POLIOMYELITIS IN CHIMPANZEES

Studies in Homologous and Heterologous Immunity Following Inapparent Infection*

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The development of active immunity to poliomyelitis in chimpanzees following inapparent infection has been reported previously from this laboratory (1, 2) and by Howe, Bodian, and Morgan (3). The present series of experiments is an extension of our earlier ones, designed to test further the immunity of chimpanzees to homologous and heterologous strains of virus introduced orally and subcutaneously. Since these animals rarely develop definite signs of poliomyelitis when infected by the oral route, the test of infection as before has been the development of an intestinal carrier state following ingestion of virus or its introduction into the skin. The measure of immunity has been whether or not an animal again becomes a carrier when challenged with homologous or heterologous strains of virus introduced by these same routes. In addition, the development of neutralizing antibodies in the sera of infected animals has been correlated both with infection and immunity.

Materials and Methods

The details of the procedures employed have been outlined previously (1), and only a brief account will be given here.

Chimpanzees.—Eighteen chimpanzees were used; they were exposed to various strains of poliomyelitis virus a total of 54 times. Eight of these animals were subjects in previous similar experiments (1). Nine of the animals, ranging in age from 12 months to about 4 years, were born and had always lived in the Yerkes Laboratory of Primate Biology, Orange Park, Florida,[1] before coming to our laboratory. The other nine, aged approximately 2 to 4 years, were obtained from a dealer who had imported them from Africa.

Virus.—The following virus strains were used:

1. The *Y-SK strain* was used as monkey CNS serial passage, and as cotton rat CNS and mouse CNS (4). In our laboratory it has a titer of about $10^{-2.5}$ in mice, $10^{-3.5}$ in cotton rats, and has gone as high as 10^{-6} in monkeys (5). The murine-adapted strain retains its serological identification with the parent monkey virus. The material given to the chimpanzees was of 4 types which are listed in conformity with the recommendations of the Committee on Nomenclature of the National Foundation for Infantile Paralysis (6): (*a*) monkey CNS (Y-SK-M_{25}); (*b*) cotton rat CNS (Y-SK-$M_{22}CR_5$); (*c*) mouse CNS (Y-SK-$M_{23}CR_9m_{10}$); and (*d*) a mixture of above monkey, cotton rat, and mouse CNS tissues.

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2. *NC*, a human strain, was used in the form of a pool of stool specimens collected from 12 patients during the North Carolina epidemic of 1944. As pointed out previously (1), the pool may represent one or more strains of virus. The material fed to chimpanzees was purified and concentrated by ultracentrifugation (7). Monkeys paralyzed after inoculation with NC, and hyperimmunized by intraperitoneal and intramuscular inoculations of virus failed to produce serum which could neutralize the Lansing strain.

3. *Far East* (FE) strain, generation 1 monkey cord, was composed of a pool of 10 per cent spinal cords from monkeys infected with CNS or stools from human cases occurring in Japan, China, the Philippine Islands, and the Malay Peninsula in 1945.

4. *Pittsfield* strain, isolated during a small outbreak in a boys' camp during 1946, was used as a pool of known positive stools collected from patients and carriers. This strain had been shown to be unrelated immunologically to the Lansing and Y-SK strains (8).

5. *Texas* strain, isolated from flies trapped during an epidemic in southern Texas in 1948, was used as 10 per cent spinal cords of monkeys infected with the virus (first passage in monkeys).

6. *Egg-adapted FA mouse encephalomyelitis* virus was derived from a strain picked up spontaneously in mice on intracerebral passage of the Y-SK strain. The circumstances of its isolation and identification have been described elsewhere (9).

7. *Lansing* strain mouse CNS was used in the neutralization tests. The strain, in its 240th mouse passage, was kindly supplied to us by Dr. Charles Armstrong of the National Institutes of Health. It had been passed 25 times in our laboratory before use in the present experiments.

Administration of Virus to Chimpanzees.—Virus was either fed or, in a few instances, inoculated intra- and subcutaneously. When fed, 1 to 5 ml. was placed in bananas or in milk and given as the first meal of the day. When virus was given intra- and subcutaneously it was administered in several piqûres, about half going into and half under the skin. Nembutal anesthesia (the drug being administered subcutaneously, or orally in bananas, milk, or chocolate bar) was necessary in order to bleed or to administer virus parenterally to some of the larger animals.

Testing of Chimpanzee's Stools for Poliomyelitis Virus.—Each chimpanzee was housed in an individual cage under which there was a movable pan for the collection of excreta. The pans were cleaned and hosed thoroughly each day; an effort was made to collect fresh fecal material at appropriate times and the specimens were frozen on dry ice immediately after collection. The previously reported method of concentration and purification by ultracentrifugation was carried out (7). The resulting clear suspensions were inoculated intracerebrally into rhesus, *M. mulatta*, or green African monkeys, *C. aethiops sabeus*, (usually one animal for each specimen) and when the Y-SK strain was used, sometimes into twelve mice and four to six cotton rats in addition. It was found that by adding penicillin and streptomycin to the final inoculum, a much lower percentage of monkeys developed signs of brain abscess, and none of the animals died of this cause.

Definition of an Intestinal Carrier State.—As has been shown in the early part of this work (1), virus fed to immune chimpanzees may be recovered in their stools but only for a few days following the feeding, which indicates that passive transfer through the gut is relatively brief. In some new animals which became carriers of virus there was an interval of several days between the elimination of fed virus and of the newly formed virus resulting from the infection. When such an interval was present, the virus fed could not be detected beyond the 3rd day after feeding. This is consistent with the passage of carmine through these animals, for the dye could be detected in the stools only for a period of 3 days, and on the 3rd day the stools were faintly colored. In a single experiment with an agent which does not propagate in the chimpanzee, FA type of mouse encephalomyelitis virus, the virus was found 5 to 7 days after its feeding but not thereafter. Consequently we have considered in this report that all

poliomyelitis virus isolations 8 days beyond the last day of virus feeding are indicative of virus multiplication and a true carrier state.

Neutralization Tests.—Chimpanzees were bled on arrival in the laboratory and at indicated intervals through the experiment. Some difficulty was encountered in handling the larger animals, and there are, therefore, gaps in the serum collections indicating insurmountable resistance on the part of the chimpanzee to be bled.

Inasmuch as the Y-SK strain has been demonstrated to be related serologically to the Lansing strain, the latter was used in most of the neutralization tests because of its more regular titer and shorter incubation period. When Lansing virus is used, the surviving mice can be discarded after a period of 30 days, while a 60 day observation period is necessary with the Y-SK strain in certain of its passages.

The neutralization tests were carried out using a 2 to 10 per cent suspension of Lansing mouse CNS virus, and serial tenfold dilutions of serum, each in 0.2 or 0.3 cc. amounts. After thorough shaking, the mixtures were incubated at 37°C. for 1½ hours following which they were transferred to an ice water bath and inoculated immediately. Eight Swiss mice, 3 to 4 weeks old, were used in each test. With some of the sera, neutralization tests using both Lansing and Y-SK strains as antigens were carried out. It was found that the strain used in the neutralization test did not influence the level of antibodies found. A modification introduced into most of the latter tests was incubation of the virus-serum mixtures at room temperature for 1 hour, rather than at 37°C. for 1½ hours. Although the amounts of virus used in the neutralization tests varied from 1.5 to 2.0 logarithms, this appeared to be without significant effect on the serum titers.

