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SHORT COMMUNICATION

LIMITED IN VIVO SUSCEPTIBILITY OF THE SOUTH AMERICAN MONKEY -CEBUS APELLA- TO TYPE 1 POLIOVIRUS: PHYSICAL AND HISTOPATHOLOGICAL OBSERVATIONS AFTER INTRASPINAL INOCULATION

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Type 1 polioviruses (an attenuated strain, Sabin 1 LSc 2ab and a virulent strain, Mahoney) were inoculated intraspinally into the South American Cebus monkey *Cebus apella*. Neither physical symptoms nor histological changes in the central nervous system were observed after inoculation of attenuated Sabin strain. But the virulent Mahoney strain caused flaccid paralysis in two of three monkeys. In these two paralyzed monkeys, definite specific histological changes were observed with spreading of the lesions to places far from the inoculation site, i.e., the cervical cord and brain. These results suggest that *Cebus apella* has limited susceptibility to type 1 poliovirus.

There are several reports on the susceptibilities of South American monkeys to polioviruses (Melnick and Paul, 1943; Jungeblut and Rodaniche, 1954; Kaplan and Melnick, 1955; Kaplan, 1955a; 1955b). In an in vivo study Melnick and Paul (1943) found that the South American ringtail monkey, *Cebus capuchina*, has limited susceptibility to virulent poliovirus inoculated intracerebrally.

In in vitro studies, Kaplan and Melnick (1955) and Kaplan (1955a, 1955b) observed that kidney cell cultures of *C. capuchina* supported multiplication of virulent type 1 poliovirus without showing noticeable cytopathic changes. But they did not obtain evidence for the propagation of virulent type 2 and 3

1 This work was done during implementation of the Japan-Brazil Technical Cooperation Project on Viral Vaccine Production.

TABLE 1. *Characterization of Sabin 1 and Mahoney strains in vitro*

[rct marker tests]

Strain	Test No.	Cells[a]	PFU/ml at			Log difference	
			36 C	39 C	39.5 C	36/39	36/39.5
Sabin 1	1	GMK2	7.30	1.70	≦0.39	5.60	≧6.91
	2	pMK	7.50	4.40	2.30	3.10	5.20
	3	GMK2	7.70	2.40	≦0.69	5.30	≧7.01
	4	pMK	7.55	3.90	≦0.39	3.65	≧7.16
Mahoney	1	GMK2	8.24	7.96	7.74	0.28	0.50
	2	pMK	7.97	7.51	6.93	0.46	1.04
	3	GMK2	8.00	7.74	8.01	0.26	−0.01
	4	pMK	8.87	7.00	6.88	1.87	1.99

[d merker tests]

Strain	Test No.	Cells[a]	PFU/lm at		Log difference
			0.225% $NaHCO_3$	0.03% $NaHCO_3$	0.225/0.03
Sabin 1	1	GMK2	7.33	≦3.20	≧4.13
	2	GMK2	7.60	5.32	2.28
	3	pMK	7.51	5.59	1.92
Mahoney	1	GMK2	8.49	8.10	0.39
	2	GMK2	8.43	8.19	0.24
	3	pMK	8.40	8.20	0.20

[a] GMK2: Green monkey kidney cell line.
pMK: Primary culture of Rhesus monkey kidney cells.

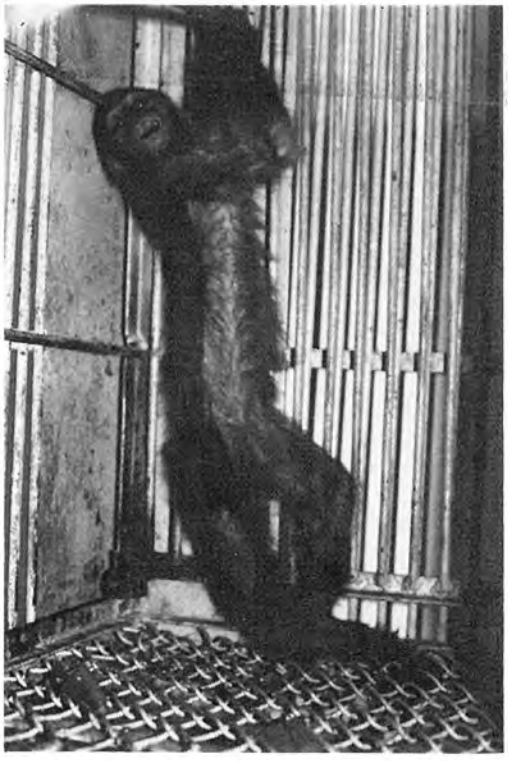
viruses in the cultures. For development of technology for oral poliovaccine production in Brazil, it is essential to examine the susceptibilities of Brazilian monkeys to polioviruses to determine these species are suitable for use in production of vaccine-virus and/or for test purposes.

