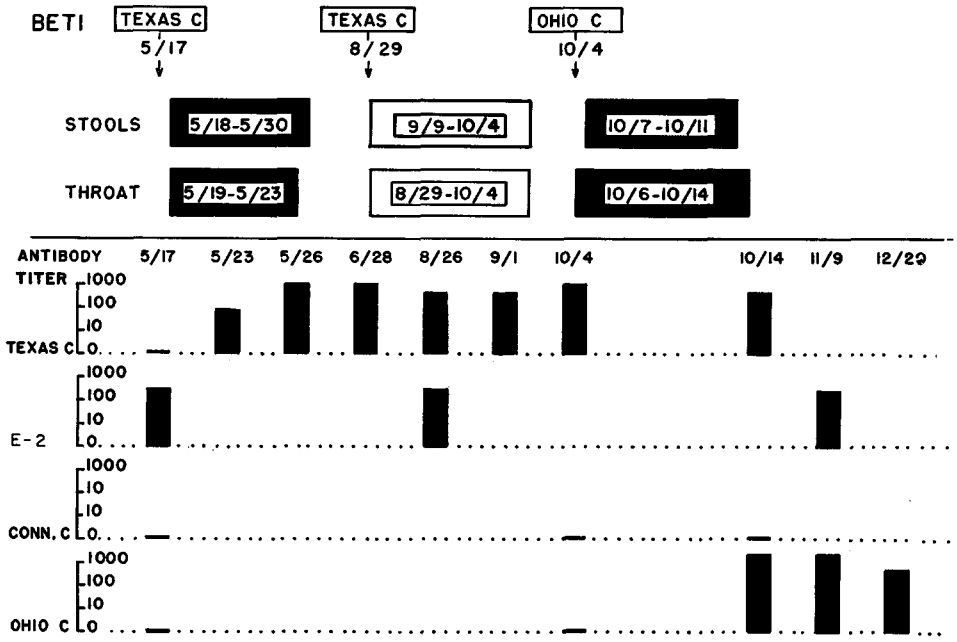
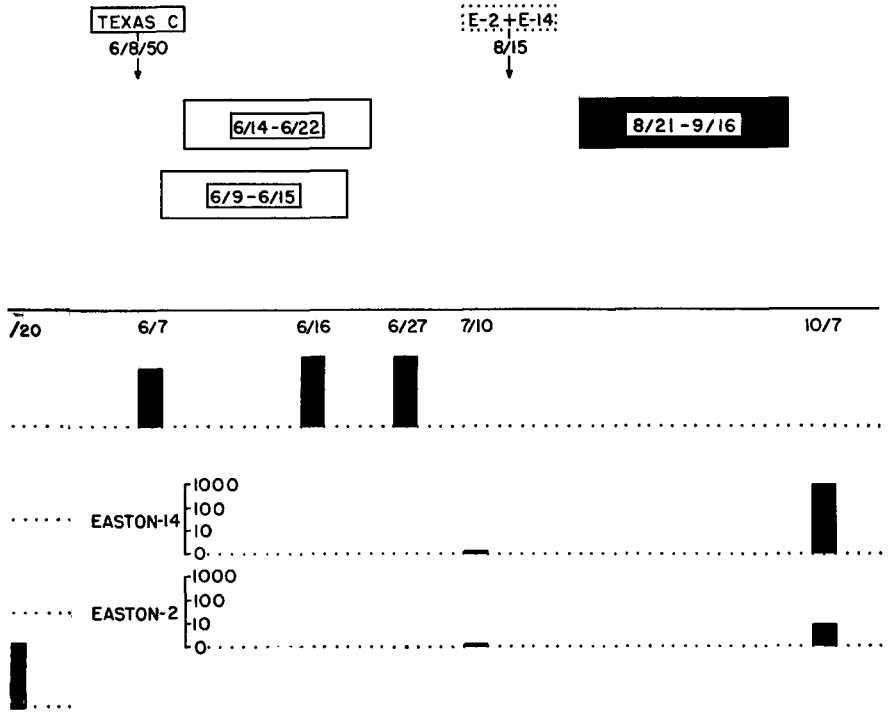
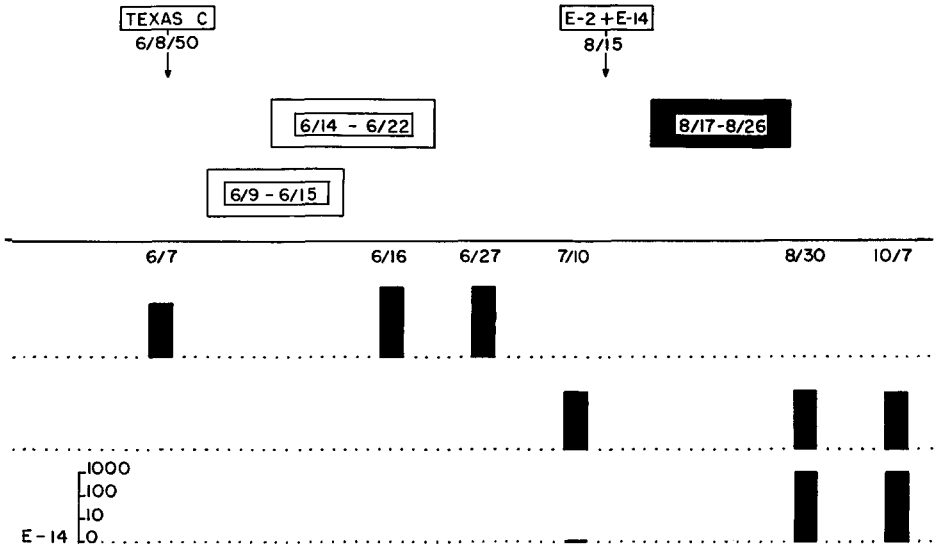


FIG. 4. Isolation of virus from the stools, throat, and blood of chimpanzee Beti,



·y, correlated with the development of neutralizing antibodies. Legends as in Fig. 1.



correlated with the development of neutralizing antibodies. Legends as in Fig. 1.

4th, 5th, and 6th days but not thereafter. The finding of virus in the blood at any time is regarded as evidence of infection.

Neutralizing antibodies to both the Texas and Conn. types were absent on the day prior to oral administration of virus. However, on the 8th day they were present in high titer, 1:320, for the Conn. virus which had been fed, and they were maintained at this level for the following 2 years. Antibodies to the Texas type did not appear until the animal had been exposed to this strain some time later.

Chimpanzee Donna was fed the Texas virus on April 26, 1949, following which virus was detected in the stools for the next 3 weeks. Virus was not detected in the throat on the day following its administration but was found there from the 2nd through the 6th day. The

TABLE IV

Typical Protocol of Neuralization Tests with Sera of Chimpanzee Beti

Serum	Dilutions of serum				Dilution at which 50 per cent of mice were protected (neg. logarithm)
	1:2	1:10	1:100	1:1000	
May 17, 1949	6/6(2)*	6/6(3)			0
23	0/9	0/8	3/7(3)	9/9(5)	2.1
26	0/9	0/9	0/9	4/9(2)	3.1
June 2			1/9(0)	1/6(1)	3.0+
28			0/9	4/9(1)	3.1
Aug. 26			0/9	3/3(3)	2.5
Sept. 1			0/9	8/8(7)	2.5
Oct. 4			0/7	4/9(4)	3.1
14			0/9	9/9(7)	2.5
		10^{-6}	10^{-7}	10^{-8}	ID_{50}
Virus control (Texas-1)		8/8	8/8	0/8	$10^{-7.5}$

The serum was diluted in sterile, distilled water, the dilution indicated being that after the addition of virus. Virus was diluted in 10 per cent normal horse serum inactivated at 56°C. for 2 hours; it was added in a concentration of 10^{-5} so that the final concentration was $10^{-5.3}$. The mixtures of serum and virus were incubated at room temperature for 1 hour and then inoculated subcutaneously into infant mice.

* 6/6(2) indicates that of 6 mice inoculated, 6 succumbed to disease, 2 with observable paralysis before death.

blood was not examined for virus. 3 weeks after virus feeding, a serum-neutralizing antibody titer of 1:320 was found against the Texas virus. A similar antibody titer was found for the High Point strain which is antigenically related to the Texas type (6).

On May 17, the Conn. strain was again fed to Donna as a homologous challenge. It was not realized at this time that the animal was still excreting Texas virus. Therefore, the virus which appeared in the stools on May 18, 19, and 20, may well have been a mixture of the Texas virus which was gradually disappearing at this time plus the Conn. virus which had been fed on the 17th. At any rate, the Conn. virus did not set up an additional infection because virus could not be detected in the stools between May 21 and June 24, nor did it appear in the throat following the Conn. feeding.