Experiments with Four Chimpanzees,—Flora, Falla, Webb, and Jent

The earlier exposures to poliomyelitis virus of the four chimpanzees Flora, Falla, Webb, and Jent have already been described (1). In brief, each of these animals had become an intestinal carrier of Y-SK strain (cotton rat CNS) and all but Falla had been challenged orally or intracutaneously and found resistant to the homologous strain.

Experiment 1. Homologous Challenge with Y-SK Strain.—In the present experiments, beginning March 28, 1946, the animals were given further homologous challenge in the form of the parent monkey Y-SK strain. A 10 per cent monkey cord suspension, which represented the Y-SK strain before its adaptation to rodents, was fed on 2 successive days in 4.5 ml. amounts. Stools were collected and tested as before.

From Table I, in which only new data described in this paper are presented, it can be seen that all four animals were again resistant to homologous challenge; the carrier state was not demonstrated in any one. No change in the titer of neutralizing antibodies to the Lansing strain was demonstrated in the July (3 months after feeding) sera of Flora, Webb, or Falla after their exposure to the parent monkey Y-SK strain.

Experiment 2. Heterologous Challenge with NC Strain.—Having resisted homologous challenge, carried out 3 to 8 months after the initial exposure to virus, the chimpanzees were given a different strain approximately 5 months after their previous exposure to virus. All four animals were fed NC strain, each receiving 1 to 1.5 ml. in bananas, daily for 3 days beginning August 16, 1946.

TABLE I

Experiments 1 and 2. Homologous and Heterologous Challenge by the Oral Route of Four Chimpanzees Infected with Y-SK Poliomyelitis Virus 1 Year Previously

The response of these animals to earlier exposure to the Y-SK strain appears in reference 1.

Chimpanzee	Date	Virus	Test in monkeys for virus in chimpanzees' stools*	Serum titer of Lansing antibodies‡
		1. Homologous challenge. Y-SK strain		
Webb	Nov. 16, 1945			1:100
	Mar. 28–29, 1946	9 ml. monkey CNS	0/1	
	Apr. 3–26		0/3	
	July 3			1:100
Flora	Aug. 10, 1945			1:75
	Mar. 28–29, 1946	9 ml. monkey CNS	0/1	
	Apr. 3–26		0/3	
	July 7			1:100
Jent	Nov. 15, 1945			1:100
	Mar. 28–29, 1946	9 ml. monkey CNS	0/1	
	Apr. 3–26		0/3	
	July 5			1:100
Falla	Mar. 28–29, 1946	9 ml. monkey CNS	0/1	
	Apr. 3–26		0/3	
	July 5			1:100
		2. Heterologous challenge. NC strain		
Webb	Aug. 16–18, 1946		0/1	
	Aug. 27–Sept. 14	4 ml. human stool	1/4	
	Oct. 24			1:100
Flora	Aug. 16–18, 1946		0/1	
	Aug. 27–Sept. 14	4 ml. human stool	1/3	
	Oct. 2			1:500
Jent	Aug. 16–18, 1946		0/1	
	Aug. 28–Sept. 6	4 ml. human stool	2/2	
	Oct. 2			1:250
Falla	Aug. 16–18, 1946	4 ml. human stool	1/1	
	Aug. 27–Sept. 14		1/3	

* Denominator indicates number of tests in monkeys; numerator, number of positive tests.

‡ Serum antibody titers given in this and subsequent tables represent the 50 per cent end-point of serum dilutions against 30 to 100 ID^{50} doses of virus.

The results are also tabulated in Table I. During feeding, Falla alone excreted virus. However, all four animals again became carriers of the new (NC) strain, virus being found in their stools 23 to 27 days (Falla), 15 to 19 days (Webb and Jent), and 9 to 13 days (Flora) after feeding. The pre- and post-feeding sera of three of the animals were tested for neutralizing antibodies against the Lansing strain. Two of the three chimpanzees (Flora and Jent) were found to have a slight and probably insignificant increase in Lansing antibodies. As mentioned earlier, the strain with which they were now infected, appears to be unrelated to the Lansing type.

A summary of experiments with Falla, Webb, Jent, and Flora is given in Fig. 1, in which the present data have been added to those reported in our earlier paper (1). All four animals developed the carrier state on their first exposure to Y-SK virus, all resisted homologous challenge, and all became carriers again when challenged with NC, an heterologous strain.

Experiments with Three Other Chimpanzees,—Hickory, Catawaba, and Jada

The first experiences of Hickory and Catawba with poliomyelitis virus have also been reported in detail (1). They will be summarized in brief because the animals were again employed.

These animals had been fed poliomyelitis virus on three occasions,—first in July, 1944 (fly-contaminated food collected in epidemic areas in North Carolina and New York, second in April, 1945 (Y-SK strain), and third in May, 1945 (NC strain). Hickory became an intestinal carrier following each exposure, but Catawba only after the first exposure in July, 1944. Neutralization tests revealed that Catawba had antibodies to the Y-SK strain before the exposure to this strain in April, 1945, but that Hickory developed such antibodies only following her exposure to the Y-SK strain.

Experiment 3. Homologous Challenge with Y-SK Strain.—In January of 1947, after an 18 month sojourn in Florida, Hickory and Catawba were returned to our laboratory in New Haven and found to have retained their antibody levels to the Y-SK strain. They were challenged again with homologous and heterologous strains of virus. First they were fed Y-SK mouse CNS: 15 ml. of a 10 per cent suspension in bananas over a 3 day period. A control chimpanzee, Jada, never before exposed to virus, was fed an aliquot of the same material at the same time.

Neither Hickory nor Catawba excreted virus during feeding or thereafter; nor did their serum antibody level change (Table II). Jada's stools, however, contained virus during feeding and several weeks later, and neutralizing antibodies appeared in her serum. This animal developed fever and an upper respiratory infection 13 days after being fed virus. Throat swabs and stools collected on that day were negative for poliomyelitis virus when tested in monkeys, but a stool pool collected from the 14 to 18th day after feeding was positive in a monkey, but negative in mice and cotton rats. Jada seemingly

