THE INFECTION OF CHIMPANZEES WITH ECHO VIRUSES*

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(Received for publication, July 10, 1957)

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The "echo" viruses share certain properties with other enteric viruses, namely the polioviruses and Coxsackie viruses, and yet exhibit no pathogenicity for common laboratory animals. The present studies were undertaken to learn whether echo virus Types 6 and 4 which cause aseptic meningitis in human beings (1–5) would infect the highest laboratory primate available, the chimpanzee, and whether the exposure would give rise to an illness.

In the study being reported, only inapparent infections were demonstrated, the patterns of infection being similar to those found earlier for the poliomyelitis (6, 7) and Coxsackie viruses (8). The infections were characterized by virus carriage in the stools and in the throat of the fed animals, and antibody responses in all animals infected.

Materials and Methods

Virus Used.—Type 6 D'Amori strain was used in its 10th passage in monkey kidney tissue culture (TC). The virus stock had a titer of $10^{7.7}$ TCD_{50} per ml. Type 4 Pesascek virus was used at the 9th and 10th passage levels in monkey kidney after having had 9 earlier passages in monkey testis. The titers of these two stocks were $10^{6.0}$ and $10^{6.3}$ per ml., respectively. Both virus strains had been isolated from cases with the aseptic meningitis syndrome in New England (1, 9).

Exposure to Virus.—Five chimpanzees, each about 2 years old, were exposed to large doses of both viruses, two orally and three parenterally. The interval between the exposures was 2½ months. Each animal was infected by the same route in the two exposures.

Type 6 Exposure: On 2 consecutive days, 10 ml. of undiluted TC fluid were given to each of two animals, being mixed with fresh banana before breakfast. The total TCD_{50} fed was $10^{9.0}$ for each chimpanzee. Each of 3 animals was inoculated intramuscularly with 1.0 ml. and intracutaneously with 0.6 ml. of undiluted TC fluid. The latter was divided into six piqûres of 0.1 ml. each on the thigh. The total TCD_{50} given for each animal was $10^{7.9}$.

Type 4 Exposure: Almost the same routines were followed as with the Type 6 virus, except that 20 ml. of the undiluted TC fluid was fed on two occasions. The total TCD_{50}

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

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of virus given was $10^{7.8}$. The 3 parenterally inoculated chimpanzees each received $10^{6.2}$ TCD_{50} administered in the same way as with the Type 6 virus.

Observation of animals.—Rectal temperatures were taken daily in the forenoon for a period of 2 weeks after exposure. The chimpanzees were examined daily for other suggestive signs; *i.e.*, pharyngeal injection, anorexia, lymphoadenopathy, skin rash, loose bowels, etc.

Virus Isolation.—Stools, throat swabs, and blood specimens were collected almost daily for 12 days after Type 6 infection and 13 days after Type 4 infection, and weekly thereafter, as deemed necessary. Chimpanzee 4-2 was intractable and attempts to isolate virus from its throat were abandoned after collecting two postinfection specimens.

Fresh stools were collected each morning and frozen at −20°C. for further processing. 3 gm. of each stool specimen were suspended in 27 ml. of water by mechanical shaking for an hour. After the centrifugation at 3,000 R.P.M. for 60 minutes, penicillin and streptomycin were added to the supernate in the concentrations of 500 units/ml. and 500 μg/ml., respectively. These specimens were used immediately or kept frozen at −20°C. until tested.

One cotton swab was used to swab the throat of each chimpanzee. It was extracted as follows in 1.0 ml. of Hanks's balanced salt solution (BSS) containing antibiotics (penicillin, 200 units/ml.; streptomycin, 200 μg./ml.; tetracycline, 25 μg./ml; and mycostatin, 100 units/ml.). Each cotton swab was transferred from the wooden applicator into the barrel of a 1.0 ml. syringe. Then 1 ml. of BSS was swished back and forth several times through the swab, each time squeezing the swab with the plunger.