A second homotypic challenge was carried out with the Conn. virus on August 29. Virus was detected in only one sample, that collected on September 1, but not thereafter, nor was

it present in the throat after this feeding. The antibody levels remained unchanged. Thus Donna had been rendered immune by its first experience with the Conn. virus. The virus which appeared transiently in the feces following challenge is interpreted as passive transfer of the agent through the alimentary tract.

On October 4, 1949, chimpanzee Donna was challenged with the heterotypic Ohio strain. Following its oral administration, virus was detected in the feces between the 3rd and 7th day after the feeding; it was not present in the throat on the 1st day after feeding, but persisted from the 2nd through the 8th day. Thus, in a primary infection with the Ohio type of C virus, virus persisted in the throat as long as in the stools, while with the other C virus types involved in this study, the duration of excretion in the stools was extended well beyond that in the throat. Antibodies to the Ohio type were absent before the feeding of this strain but were present in high titer, 1:1000, 10 days after the feeding. As with antibodies to the previously fed C viruses, the Ohio antibody was maintained during the entire period of observation.

Several months later, in June, 1950, Donna was again challenged orally with the Conn. virus. Virus was not detected in the throat or the stools during the next 2 week period, and the antibody level to the Conn. virus remained unchanged.

On August 15, 1950, Donna was fed a mixture of Easton-2 and Easton-14 strains, each belonging to a distinct antigenic type different from the 3 types to which she had previously been exposed. Virus persisted in the stools for the next 2 week period. (Examinations of throat swabs and blood at this time were not carried out.) Antibodies to both strains were absent prior to the feeding but 2 weeks later were present to both the Easton-2 and the Easton-14 types.

In general, the pattern of the host-virus relationship just described was encountered in each of the other three chimpanzees. After virus is administered *via* the oral route to the animals, a viremia may be detected for a few days. In addition, virus can be recovered from the throat commencing within 2 to 3 days following ingestion of virus; the agent persists in the throat for about a week. Within 2 to 4 days virus is also excreted with the stools, this virus probably being the major portion of that fed the animal; following this initial virus excretion, virus can be found in the stools for 2 to 3 weeks, or even longer, presumably newly formed virus. Neutralizing antibodies to the virus given orally make their appearance within 2 weeks and maintain the same titer for at least 1 to 2 years.

The response of the chimpanzees is such that if several weeks or months after the initial oral administration of virus, the homologous strain is again administered, virus can again be detected in the feces; this time, however, for a period of only 3 to 4 days during which it is presumed that the virus is passively transported through the alimentary canal. Under such conditions, a true carrier state does not develop, and the isolation of virus from the blood or throat is not possible. If now, however, the chimpanzee is given a heterotypic instead of a homotypic virus, the animal responds as though it had encountered virus for the first time, with the development of a true carrier state and of new antibody to the heterotypic virus. A chimpanzee which has thus been exposed to two different C viruses is now immune to these two viruses; yet, if a third

and antigenically distinct C virus is fed to the animal, it will again react to the agent like a new animal. As noted above, only with animals given the Ohio type could virus be recovered from the throat as long as in the stools.

The amount of virus necessary to initiate infection deserves comment. In general, large doses were used in these experiments. However, only two of the animals were fed the Easton-2 and Easton-14 mixture on August 15, 1950. Two other animals in the room, Becky and Alamo, were not fed virus at this time, yet one of them, Becky, became a carrier of the Easton-2 and Easton-14 viruses from August 21 through September 16, and also developed antibodies to both agents. Alamo did not become infected: virus was not detected in its feces nor were antibodies present at this time or subsequently. The fact that Becky excreted virus as readily as did the two animals which were fed the large doses would indicate that a very small amount of virus is enough to initiate infection by the oral route. This is borne out in subsequent experiments with chimpanzees 21 to 24, and will be referred to again later.

Quantitative Determinations of Virus in the Throat and in the Feces of Infected Animals.—To determine the fate of the virus fed and to evaluate the contribution of newly developed virus to the total recovered from an animal, virus titrations were carried out on those stool samples which proved positive in the qualitative test. The data are summarized in Figs. 5 to 8. Again, the data for only one animal will be presented in detail, for the other chimpanzees show similar patterns.

Donna (see Fig. 6) was first fed Conn. virus on March 2, 1949; and as only qualitative determinations were made after that feeding, the results were not entered in the chart. However, following the first oral administration of Texas virus on day 55, quantitative determinations were carried out. Relatively large amounts of virus were detected in the stools immediately following the virus feeding, the feces having a titer of $10^{-2.7}$. This subsequently dropped to 10^{-2} over the next 10 day period and then maintained itself at about 10^{-1} through the 25th day, after which it was no longer detectable. It was surprising to find that the virus titer of the throat swab extract was of the same order of magnitude, also being about 10^{-3}, for the short period of time it was detected in the throat.

A difference in the virus excretion patterns could be readily ascertained following feeding of homotypic or heterotypic challenge viruses. Homotypic challenge was followed by passive transfer of virus through the alimentary tract, with low titer being found in the stools for a relatively short period. Heterotypic challenge was followed by active infection as manifested by the events detailed above. For example, in the period following day 180, when the Conn. strain was refed, virus appeared in the stools in low titer and only for a few days and could not be found in the throat. The Ohio type was fed on day 215, and the equal persistence of virus both in the throat and feces has been noted above. In this connection, it is noteworthy that Ohio virus in the throat actually had a higher titer than in the feces. Following feeding of the heterotypic Easton-2 and Easton-14 strains, Donna maintained a titer in the feces of 10^{-2} for a period of 10 days, and then virus persisted for another week or so at low titer—furnishing ample evidence that it was actually multiplying during this period.

The results with chimpanzee Becky show, in the period following the first Texas virus feeding, the usual appearance of fecal virus and the gradual de-

crease in titer of this virus. Later, however, there was an increase of virus concentration in the stools, starting on day 15 and continuing through day 25. The brief appearance of virus in the stools following its feeding to an immune animal, as shown when Texas virus was fed on day 125 and again on day 405 to this animal, is remarkably different from the response seen when virus is first fed. Again with this animal we note that following the oral administration of the Ohio type, virus is present in as great a concentration in the throat as it is in the feces and persists in both places for about the same length of time. It was Becky who became "spontaneously" infected in the laboratory when Easton-2 plus Easton-14 strains were fed to her roommates. It will be noted that the virus titer found in her feces following the exposure of her roommates was of the same order of magnitude as that present in Beti and Donna, the animals which were fed the two agents.

The results with chimpanzee Alamo following its first exposure to the Texas virus are noteworthy. The titrations showed that there was a marked excretion of virus for a few days after feeding, following which it could not be detected for a period of 5 days. Virus then reappeared in the stools at low titer and was maintained there for a period of about 20 days. The second phase of virus excretion may be interpreted as a result of multiplication of the agent in the host.

Search for Virus-Neutralizing Substances in Stools and in the Throat of Chimpanzees Following Infection.—In these experiments consideration was given to the strain which produced the infection, in that type-specific antibodies were searched for in the stool and throat samples collected from animals at a time when high antibody levels against the particular strain involved were present. For example, on May 30, 1949, Alamo had a serum-neutralizing antibody titer of greater than 1:1000 for Conn. virus, and yet its stools collected on that day had no inhibitory effect on the virus, when fecal extracts were used as "serum" in neutralization tests. The same was true when antibodies were looked for in the throat, as shown in Table V. Examinations of 12 specimens of feces and 22 specimens from the throat, taken soon or late after infection with three different types of C virus, were made. There was no indication of any neutralizing substances in any of these materials. Similarly negative results had previously been obtained in a search for neutralizing substances against poliomyelitis virus in the stools of chimpanzees following subclinical infection (5).

The Possible Influence of Coxsackie Infection on Subsequent Infection with Poliomyelitis Virus.—Numerous examples are now on record of patients excreting simultaneously both a C virus and a poliomyelitis virus. The possibility has been discussed of the potential influence of one infection upon the other, particularly whether the superimposition of a C virus infection on a poliomyelitis infection might turn a non-paralytic into a paralytic disease (3). To study this problem adequately in the laboratory would mean the use of a