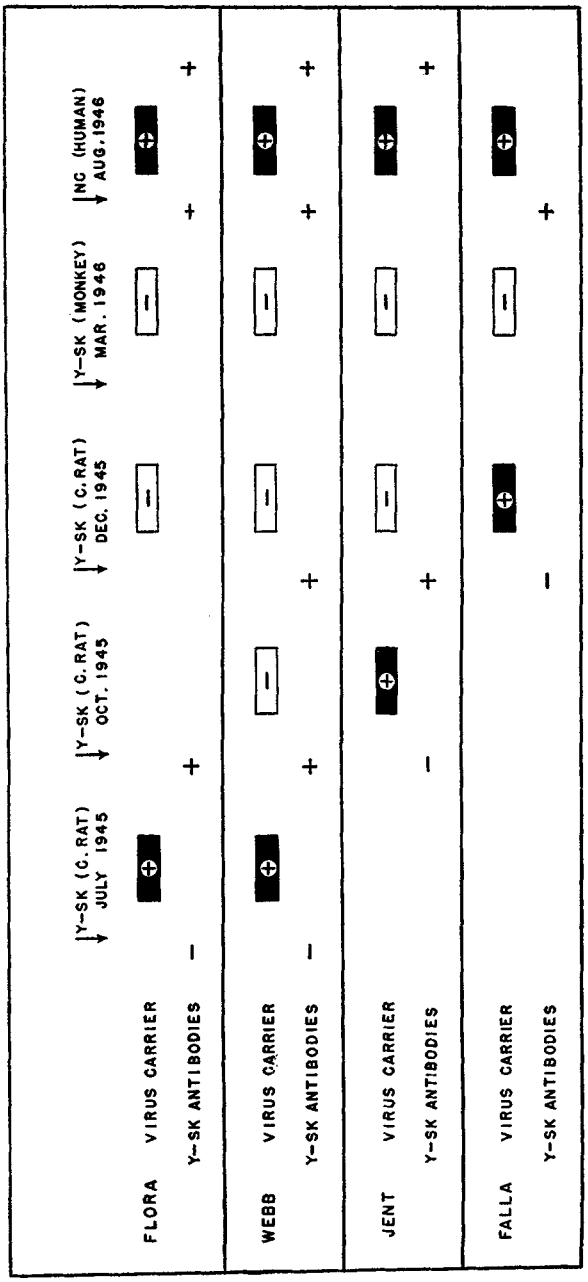

FIG. 1. Results of Experiments 1 and 2. Demonstration in chimpanzees of (*a*) intestinal immunity to homologous Y-SK strain, and (*b*) lack of such immunity to heterotypic NC strain of poliomyelitis virus. A carrier state is indicated by a black rectangle. The development of Y-SK antibodies is shown in relation to indicated exposures to virus. In these and subsequent figures the term "Y-SK antibodies" is used even though the homotypic Lansing strain may have been used in the neutralization test.

recovered from this mild illness which may possibly have been abortive poliomyelitis, but her temperature remained at subnormal levels (95–97°F.) for the subsequent few days. She died suddenly during the night on the 24th

TABLE II

Experiments 3 and 4. Homologous Challenge by the Oral Route of Hickory and Catawba, after 21 Months Interval (1), with Y-SK Strain, and Heterologous Challenge with FE Strain

Chimpanzee	Date	Virus	Test for virus in chimpanzees' stools in			Serum titer of Lansing antibodies
			Mice	Cotton rat	Monkey	
1. Homologous challenge. Y-SK strain						
	1947					
Hickory	Jan. 23–25	15 ml. mouse CNS	0/12	0/4	0/1	1:75
	Feb. 5–26		0/36	0/13	0/3	
	Mar. 13					1:75
Catawba	Jan. 23–25	15 ml. mouse CNS	0/12	0/4	0/1	1:75
	Feb. 5–26		0/36	0/14	0/3	
	Mar. 13					1:75
Jada (control)	Jan. 23–25	15 ml. mouse CNS	1/12	0/4	1/1	0
	Feb. 5–16		0/38	0/20	1/4	
	Feb. 15					1:50
2. Heterologous challenge. FE strain						
Hickory	Mar. 14–17	18 ml. monkey CNS			0/1	1:75
	Mar. 23–Apr. 9				1/3	
	Apr. 29					1:75
Catawaba	Mar. 14–17	18 ml. monkey CNS			1/1	1:75
	Mar. 25–Apr. 9				0/4	
	Apr. 29					1:75
Becky (control)	Mar. 14–17	17 ml. monkey CNS			1/1	1:9
	Mar. 23–Apr. 29				6/7	
	Apr. 29					1:600

day after being fed virus. At autopsy, a diffuse hemorrhagic bronchiolar pneumonia was found. The CNS on gross examination was soft (postmortem autolysis); sections taken at various cord levels were negative for poliomyelitis lesions. CNS and colon contents were tested for poliomyelitis virus in monkeys and rodents with negative results.

Experiment 4. Heterologous Challenge with Far East (FE) Strain.—Hickory and Catawba, having been demonstrated to possess immunity to homologous

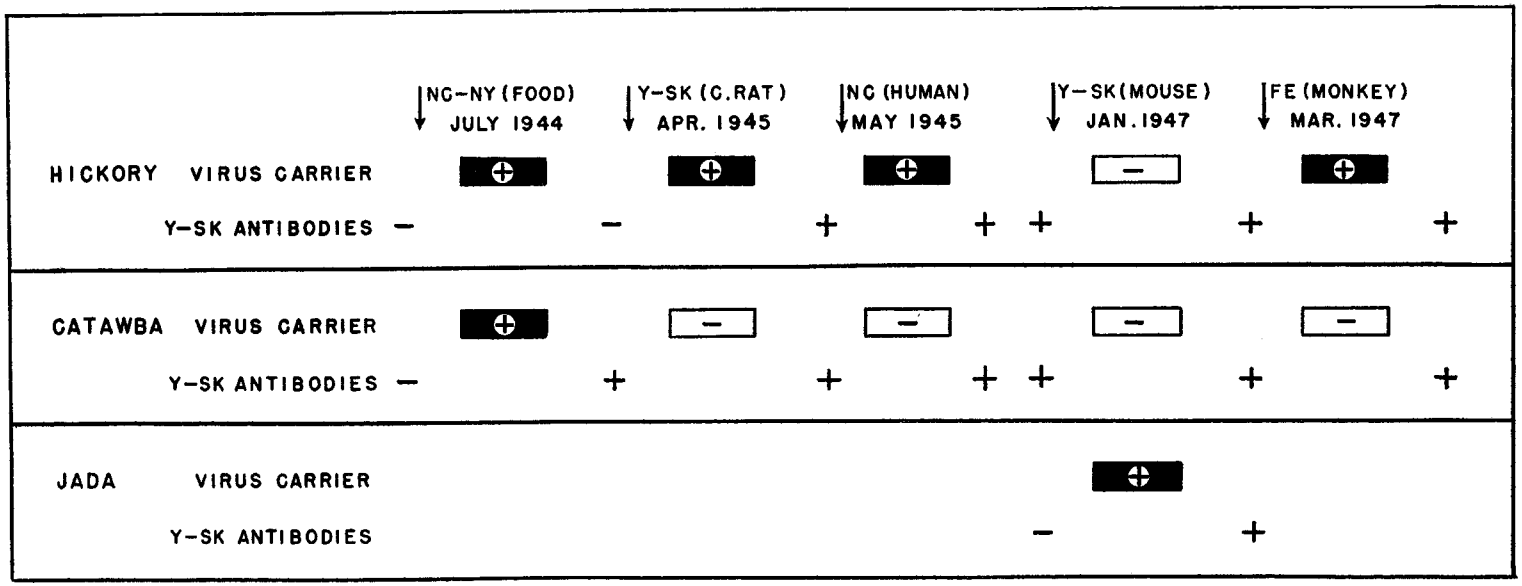

FIG. 2. Results of Experiments 3 and 4.

challenge with Y-SK strain, were given an heterologous challenge with FE strain, being fed 18 ml. of infected monkey CNS over a 3 day period. A control chimpanzee, Becky, who had never been exposed to virus previously, was fed FE strain at the same time.

The results are shown in Table II. Hickory once more became a virus carrier (her fourth time as such); Catawba once more resisted reinfection; and Becky became infected as a result of her first known exposure to poliomyelitis virus. The antibody level of the serum of Hickory and Catawba against the Lansing strain was unaffected by exposure to the FE strain.