Here we report preliminary results of physical and histopathological observations on the Brazilian monkey, *Cebus apella*, after intraspinal inoculation of either attenuated or virulent type 1 poliovirus.

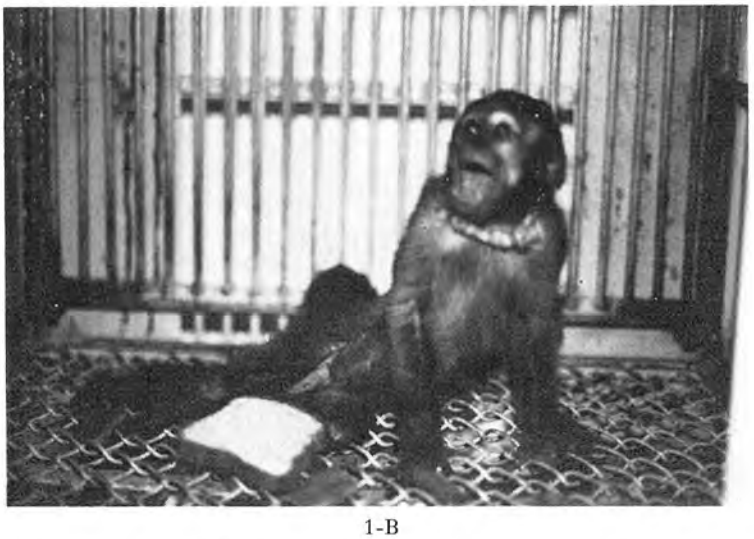
The Sabin and Mahoney strains of type 1 poliovirus were obtained from the Department of Virology, Oswaldo Cruz Foundation, and were passaged once in a green monkey kindey cell line (GMK-2) at 33.5 C and 36 C respectively. As shown in Table 1, these viruses were characterized as attenuated and virulent respectively by reproductive capacity at different temperatures (rct) (Benyesh-Melnick and Melnick, 1959; Lwoff and Lwoff, 1958) and delayed (d) (Vogt et al., 1957; Hsiung and Melnick, 1958) marker tests in vitro.

In in vivo tests, groups of three *Cebus apella* monkeys weighing 1.0–1.3 kg were inoculated intraspinally with $10^{6.5}$ $TCID_{50}$ in 0.1 ml of the Sabin or Mahoney strain of virus by the method of Sabin (1959) and then observed for 17 days. One of the two monkeys that developed severe paralysis after inoculation of the Mahoney strain was sacrificed at the time of the peak of disease.

For histological examination, 40 sections



1-A



1-B

FIGURE 1. Paralytic symptoms in Cebus apella. Monkey No. 5 inoculated with the Mahoney strain in the lumbar enlargement suffered from flaccid paralysis of both legs on the 4th (1-A) and 5th (1-B) days of observation.

TABLE 2. *Clinical observations*

Virus strain	No.	Sex	Body weight (kg)		Physical examination[a]																Paralysis
			Pre.	Post.	1	3	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Sabin 1	1	m	1.0	1.3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	No
					00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	
	2	m	1.3	1.3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	No
					00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	
	3	f	1.3	1.1	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	No
					00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	
Mahoney	4	m	1.2	1.1	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	Yes
					00	00	01	02	02	02	02	02	02	02	02	02	02	02	02	02	02	
	5	f	1.2	1.2	00	00	00	00	00	00	00	00	00	00	Sacrificed							Yes
					00	02	03	34	44	44	44	44	44	44								
	6	f	1.1	1.0	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	No
					00	00	00	00	00	01	01	01	00	00	00	00	00	00	00	00	00	