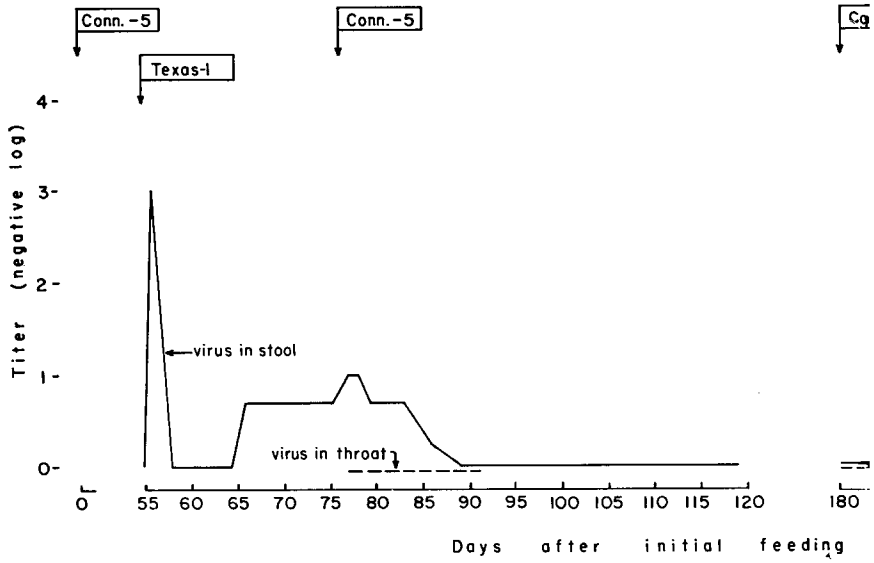


FIG. 5. ID_{50} titers of virus present in stools and throat in relation to exposure to virus
In Figs. 5 to 8 the arrows indicate the dates the virus enclosed in the blocks was fed.
line of virus in the throat. The dot-dash line represents the assumed titer in those instances

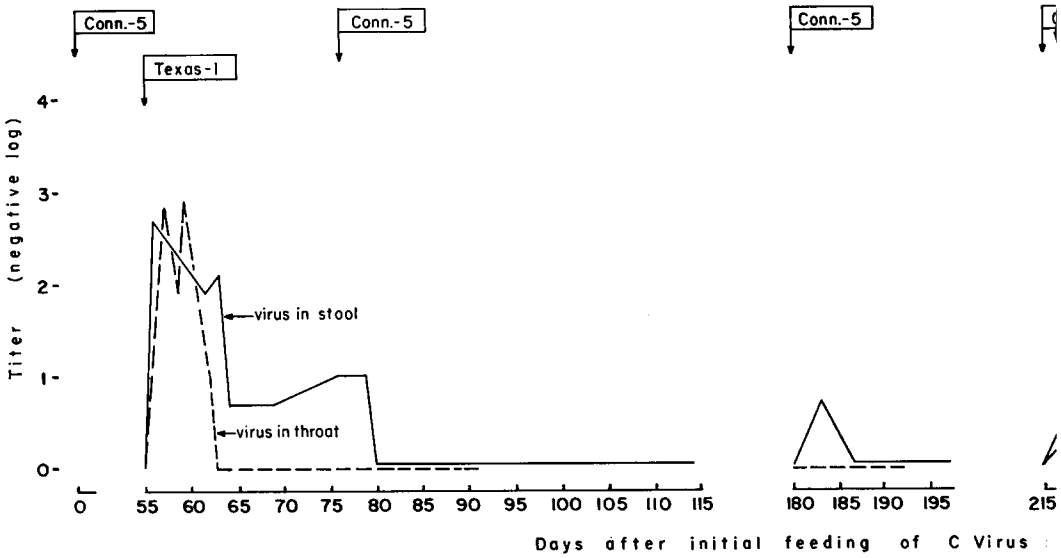
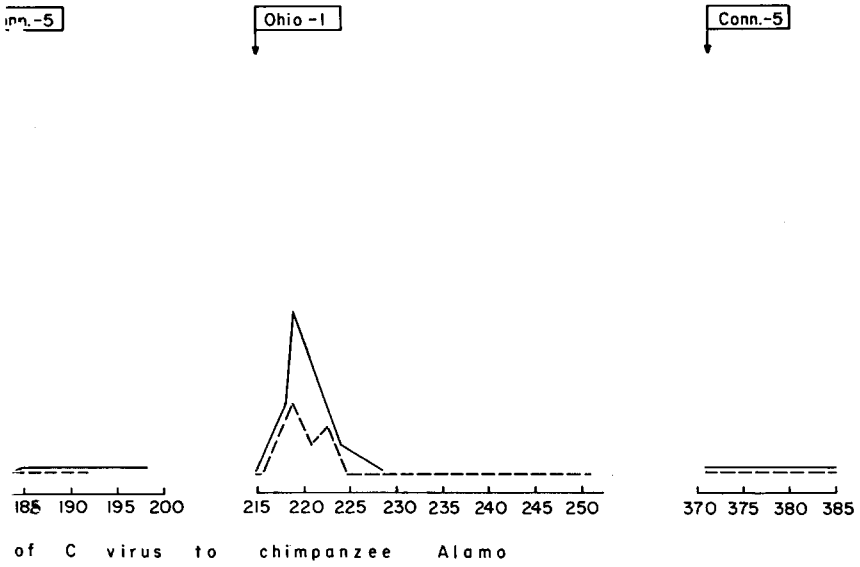
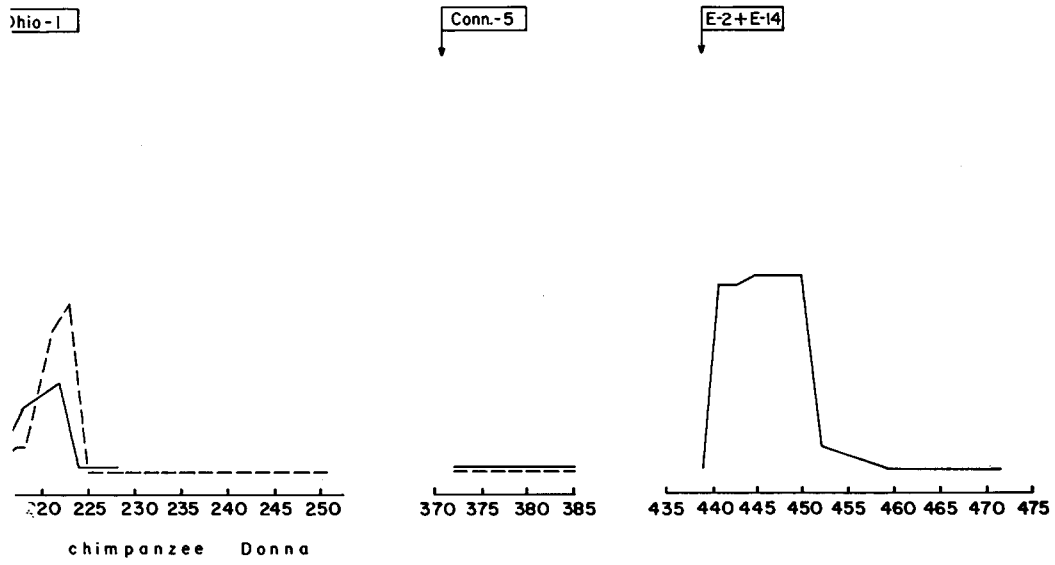


FIG. 6. ID_{50} titers of virus present in stools and throat in relatio



for chimpanzee Alamo.
The solid line represents the presence and amount of virus in the stools, and the broken
in which virus was found but not titrated.



to exposure to virus for chimpanzee Donna. Legend as in Fig. 5.

TABLE V

Search for Virus-Neutralizing Substances in Stools and Throat

Chimpanzee and date	Results of tests for neutralizing substances in		
	Serum (50 per cent protection end-point neg. logarithm)	Stools	Throat
Alamo			
May 30, 1949	3.0 + C	−C	
Sept. 10	3.0 + C		−C
16	3.0 + C	−C	
Oct. 14	3.0 + O		−O
Donna			
May 5	2.5 C		−C
12	2.5 C		−C
18	2.5 C		−C
20	2.5 C		−C
28	2.5 C		−C
30	2.5 C	−C	−C
Aug. 31	2.5 C		
Sept. 10	2.5 C		−C
16	2.5 C	−C	
Becky			
May 7	2.0 + T		−T
12	3.0 + T		−T
18	3.0 T		−T
23	2.5 T		−T
28	2.5 T		−T
June 1	2.5 T	−T	
27–29	2.5 T	−T	−T
Sept. 16	2.5 T	−T	
Oct. 14	2.5 O		−O
17	2.5 O	−O	
Nov. 9	3.0 + O		−O
Beti			
May 21	2.3 T		−T
31	3.0 T	−T	
June 27–29	3.0 T	−T	
Sept. 2	2.5 T		−T
10	2.5 T		−T
16	2.5 T	−T	
Oct. 14	3.0 + O		−O
17	3.0 + O	−O	
Nov. 9	3.0 + O		−O
Total.....	33/33	0/12	0/22

Negative sign indicates absence of neutralizing substances.
C = Conn.-5; O = Ohio-1; T = Texas-1.
For strain of virus and date fed see Table II.

large number of chimpanzees, for the natural response of these animals to poliomyelitis virus is that of inapparent infection. It would be desirable to know the incidence of paralytic disease in chimpanzees receiving varying doses of poliomyelitis viruses and of C viruses, the two kinds of agents being administered under different time relationships.

