

Poliomyelitis Virus in Blood of Orally Infected Monkeys and Chimpanzees.* (19398)

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Although poliomyelitis virus has occasionally been isolated rather late in the experimental disease from the blood of paralyzed monkeys infected by various routes(1-3), viremia in human poliomyelitis, in spite of many attempts, has been demonstrated only once—early in the course of an abortive or non-paralytic attack(4). A possible explanation for the negative results with the human disease is that in the naturally occurring infection transient viremia occurs early, in the incubation period, or during the first phase, and by the time clinical signs of the second phase appear (paralytic or non-paralytic), it is too late to demonstrate virus in the blood. This line of reasoning prompted the present experiments, in which animals were infected by a natural route, *i.e.* orally, and blood specimens, collected during the incubation period, were tested for the presence of poliomyelitis virus.

Materials and methods. Cynomolgus monkeys (*M. irus*, *M. cynomolgus*) and chimpanzees (*Pan satyrus*) were used because of their known susceptibility to oral infection(5-8). Four separate experiments were carried out. In the first 3, the animals were fed the Y-SK strain (Lansing type) of poliomyelitis virus in the form of pooled monkey cords (approximately 30th passage); in the fourth, the Egypt strain, a Brunhilde type, was used in a pool of early passage monkey cords. The monkeys received 5 ml of 20% virus suspension with mashed banana on one occasion only, while the chimpanzees were fed 5 to 15 ml of the same material twice, 3 hours apart. In the first experiment, carried out in Mar. 1951, 13 monkeys were fed virus; in the second (Oct. '51), 26 monkeys were fed poliomyelitis virus, and in addition some of these received the Texas strain of Coxsackie virus and/or cortisone, 25 mg daily for 12 days. In the

third experiment (Nov., '51), 4 chimpanzees were fed the Y-SK strain of virus alone, and in the fourth (Dec., '51), 15 cynomolgus were given the Egypt strain of virus. Twenty-four hours after virus feeding, 5-10 ml of blood were withdrawn from the femoral artery or vein of each animal. This was repeated daily for 5-7 days with most of the animals, although some were bled only on alternate days. All bloods were heparinized, placed in sterile lusteroid tubes within larger tubes, and spun at 2000 rpm before being frozen on dry ice. When prepared for inoculation, the middle section of the frozen blood was cut out, so that the 1 cc inoculum contained equal amounts of plasma and RBC, and the entire buffy coat, or at least the majority of the WBC from about 6 cc of blood. Tests for virus in these samples were carried out by intracerebral inoculation of rhesus monkeys, one animal for each specimen. If the test monkey failed to show signs of disease after 3-4 weeks, it was reinoculated with the same material a second time. Test animals were observed for 4 weeks after the last virus inoculation; those which developed paralysis and any which showed questionable signs were sacrificed for histological study.

Results. A summary of the findings appears in Table I. In the 1st experiment, 4 of 13 monkeys became paralyzed after feeding, the incubation periods ranging from 11 to 32 days. All of the bloods which had been collected from the paralyzed animals were tested, but only one monkey (Cy 5372) proved to have viremia: a specimen of its blood collected 5 days after virus feeding, on the 6th day before the appearance of paralysis, produced typical poliomyelitis in a rhesus monkey. In the second experiment, only 1 of the 26 animals developed paralysis; this was Cy 5965, which had been given daily injections of cortisone for 12 days following virus feeding. Blood specimens from this animal, collected 4 and 5 days after feeding, on

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TABLE I. Viremia in Chimpanzees and Monkeys After Ingestion of Poliomyelitis Virus.

Date	Strain fed	No. animals paralyzed	Animal	Results of tests for virus in blood							Day of paralysis
				Days after virus ingestion							
				1	2	3	4	5	6	7	
3/51	Y-SK	4/13*	Cy 5372	—	—	—	—	+			11
			5367	—	—	—	—	—			32
			5423	—	—	—		—			11
			5432	—			—	—			12
10	"	1/26	Cy 5965		—		+	+			11
			5795					+		} No apparent disease	
			5803				+	+			
11	"	0/4	Chimpanzees								} No apparent disease
			Karin	—	—	—	+	+	—	—	
			Ingrid	—	—	—	—	+	—	—	
			Asta	—	—	—	+	+			
			Lisa	—	—	—	—	—			
12	Egypt	10/15†	Cy 5935	—		—	+	+	+		8
			5938	—	—		Inc.	+	+		9
			5805	—	—	—	+	+	+		9

* Numerator indicates number paralyzed; denominator, number fed.

