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Fall of Infectivity and Immunizing Power of Poliomyelitic Tissue at Intervals after Complete Paralysis.*

MAURICE BRODIE.

From the Department of Bacteriology, McGill University, Montreal.

Monkeys infected with the virus of poliomyelitis, develop after the height of paralysis, sub-normal temperature, difficulty in deglutition, inanition and bed sores and so it is extremely difficult to maintain them alive for long periods of time. However, by wrapping them in blankets, bottle feeding and other measures, it was possible to keep a small number alive for varying intervals after complete paralysis. The cord of these animals was tested for infectivity by titrating a 5% or 10% suspension made up of 6 to 8 segments. During the course of this work, the infectivity of cord removed at the height of paralysis varied between 0.000625 and 0.0025 cc. of a 5% suspension. The results are shown in Table I.

TABLE I.

Cord Specimen: Interval after paralysis days	Infective dose in cc. of 5% suspension
2 (specimen 1)	0.01
2 (" 2)	0.005
4 (" 1)	0.1
4 (" 2)	0.1
6	0.15
8	0.2
11–12	0.5
16	0.8
19	3 cc. of a 15% suspension
21	failed to infect
23	" "

Further tests were made with 23-day-old cords, using the double inoculation technique.[1] One animal was inoculated intracerebrally with 1 cc. of a 20% suspension and 10 days later it again received 1 cc. intracerebrally and also 6 cc. intraperitoneally. This animal developed no symptoms of the disease. A second monkey was given 23-day cord which had been washed as free as possible of antibody. The animal from which it had been obtained was, after bleeding, infused with 2 liters of Ringer's solution. This, rather

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[1] Flexner, S., *Science*, 1931, **74**, 520.

than isotonic gum acacia solution was used, for it was found that the latter sometimes reduced the infective power of spinal cord. One cc. intracerebrally and 6 cc. intraperitoneally of a 20% suspension and repeated in 11 days, gave no manifestations of poliomyelitis. It seems that the cord is no longer infective after 21 to 23 days.

The infectivity of the cord declines rapidly for the first few days after complete paralysis and then more gradually until the end of the third week, when it is no longer infective. These results agree in a general way with those of Levaditi and Landsteiner,[2] who gave infection with cord obtained 4 days after paralysis, and with those of Flexner and Clarke,[3] Levaditi and Landsteiner,[2] Levaditi and Lépine,[4] and Landsteiner and Levaditi,[5] who found that at intervals longer than 3 weeks after paralysis, cord was no longer infective. Flexner and Clarke,[3] on the other hand, at times failed to infect with cord obtained a few days after paralysis, and Leiner and Wiesner[6] with cord obtained 6 days after paralysis.

A small series of animals were inoculated, intracutaneously, with some of these cords, in order to determine their immunizing power and to compare it with that obtained with acute cord. The first 2 received cord tissue in which virus was not demonstrated, one obtained 35 days after the height of paralysis, the other 23 days after paralysis and which had been washed as free as possible of antibody. The next 3 were given cord tissue obtained at 8, 4 and 2 days, respectively, after paralysis. The neutralizing power of the serums was determined in terms of minimal completely paralyzing doses and with the following results (Table II).

The immunizing power of spinal cord decreases markedly as the period after complete paralysis is prolonged and there is a relationship between the infectivity and immunizing power. Cord that proved non-infective failed to immunize, whereas cord which proved infective also immunized. That large quantities of cord, obtained 23 days after paralysis and washed as free as possible of antibody, failed to immunize, even though over neutralized virus-serum combinations give some immunity,[7] suggests an absence of

[2] Levaditi, C., and Landsteiner, K., *Comp. Rend. Soc. de Biol.*, 1910, **68**, 311.

[3] Flexner, S., and Clarke, P. F., *J. Am. Med. Assn.*, 1911, 56, **8**, 585.

[4] Levaditi, C., and Lépine, P., *C. Rend. des Seances de la Soc. de Biol.*, 1931, **34**.

[5] Landsteiner, K., and Levaditi, C., *Annales de l'Inst. Pasteur*, 1910, **24**, 833.

[6] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1910, **23**, 91, 817.

[7] Brodie, M., *J. Exp. Med.*, 1932, **56**, 493.

TABLE 2.

Cord Specimen days after paralysis	No. Monkey	Gm. intra-cutaneously	Neutralization Test 1				Neutralization Test 2				Neutralization Test 3			
			Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result	Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result	Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result
35	148	2	148	1.	1	Par. 7 day								
23	204	1.5	204	0.9	1	Par. 8 day								
8	202	1.2	202	0.9	1	No paralysis	202	0.9	2	Par. 15 day				
4	294	1.2	294	0.9	2	" "	294	0.9	4	Par. 10 day				
2	299	1.2	299	0.9	16	" "	299	0.9	32	No paralysis	299	0.9	48	Par. 17 days
Height of Paralysis	233	1	233	0.9	16	" "	233	0.9	32	" "	233	0.9	48	" 17 "

CONTROLS

No. Monkey	MCP Doses Virus	Saline or Normal Monkey Serum cc.'s	Result
307	1	0.9 saline	Paralysed 6 days
283	2	0.9 "	" 6 "
296	1	0.9 "	" 6 "
348	1	0.9 serum	" 5 "
273	1	0.9 "	" 13 "

virus rather than its neutralization by antibody. The optimal time to collect cord tissue for both infection and immunization is at the height of paralysis.

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Active Immunization of Human Beings with Tetanus Toxoid.

EDITH M. LINCOLN AND CHARLES K. GREENWALD.
(Introduced by W. H. Park.)
From the Bureau of Laboratories, Department of Health, New York.

Apparently the first attempt at active immunization of man was made by Louis Bazy[1] working with Vallee. He gave iodized tetanus toxin obtained from Ramon at the Pasteur Institute to wounded soldiers. Three injections were given 5 days apart and 10 days after the last injection the men were bled and 1 cc. of the serum of each individual examined at the Pasteur Institute was found to contain from 10 to 100 "units" of tetanus antitoxin. In 1927 Ramon and Zoeller[2] used tetanus anatoxin, toxin treated with formaldehyde, and then incubated. They injected several hundred subjects. The greatest immunity was obtained by giving 0.5 cc. of anatoxin, 1 month later 1 cc. and 2 weeks later 1.5 cc. The immunity acquired was apparently so great that 1 cc. of the subject's serum neutralized 500 to 1,000 minimum lethal doses for a guinea pig. Twelve to 18 months later the immunity was still higher. After 1 to 2 years a single injection of anatoxin, the so-called "injection de rappel", resulted in a great increase in antitoxic power of the serum. 1 cc. of the serum neutralized 10,000 minimum lethal doses. The process of active immunization can not be hurried by shorter intervals between injections or large doses of anatoxin.

In May, 1930, we assembled 18 volunteers, 5 adults 18 years and over, and the rest children varying from 5 years up, who were bled and no trace of tetanus antitoxin found in their blood sera. At weekly intervals 3 doses of tetanus toxoid, made by treating the toxin with 0.4% formalin and incubating at 38° for 10 days, were injected intramuscularly. The injections were painful for about 5

[1] Bazy, L., *La Medecine*, 1926, **8**, 26.

[2] Ramon and Zoeller, *Comp. Rend. des Seances et Mem. de la Soc. de Biol.*, 1929, **100**, 92.