Preliminary attempts were made with the 4 animals studied here. This was done in two ways: Donna and Alamo, before exposure to poliomyelitis virus, had had a prior infection with Conn. C virus. On April 26, 1949, they were infected with Texas C virus, and both animals became carriers of the Texas C virus and developed antibodies to it. On May 17, they were given orally an additional dose of Conn. C virus which, it is probable, went passively through the alimentary tract and was eliminated completely within the next 3 or 4 days. On May 26, Donna and Alamo were fed Y-SK poliomyelitis virus, and both animals developed inapparent poliomyelitis infection. With Donna, poliomyelitis virus was first detected in the feces on June 14, and excretion persisted through June 29. With Alamo, poliomyelitis virus was not detected in the stools until June 27 and 29, 4 weeks after its feeding. Neither animal had Lansing antibodies prior to the feeding of Y-SK virus but both developed specific antibodies within 2 weeks after the ingestion of poliomyelitis virus *before* virus appeared in the stools. Neither animal showed any evidence of illness during this period, nor did either of them develop fever. These results are shown in Figs. 9 and 10.

A second experiment was carried out with two animals which were fed first the Texas C virus, of which they became carriers, and subsequently the Texas strain of poliomyelitis virus (Brunhilde type), as illustrated in Figs. 11 and 12. The reason for making this selection was that both agents had been isolated from the same batch of flies collected during an epidemic (12). One animal, Becky, failed to become an intestinal carrier of poliomyelitis virus whereas the second animal, Beti, did. The latter excreted virus during a 3 week period, beginning about 10 days after feeding. The reason for the difference in response of the two animals is obvious, since this was Beti's first experience with this type of poliomyelitis virus, while for Becky it was an homologous challenge since this animal had previously been infected with a Type 1 (Brunhilde) poliomyelitis virus. Becky's antibodies against the Lansing strain did not change as a result of this infection. Previous studies have shown that once a chimpanzee has been infected with one type of poliomyelitis virus, it is uncommon for reinfection to occur with the same type (5, 14).[2]

In a second series, four new chimpanzees (Nos. 21 to 24) were employed in the same kind of experiment with Leon poliomyelitis virus and Easton-2 and Easton-14 C viruses. All four animals were fed poliomyelitis virus but only two received the C viruses. In this experiment, as in the experiments with the Y-SK and Texas strains of poliomyelitis virus, there was no

[2] In a continuation of the study of the relationship of Coxsackie and poliomyelitis viruses after ingestion in chimpanzees, a third antigenic type of poliomyelitis virus, (Leon, Type 3), was fed to the four animals, and C viruses to two. None of the animals became carriers of the Type 3 poliomyelitis virus. This was confirmed by the absence of neutralizing antibodies to this virus in serum samples taken about 2 months after feeding. The effect of one virus upon the course of development of the other could not be determined by this experiment since neither the control animals (fed Leon alone) nor the experimental animals (fed Coxsackie and Leon) became infected with Leon poliomyelitis virus. There have been few attempts to infect with Type 3 virus chimpanzees previously infected with Types 1 and 2 poliomyelitis viruses (5, 14), and it is for this reason that these results are recorded here.

influence of one virus upon the other. None of the animals had fever or clinical illness. The stools were not tested for the presence of Leon virus; however, two of the animals, one given both viruses and one given just poliomyelitis virus, developed neutralizing antibodies to Leon virus. The two animals fed the C viruses became intestinal carriers of these viruses and developed type-specific neutralizing antibodies.

Thus, under the limited conditions of these experiments, there was no demonstrable effect of a C virus infection on subsequent infection with poliomyelitis virus. The eight animals responded to exposure to poliomyelitis virus in precisely the same fashion as chimpanzees which had never been exposed to a C virus. This does not mean to say that we have thoroughly explored this question; in particular, other intervals of time of virus feeding and different virus doses should be investigated.

Experiments with Chimpanzees 21 to 24.—These animals were exposed to C viruses on three different occasions. They had arrived in the laboratory in August, 1950, and prior to these Coxsackie experiments, they had been used in work with poliomyelitis virus (Y-SK and Brunhilde) and also with the Egyptian strain of West Nile virus (15), as indicated in Table VII.

In the first C virus experiment, an attempt was made to compare the titer of Texas virus in chimpanzees by the oral route with its titer in mice. It was not known at the time of the feeding that these animals had both neutralizing antibodies (and complement-fixing ones in low titer) to the Texas C virus, presumably as a result of exposure before their arrival in New Haven. This experiment must therefore be considered as a titration of virus in supposedly experienced hosts. Chimpanzee 21 was fed 5 ml. of $10^{-3.5}$ concentration of infected mouse torso; chimpanzee 22, $10^{-6.0}$; chimpanzee 23, $10^{-3.5}$; and chimpanzee 24, $10^{-1.0}$, in the order listed. The recovery of virus is presented in Table VI and the antibody determinations in Table VII.

Virus was not recovered in the blood or throat of any of the four animals, as had been found previously with immune chimpanzees. However, it was detected in the stools of three animals: chimpanzee 24, for 16 days; chimpanzee 23, for 4 days; and chimpanzee 22, for 5 days. When one considers that chimpanzees 23 and 22 were fed $10^{-3.5}$ and $10^{-6.0}$ concentrations of virus-infected tissue, respectively, the titers of virus in stool must mean that it had multiplied in these animals, as well as in chimpanzee 24. Chimpanzee 23 actually showed daily tenfold increases in viral concentration in the feces between the 1st and 3rd days. Chimpanzee 22 excreted stools containing a higher concentration of virus than the food which was fed to it. The rapid termination of excretion in chimpanzees 22 and 23 may be a reflection of the partially immune status of these animals, in that previous work with poliomyelitis virus in chimpanzees also pointed to a briefer period of virus excretion in immune animals challenged with the homologous strain (5, 14). Temperatures were taken daily for 5 weeks after the virus feeding; and there was no indication of fever in any of the animals.

The virus carrier state in these animals was paralleled by the change in neutralizing antibody level. All the sera of a single chimpanzee listed in Table VII, were run in one test, using serial tenfold dilutions of serum with 16 mice per dilution. The results show that the antibody titers were constant between August 15, 1950, and February 5, 1951, the day when the chimpanzees were fed the Texas virus. Following this, the three animals which became virus carriers, chimpanzees 22, 23, and 24, showed significant rises (tenfold) in their antibody levels while that for chimpanzee 21 remained constant.

Thus, relatively small amounts of virus, only 60 times that necessary to infect 50 per cent of newborn mice by the subcutaneous route, may be sufficient to reinfect an "immune" chimpanzee by the oral route. It should be recalled that these primates had become naturally immune and their exposure was undoubtedly different from that of the chimpanzees in the first part of this paper. The latter animals were given massive doses of virus at the time of their first experience. Furthermore, in the second series of chimpanzees, the type of infection was different from that seen in a new animal; no virus was found in the blood or throat and the excretion in 2 of the 3 animals was short lived. The infection was sufficient, however, to result in a marked boost in the serum antibody titer.

On March 14, 1951, chimpanzees 23 and 24 were fed 5 ml. of Easton-2 virus plus 5 ml. of Easton-14; a 20 per cent suspension of infected mouse tissue was fed for each strain, Easton-2 having a titer of $10^{-7.0}$ and Easton-14, $10^{-7.5}$. As summarized in Tables VIII and IX, inapparent infection resulted in these animals; virus was found in the blood of one on the 2nd day, in the throat of both animals from the 2nd through the 7th and 8th day, and in the stools of both from the 2nd through the 16th and 23rd day. These two chimpanzees, 23 and 24, responded with the appearance of antibodies to both viral types.

Two animals, chimpanzees 21 and 22, were not fed these strains and were studied as controls; they were housed in the same room with the two animals which were fed virus. The results are also detailed in Tables VIII and IX. Chimpanzee 22 was found to be excreting virus 2 days after its roommates had been fed and continued doing so for at least the next 5 days; the virus titer of the stools varied between $10^{-1.8}$ and $10^{-3.0}$, which is as high a concentration as is found in animals fed large amounts of virus. Virus also appeared in the throat on the 6th and 7th day, with the titer on the 6th day being relatively high, $10^{-3.1}$. The blood was not examined for virus. The evidence for infection was completed by the antibody study, since chimpanzee 22 developed antibodies in high titer to both Easton-2 and Easton-14 viruses. Chimpanzee 21 never was found to have virus in the throat or feces. However, its antibody picture is suggestive of subclinical infection with one of the agents, since antibodies appeared (although at low titer) to Easton-2 but not to Easton-14. None of the 4 animals developed fever during the 4 weeks following the virus feeding.

The next experiment with these animals was concerned with two points—

(*a*) A comparison of the type of infection following *intramuscular* and subcutaneous inoculation of virus with that following its oral administration.

(*b*) Possible *interference* of one C virus type with multiplication of another.

On April 10, 1951, a mixture of 7 virus types was inoculated into each of four chimpanzees. Each animal received intramuscularly and subcutaneously, a 20 per cent virus suspension (infected mouse torso); for each route, the inoculum was 1 ml. and consisted of equal parts of suspensions of the following strains, each a distinct antigenic type: Nancy, Conn.-5, Ohio-1, Alaska-5, Easton-10, Fleetwood (Type 2), and Olson (Type 3). Even though these agents were inoculated directly into the muscle, none of the chimpanzees became ill, and none developed fever during the next 3 week period.