Blood was drawn usually from the forearm veins but in a few instances from the femoral vessels. The blood was mixed with an equal volume of sterile distilled water, shaken vigorously, and stored in the frozen state at −20°C. It was not treated further before testing.

Monkey Kidney Cultures.—Trypsinized *rhesus* monkey kidney cultures were employed for detection of virus by methods already described (9, 10). Five tubes were used for each specimen, each being inoculated with 0.1 ml. The cultures were checked microscopically for cytopathic changes for at least 10 days. Positive specimens after the Type 6 infection were then titrated. In doubtful cases TC fluid was transferred to fresh tubes.

On occasion, 3 oz. bottle cultures were used to detect small amounts of virus. Monolayer cultures in prescription bottles were inoculated with 1.0 ml. of the stool specimens, and 20 hours later fluids were changed with fresh medium and the cultures were again inoculated with 1.0 ml. of the same specimen.

Identification of the Isolated Viruses.—Neutralization tests were performed on a number of the isolates. For Type 6 we used a 1:100 dilution of hyperimmune rabbit antiserum which at this dilution contained 100 antibody units against the D'Amori strain, and about 10^3 to 10^4 TCD_{50} of virus as challenge. All tests for Type 6 were carried out by inoculation of monolayer cultures in tubes.

Owing to the difficulty experienced in the Type 4 neutralization tests in tubes, reduction in plaque titer in bottle cultures was adopted (10, 11). A 1:30 hyperimmune serum was mixed with about 500 and 50 PFU (plaque-forming units) of the first passage virus in monkey kidney culture, and plated under agar in bottle cultures (12).

Serum Neutralization Test.—Pre- and postinfection serum specimens were obtained. About 30 ml. of blood were taken at 2 week intervals, and the serum was stored in the frozen state. For Type 6 antibodies 0.5 ml. of D'Amori virus containing an estimated 100 TCD_{50} per 0.1 ml. was mixed with 0.5 ml. aliquots of several 2- or 4-fold serum dilutions. The diluent for both virus and serum was BSS. The mixture was incubated at room temperature for 1 hour, and then 0.2 ml. of each mixture was inoculated into each of 3 or 4 TC tubes, containing 1.0 ml. of M-E[1] maintenance medium. For the parallel titration of virus, 0.1 ml.

[1] A maintenance medium used in this laboratory since 1952. It contains 0.5 per cent lactalbumin hydrolysate, Earle's salt solution, and 1 or 2 per cent calf serum.

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of each 10-fold virus dilution was inoculated per culture. The final readings were made on the 7th day, when the control titration showed that 100 TCD_{50} was present in the test dose. The 50 per cent end-point titer was calculated by the method of Reed and Muench, and was indicated as serum dilution before mixing with virus. Serum neutralization tests against 6′, 6″, and a few other echo virus types were performed in the same way.

Because of the difficulty in demonstrating Type 4 neutralizing antibody, four different methods were employed:—Method I was the technic given above for the Type 6 neutralization. Method II was taken from the procedure in use in Syverton's laboratory at the University of Minnesota. Virus dilutions were prepared in M-E medium to contain 100 TCD_{50} in 0.5 ml., and 2.5 ml. mixed with equal volumes of 3-fold dilutions of serum also diluted in the same medium. While the mixture was incubated at room temperature for 1 hour, the fluid was drained from monkey kidney cultures. The drained cultures were then inoculated with 1.0 ml. of the serum-virus mixture, 4 tubes being used for each mixture. The procedure was similar to Method I but allowed us to use a five times greater ratio of serum to virus.