[a] right upper limb | left upper limb
right lower limb | left lower limb

0: Apparently normal
1: Weakness
2: Partial paralysis
3: Incomplete paralysis
4: Complete flaccid papalysis

in all were prepared from the central nervous system: 1 section from each side of the cerebral cortex and the thalamus, 1 section each from the midbrain, pons and cerebellum, 3 sections from the medulla oblongata, 12 sections at different levels of the cervical enlargement and 18 sections at different levels of the lumbar enlargemant. Lesions by viral infection in the hemisections of the spinal cord and brain were scored as follows (WHO Exp. Comm. Biol. Stand., 1983):

Grade 1. Cellular infiltration only
Grade 2. Cellular infiltration with minimal neuronal damage
Grade 3. Cellular infiltration with extensive neuronal damage
Grade 4. Massive neuronal damge with or without cellular infiltration

The severity of histological lesions was evaluated by calculating the lesion score (WHO Exp. Comm. Biol. Stand., 1983) as follows:

$$\text{Lesion score} = \left(\frac{\sum \text{ of lumber score}}{\text{No. of hemisections}} + \frac{\sum \text{ of cervical score}}{\text{No. of hemisections}} + \frac{\sum \text{ of brain score}}{\text{No. of hemisections}} \right) \div 3$$

As shown in Tables 2 and 3, neither physical symptoms nor histopathological changes in the central nervous system were observed in the three monkeys inoculated with the Sabin 1 strain.

Two of the three monkeys inoculated with the Mahoney strain showed typical paralysis of poliovirus infection (Fig. 1, Table 2). On histopathological examinations of the monkeys with paralysis, severe damage of neuronal cells was observed at the inoculated site, and the lesions were seen to have spread to the brain (Table 3). These features of the lesions (Fig. 2-B, C) were similar with those of lesions in Cynomolgus monkeys inoculated with same

Mahoney strain, although they varied more or less in severity.

As shown in Table 4, the neutralizing antibody titer of the sera obtained at autopsy was slightly increased. These results indicate that little or no proliferation of inoculated virus occurred in the monkeys and that there was little stimulation of the immune system. It is unknown why the neutralizing antibody titers were low in the two monkeys with paralysis after inoculation of the Mahoney strain.

Brazilian monkeys are of interest with respect to whether they can be used for studies on poliovirus. This in vivo study, summarized in Table 5, it indicated that *Cebus apella* has no susceptibility to Sabin type 1 virus and limited susceptibility to the Mahoney strain. We also found that cultures of kidney cells from this monkey have no susceptibility to any type of the Sabin strain (unpublished data). Although it seems from this study that *Cebus apella* is not suitable for neurovirulence tests for quality control of the attenuated poliovaccine, it could be used for differentiation between attenuated and virulent polioviruses.

