

## SUSCEPTIBILITY OF CERTAIN SPECIES OF PANAMANIAN MONKEYS TO THE VIRUS OF ACUTE ANTERIOR POLIOMYELITIS

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During the recent epidemic of acute anterior poliomyelitis in Panamá the author became interested in experimental studies of the etiologic agent. Three strains of poliomyelitis virus were isolated in rhesus monkeys (Rodaniche, in press). However, in view of the costliness of *Macaca mulatta* and the delay and difficulty experienced at times in obtaining specimens, it seemed advisable to attempt transmission of the virus to local monkeys. Since Melnick and Paul (1943) and Melnick (1946) had reported successful transmission to South American species of *Cebus capucina*, the corresponding Panamanian species was chosen for study. Later, experiments were also conducted with two of the four local subspecies of the howler monkey and with juvenile specimens of the black spider monkey. Mackay and Schroeder (1935) were unsuccessful in their attempts to transmit the virus to the South American spider monkey, *Ateles ater*, but we hoped that perhaps infant and juvenile specimens might prove more susceptible.

The species of Panamanian monkeys utilized in these studies according to the latest classification by the Smithsonian Institution were:

- Whiteface monkey—*Cebus capucinus imitator*
- Howler monkey—*Alouatta palliata palliata*  
*Alouatta palliata aequatorialis*
- Black spider monkey—*Ateles fusiceps robustus*

Whiteface monkeys were available in fair numbers, as this animal has a wide sale as a pet and more intensive efforts are made, therefore, to capture it. Unfortunately, many specimens are maintained alive in the laboratory only with difficulty due to the heavy infestations with parasites to which they are prone. Relatively few howler monkeys were available. Although this is one of the most abundant species in Panamá, little effort is made to capture it alive as there is no market for it as a pet. Due to dietary idiosyncracies the howler is troublesome to maintain in the laboratory for more than a few weeks. However, if patience is exercised in training it to a laboratory diet, it thrives well.

Virus was successfully transmitted to *Cebus capucinus imitator*, *A. palliata palliata* and *A. palliata aequatorialis*. To the best of our knowledge this is the first report of successful transmission to the latter species. Only two *Ateles fusiceps robustus* were inoculated. As these gave negative results, and in view of the previous report of Mackay and Schroeder (1935), investigation of these animals was abandoned.

### VIRUS STRAIN

The virus utilized in the experiments here reported was a strictly monkey-pathogenic strain (non-transmissible to rodents), obtained from the spinal

cord of a 2 year old male infant (A.A.) who died of clinically typical poliomyelitis, confirmed by post-mortem pathological studies, in the Santo Tomas Hospital, Panamá, R. P. in August of 1950 at the beginning of the outbreak. Primary isolation was made in *M. mulatta*.

#### METHOD

Inoculation was made in most instances by the combined intracerebral, intranasal and intraperitoneal routes as Melnick and Paul (1943) reported greater success by this method. Intracerebral dosage was graduated from 0.5 to 1 cc according to the weight of the monkey and injection was always unilateral. Ten per cent centrifuged suspensions of cord tissue in physiologic saline solution were employed. Temperatures were taken rectally once daily at approximately 10 A.M. The majority of animals were sacrificed soon after the onset of paralyzes. The usual criteria for pathological diagnosis were employed: focal, diffuse and perivascular round cell infiltration most prominent in the anterior horns of the spinal cord, neuronal necrosis and neuronophagia. After each successive monkey passage, 6 adult and 6 infant white mice, 2 guinea pigs, 2 hamsters and one rabbit were inoculated intracerebrally with consistently negative results.

The rhesus monkeys were healthy young tuberculin-negative specimens weighing from 2500 to 3200 grams. Six of the 8 cebus monkeys were young adults or juveniles weighing from 900 to 2200 grams. Two infant cebus monkeys #391 and #413 weighing 690 and 525 grams respectively were used. With one exception all howlers were juvenile or infant specimens weighing from 1050 to 1595 grams. Howler #462 was a young adult weighing 4380 grams. The two black spiders were one infant and one juvenile specimen weighing 1375 and 2510 grams respectively.