With Method I the sera after Type 4 infection gave negative results at the lowest serum-dilution, 1:4. With Method II all the specimens tested over an 8 week period also gave negative results at the lowest dilution, 1:10. This would correspond to 1:2 serum dilutions for Method I. Thus, as the next step, we adopted the virus dilution technic in which the neutralization index was determined as a measure of antibody titer. Undiluted serum was mixed with equal amounts of several 10-fold dilutions of virus. After incubation at room temperature for 1 hour, 0.2 ml. of each mixture was inoculated into each of four tubes. The results were read on the 5th day. The results are presented as the logarithm of the neutralization index, that is, the logarithm of the difference between the control virus titer and the virus titered in the presence of the serum. Even with this technic antibody titers were found to be low. Therefore, as the fourth method, plaque neutralization tests were carried out. 5-fold serum dilutions from 1:2 to 1:250 were mixed with an estimated 100 PFU of virus. Equal volumes of diluted serum and virus in BSS were mixed. The mixture was incubated for 1 hour at room temperature, and 0.2 ml. of each mixture was inoculated into each of two drained bottle cultures. After an adsorption period of 1 hour at 37°C., the monolayers were covered with agar (11). The overlay contained 1.5 per cent agar, 2.0 per cent calf serum, 1:60,000 neutral red, and 0.225 per cent sodium bicarbonate in final concentration in Earle's salt solution. 440 units of penicillin, 440 μg. of streptomycin, and 110 units of mycostatin were included in each milliliter of the overlay. The bottles were left about 1 hour at room temperature for solidification of the overlay, inverted, and incubated at 37°C. The plaque counts of both bottles at each serum dilution were compared with the number of plaques in the control group without serum, 80 per cent reduction in plaque count was taken as the lower limit of significant neutralization, and the highest dilution of serum which gave such neutralization was considered as the titer of the serum.

Complement Fixation Test.—The details of the plate CF technic as used in this laboratory have already been described (12). The D'Amori antigen was in the form of undiluted TC fluid which had been harvested at the time of strong CPE (cytopathogenic effect) and centrifuged at 3000 R.P.M. for 30 minutes. The antigen was grown in washed monolayer cultures in the presence of C-E medium (0.01 per cent cysteine, 0.25 per cent glucose, Earle's salt solution). The antigen was standardized by performing box titrations with a monkey immune serum, and was found to contain 8 units per 20 c.mm. drop of TC fluid. The chimpanzee sera were tested in 2-fold falling dilutions against 8 units of echo–6 antigen and also as controls against 8 units of each of 3 types of polioantigens grown under similar conditions. Avidity scores were calculated for each dilution of serum. The CF titer of the serum

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was taken as the highest dilution of serum which fixed 2 units of complement in the presence of 8 units of antigen.

RESULTS

Type 6 (D'Amori) Infection.[2]—Neither the chimpanzees fed nor those injected with virus parenterally manifested any illness including fever, pharyngeal injection, skin

TABLE I

Isolation of Virus from Stool, Throat, and Blood after Exposure to Echo Type 6 (D'Amori) Virus

Dates of infection	Days after infection	Oral infection						Parenteral infection								
		Stool		Throat		Blood		Stool			Throat			Blood		
		No. 4-0	No. 4-1	No. 4-0	No. 4-1	No. 4-0	No. 4-1	No. 4-2	No. 4-4	No. 4-5	No. 4-2	No. 4-4	No. 4-5	No. 4-2	No. 4-4	No. 4-5
July 13–14, 1956	1	4.7*	3.5*	1.4*	3.7	–	–	–	–	–	–	–	–	–	–	–
	2	5.3	3.7	3.5	3.5	–	–	–	–	–	–	–	–	–	1.5*	–
	3	5.3	2.7	3.0	3.5	–	–	–	–	–		–	–	–	–	–
	4	4.2		3.0*	3.0	–	–	–	–	–		–	–	–	–	–
	5	2.5	3.5	2.7	2.0*	–	–	–	–	–		–	–	–	–	–
	6	2.7*	2.2	1.7	2.0*	–	–	–	–	–		–	–	–	–	–
	7	+	+*	2.7	2.0	–	–	–	–	–		–	–	–	–	–
	8	+	+	1.5	1.4*	–	–	–	–	–		–	–	–	–	–
	9	+	–	1.5	1.5	–	–	–	–	–		–	–	–	–	–
	10		–	1.7	1.2*	–	–	–	–	–		–	–	–	–	–
	11	–	+	1.4*	0.6	–	–	–	–	–		–	–	–	–	–
	12	+	+	0.6	–	–	–	–	–	–		–	–	–	–	–
	14	–	–					–	–	–						
	21		–	–	–							–	–			