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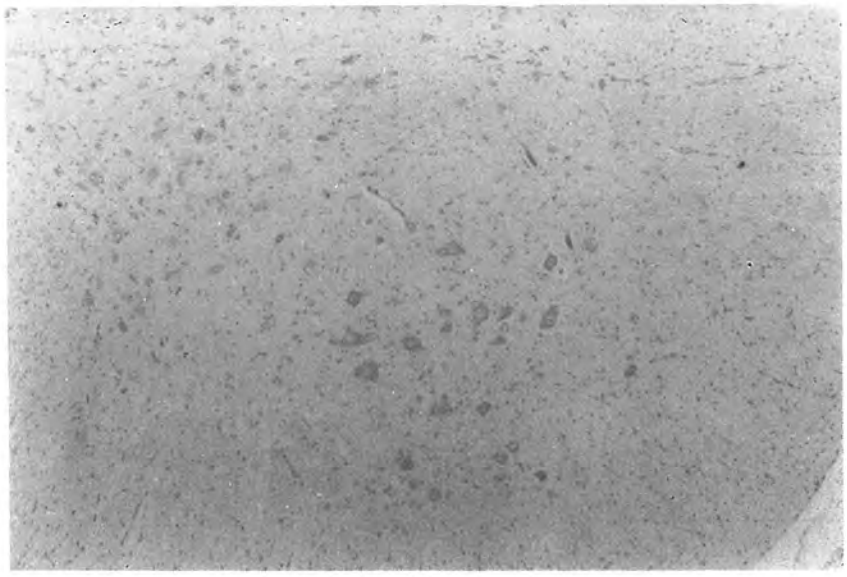
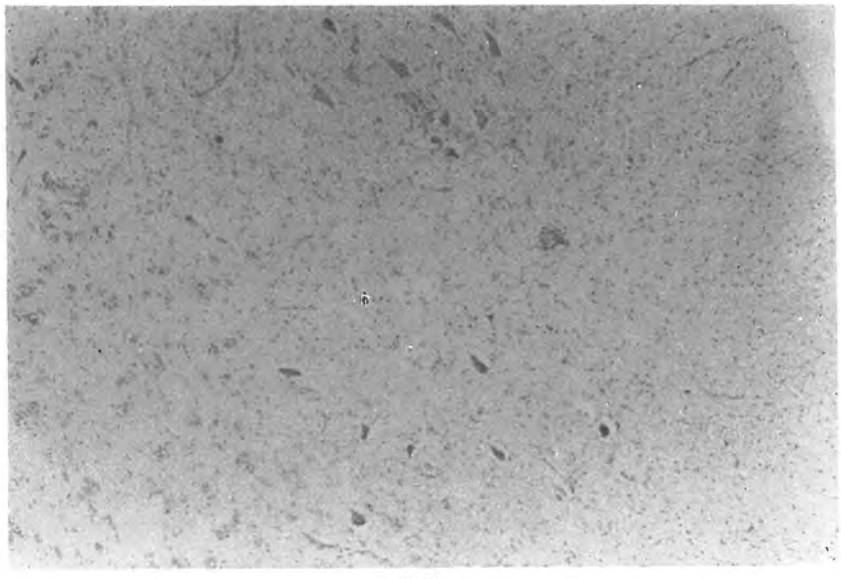
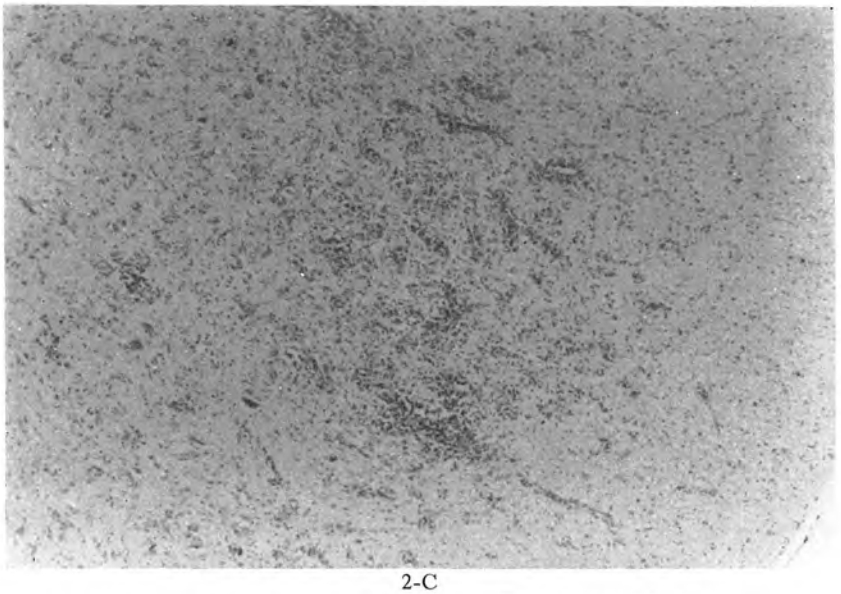