† The testing of the bloods of only 3 of the paralyzed monkeys in this group has been completed to date.

+ and — indicate positive and negative tests for virus. Inc. = incomplete test.

the 6th and 7th days before the appearance of paralysis, both yielded positive tests for poliomyelitis virus. Two other monkeys (5795, 5803), although they did not show evidence of disease, were nevertheless infected, as indicated by the development of specific neutralizing antibodies. The blood specimens of these animals, collected on the 4th and 5th days after virus ingestion, were also found to contain poliomyelitis virus. The administration of cortisone or cortisone and Coxsackie virus in this experiment did not increase the incidence of paralytic disease. Of the 4 chimpanzees fed virus in the third experiment, 3 proved to have viremia on the 4th and 5th days after virus ingestion.[†] As is usual in these animals, none developed signs of the disease. Ten of the 15 monkeys fed the Egypt strain of poliomyelitis virus in the fourth experiment developed paralysis with incubation periods ranging from 8 to 12 days. Blood specimens from several of these have been

tested and viremia has been detected on the 4th, 5th, and 6th days after virus ingestion.

All blood samples collected from chimpanzees and paralyzed monkeys during the first 3 days after feeding failed to produce poliomyelitis in the test animals; 6th and 7th day bloods from two chimpanzees were also negative.

Comment. Mention has already been made that poliomyelitis virus has been isolated from the blood of experimentally infected monkeys, late in the paralytic disease. It has been suggested that that phenomenon is part of a severe or overwhelming infection, and is perhaps a terminal event. On the other hand, the demonstration of poliomyelitis virus in the blood early in the incubation period in animals experimentally infected by the oral route with both Brunhilde and Lansing types, is a new finding. It suggests the possibility that viremia may occur in the incubation period of the human disease. Since it is known that antibodies appear in the blood of cynomolgus monkeys(9) and chimpanzees(10) as early as 7 to 10 days following ingestion of virus, the first 5-7 days after virus feeding was the period upon which attention was concentrated. Whether or not viremia occurs later in the incubation period of the experimental disease has not yet been determined. In the experi-

[†] In a subsequent experiment, as yet incomplete, these same chimpanzees which had developed viremia following infection with the Y-SK strain were fed Egypt strain (Brunhilde type). To date, 2 of the 4 have been found to have viremia on the 5th and 6th days after virus ingestion, indicating that viremia can occur in the same animals at least twice, with 2 different immunological types of virus.

ment on chimpanzees (No. 3), the viremia was transient and limited to the 4th and 5th days. In the human disease, as in orally infected monkeys, antibodies are already present at the time of onset of clinical symptoms(11,12), which suggests that the time to look for viremia in man is early in the incubation period.

Summary. Seven of 10 cynomolgus monkeys and 3 of 4 chimpanzees have been found to have viremia early in the incubation period following oral infection with poliomyelitis virus. Viremia was present in animals infected with a Brunhilde type (Egypt) as well as a Lansing type (Y-SK), and persisted in some animals over the 4th, 5th, and 6th days after virus ingestion. Virus was not isolated from blood specimens collected during the first 3 days after feeding, nor on the 7th day, in the two instances in which it was tested. The interval between viremia and the appearance of paralysis varied between 3 and 7 days in the cynomolgus monkeys.

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Measurement of Total Body Water in the Dog with Antipyrine.* (19399)

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Body water has been measured in man(1) and in other animals(2,3) by determining the volume of distribution of antipyrine, which appears to equilibrate with all compartments of body water. The originators of the method have, however, questioned the usefulness of this technic in the determination of body water in the dog, for although antipyrine is present in all tissues and organs in this species in amounts proportional to the water content, it is so rapidly destroyed in the dog that it was thought questionable that equilibration could be attained before nearly complete destruction of administered antipyrine.

In preparation for some experiments in-

volving the determination of body water in human subjects, we attempted to study the technic in dogs. In agreement with previous workers(2) we found that blood antipyrine concentrations in the dog decreased at a rate some 5 times greater than the corresponding decrease in man. Since equilibrium in man is not attained before one to 2 hours, it appeared probable that destruction in the dog might be complete before adequate mixing in the body fluids could be accomplished. However, in investigating this question further, we found that the rate of equilibration of antipyrine with body fluids in the dog was so rapid that it compensated for its rapid destruction and made feasible the determination of body water in this species by the antipyrine method.

Methods. The dogs used were mongrel

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