* Isolates which were typed. All were identified as echo Type 6 by neutralization tests. Titers are expressed as log TCD_{50} per gram stool, per throat swab, and per milliliter blood, respectively. + indicates positive isolation, but stool was not titrated.

rash, etc., during the period of observation. The recovery of virus is shown in Table I. Virus could be isolated from the upper and lower ends of the alimentary tract for 12 days in the orally infected animals, but not from the parenterally infected animals, except for a single blood specimen taken 2 days after exposure. This cannot be accepted as evidence of true viremia following viral multiplication as is usually observed in Coxsackie or virulent poliovirus infections in primates (8, 13, 14). Because chimpanzee 4-5 already had neutralizing antibodies in its pre-inoculation serum, the negative results in the blood of this animal are of little significance.

The amount of virus fed to each animal was 0.5×10^9 TCD_{50} on each of 2 successive days. On the morning after the second feeding, only a small fraction of the virus fed could be recovered in the stools and throat. The titers were relatively high, $10^{3.5}$ to $10^{4.7}$ for feces, and $10^{1.4}$ to $10^{3.7}$ for the throat swab extracts. Larger amounts of

[2] We wish to acknowledge the assistance of Dr. T. Kuwata, Chiba University, Chiba, Japan, who participated in the initial part of this study.

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virus were excreted during the first 4 to 5 days, then the virus levels in these sites declined gradually during the next week. There seems little doubt that the virus had multiplied after the oral exposure, particularly in view of the control experiments in

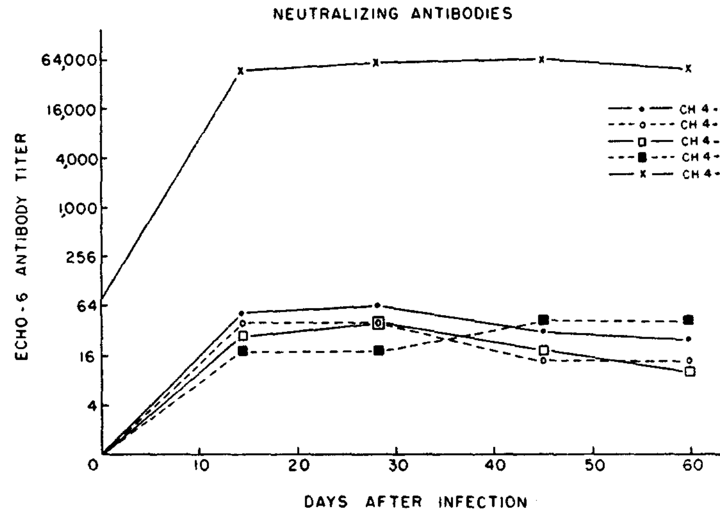

FIG. 1. Development of neutralizing antibodies in chimpanzees after inoculation with echo virus Type 6. Those animals having their first experience with this virus made similar antibody responses. Chimpanzee 4-5, with pre-existing antibodies to echo-6 or a related virus, responded to "boostering" with high antibody production; this animal was the only one whose postinoculation serum neutralized echo-6′ and echo-6″ also (see Fig. 2).