2-A

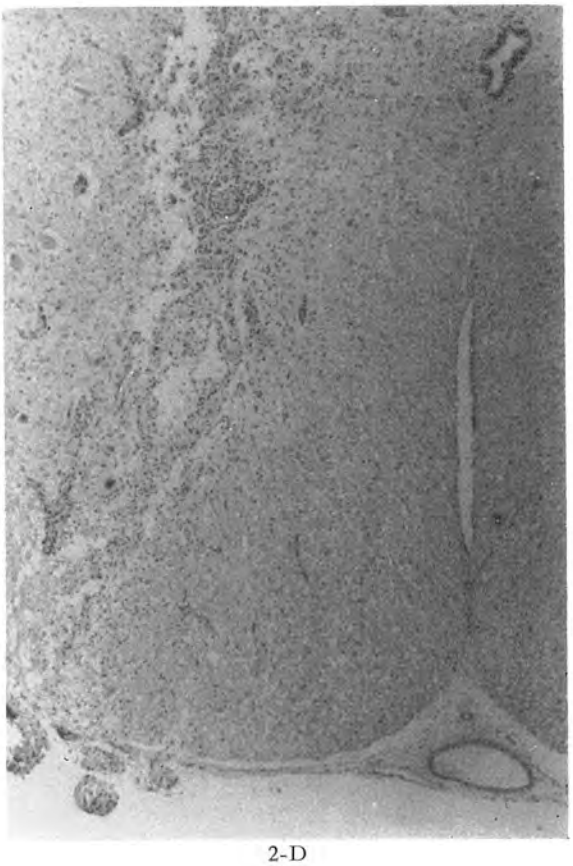


2-B

FIGURE 2. The lesions in lumbar enlargment of Cebus apella. 2-A: No histological change (grade O), Monkey No. 1. 2-B: Slight neuronal damage and perivascular cuffing (grade 2), Monkey No. 4. 2-C: Massive neuronal damage with infiltration of mononuclear cells (grade 4), Monkey No. 4. 2-D: Needle trauma, Monkey No. 2.



2-C



2-D

TABLE 3. *Histopathological examinations*

Monkey No.		Valid		Histological lesions: Lumbar cord 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Ave.[e]
Sabin 1	1	+[a]	L[c]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			R[d]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	+	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	−[b]	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mahoney	1	+	L	3	3	3	4	2	4	3	4	—	4	4	3	2	2	4	0	0	0	2.46
			R	1	3	3	3	4	2	1	2	4	4	4	4	2	1	2	1	0	0	
	5	+	L	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	2	0	2	3.50
			R	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	0	2	
	6	+	L	0	1	0	3	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0.53
			R	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

[a] Virus was inoculated correctly into the anterior horn of the spinal cord.
[b] Needle trauma at the inoculation site was not confirmed.
[c] L=Left
[d] R=Right
[e] Ave.=Average
[f] M.o.=Medulla oblongata

TABLE 4. *Poliovirus neutralizing antibody titers determined in samples collected from Cebus monkeys before and after intraspinal inoculations of the Sabin type 1 and Mahoney strains*

Virus strain	Monkey No.	Neutralizing antibodies to 100 $TCID_{50}$ of Sabin virus: Type 1 Before	Type 1 After	Type 2 Before	Type 2 After	Type 3 Before	Type 3 After
Sabin 1	1	0	4.0[a]	0	NT[b]	0	NT
	2	0	2.8	0	NT	0	NT
	3	0	4.0	0	NN	0	NT
Mahoney	4	0	5.6	0	NT	0	NT
	5	0	5.6	0	NT	0	NT
	6	0	1.4	0	NT	0	NT

[a] Reciprocal dilution of serum.
[b] Not tested.

of poliomyelitis

Cervical cord												Ave.	M.o.[f]			Brain					Ave.	Lesion score	
1	2	3	4	5	6	7	8	9	10	11	12		1	2	3	Cb[g]	P[h]	M[i]	T[j]	Co[k]			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0			
0	0	0	0	0	0	0	0	0	0	2	0	0.08	2	0	1	0	2	1	0	1	0.68	1.07	
0	0	0	0	0	0	0	0	0	0	0	0		2	1	0	0	0	1	0	0			
0	0	0	0	2	1	2	0	0	0	1	2	0.83	2	0	0	2	0	0	0	0	0.87	1.74	1.00
0	0	0	—	1	3	1	2	0	0	0	4		2	0	2	0	2	0	2	0			
0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	0	0	0	0	0	0	0.06	0.20	
0	0	0	0	0	0	0	0	0	0	0	0		1	0	0	0	0	0	0	0			

[g] Cb=Cerebellum
[h] P=Pons
[i] M=Midbrain
[j] T=Thalamus
[k] Co=Cortex
— =Tissue lost

TABLE 5. *Summary of physical and histological examinations in 6 Cebus apella inoculated intraspinally with the Sabin type 1 or Mahoney strain of poliovirus*

Virus strain	Inoculation		Results (No. of monkeys with positive/valid)	
	$TCID_{50}$/0.1 ml	No. of monkeys	Paralysis	Histopathological change
Sabin 1	6.5	3	0/2	0/2
Mahoney	6.5	3	2/3	3/3

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