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Nature of Immunity in Poliomyelitis.

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The evidence increases that circulating antibodies play a minor part in the resistance to poliomyelitis both in man and in monkeys. Antisera have commonly failed to prevent the disease[1] even when used in large quantities or to modify its course when used therapeutically;[2] the disease has developed both in monkeys and in man even though they possessed neutralizing blood of high titer;[3] antibodies have been found in 75 to 85% of normal adults regardless of previous poliomyelitis;[4] neither resistance nor susceptibility to the disease in man or monkey presents any close correlation to the presence or absence of demonstrable antibodies.[5] In consequence of these findings, some authors have attempted to seek an answer to the problem of immunity in this disease on an hormonal basis,[6] with conflicting results, others have raised the query of nutritional deficiencies,[7] while still others have employed the term "tissue immunity"[8] to indicate the resistance, due to obscure factors, apparently independent of humoral antibodies. In attempting to unravel the varying reports on this type of resistance, one must bear in mind the primary cellular basis of immunity and anaphylaxis, the many instances of interference phenomena[9] sometimes entirely non-specific, as well as the possibility of closely bound antibodies such as those demonstrated with the Shope rabbit papilloma virus.[10]

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Recently Howe and Bodian[11] have summarized their extensive investigations on neural mechanisms in poliomyelitis in a telling monograph. Closely related to the problem of our paper is their demonstration that a second attack of poliomyelitis can be induced in rhesus monkeys either with homologous or heterologous strains of virus provided a different portal of entry is employed, thus bringing the virus in contact with nervous tissue not previously involved with the virus. They suggest that their experimental data support the conception "that immunity to poliomyelitis in man is not the result of immunization of the nervous system but rather some process which prevents infective quantities of active virus from reaching nervous tissue."

We have been interested in determining what difference, if any, could be shown in the persistence and propagation of poliomyelitis virus along peripheral nerves of strongly immune as compared with normal monkeys. A preliminary paper on this point was presented at a meeting of the Society of American Bacteriologists in 1941.[12]

Four rhesus monkeys, which had recovered from a paralytic attack of poliomyelitis with MV virus and had resisted a second intracere-

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[10] Kidd, J. G., *J. Exp. Med.*, 1939, **70**, 583.

[11] Howe, H. A., and Bodian, D., *Neural Mechanisms in Poliomyelitis*, The Commonwealth Fund, New York, 1942.

[12] Rasmussen, A. F., Jr., and Clark, Paul F., *J. Bact.*, 1941, **42**, 143.

bral inoculation, were available. The other immune animals were obtained by inoculating a mild "Kessel strain" (McK) intracerebrally into rhesus monkeys which had been used previously, but which had not shown signs of disease. The animals recovering from infection with this strain were then reinoculated with MV virus, the result in most instances being a second attack with more complete paralysis or paralysis of limbs not previously involved. The survivors were used in these experiments.

Inoculations were made into the sciatic nerve just above the knee, excepting in monkey 419, series IV, in which the muscles of the leg were so atrophied that the ulnar nerve at the elbow was used. In each instance, the sciatic nerve employed was supplying functional muscle groups. In series I, 0.5 ml of 5% virus suspension was injected at several points so that the nerve sheath ballooned markedly. After it became evident that this method did not insure consistent infection, satisfactory results were obtained by the method of Bodian and Howe;[13] the nerve was sectioned with sharp scissors, and the proximal end soaked in 5% virus suspension for 4-10 minutes. Two to 5 days after inoculation, the immune animals and the similarly inoculated controls were sacrificed, and 0.5 to 2 g portions of cervical cord, lumbar cord, sciatic nerve proximal to the sciatic notch, and sciatic nerve at the site of inoculation were removed aseptically with separate sets of sterile instruments. Each tissue was then tested for virus by injecting 1.0 ml of a 10% suspension into the brains of normal monkeys. The effectiveness of the intrasciatic inoculation and the resistance of the immune monkeys was determined by using normal and immune animals in each series as controls. (For details, see Table I.)

In series I, in which the method of inoculation was ineffective, only one of the tissues reinjected, the sciatic nerve at the site of the inoculation of the normal control, Monkey 412, contained demonstrable virus. In the remaining experiments, Series II, III, and IV, in which the "nerve soak" method was used, the normal controls were paralyzed in from 4 to 5 days, while the immune controls were uniformly resistant to infection. In the normal animals which were inoculated and later sacrificed, the virus was demonstrated at progressively higher levels as time advanced and the lesions developed; the virus was never found in the immune animals.