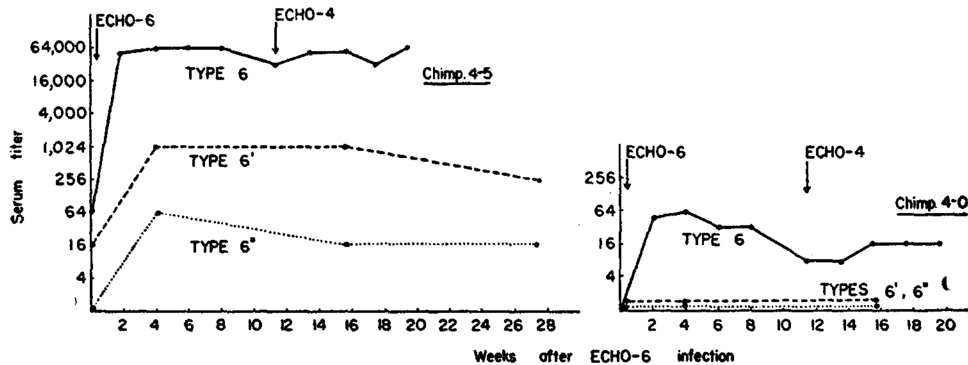

FIG. 2. Neutralizing antibodies against echo virus types 6, 6′ and 6″ in chimpanzees 4-5 and 4-0.

the stability of the D'Amori virus at 37°. Undiluted tissue culture fluid which titered $10^{7.7}$ per ml., decreased in titer to $10^{2.5}$ per ml. in 1 week, and was entirely negative in 2 weeks.

Neutralizing Antibodies.—As can be seen in Fig. 1, all animals developed neutralizing antibodies, with peak titers being reached within 2 to 4 weeks. The antibodies

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persisted at their maximum levels for more than 6 months, and were not influenced by the subsequent exposure to Type 4 virus. The antibody response was almost the same in the fed and injected animals except for chimpanzee 4-5. This parenterally infected animal had neutralizing antibodies at a level of 1:64, in its pre-inoculation serum. The titer rapidly rose to reach 1:50,000 within 2 weeks, which happened to be even higher than the titer of a hyperimmune serum being used by us as a reference standard.

As shown in Fig. 2, chimpanzee 4-5 developed antibodies which also neutralized the antigenic variants, echo 6′ and 6″ viruses. The pre-inoculation serum of 4-5

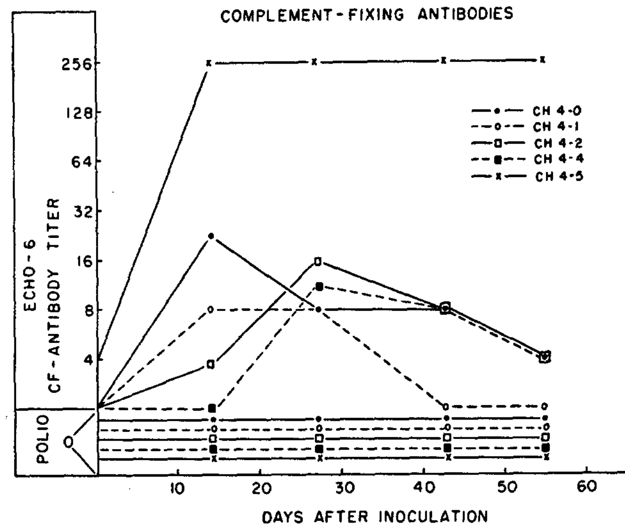

FIG. 3. Development of complement-fixing antibodies in chimpanzees after inoculation with echo virus Type 6. The animals which lacked previous experience with this virus all made CF responses reaching a level of 8 to 16, and falling after 40 to 56 days. Chimpanzee 4-5, with pre-existing neutralizing antibodies, also had pre-existing CF antibodies; these rose to a level of 256, which was maintained throughout the period of observation.

exhibited a titer of 1:16 against echo-6′ virus, and the peak titer after inoculation rose to 1:1024. Against echo-6″ virus, no antibodies were found before inoculation, but afterwards the 4 week serum had a titer of 1:64, and was still 1:16 in the 28th week. In the other chimpanzees no neutralization occurred at the lowest dilution of serum tested, 1:4, against these viruses, even after second infection with echo-4 virus.

Pre, 2 week, and 6 week sera of chimpanzee 4-5 were also tested against echo-1 (Farouk) and echo-5 (Noyce) viruses with no neutralization being detected at 1:4 serum dilution. Polio antibodies were present to varying degrees (greatest for Type 2) in view of the fact that the animals had been vaccinated with commercial vaccine some months before being used for the present experiments. The level of polio antibodies was not influenced by the echo virus infections.