The data on virus recovery are presented in Table X. Virus was sought in the blood of 3 animals and found in all on the 1st and 3rd days. It was found in the throat of all 4 animals, reaching titers of 10^{-2} to 10^{-3}. In one chimpanzee, virus was not recovered in the pharynx on

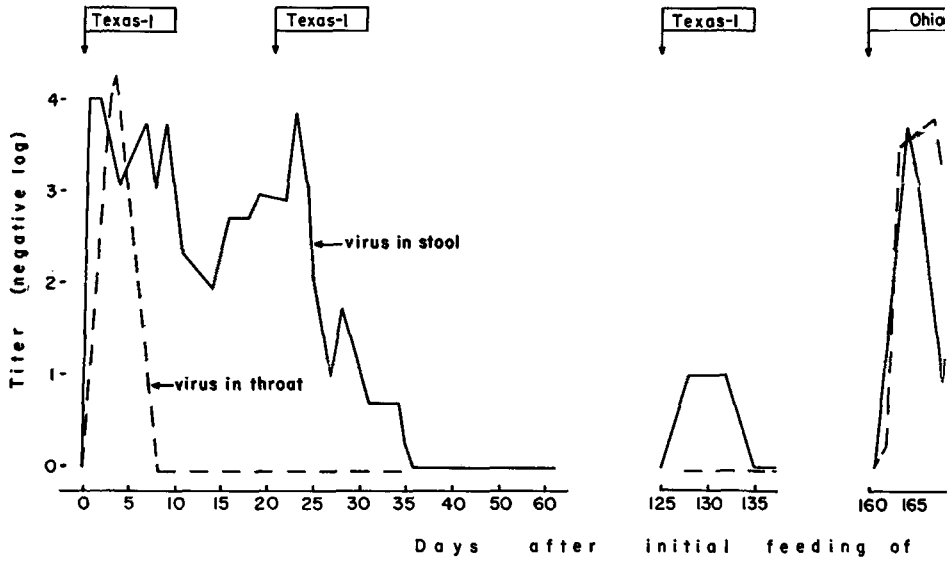


FIG. 7. ID_{50} titers of virus present in stools and throat in relat

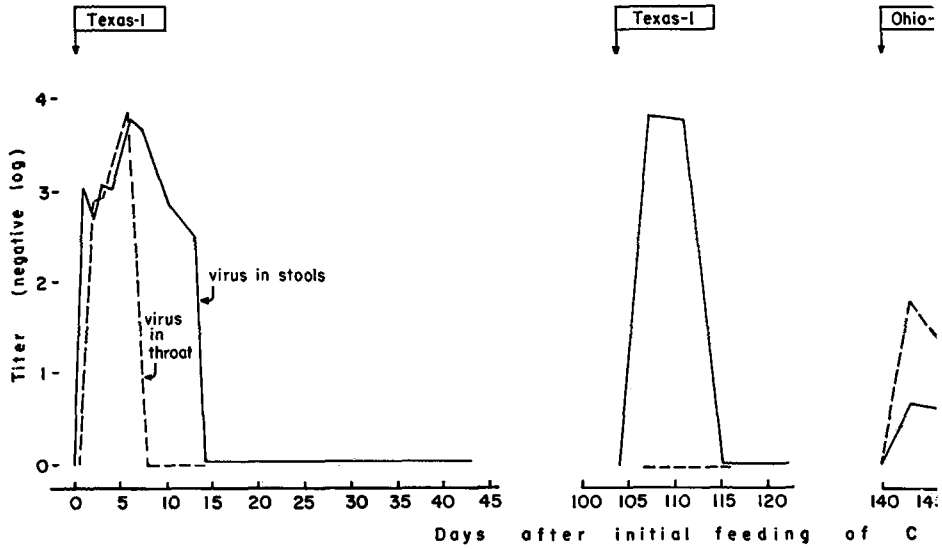
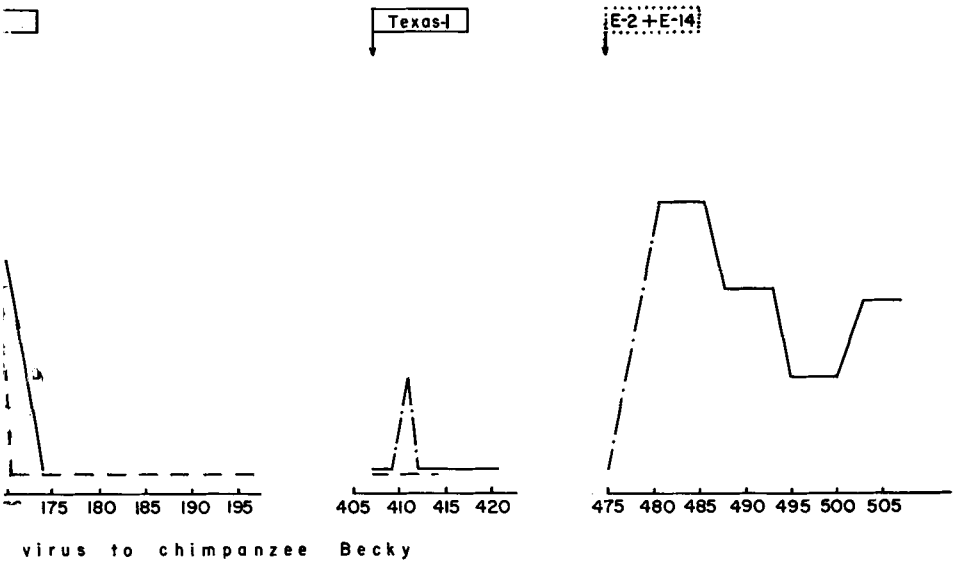
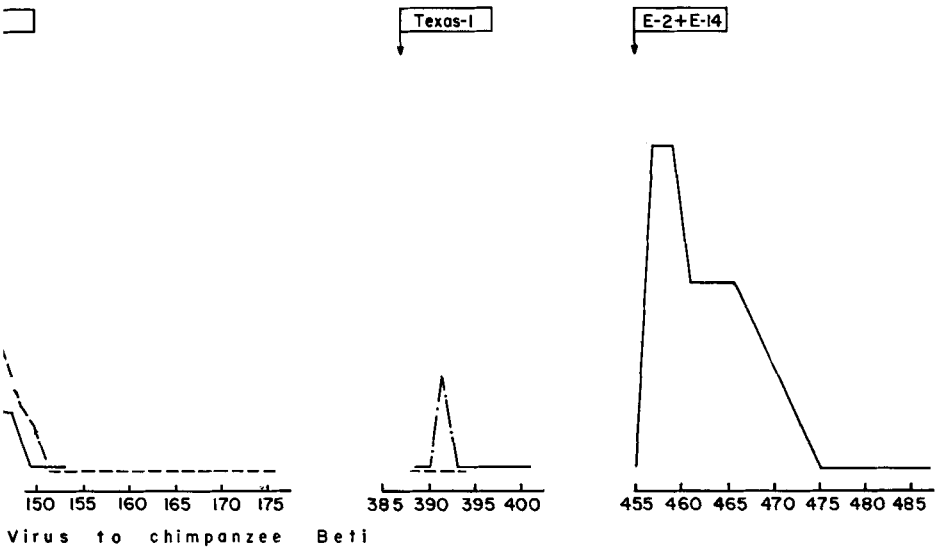


FIG. 8. ID_{50} titers of virus present in stools and throat in rela



n to exposure to virus for chimpanzee Becky. Legend as in Fig. 5.



io.: to exposure to virus for chimpanzee Beti. Legend as in Fig. 5.

the 1st day after the injection, but was regularly present for the next 8 days. Virus was detected in the stools of all four animals,—for a brief period of only 4 days in one, and for periods up to 28 days in the others. It is noteworthy that in one animal, chimpanzee 22, virus was regularly detected through the 14th day; then it could not be found for the next 4 days but was found again during the next week (20th to 28th days). In view of the fact that no attempt was made to type the viruses being isolated from these animals, it may be that virus