A summary of the total known experiences of Hickory and Catawba with poliomyelitis virus is given in Fig. 2. These animals behaved differently as far as resistance to reinfection with heterologous strains is concerned. Catawba, on the one hand, developed a broad immunity, probably as a result of her first exposure to virus, and subsequently resisted homologous and heterologous challenge. In view of the nature of the fly-contaminated food to which she was first exposed, she may have had experience with several strains originally (1). Hickory, in contrast, became a carrier 4 times. She did resist *homologous* challenge with Y-SK strain, however.

Experiments with Chimpanzee Becky

Becky, whose experience is summarized in Fig. 3, had become a carrier on her first exposure to virus (Experiment 4 above, FE strain, March 14 to 17, 1947) when she served as a control for heterologous challenge of Hickory and Catawba. On her arrival in the laboratory, January 21, 1947, her serum contained no neutralizing antibodies to the Lansing or Y-SK strain, but before the FE strain was fed to her, she already had serum-neutralizing antibodies to Lansing strain (1:9) and to Y-SK strain (1:10). The appearance of antibodies before any virus was given, suggests that this chimpanzee had been infected with Y-SK or Lansing virus accidentally in the laboratory, as both of these strains were in use in adjacent animal quarters at the time. Such accidental infection of chimpanzees has been reported previously by Howe and Bodian (11). Control prefeeding stools of February 11 to 15 and March 13 to 14 were tested in 3 monkeys, but were negative for poliomyelitis virus.

While a carrier of FE strain, Becky's antibody titer rose from 1:9 to 1:600 against the Lansing strain, and from 1:10 to 1:500 against Y-SK. It cannot be said certainly whether or not this increase would have occurred if the FE carrier state had not been established. However, in contrast, Hickory, who had Lansing antibodies at the time she was fed FE and also became an FE carrier, showed no change in titer as a result of experience with FE strain. From the change in titer of Becky's serum antibodies, it would appear that Becky had been accidentally infected about March 1, 1947, with the Lansing, Y-SK, or some homotypic strain.

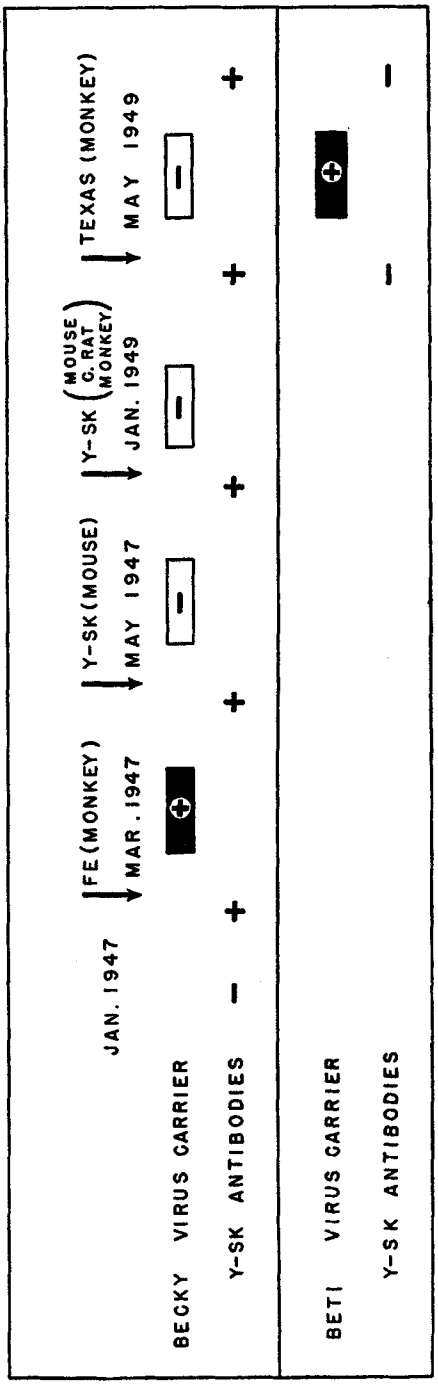

FIG. 3. Results of Experiments 5 to 7.

Experiment 5. Challenge with Y-SK Strain.—During March and April 1947, Becky had been a carrier of FE strain, and she also had developed at about the same time antibodies against Y-SK strain. From April 30 to May 2 she was challenged by the oral administration of 10 ml. daily of 10 per cent Y-SK monkey and mouse cord suspension in milk. Subsequently it was discovered that on the day that Y-SK was fed, Becky was still excreting virus in her stools as a result of infection with FE strain. During the month following Y-SK feeding she continued to excrete virus, and the question arose as to whether it was still FE strain, or whether a second infection had been established with Y-SK strain. As we have never been able to infect a chimpanzee with Y-SK in the presence of neutralizing antibodies against that strain, it seemed likely that Becky continued to be an FE carrier. This was proved to be the case while attempting to identify the virus in her stools excreted between May 19 and 23, 3 weeks after being fed Y-SK. This was done in the following manner:

Rhesus 35-84 was inoculated intracerebrally with Becky's stool collected between May 19 and 23, 1947. The monkey developed typical poliomyelitis, and the diagnosis was confirmed by the presence of characteristic histological lesions. Its cord was passed to *Rhesus* 44-26, which also developed typical poliomyelitis. Rh 44-26 was allowed to convalesce, and during late convalescence was hyperimmunized by repeated intraperitoneal and intramuscular injections of 10 per cent cord suspension of *rhesus* 35-84. Bleedings from *rhesus* 44-26 were collected at suitable intervals, and a neutralization test was set up against Lansing virus. Sera collected before inoculation, during convalescence, and after hyperimmunization were set up against several dilutions of virus. The results indicated no neutralization of Lansing virus by any of the sera, including the undiluted, hyperimmune serum. Following such a course of immunization of a convalescent Y-SK monkey, we have always found Y-SK and Lansing antibodies present in high levels (over 1:100 and usually 1:1000).

It was concluded that *rhesus* 44-26 which showed characteristic lesions of poliomyelitis at autopsy, had not been infected with Y-SK strain, and Becky's stool of May 19 to 23, therefore, did not contain Y-SK virus. At the time of her first Y-SK feeding, Becky was apparently excreting FE strain, and continued to do so for another month.

Experiment 6. Homologous Challenge with Y-SK Strain.—Twenty months after her last exposure to this strain, Becky, in January, 1949, received 9 ml. of a 33 per cent suspension of virus (monkey-mouse-cotton rat cord). Her titer of neutralizing antibodies before feeding was $> 1:100$; 3 months later it was the same and virus was not detected in her stools at any time during the month following feeding. She was thus never demonstrated to be a Y-SK carrier, although presumably she was accidentally infected with this strain, for she developed antibodies against it early in the course of her stay in our laboratory, and resisted infection with it twice subsequently.

Experiment 7. Heterologous Challenge with Texas Strain.—One further virus exposure was given to Becky in May, 1949. At that time she was fed Texas 1948 strain, receiving 6.5 ml. of cord suspension. She did not become a carrier

as a result of this heterologous challenge, but it should be recalled that she had previously been infected with two different immunologic types of poliomyelitis virus. Her antibody level against Lansing strain remained fairly constant from April, 1947, through June, 1949, varying only between 1:100 and 1:500. A control animal, Beti, was fed the Texas strain together with Becky and, as seen in Table III and Fig. 3, she became a carrier of this virus. Lansing antibodies did not result following this infection.