Complement-Fixing Antibodies.—Development of CF antibodies in all five chimpanzees is shown in Fig. 3. Again chimpanzee 4-5 manifested evidence of prior echo-6

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infection, for a low level of antibody was present in its pre-inoculation serum. By the end of 2 weeks the titer rose to a level of 1:256, which was maintained for at least 8 weeks. With other chimpanzees their peak titers, ranging from 1:8 to 1:24, were reached in 2 to 4 weeks. By the 8th week they dropped to 1:4 or were negative. No CF antibodies to the polioviruses developed, even though the animals had been

TABLE II

Isolation of Virus from Stool, Throat, and Blood after Exposure to Echo Type 4 (Pesascek) Virus

Dates of infection	Days after infection	Oral infection						Parenteral infection					
		Stool		Throat		Blood		Stool		Throat		Blood	
		No. 4-0	No. 4-1	No. 4-0	No. 4-1	No. 4-0	No. 4-1	No. 4-4	No. 4-5	No. 4-4	No. 4-5	No. 4-4	No. 4-5
Oct. 2-3, 1956	Pre	−	−	−	−			−	−	−	−		
	1	+*	+*	−	+	−	−	−	−	−	−	−	+*
	2	+	+	−	+	−	−	−	+*	−	−	−	−
	3	+	+	+	+	−	−	−	+	−	−	−	−
	4	+	−	+*	+*	−	−	−	−	+*	−	−	−
	6	−	−	+	+	−	−	−	−	−	−	−	−
	7	−	−	+	+	−	−	−	−	−	−	−	−
	8	+	−	+	−	−	−	−	−	−	−	−	−
	9	+	−	+	+	−	−	−	−	−	−	−	−
	10	+	−	+	+*	−	−	−	−	−	−	−	−
	11	−	−	+	−	−	−	−	−	−	−	−	−
	13	−	−	+	−	−	−	−	−	−	−	−	−
	20			+*	−					−	−		
	28			+	−								
	35			+	−								
	42			−	−								
	57			−	−								

Chimpanzee 4-2 was infected parenterally, but was not tested for virus excretion.

* Isolates which were typed. All were identified as echo Type 4 by neutralization tests.

+ indicates more than 40 TCD_{50} per gram stool, 4 TCD_{50} per throat swab, and 8 TCD_{50} per milliliter blood.

sensitized by receiving a course of commercial poliovaccine several months prior to the echo-6 exposure.

Type 4 (Pesascek) Infection.—The animals were exposed to echo-4 virus 10 weeks after the echo-6 had been given. Again no overt illness occurred during the period of observation. A picture similar to that of echo-6 infection is shown in Table II. Titration of the positive specimens was not carried out, but the duration of excretion, particularly in the throat, was adequate to show that virus multiplication had occurred. For interpretation of the data in Table II it may be useful to recall, as described under Materials and Methods, that the dose of echo-4 was less than 10 per cent of that of echo-6. Thus the duration of virus excretion, especially in chimpanzee 4-0, is all the more significant.

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TABLE III

Neutralizing Antibodies against Echo Type 4 Virus

Chimpanzee No.	Time of bleeding	Titers by Methods I and II* (serum diluted, virus held constant)	Log neutralization index (virus diluted, serum held constant)	Titer by plaque method
	wks.			
4-0	Pre	0	0.50	<1:2
	2	0		
	4	0	0.83	<1:2
	6	0		
	8	0	0.33	1:10
	16		0.73	1:10
	19			1:10
4-1	Pre	0	0.17	<1:2
	2	0		
	4	0	0.0	1:10
	6	0		
	8	0	0.50	
	16		1.00	1:50
	19			1:50
4-2	Pre	0	0.25	<1:2
	2	0		
	4	0	0.50	
	6	0		
	8	0	0.73	
	16		1.00	1:10
	19			1:50
4-4	Pre	0	0.50	<1:2
	2	0		
	4	0	0.33	1:10
	6	0		
	8	0	0.83	
	16		1.00	1:50
	19			1:250
4-5	Pre	0	0.0	<1:2
	2	0		
	4	0	1.00	1:10
	6	0		
	8	0	1.17	1:50
	16		1.50	1:50
	19			1:50

* Zero indicates <1:4 for Method I, and <1:10 for Method II. Because of the 5-fold greater serum to virus ratio in Method II, this would correspond to a 1:2 serum dilution in Method I. Also, dissociation upon dilution in Method II is not possible, because the serum-virus mixture is added to drained tubes (see Materials and Methods).