#### RESULTS

A fairly detailed tabulation of results obtained is presented in Table I.

##### *Susceptibility of Cebus capucinus imitator*

This species was found to be quite susceptible to the A.A. monkey-pathogenic strain of poliomyelitis but only after various passages of this strain in the rhesus. One cebus inoculated with human cord, two with first passage rhesus cord and one with second-passage rhesus cord failed to develop symptoms or pathological changes suggestive of poliomyelitis. Infection was obtained for the first time with third rhesus passage cord in both whitefaces injected. After the third passage in rhesus monkeys the virus was passed twice in succession in howler monkeys, and one cebus was inoculated with howler cord after each passage. Positive results were obtained in both cebus monkeys thus inoculated with howler cord. Symptoms in cebus monkeys were less severe than in either *M. mulatta* or *Alouatta palliata*. After an incubation period of 4 to 5 days febrile temperatures were noted in 3 of the 4 monkeys giving a positive result, followed in 2 days by the onset of paralyzes which affected one or both posterior ex-

TABLE I

*Susceptibility of Various Species of Panamanian Monkeys to the A.A. Strain of Acute Anterior Poliomyelitis*

INOCULUM UTILIZED	NO. OF PREVIOUS MONKEY PASSAGES*	NO. DAYS CORD WAS PRESERVED IN GLYCERINE PRIOR TO FURTHER PASSAGE	SPECIES AND NO. OF MONKEYS INOCULATED.	1ST DAY OF FEVER	1ST DAY OF PARALYSIS	EXTENT OF PARALYSIS	PATR. OF MONKEY	PATHOLOGICAL CHANGES IN SPINAL CORD
Human cord (AA)	0	3	Rhesus # 334	6	9	2H	S9	Pos.
			Whiteface # 391	—	—	—	D47	Neg.
Human cord (AA)	0	264	Rhesus # 426	10	14	1H	Alive Retested† D27	—
			Howler # 486	—	—	—		Neg.
			Howler # 487	—	—	—		
Cord of Rhesus # 334	1	2	Rhesus # 333	10	11	2H & 1F	S14	Pos.
			Whiteface # 394	—	—	—	Alive	—
			Whiteface # 413	—	—	—	D38	Neg.
Cord of Rhesus # 333	2	181	Rhesus # 425	4	6	2H	S8	Pos.
Cord of Rhesus # 333	2	285	Whiteface # 455	—	—	—	D19	Neg.
			Howler # 492	—	15	2H & 2F	S16	Pos.
Cord of Rhesus # 425	3	6	Whiteface # 452	5	7	2H	S8	Pos.
			Whiteface # 453	—	8	1H	Alive	—
			Howler # 462	5	8	2H & 2F	S8	Pos.
			Spider # 442	—	—	—	Alive	—
			Spider # 454	—	—	—	Alive	—
Cord of Howler # 462	4	26	Whiteface # 457	4	6	1H	D48	NT
			Howler # 482	4	6	2H & 2F	S6	Pos.
			Rhesus # 429	4	7	2H	Alive	—
Cord of Howler # 482	5	51	Whiteface # 480	5	7	2H	Alive	—
			Howler # 491	7	11	2H & 2F	D17	Pos.
			Howler # 486	6	8	2H & 2F	S8	Pos.

\* The first three passages were effected in rhesus monkeys. Passages no. 4 and 5 were made in howler monkeys.

† Re inoculated 46 days after first inoculation.

H indicates hindleg. F indicates foreleg. S9 indicates sacrifice of animal in 9 days. D47 indicates death of animal in 47 days.

tremities. No paralyzes of the upper extremities were observed. Melnick and Paul (1943) suggest that positive results in cebus monkeys may be favorably influenced by the proximity of the virus to its human origin. Our results with the A.A. strain isolated from human cord indicate that previous passages in the rhesus monkey favored infection of the cebus monkey.