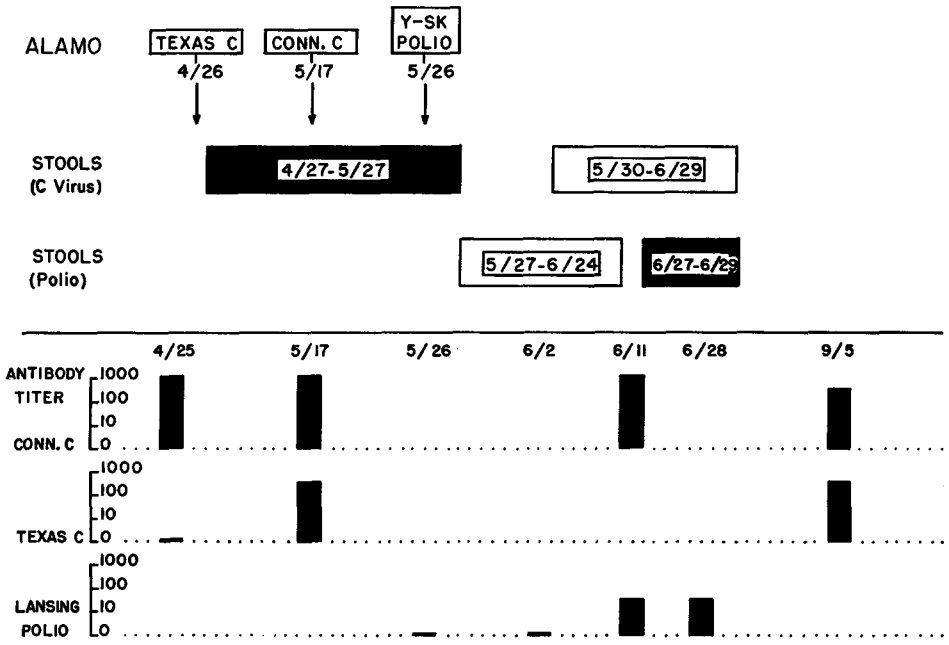


FIG. 9. Virus isolation and neutralizing antibody titers following the feeding of *both* poliomyelitis and C viruses to chimpanzee Alamo. Legends as in Fig. 1.

excretion on days 20 to 28 may have resulted from an infection with a type having a longer latent period than the type (or types) which were recovered soon after the injection.

The antibody determinations, presented in Table XI, indicate clearly that the 4 animals each responded with antibodies to all 7 types inoculated. Thus, even though 7 types were inoculated simultaneously, all produced infection and no interference was noted. The antibody levels present 3 weeks after the virus injection were about the same for all types, except for Type 2 which were consistently higher than the others. At the time of the inoculation antibodies to each of these types were absent, except for chimpanzee 22 which had low levels of antibodies to Easton-10, Alaska-5, and Type 2 strains. From their earlier experiences, three of the four animals already had antibodies to Texas-1, Easton-2, and Easton-14 in high titer (prior to the viral inoculation). The specificity of the neutralizing antibody response was emphasized by the

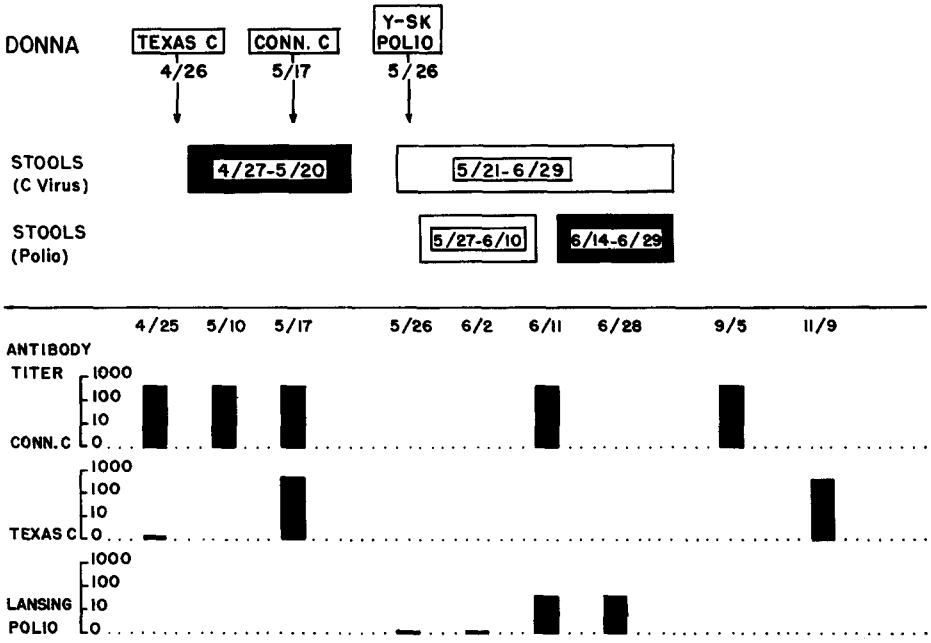


FIG. 10. Virus isolation and neutralizing antibody titers following the feeding of *both* poliomyelitis and C viruses to chimpanzee Donna. Legends as in Fig. 1.

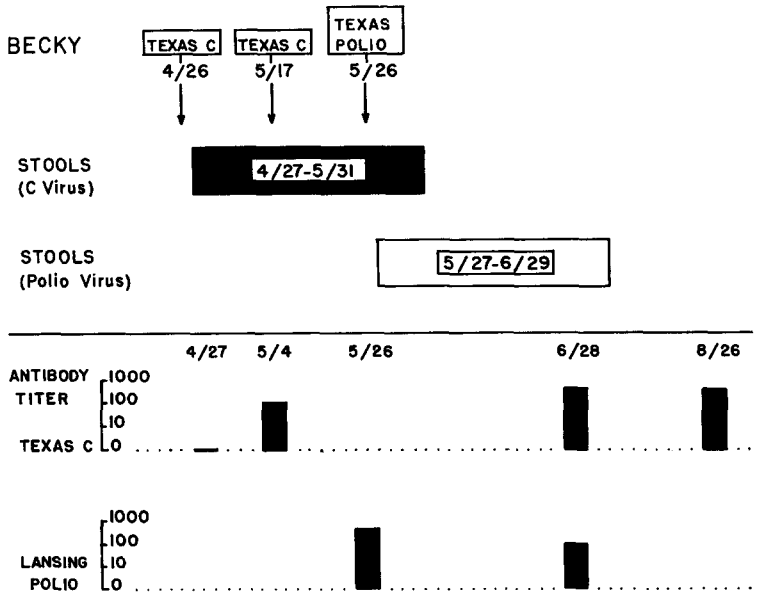


FIG. 11. Virus isolation and neutralizing antibody titers following the feeding of *both* poliomyelitis and C viruses to chimpanzee Becky. Legends as in Fig. 1.

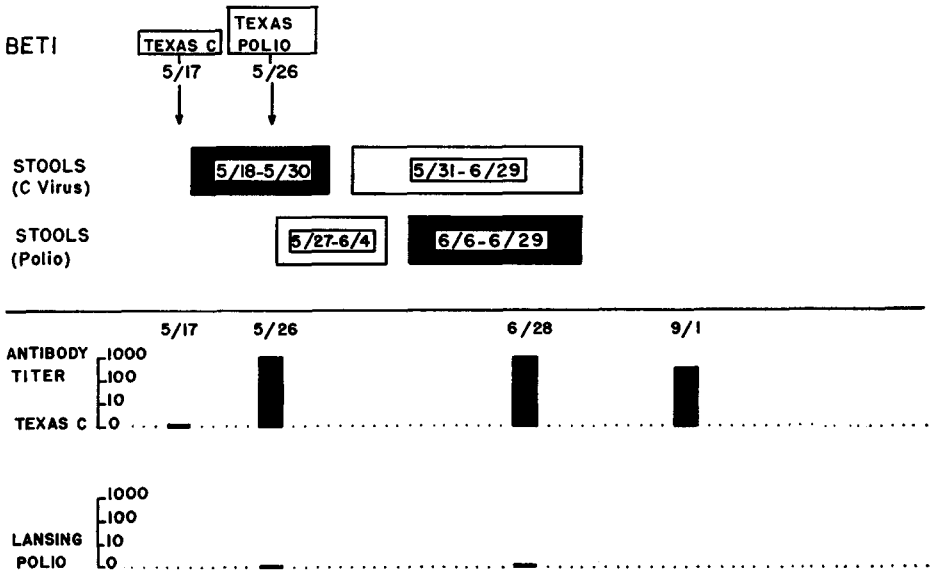


FIG. 12. Virus isolation and neutralizing antibody titers following the feeding of *both* poliomyelitis and C viruses to chimpanzee Beti. Legends as in Fig. 1.

TABLE VI

Chimpanzees 21 to 24—Tests for Virus in Stools, Throat, and Blood after Feeding Texas-1 Virus

Virus	Date	Titer of virus* isolated from											
		Stools				Throat				Blood			
		Ch. 21	Ch. 22	Ch. 23	Ch. 24	Ch. 21	Ch. 22	Ch. 23	Ch. 24	Ch. 21	Ch. 22	Ch. 32	Ch. 24
Texas-1‡	Feb. 5, 1951												
	6	0	0	1.0	3.3	0	0	0	0	0	0	0	0
	7		2.1	2.5				0	0	0	0	0	0
	8	0		3.5	3.5		0	0	0	0	0	0	0
	9		1.1	+				0					
	10	0	+	0	1.8	0	0			0	0	0	0
	12		0		+		0	0	0				
	13	0	0	0	+								
	14					0	0		0				
	15			0	+								
	19				1.5								
	21	0	0	0	0.5								
	24	0	0	0	0								
	28	0			0								

* Negative logarithm of LD_{50}. 0 indicates no virus detectable. + indicates virus present, titration not done.