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Among the parenterally infected animals, 4-5 showed virus in the stools 2 to 3 days after inoculation and 4-4 in the throat on the 4th day. Again one blood specimen was positive, a sample taken 1 day after inoculation of virus. In the fed group, chimpanzee 4-0 secreted virus for 35 days in the throat, but only 10 days in the stool and, even here, with some intermittency. However, less than 40 TCD_{50} in 1 gm. of stool would not give a positive test in the present experiments, because of the dilution factor in preparing the stool extract. With 4-1, virus excretion was more brief, that is, for 10 days in the throat and only 3 days in stools.

Neutralizing Antibodies.—The results of neutralization tests carried out by the four methods described under Materials and Methods are shown in Table III. By the conventional test (Method I) all the specimens tested were negative at the lowest dilu-

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TABLE IV

Neutralizing Antibody Response in Chimpanzees Fed Echo Types 2, 3, and an Untypable Strain (Co)

Chimpanzee	Virus	Serum titers		Reference antisera	
		Pre-exposure	1 month after exposure	Type 2	Type 3
A	Echo-2 fed	0	32	256+	0
	Echo-2 recovered from stools	0	64	512	0
B	Echo-3 fed	0	32		512
	Echo-3 recovered from stools	0	32	0	64
C	Co fed	0	96	0	0
	Co recovered from stools	0	32	0	0

tion tested, 1:4. Method II was then employed, in which larger amounts of serum were mixed with 100 TCD_{50}, and added to drained cultures, as recommended by Syverton. Possible dissociation of the antigen-antibody complex which might occur in the ordinary method (in which 0.2 ml. of the virus-serum mixture is added to 1 ml. of fluid) is thus avoided. Again all sera tested gave negative results at the lowest dilution tested, 1:10 (equivalent to 1:2 of Method I).

In the next tests, the neutralization index was determined, using undiluted serum, and varying dilutions of virus from 1 to 1000 TCD_{50}. In general, the indices were small, but tended to increase towards 8 and 16 weeks after infection. Tests for neutralizing antibodies by the plaque reduction method gave better results, with antibody levels of 1:10 to 1:250 being observed.

There was no significant effect of the exposure to echo-4 virus on the pre-existing Type 6 antibody. Similarly the echo-6′ and 6″ antibodies in chimpanzee 4-5 were not influenced by the echo-4 exposure.

Trials with Types 2, 3, and an Untypable Echo Virus.—Three other chimpanzees

were each fed an echo virus, each animal receiving 4 to 12 ml. of undiluted TC fluid.[3] The animals excreted virus for up to 3 weeks after the exposure. As shown in Table IV, the animals developed specific antibody against the ingested virus. Because the outcome confirmed the pattern established with echo types 4 and 6, the experiments on these 3 animals will not be reported here in any greater detail.

DISCUSSION

Infection of chimpanzees with echo viruses followed the pattern established with the other enteric viruses, both poliomyelitis and Coxsackie (6–8). No apparent illness occurred, but infection was readily demonstrated by the presence and persistence of virus in the throat and in the feces. It is noteworthy that with these *enteric* viruses, in the orally infected animals virus was found in the throat as long as in the intestines for Type 6, and even longer for Type 4. In the chimpanzees fed Type 4, virus persisted in the throat for 10 and 35 days but in the feces of these animals for 3 and 10 days, respectively. This recalls a similar observation with the Coxsackie viruses, which was limited to type B2, in which carriage of virus in the chimpanzee throat equalled that in the lower alimentary tract (8).