Experiments with Chimpanzees Rosebud and Mary Lou

Experiment 8. First Exposure to Virus.—Rosebud and Mary Lou arrived in the laboratory in February, 1947. Their first known exposure to virus was in May, 1947, when each was fed virus in the form of naturally infected human stools. Rosebud received the Pittsfield 1946 strain, Mary Lou the North Carolina 1944 strain. Feedings were of 4 ml. amounts, daily for 3 days.

Both chimpanzees became virus carriers and excreted virus for at least 3 and 5 weeks respectively which was the period covered by the stool tests (Table IV). Neutralization tests on pre- and postfeeding sera indicated that Rosebud's serum contained no neutralizing antibodies against the Lansing strain either before or after her being a Pittsfield strain carrier. Mary Lou, on the other hand, already had such Lansing neutralizing antibodies which had risen from a titer of 1:8 to 1:100 previous to her first known virus exposure. The fact that Lansing antibodies were present *before* ingestion of NC virus suggests that Mary Lou, like Becky, became accidentally infected with Y-SK or a homotypic strain in our laboratory.

Experiment 9. Challenge with Y-SK Strain.—After an interval of 8 months, both Rosebud and Mary Lou were given heterologous challenge with Y-SK strain, each being fed 6 ml. daily for 2 days on January 16 and 17, 1948. Neither animal excreted virus in the stools following this virus exposure (Table IV). For Mary Lou, this was not surprising, since she already had a high titer of Lansing (or Y-SK) antibodies at the time of challenge. Rosebud, on the other hand, had no antibodies against Lansing virus at the time she was fed the related Y-SK strain, but did develop them some time during the ensuing 4 months. Because of extreme difficulty in handling this animal, she was not bled until June 5, 1948. Her serum of this date had a titer of 1:550 against Lansing virus (Table IV). This indicates that, by the apparently more sensitive serological method of testing, Rosebud *did* become infected with Y-SK strain, and our failure to demonstrate virus in her stools was due perhaps to the low level of virus excretion or to some technical deficiency.

Experiment 10. Homologous Challenge with Y-SK Strain.—As further test of her immunity, Rosebud was given an homologous challenge with Y-SK virus 1 year later, on January 6, 1949 (Table IV). Her antibody titer before and after this challenge was $>$ 1:100, and she did not excrete virus during the

TABLE III

Experiments 5, 6, and 7. Chimpanzees Becky and Beti. Experiences with Different Strains of Poliomyelitis Virus by Oral Administration

Chimpanzee	Date	Virus	Test in monkeys for virus in chimpanzees' stools	Serum titer of antibodies against	
				Lansing	Y-SK
1. First virus exposure. FE strain					
Becky	Jan. 21, 1947			0	0
	Feb. 11–15		0/2		
	Mar. 13		0/1	1:9	1:10
	Mar. 14–17	17 ml. FE monkey CNS			
	Mar. 23–Apr. 29		6/7		
2. Second virus exposure. Y-SK Strain					
Becky	Apr. 29			1:600	1:500
	Apr. 30	30 ml. monkey-mouse CNS			
	Apr. 30–May 23*		4/4		
	June 11			1:500	1:500
	July 31				1:200
	Sept. 23				1:100
2. Third virus exposure. Y-SK strain					
Becky	Sept. 15, 1948			>1:100	
	Jan. 10, 1949			>1:100	
	Jan. 11–13	9 ml. monkey-rodent CNS			
	Jan. 17–Feb. 9		0/3		
	Apr. 27			>1:100	
4. Fourth virus exposure. Texas 1948 strain					
Becky	Apr. 27, 1949			>1:100	
	May 26	6.5 ml. monkey CNS		1:550	
	June 6–30		0/3		
	June 28			1:100	
Beti (control)	May 26, 1949	6.5 ml. monkey CNS		0	
	June 6–29		2/4		
	June 28			0	

* Although given Y-SK on April 30, Becky continued to excrete FE strain during the period April 30 to May 23, 1947.

month following feeding. The material given her at this time was a 33 per cent mixture of monkey, mouse, and cotton rat cord, which had a titer of $> 10^{-4}$ in monkeys.

A summary of the results with Rosebud and Mary Lou is given in Fig. 4. Rosebud apparently became a carrier of two unrelated strains, Pittsfield and

TABLE IV

Experiments 8, 9, and 10. Chimpanzees Rosebud and Mary Lou. Oral Exposure to Human Source of Virus Followed by Challenge with Y-SK Strain

Chimpanzee	Date	Virus	Test for virus in chimpanzees' stools			Serum titer of Lansing antibodies
			Mice	Cotton rat	Monkey	
1. First virus exposure. Pittsfield strain						
Rosebud	Mar. 10, 1947					0
	May 12–14	12 ml. human stool				
	May 14–June 7				3/3	
	June 23					0
2. Heterologous challenge. Y-SK strain						
Rosebud	Jan. 15, 1948					0
	Jan. 16–17	12 ml. monkey-rodent CNS				
	Jan. 17–Feb. 14		0/48	0/16	0/6	
	June 5					1:550
3. Second challenge. Y-SK strain						
Rosebud	Jan. 6, 1949					>1:100
	Jan. 11–13	9 ml. monkey-rodent CNS				
	Jan. 17–Feb. 9				0/4	
	Apr. 8					>1:100
1. First virus exposure. NC strain						
Mary Lou	Mar. 20, 1947					1:8
	Apr. 30					1:100
	May 12–14	12 ml. human stool				
	May 14–June 23				4/4	
	June 23					1:100
2. Heterologous challenge. Y-SK strain						
Mary Lou	Jan. 15, 1948					>1:100
	Jan. 16–17	12 ml. monkey-rodent CNS				
	Jan. 17–Feb. 14		0/31	0/12	0/6	
	May 5					>1:100

Y-SK, and resisted homologous challenge with the latter. Mary Lou, on the other hand, was demonstrated to have become a carrier only of NC strain. However, from the antibody determinations, it would appear that she was