‡ Chimpanzees 21 to 24 were fed virus diluted to $10^{-8.5}$, $10^{-6.0}$, $10^{-3.5}$, 10^{-10}, respectively.

TABLE VII

Neutralizing Antibodies to Texas-1 Coxsackie Virus in Chimpanzees 21 to 24 Following Exposure to Different Viral Agents

Date	Virus	Route	Serum titer* *vs.* 100 ID_{50} Texas strain			
			Ch. 21	Ch. 22	Ch. 23	Ch. 24
Aug. 15, 1950	Poliomyelitis Y-SK	Oral	1.5+	Inc.	1.5+	1.5+
Nov. 4	" Brunhilde	"	2.5	2.5	3.5	2.5
Dec. 13	West Nile, Egypt strain	Intracutaneous	2.5	2.5	3.5	2.5
Feb. 5, 1951	Coxsackie, Texas-1‡	Oral	2.5	2.5	3.5	2.5
Mar. 1			2.5	4.0	4.0+	3.5

* Negative logarithm of serum dilution at which 50 per cent of mice were protected.

‡ Chimpanzee 21 was fed $10^{-8.5}$ concentration of infected mouse torso; chimpanzee 22, $10^{-6.0}$; chimpanzee 23, $10^{-3.5}$; and chimpanzee 24, $10^{-1.0}$. The virus was titrated in infant mice the day it was fed to the chimpanzees and had a titer of $10^{-7.8}$.

TABLE VIII

Isolation of Virus from Chimpanzees 21 to 24 from Stools, Throat, and Blood after Feeding 2 Strains of Coxsackie Virus

Virus	Date	Titer of virus isolated from									
		Stools				Throat				Blood	
		Ch. 21	Ch. 22	Ch. 23	Ch. 24	Ch. 21	Ch. 22	Ch .23	Ch. 24	Ch. 23	Ch. 24
Easton-2 and Easton-14 fed to Ch. 23 and 24	Mar. 14, 1951										
	16	0	1.8	+		0	0	1.5	+	+	0
	17		+		+						
	19		2.0	1.5	+	0	0	2.5	+	0	0
	20	0		+	1.0		3.1				
	21		3.0		+		0.5			0	0
	22					0	0	0.5	+		0
	24						0	0.5	0	0	0
	25	0	0								
	26			+	0						
	27	0	0								
	28		0	0.5	+						
	29			+							
	30			+	+						
	Apr. 3			0	+						
	4			0	+						
	6				+						

finding that these sera with antibodies to 10 C virus types failed to neutralize other types such as Texas-13 and Israel-7 (16).

Failure of Cortisone to Reactivate a Virus Carrier State.—The possibility of lighting up a latent infection with cortisone was considered, using chimpanzees

TABLE IX

Neutralizing Antibodies to Easton-2 and Easton-14 Strains Following Feeding of These Strains to Chimpanzees 23 and 24 on March 8, 1951*

Antibodies to strain	Date	Serum titer Neg. logarithm			
		Ch. 21	Ch. 22	Ch. 23	Ch. 24
Easton-2	Mar. 8	0	0	0	0
	Apr. 7	1.0	2.5	2.5	2.5
	27	1.5	2.5		
Easton-14	Mar. 8	0	0	0	0
	Apr. 7	0	3.0	2.5	2.5
	27	0	3.0		

* Chimpanzees 21 and 22 were kept in the same room and were infected by contagion.

TABLE X

Isolation of Virus from Chimpanzees 21 to 24 from Stools, Throat, and Blood after Intramuscular and Subcutaneous Inoculations of 7 Heterotypic Strains of Coxsackie Virus

Virus	Date	Titer of virus isolated from										
		Stools				Throat				Blood		
		Ch. 21	Ch. 22	Ch. 23	Ch. 24	Ch. 21	Ch. 22	Ch. 23	Ch. 24	Ch. 21	Ch. 22	Ch. 24
Nancy, Conn.-5,	Apr. 10, 1951											
Ohio-5, Easton-	11	0.5	1.5	0	+		+	0	0.5	2.0	1.0	1.0
10, Alaska-5,	13		0.5			2.7	2.7	1.0	+	1.1	+	+
Type 2, Type 3	14	+	0.5	+	0.5	2.0	2.9	1.5	+			
	16		1.5									
	17	0	+	0.5	+	+	+	0.5	+			
	19		+	+		0	+	+				
	21	0	+	+	+		0	0				
	24	0	1.5		+							
	26		0		+							
	28	0	0		+							
	30		+		+							
	May 8		+									
	June 4	0	0	0	0							
	6	0	0	0	0							
	12	0	0	0	0							

21 to 24 as experimental subjects. These animals had been infected with all 3 types of poliomyelitis virus and with 10 types of C virus during the past year. They had had their last viral exposure on April 10, 1951, and the last positive stool specimen for C virus for any member of the group was on May 8.

Between May 30 and June 11, they were inoculated daily or every other day with 75 to 200 mg. of cortisone.[3] Stool samples were collected between June 4 and 13 and the specimens on each chimpanzee were run separately, a total of 8 monkeys and 300 infant mice being used to test for poliomyelitis and C viruses. The results were negative.

TABLE XI

Neutralizing Antibodies to Seven Coxsackie Virus Types Following Their Simultaneous Inoculation by the Intramuscular and Subcutaneous Routes, April 10, 1951

Antibodies to strain	Date	Serum titers Neg. logarithm			
		Ch. 21	Ch. 22	Ch. 23	Ch. 24
Ohio-1	April 7	0	0	0	0
	27	2.5	3.0	2.5	2.5
Conn.-5	April 7	0	0	0	0
	27	2.5	3.0	2.5	2.5
Nancy	April 7	0	0	0	0
	27	2.5	2.5	2.5	2.5
Easton-10	April 7	0	1.5	0	0
	27	2.5	3.0+	2.0	2.0
Alaska-5	April 7	0	1.0	0	0
	27	2.5	1.5	2.5	2.5
Fleetwood (Type 2)	April 7	0	0.5	0	0
	27	3.0+	3.0+	3.0+	3.0+
Olson (Type 3)	April 7	0	0	0	0
	27	2.5	2.5	2.5	2.5

DISCUSSION

The observations here recorded show that chimpanzees are readily infected on being exposed to C viruses by the oral route but fail to exhibit any signs of illness. Even direct injection of these viruses into the muscle produced no evidence of disease. The pattern of response was fairly typical in the 8 animals studied, and is shown diagrammatically in Fig. 13. Following the feeding of a C virus, as Conn.-5, virus appears in the blood for a few days. Virus can generally be recovered from the throat, starting a few days after the exposure and continuing for 2 to 7 days. Some, if not the bulk, of the ingested virus is excreted within 3 to 5 days, and this is followed by new virus, "manufactured"

[3] These animals were inoculated by Dr. Robert McCollum in connection with his attempts to induce viral hepatitis in them.