*Susceptibility of Alouatta palliata*

Howler monkeys became available for this study only after three passages of the A.A. human cord strain had been effected in *M. mulatta*. A single howler #462 (see Table I) was inoculated with this third passage rhesus cord virus with a successful result. In order to test the susceptibility of this species to virus in human cord, we were obliged to resort to inoculation with the original human cord (A.A.) after more than 8 months of preservation in glycerine solution. One *M. mulatta* and two juvenile howlers were injected. One of the howlers died of other causes in 27 days. Its cord was negative for characteristic pathological changes. The other howler remained alive and well and was reinoculated 46 days later with a suspension of the cord of howler #482 (see Table I). It developed a rapid and severe infection indicating absence of immunity. The rhesus, on the other hand, developed a mild infection with fever and paralysis of one hindleg ten days after inoculation.

Due to scarcity of animals no attempt was made to pass first generation rhesus cord virus to the howler. However, second rhesus passage virus preserved 285 days in glycerine solution gave a positive result in the one howler injected, #492, characterized by a quadriplegia which developed 15 days after inoculation. A cebus simultaneously inoculated gave a negative result.

Three howler monkeys proved fully susceptible when inoculated with virus in homologous cord tissue in the 4th and 5th monkey-passages, the first three of which were effected in the rhesus and the last two in the howler monkey. Two cebus and one rhesus monkey were similarly successfully infected with howler cord virus. It is to be noted that all of five howler monkeys inoculated with second to fifth monkey passage virus developed the paralytic disease, including 4 specimens identified as *Alouatta palliata palliata* and one, #492, identified as *A. palliata aequatorialis*. The incubation period varied from 4 to 11 days followed by one or more days of fever. One animal presented paralysis on the 15th day without a previous rise in temperature having been observed. In two howlers light tremors of the head and incessant crying were noted at the beginning of the febrile period. In all 5 animals there was almost simultaneous involvement of all four extremities. Characteristically, the monkey would be noted to show apathy and inappetence one day and the next day would be found lying prostrate on the floor of the cage. The hindlimbs usually showed more extensive paralysis than the forelimbs. Four of the five animals were sacrificed within one to two days of the onset of paralysis. The fifth #491 became progressively weaker, showed gasping difficult respiration and died 17 days after inoculation, 6 days after the onset of paralysis.

*Inability to infect Ateles fusciceps robustus*

Only two black spider monkeys were used in the present experiments, one juvenile and one infant. These were inoculated with third rhesus passage cord with a completely negative result. As two whiteface and one howler monkey similarly inoculated all developed symptoms, it seemed of little value to pursue attempts to infect this species.

## DISCUSSION

The results here reported indicate that the Panamanian whitface monkey, *Cebus capucinus imitator*, and at least two subspecies of the howler monkey, *Alouatta palliata*, are susceptible to infection with the virus of acute anterior poliomyelitis. However, the howler would appear to be preferable for experimental work since it showed a more uniform susceptibility and developed a much severer and more easily recognizable infection. If we can judge by a single experiment using one rhesus monkey, two howlers and one virus strain, the howler would seem to be less suitable than *M. mulatta* for use in primary isolation of virus from human nervous tissue. No tests were made of the susceptibility of these species to single routes of injection or to virus in stool suspensions due to the scarcity of animals. We hope to be able to test this phase of the experimental disease in the future, if we can obtain an adequate supply of monkeys and if there are sufficient human cases available. In the experiments reported here, age apparently did not influence susceptibility of the howler to any important degree as one adult, three juvenile and one infant animals all proved equally susceptible.

## CONCLUSIONS

1. Monkey-pathogenic poliomyelitis virus proved infective to two species of Panamanian monkeys, *Cebus capucinus imitator*, the whitface monkey, and *Alouatta palliata*, the howler monkey. This is the first report of successful transmission to the latter genus.
2. Howler monkeys were infected with second and third rhesus passage cord and with howler cord suspensions in the 4th and 5th monkey passages. Virus in howler cord could also be transmitted to *M. mulatta* and *C. capucinus*.
3. In a single experiment we failed to infect *A. palliata* with human cord virus which had become somewhat reduced in virulence after 8 months preservation in glycerine solution.
4. Infection in the howler monkey was invariably severe with rapid development of quadriplegia after a variable incubation period.
5. The uniform susceptibility of *Alouatta palliata* to monkey passage virus and the marked clinical manifestations elicited by infection in this animal should make this species of value in experimental studies.

## REFERENCES

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