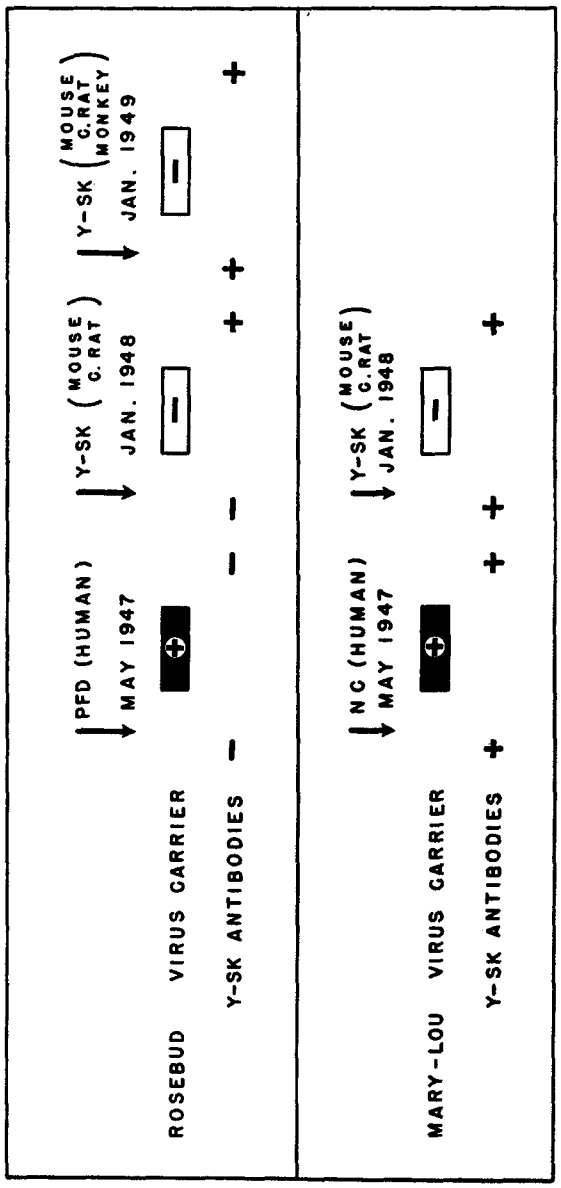

FIG. 4. Results of Experiments 8 to 10.

accidentally infected with a Lansing homotypic strain about March 10, 1947, soon after her arrival in the laboratory. The NC strain was not fed until May 12, 1947. She subsequently resisted challenge with Y-SK strain, in accordance with previous results in chimpanzees with Lansing or Y-SK antibodies.

Experiments with Chimpanzees Pinta, Nina, and Santa Maria

Experiment 11. Exposure to FA Type of Mouse Encephalomyelitis Virus.—In January, 1946, three chimpanzees were transferred to the poliomyelitis laboratory from "isolation quarters" five stories below, where for some months they had been housed while being used in experiments with the virus of infectious hepatitis. On January 29 they were bled for serum, and control stools were collected. On the same day, two of the animals were given virus and one was kept as a control. The virus was a strain of FA mouse encephalomyelitis grown in embryonated chicken eggs. A 20 per cent suspension of chick embryo was used. Pinta received 3 ml. intra- and subcutaneously in piqûres on the left arm and thigh; this dose was repeated twice 3 and 4 days later. Maria was fed 6 ml. of the same material on 4 consecutive days beginning January 29, and again on 3 consecutive days beginning February 4. Nina, the control chimpanzee, received no virus.

Table V indicates the results obtained. Pinta, who was inoculated intra- and subcutaneously, did not excrete FA virus in her stools during any of the periods tested. This is in contrast to our experience with the Y-SK strain of poliomyelitis virus, which, when inoculated intracutaneously, has produced an intestinal carrier state in three of three chimpanzees on their first exposure (1). Maria, who ingested FA virus daily over one 4 and one 3 day period (January 29 to February 1, February 4 to 6) was found to excrete it during the first feeding period and in the pool of stools collected on the 5th, 6th, and 7th days after the second feeding. Neutralization tests against Lansing and FA strains were carried out on Pinta's serum collected before and 2 months after cutaneous inoculation of FA virus. Neither serum neutralized Lansing virus. The preinoculation serum did not neutralize FA and the undiluted postinoculation one neutralized only one log of virus (neutralization index of 10) which is not considered significant. Samples of Maria's serum, collected before and 6 months after being fed FA virus, contained no neutralizing antibodies against FA.

Experiments 12, 13, and 14. Exposure and Challenge with Y-SK and NC Strains of Poliomyelitis Virus.—A summary of experiments with Nina, Pinta, and Santa Maria appears in Fig. 5; the details of the experiments are presented in Table V. The response of these animals, for some unknown reason, was not as regular as with previous animals. Thus Pinta and Maria are the only animals in this series of eighteen who failed to become carriers on their first

TABLE V

Experiments 11, 12, 13, and 14. Chimpanzees Pinta, Nina, and Santa Maria. Exposures to Mouse Encephalomyelitis Virus (FA) and Two Types of Poliomyelitis Virus

Chimpanzee	Date	Virus	Test for virus in chimpanzees' stools			Serum titer of antibodies against	
			Mice	Cotton rat	Monkey	Lansing	FA
1. Exposure to FA encephalomyelitis virus							
Pinta	Jan. 29, 1946					0	0
	Jan. 29–Feb. 5	9 ml. egg cutaneously	0/24	0/4	0/1		
	Feb. 5–Mar. 1		0/46				
	Apr. 1					0	0
Santa Maria	Jan. 29, 1946					0	0
	Jan. 29–Feb. 1	24 ml. egg orally	3/12	0/4			
	Feb. 2–4		8/10				
	Feb. 4–6	18 ml. egg orally					
	Feb. 5–8		0/12				
	Feb. 11–13		8/22				
	Feb. 18–Mar. 1		0/12				
	Aug. 9						0
2. First exposure to poliomyelitis virus Y-SK strain							
Pinta	Apr. 1, 1946					0	
	Apr. 1–2	8 ml. cotton rat CNS orally	10/12	3/4	0/1		
	Apr. 7–May 3		0/47	0/17	0/4		
	July 5					0	
Santa Maria	Apr. 1–2, 1946	8 ml. cotton rat CNS orally	5/23	0/9	0/1		
	Apr. 7–May 3		0/48	0/18	0/4		
Nina	Jan. 29, 1946					1:5	
	Apr. 1–2	8 ml. cotton rat CNS orally	9/12	1/4	0/1		
	Apr. 7–May 3		0/36	0/14	1/4		
	July 5					1:50	
3. Homologous challenge. Y-SK strain							
Pinta	July 5, 1946					0	
	July 12	2 ml. mouse CNS cutaneously					
	July 15–Aug. 3		0/43	0/12	0/4		
	Aug. 9					1:50	

TABLE V—*Concluded*

Chimpanzee	Date	Virus	Test for virus in chimpanzees' stools			Serum titer of antibodies against	
			Mice	Cotton rat	monkey	Lansing	FA
3. Homologous challenge Y-SK strain—*Continued*							
Santa Maria	July 12–13, 1946	6 ml. mouse CNS orally	0/10	0/4	0/1		
	July 15–Aug. 13		0/32	0/13	0/3		
	Aug. 9					1:50	
Nina	July 5, 1946					1:50	
	July 12–13	6 ml. mouse CNS orally	0/11	0/4	0/2		
	July 15–Aug. 3		0/23	0/8	0/3		
	Aug. 9					1:50	
4. Heterologous challenge. NC strain							
Pinta	Aug. 16–18, 1946	4 ml. human stool orally			0/1	1:50	
	Aug. 27–Sept. 14				0/4		
	Oct. 2					1:50	
	Dec. 11					1:30	
Santa Maria	Aug. 16–18, 1946	4 ml. human stool orally			0/1	1:50	
	Aug. 27–Sept. 14				1/3		
	Feb. 8, 1947					1:100	
Nina	Aug. 16–18, 1946	4 ml. human stool orally			0/1	1:50	
	Aug. 27–Sept. 14				0/3		
	Oct. 2					1:50	

exposure to poliomyelitis virus. They were also the only two animals in the series who had been given a primary exposure (non-infectious) to the FA type of mouse encephalomyelitis virus. Not only did Pinta fail to become a carrier, but she did not develop neutralizing antibodies, indicating that infection with Y-SK strain did not occur. Subsequently, Pinta did develop neutralizing antibodies on challenge with Y-SK, but at no time was she demonstrated to be a carrier of this strain, or of any other. Nina, on the other hand became a carrier of Y-SK, developed neutralizing antibodies, and resisted both homologous and heterologous challenge subsequently. Maria developed neutralizing antibodies as a result of either her first or second exposure to Y-SK strain, but was only demonstrated to have become a carrier when