Evidence of virus invasion as seen by microscopic examination of sections of the cords of the animals sacrificed in Series IV agreed essentially with the animal titrations, no sign of recent virus activity

[13] Bodian, D., and Howe, H. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 170.

TABLE I.

Series	Monkey No.	Method of inoculation (M V virus)	Subsequent course	Tissues of sacrificed animals reinjected (1 ml of 10% suspension intracerebrally)			
				Cervical cord	Lumbar cord	Sciatic nerve prox. to sciatic notch	Sciatic nerve at site of inoculation
I	167 Immune	0.5 ml 5% susp. inj. into sciatic nerve	Sacrificed 2 days		417 N P*	415 N P	410 N P
	412 Normal		" " "		418 N P	416 N P	411 P† 11 days
	223 Immune		Control N P				
	413 Normal		" N P				
II	421 Immune	Prox. end of sectioned sciatic nerve soaked in 5% susp. 4 min.	Sacrificed 5 days	470 N P	469 N P	468 N P	450 N P
	464 Normal		" " "	467 N P	466 P 9 days	465 N P	449 N P
	419 Immune		Control N P				
	459 Normal		" P 5 days				
III	431 Immune	Prox. end of sectioned sciatic nerve soaked in 5% susp. 10 min.	Sacrificed 3 days	439 N P	438 N P	437 N P	434 N P
	463 Normal		" " "	448 N P	446 P 12 days	445 N P	441 P 34 days
	430 Immune		" 5 "	471 N P	457 N P	475 N P	462 N P
	461 Normal		" " "	472 P 13 days	458 P 8 days	474 P 12 days	480 N P
	428 Immune		Control N P				
	454 Normal		" P 5 days				
IV	419 Immune‡		Sacrificed 5 days	C 12 N P	C 10 N P	C 3 N P	480 N P
	C 21 Normal		" 4 "	C 47 P 11 days	C 59 P 5 days	C 45 P 13 days	C 44 N P
	445 Immune		" 3 "	C 43 N P	C 20 N P	C 30 N P	C 23 N P
	471 Normal		" " "	C 22 N P	Died	C 15 N P	C 14 N P
					C 25 T.B. 11 days		
	428 Immune		Control N P				
	C 28 Normal		" P 4 days				

*N P = No poliomyelitis.

†P = Typical paralytic poliomyelitis.

‡In this monkey, the muscles of the lower legs were almost completely atrophic and the ulnar nerve at the elbow was used for inoculation.

All inoculations were made under ether anesthesia.

appearing in any of the sections from the immune animals, while those from the normal animal C-21, sacrificed 4 days after inoculation, revealed a striking degree of neuronecrosis and degeneration in the lumbar cord with less extensive damage in the cervical enlargement.

In the 4 previously normal animals in which virus was found at various levels in the spinal cord, virus was also recovered from the sciatic nerve in 3 of 8 attempts, once from the site of inoculation, and twice from the nerve proximal to the sciatic notch; in an equal number of attempts, virus was not recovered from the sciatic nerves of any of the immune animals. While virus was found in the spinal cords of 4 of 5 normal monkeys 3 to 5 days after inoculation, it was never found in the spinal cords of immune animals inoculated in the same manner.

These findings raise a question with reference to the interpretation of the results of Howe and Bodian. Perhaps there is no inconsistency since our animals were all infected several times and were solidly immune. The infection was therefore widespread and the immunity was general with no evidence of susceptibility by a different portal of entry, as they describe. Recently, findings somewhat similar to ours have been presented by Habel,[14] who found that rabies virus could be recovered from the brain, cord, and peripheral nerves of normal animals 12 to 25 hours after inoculation into the gastrocnemius muscle, but could not be obtained from nervous tissues of immune animals similarly inoculated.

Lack of monkeys made antibody studies in our series impossible, although it is assumed that neutralizing bodies were present in the blood of most of the immune animals. The experiments offer no definitive information as to the basis of the resistance in the recovered animals, but they do suggest the existence of some mechanism for the arrest of the virus, intimately associated with nervous tissue. Is this possibly due to the removal of some component essential for the proliferation of the virus, or is some inhibiting body present which prevents neuronal entrance?

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14 Habel, K., *U. S. Pub. Health Rep.*, 1941, **56**, 692.