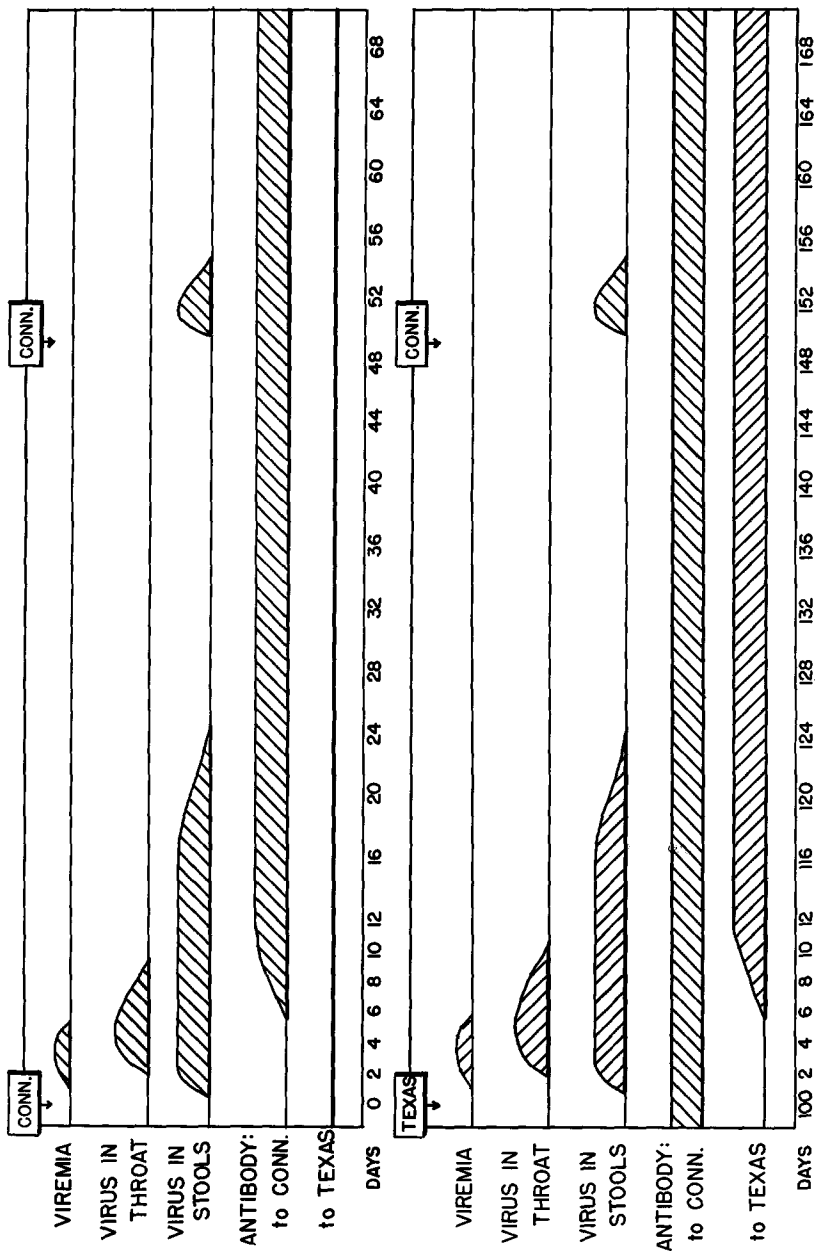


FIG. 13. Inapparent response of the chimpanzee to oral infection with Coxsackie viruses. Demonstration of homotypic immunity and heterotypic susceptibility. See text for details.

in the susceptible animals, for a period of 2 to 4 weeks. Antibodies appear within 2 weeks and maintain their titer for at least 2 years.

Several weeks after the first feeding, if the same strain is refed, then virus can be found in the stools for about 5 days, having passively travelled through the alimentary tract. No true carrier state has occurred in such animals, and it has not been possible to isolate virus from the blood or throat. However, if a heterotypic strain, as Texas, is now fed, then the chimpanzee responds as it did when the Conn. virus was first fed, and a new antibody (Texas type) appears in the blood. Such an animal is now immune to both types, but still can respond like a new animal when fed a third type, *e.g.*, Ohio. The failure to demonstrate cross-immunity is consistent with the sharp separation of the Conn.-5, Texas-1, and Ohio-1 types by neutralization (6), complement fixation (17), and cross-immunity tests carried out in infant mice born of vaccinated mothers (18).

The amounts of virus fed the chimpanzees were probably in huge excess over that needed to induce infection. The reasons for this assumption are twofold: (*a*) feeding as little as 60 ID_{50} subcutaneous murine doses of Texas-1 virus was sufficient to infect even a partially immune chimpanzee (No. 22), and (*b*) during these experiments, two instances of natural infection by contagion occurred in the laboratory, and in these cases the amounts of virus involved must have been far less than the 20 per cent suspension usually fed.

In most instances, the duration of virus excretion in the stools after feeding exceeded that in the throat. This was true with Texas-1, Conn.-5, and the Easton strains. However, the Ohio-1 strain in all 4 chimpanzees produced an infection characterized by prolonged excretion in the throat—so that the duration of virus in both throat and lower bowel was comparable. In fact, the high titers of virus found in the throat for the Ohio-1 virus make one think of the possibility that this agent localized in the throat rather than in the intestines, and that virus in the stools might have had its source in virus produced in the throat and then swallowed. On the other hand, this cannot be the mechanism for the other types, for with these there is a sharp rise of virus titer in the throat and almost as precipitous a decline, while at the same time a high titer and relatively long duration of excretion of virus persist in the stools.

The early viremia which occurs after the ingestion or injection of virus may be the important factor in the early antibody response in this infection. With virus being circulated to antibody-producing centers, a rapid appearance of humoral immune bodies results, with a quenching of the viremia. It is noteworthy that in another experimentally produced alimentary infection of chimpanzees, poliomyelitis, almost the same course of events has been recorded (5, 14, 19, 20).

In chimpanzees 21 to 24, we had occasion to study the effect of naturally induced antibodies on subsequent infection with a homotypic strain. Even though these animals had serum antibody titers of $10^{-1.5}$ to $10^{-3.5}$ to the Texas-1 virus, they did not resist infection completely with this agent, for three of the chimpanzees became intestinal carriers when challenged. However, the normal pattern of infection was altered, in that virus failed to appear in the blood or in the throat, and the agent was confined to the intestine. This suggests that virus proliferation may take place somewhere in the alimentary tract and that a viremic phase is unnecessary for virus multiplication. Following such alimentary infection, the animals showed a 10- to 100-fold increase in titer of neutralizing as well as complement-fixing antibody (1). In poliomyelitis, Bodian (20) has obtained similar data on chimpanzees which received passive antibody before poliomyelitis virus feeding.

The finding of alimentary infection in chimpanzees 22 to 24 with a C virus type to which antibodies were naturally present is consistent in part with observations in animals during the late stages of the primary infection. In animals fed a C virus, with which they have had no previous experience, antibodies develop quickly to high titer. In spite of this, virus excretion may continue to take place for a few weeks after peak antibody development, a happening that points to a site of virus synthesis out of reach of humoral antibodies. In this connection it is noteworthy that in chimpanzees Alamo and Donna after their feeding of poliomyelitis virus, fecal virus was first detected *after* antibodies had already appeared in the blood.

Another similarity in the response of these animals to C viruses and to poliomyelitis virus is the fecal excretion observed when virus is injected, subcutaneously in the case of poliomyelitis (21), and both subcutaneously and intramuscularly for the C viruses. A viremia was present for at least 3 days after injection of C virus, and this may have participated in carrying virus to the alimentary tract where it localized. This does not exclude the possibility of C virus multiplying in several tissues in the chimpanzee as happens in mice (22), but it does point to a way by which the alimentary tract may become involved following upon injection of virus as well as through feeding it.

SUMMARY

Following oral administration of Coxsackie viruses (C viruses) to susceptible chimpanzees, these agents can be isolated from the throat for a period of approximately a week, from the blood for a few days, and from the stools for 2 to 3 weeks or even longer. Animals so infected respond with the formation of specific neutralizing antibodies which are maintained for at least 1 to 2 years. Such chimpanzees are immune when challenged orally with homologous strains of virus. They then excrete virus in the stools for 3 or 4 days (passive

transfer); no virus can be recovered from the throats and blood of these animals, and neutralizing antibody levels remain unchanged.

Animals immune to one antigenic type of C virus can be infected by feeding a different antigenic type. Following such a heterotypic challenge, virus can again be isolated from the throat, blood, and stools; neutralizing antibodies develop to the new strain.

Antibodies to the Texas-1 type of C virus were already present in four chimpanzees upon their arrival in the laboratory from Africa. It was possible to set up intestinal carriage of the Texas-1 virus in these animals and to demonstrate a 10- to 100-fold increase in titer of neutralizing, as well as complement-fixing, antibody.

Quantitative titrations of the amount of virus present in the stools and throat were performed. Immediately after the first feeding of virus relatively large amounts can be detected in the stools; the titer drops, but may be maintained for as long as 25 days at 10^{-2} to 10^{-3}, furnishing evidence that virus multiplication has taken place. Virus in the throat reached the same order of magnitude at first as in the stools but there was a rapid decline to zero in a few days. The Ohio-1 virus differed from the others in that it persisted in the throat as long as in the stools, and in several instances reached a higher titer in the throat. Following homotypic challenge with all types, virus could be detected in the stools for only a relatively short period of time and at low concentration.

Virus-neutralizing substances could not be detected in the stools or throat swabs of convalescent animals, at a time when their serum-neutralizing antibody titers were high.

Under the limited conditions of the present experiments, C viruses had no effect on the infection of chimpanzees with three different antigenic types of poliomyelitis virus. Both poliomyelitis and C viruses set up independent infections without apparent interaction between them. No enhancement of the poliomyelitis infection took place, as was plain from the fact that none of the infected chimpanzees became paralyzed.

A titration of Texas-1 C virus in four chimpanzees revealed that a suspension of infected tissue diluted to $10^{6.0}$ could cause the development of the carrier state; accidental infection of control animals with other Coxsackie types indicated also that very little virus may be necessary to initiate infection.

Seven distinct antigenic types of C virus were inoculated subcutaneously and intramuscularly at the same time into four chimpanzees. The response was the same as that following feeding; virus could be recovered from the throat, blood, and stools, and the animals developed neutralizing antibodies to all seven types of C virus. There was no detectable interference among the viruses.

Cortisone did not bring about the reappearance of the virus excretion in

chimpanzees previously infected and shown to be intestinal carriers of poliomyelitis and Coxsackie viruses.

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