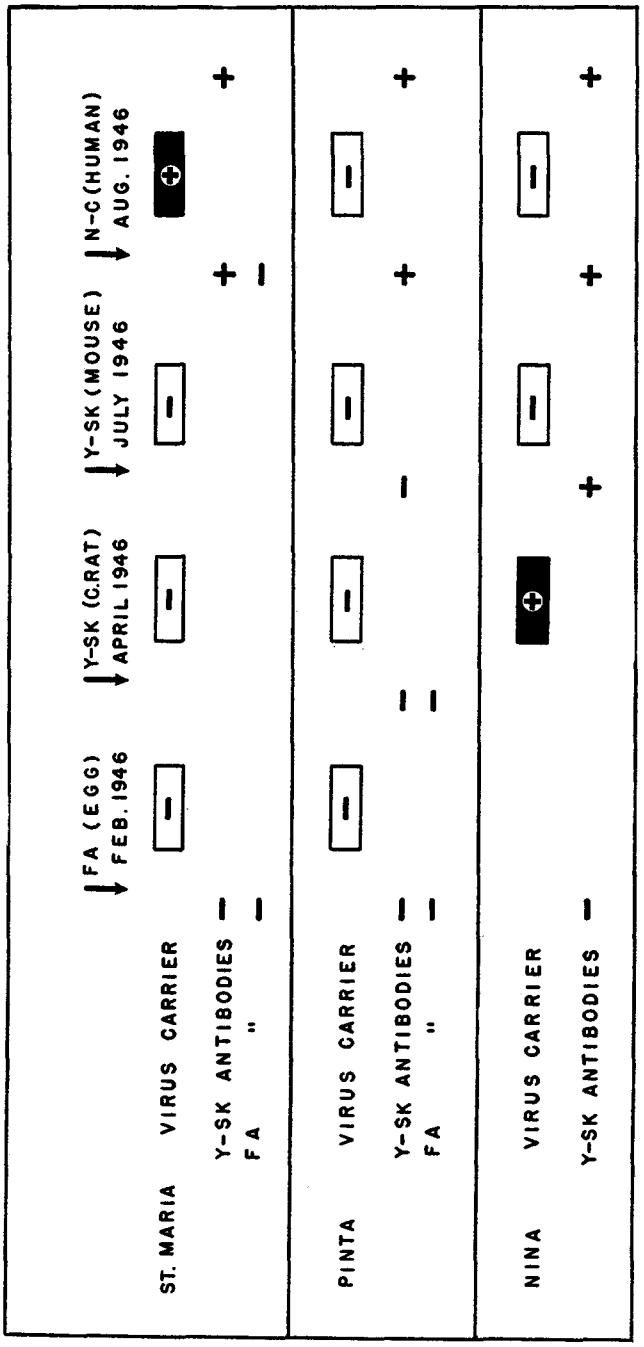

FIG. 5. Results of Experiments 11 to 14.

given heterologous challenge with NC strain. All three animals were older (being about 4 years of age) than the rest in the series, which may possibly be one reason that their responses were different.

Experiments with Chimpanzees Donna and Alamo

Experiment 15. First Exposure to Poliomyelitis Virus. Time at Which Antibodies Develop.—Donna and Alamo arrived in the laboratory in February, 1949, but were not fed the Y-SK strain until May 26, 1949. No Lansing or Y-SK antibodies had developed in this interval.

TABLE VI

Experiment 15. Chimpanzees Donna and Alamo. First Exposure to Poliomyelitis Virus(Y-SK). The Time at Which Neutralizing Antibodies Develop Following Oral Administration of Virus

Chimpanzee	Date	Virus	Test in monkeys for virus in chimpanzees' stools	Serum titer of Lansing antibodies
	1949			
Donna	May 26	9 ml. monkey CNS		0
	June 2			0
	June 6–10		0/1	
	June 11			1:55
	June 14–29		2/2	
	June 28			1:65
Alamo	May 26	9 ml. monkey CNS		0
	June 2			0
	June 6–10		0/1	
	June 11			1:55
	June 14–29		1/3	
	June 28			1:65

Both animals became carriers (Table VI), and developed antibodies to the Lansing strain. In order to determine the interval between ingestion of virus and the appearance of antibodies, neutralization tests were carried out on the serum of Donna and Alamo collected on the 7th and 16th days after virus was fed. For both animals, the tests were negative for the preinoculation serum, and for the serum collected 7 days after ingestion of virus. The first serum to contain antibodies was that collected on the 16th day; the LD_{50} titer for the sera of both animals was 1:55. By the 34th day, the titer for both was of the same order of magnitude, 1:65. Thus, these two chimpanzees developed neutralizing antibodies sometime between the 7th and 16th days after ingestion of virus and *before* virus was excreted in detectable amounts in their stools (Fig. 6). The early development of antibodies is similar to that reported by von Magnus and Melnick who followed the development of neutralizing anti-

bodies in orally infected *cynomolgus* monkeys (12, 13). The serum titers increased very little between the 17th and 34th days. As with natural infection in man (14, 15), the chimpanzees continued to excrete virus in their stools in the presence of circulating antibodies.

Attempts to Demonstrate Neutralizing Antibodies in Stools Following Alimentary Carriage of Poliomyelitis Virus

Experiment 16.—To determine whether or not antibodies are present in the feces in poliomyelitis requires that proper consideration be paid to the

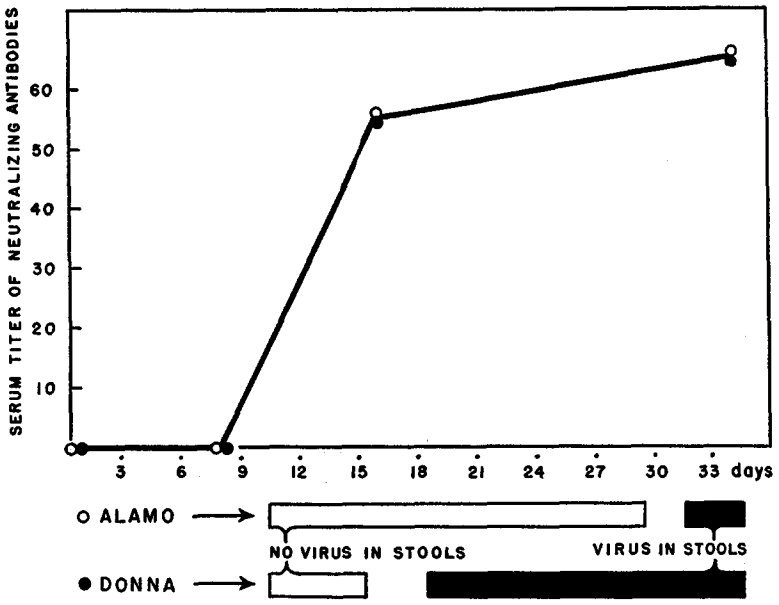

FIG. 6. Appearance of antibodies in serum before detectable excretion of virus in stools. Chimpanzees Alamo and Donna were infected by the oral administration of the Y-SK strain.

strain which produced the disease. An effort was made to answer this question by searching for antibodies in the stools of some of the chimpanzees infected with Y-SK strain.