Unlike poliomyelitis virus, which readily finds its way from the skin to the gut in the chimpanzee (15), the echo viruses studied were not readily excreted into the gut after parenteral infection. In two animals infected with Type 4, virus was found briefly in the alimentary tract but this was not followed by long term carriage.

The antibody response in the animals was prompt with respect to Type 6. As in other viral infections, neutralizing antibodies persisted at high titer for the 6 months of the study, while CF antibodies already were at borderline level 8 weeks after infection. In accord with other observations on echo-4 virus, we have found that its antibodies are difficult to detect. This has been true not only with postinfection sera, but also with sera of hyperimmunized animals. With the latter sera, we have also found that 50-fold higher titers can be obtained by using the more sensitive plaque reduction method.

The one animal, No. 4-5, with pre-existing Type 6 antibodies before we fed it virus is worthy of special comment. First it indicates that chimpanzees may become infected naturally by this echo virus. Of course, we have no way of determining at this time whether its first infection occurred in the wilds of Africa or whether it became infected during its contacts with man in its period of captivity. It seems unlikely that the animal became infected in our laboratory because it was the only one of 5 animals with antibodies and yet all 5 animals had the same laboratory exposure prior to the experiments reported.

Second, chimpanzee 4-5 was the only animal which produced such extraordinarily high antibody titers, 1:50,000. Although this was an anamnestic response, the level

[3] Dr. Bernardo Epstein, Rockefeller Foundation Fellow in 1954, participated in these studies.

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of antibody reached was as high as, if not higher than, the best levels reached by hyperimmunization of rabbits or monkeys.

Third, the antibody response of this chimpanzee alone was broad. In addition to the high level of Type 6 antibodies, antibodies also developed to antigenic relatives of the prototype D'Amori strain, namely echo Types 6′ and 6″. Antigenic variants exist within several echo types. The possibility exists that they may develop in a host which is exposed to one type and which gives rise to antibodies against the specific type as well as to antigenic variants. The stool specimens of these chimpanzees have been made available to Dr. David Karzon, University of Buffalo, who is determining whether the virus excreted late after infection differed antigenically from that excreted immediately after the exposure.

The polioviruses and the Coxsackie viruses are characterized by possessing group CF antigens. One of the purposes of the present experiment was to determine whether chimpanzees infected with an echo virus made complement-fixing CF antibodies which showed group reactivity with poliovirus antigens. The animals in question had previously been inoculated with commercial poliomyelitis vaccine, and had responded, but only to Type 2. None of the 5 animals had CF antibodies to poliovirus in their sera before exposure to echo-6 and none of them, after exposure to the echo virus, made poliomeylitis antibodies, when tested against unheated or heated poliovirus antigens.

SUMMARY

The oral and parenteral infections of chimpanzees receiving echo Types 6 and 4 viruses successively are described. The two infections, spaced 2½ months apart, and given by the same route in each animal, failed to induce overt disease. The inapparent infections were demonstrated by virus excretion in the throat and the stools and the development of neutralizing antibodies. Complement-fixing antibodies also appeared after Type 6 infection, but fell more rapidly than the neutralizing antibodies.

After oral infection, echo-6 virus was found for equal periods in both the throat and feces, but echo-4 persisted in the throat for much longer periods than in the lower bowel. Almost no virus carriage occurred after parenteral inoculation. No true viremia was exhibited in any of the animals.

One of the chimpanzees had neutralizing antibodies against Type 6 virus in its pre-inoculation serum. It responded extraordinarily to the Type 6 exposure, developing antibody levels of 1:50,000 to echo-6, of 1:1024 against the echo-6′ variant, and of 1:64 against the echo 6″ variant.

Although Type 4 antibodies developed after the exposure, they proved difficult to measure by ordinary methods. However, they could be satisfactorily assayed by the plaque reduction method.

Three other chimpanzees fed echo-2, echo-3, and an untypable echo virus, respectively, yielded results confirming those established with Types 4 and 6.

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