In these tests we employed aqueous extracts of stools; and in addition, the "gamma globulin" concentrates from such stool extracts. This latter fraction was concentrated by precipitation with alcohol in the cold according to the methods used by Cohn for concentrating gamma globulin from serum. A further attempt was made in some instances to determine whether any masked antibody was present by separating the original extract into a fraction containing small protein molecules and one containing large protein "molecules" by sedimentation of the latter in the ultracentrifuge. Ea th of the fractions was tested for antibody content.

TABLE VII

Experiment 16. Search for Virus-Neutralizing Substances in Stools

Chimpanzee	History	Date	Results of tests for antibody in				
			Serum (Titer)	Untreated stool extract	Gamma globulin concentrate from stools	Ultracentrifuged stools	
						Supernate	Sediment
Becky	Before exposure to Y-SK strain	Jan. 21, 1947	0	—			
	2 mos. after infection with Y-SK strain	June 30	1:200	—	—	—	—
	5 mos. after infection with Y-SK strain	Sept. 23	1:100	—	—		
Falla	Before exposure to Y-SK strain	Dec. 10, 1945	0	—		—	—
	6 mos. after infection with Y-SK strain	July 5, 1946	1:100	—		—	—
Hickory	2 yrs. after infection with Y-SK strain	Feb. 26, 1947	1:75	—	—		

TABLE VIII

Response of Eighteen Chimpanzees to Oral (or Intracutaneous) Administration of Poliomyelitis Virus

Group 1 received Y-SK strain first. Group 2 received other strains immunologically unrelated to Y-SK, on their first exposure, but subsequently four animals in this group received two challenges with Y-SK virus.

Group	First exposure to virus		Homologous or homotypic challenge		Heterotypic challenge	
	No. infected	Per cent	No. infected	Per cent	No. infected	Per cent
1	10/12	83	0/7	0	5/7	71
2	6/6	100	1/5	20	2/3*	67
Totals.....	16/18	89	1/12	8	7/10	70

* These animals include Hickory, Catawba, and Rosebud. Not included are Mary Lou and Becky because it appears that these two animals accidentally became infected with the Y-SK, or a related strain, before the "heterotypic" challenge with Y-SK virus.

As shown in Table VII, all tests for homologous antibody in chimpanzee stools from 2 months to 2 years after inapparent poliomyelitic infection were negative, even though all the postinfection serum samples taken at the same

times were positive. These results with chimpanzees do not indicate that specific antibodies are present in the stools following intestinal carriage of virus.

DISCUSSION

The results reported in the above experiments are summarized in Table VIII. It is clear that, as has been observed previously (1–3), chimpanzees readily become infected on being exposed to poliomyelitis virus by oral and cutaneous routes, but only occasionally develop obvious signs of disease after such peripheral administration. Thus thirteen of fifteen animals in the present series became healthy carriers on being fed virus, and an additional one did so after cutaneous inoculation. These results, when added to those reported previously with two other animals, who both became carriers following cutaneous injection of virus (16, 17), bring the total number studied in this laboratory to eighteen. Sixteen, or 89 per cent of these, became intestinal carriers on their first exposure to poliomyelitis virus.[2] On being challenged with a strain of which they had previously been carriers (or, in the case of the non-Lansing strains, presumably one of the same type), one of twelve (8 per cent) became reinfected. However, when challenged with a heterotypic strain with which they had had no previous experience, seven of ten animals (70 per cent) became carriers again. This suggests that infection with a certain strain of poliomyelitis virus conveys a high degree of immunity to that strain, but not to heterotypic strains. Essentially similar findings have been obtained by Howe, Bodian, and Morgan (3).

Interpreted in the light of possible mechanisms of immunity in the human population, the results with chimpanzees suggest that perhaps repeated asymptomatic infection with various strains of virus is the basis of the increasing immunity which occurs in man with increasing age. It has been repeatedly demonstrated that during epidemic times many healthy intestinal carriers exist, especially in the younger age groups, and it is only on the basis of large numbers of such carriers that one can at present account for the presence of poliomyelitis virus in urban sewage (10). In man then a carrier state may follow upon each exposure to a different strain until resistance to a wide variety of strains is finally achieved. However, in view of the occurrence of adult carriers it has been suggested (3, 18) that the duration of immunity to alimentary carriage may not be long lived, even to homotypic strains, and that repeated carrier states even to homotypic types are necessary for the maintenance of high antibody levels and immunity to the paralytic form of the

[2] The two animals who failed to become carriers had been exposed, one by the oral route and one by the cutaneous route, to the FA type of mouse encephalomyelitis virus. No significant amount of antibodies to either the FA or the Y-SK strain was present before the exposure to the Y-SK strain.

disease. In this connection Howe, Bodian, and Morgan have obtained infection with homologous or homotypic strains in four chimpanzees, and we have had one animal (Hickory) who became a carrier on four occasions, three of which were with non-Lansing types. It is entirely likely that two, if not all three, of these strains belong to the common Brunhilde type (3, 19). In these five animals exhibiting infection with a homotypic strain, the carrier state was relatively short lived, lasting only for a few days, suggesting that the previous infection, even though not conferring a solid immunity to alimentary carriage, did contribute to early cessation of the reinfection.

In our chimpanzee experiments, the development of neutralizing antibodies after exposure to poliomyelitis virus has generally been correlated with the intestinal carriage of virus. Consequently we have assumed that the most likely explanation of the development of neutralizing antibodies in the absence of a demonstrable carrier state following exposure to virus, which occurred in two animals, is that the carrier state was transient, or the level of virus excretion was low, or that both these factors as well as other technical ones were in operation. Another alternative, however, is that infection (virus multiplication and antibody formation), may have occurred without the development of the carrier state.

Six animals who received strains other than Y-SK as their first exposure to poliomyelitis virus, became carriers, but only one (Catawba) developed Lansing and Y-SK antibodies following this infection. In view of the possibility that the material which she received contained more than one immunological type of poliomyelitis virus, no decision can be reached as to whether or not she was also accidentally infected in the laboratory with a Lansing type of virus. Such "spontaneous" infection of uninoculated animals apparently occurred in two of our chimpanzees (Mary Lou and Becky) and has been observed previously by Howe and Bodian (11). In any case, that the antibody response was *specific* is indicated by Catawba's (and Becky's and Mary Lou's) subsequent resistance to challenge with the Y-SK strain.

SUMMARY

The response of eighteen chimpanzees to poliomyelitis virus administered orally and cutaneously has been studied. There were no signs of weakness or paralysis in any of the animals. Sixteen of the eighteen (89 per cent) became infected as measured by intestinal carriage of virus. Only one of twelve was reinfected when challenged with an homologous (or homotypic) strain by the same routes, but seven of ten were reinfected on heterologous challenge. A correlation between the development of humoral antibodies and resistance to reinfection was demonstrated.

During the course of these experiments, two chimpanzees acquired inapparent poliomyelitis by accidental contagion in the laboratory.

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