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Neuroinvasiveness of Poliomyelitis Virus in Relation to Vitamin D Nutrition.*

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Recently, the question has been raised as to whether or not poliomyelitis virus can spread along the peripheral nerves of normal rhesus monkeys. It has been stated that poliomyelitis virus has but a slight affinity for the exposed axons of peripheral medullated nerves and that the myelin surrounding the axis cylinders of healthy monkeys may be regarded as capable of stopping the spread of virus into the central nervous system.[1] It has, furthermore, been claimed that for such spread to occur (*i. e.*, for successful infection) it was necessary that the monkeys be deficient in vitamin D.[1] Thus, it appeared possible that the success with which some investigators (Fairbrother and Hurst,[2] Demme,[3] Hurst,[4] Sabin and Olitsky,[5] Bodian and Howe[6]) obtained infection by the intrasciatic route, as compared with the high incidence of failure reported by others (Flexner and Amoss,[7] Harrison and Woolpert,[8] Toomey,[1, 9]), might depend upon the status of vitamin D nutrition in the inoculated animals. Since we intended to use the intrasciatic route of infection in another investigation, we were led to inquire whether successful infection by this route was dependent on the existence of vitamin D deficiency in the inoculated monkeys.

Experimental. One of us (A.B.S.) has previously observed that monkeys arriving in the laboratory a short time after their long sea voyage to this country may exhibit roentgenologic and histologic evidence of deficiency of vitamins C and D. Guest and Rapoport[10]

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Toomey, J. A., *Am. J. Dis. Child.*, 1940, **60**, 548.

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3 Demme, H., *Deutsch. Z. f. Nervenh.*, 1930, **116**, 156.

4 Hurst, E. W., *J. Path. Bact.*, 1930, **33**, 1133.

5 Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.*, 1937, **108**, 21.

6 Bodian, D., and Howe, H. A., *Brain*, 1940, **63**, 135; PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 170.

7 Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, **25**, 525.

8 Harrison, J. A., and Woolpert, O. C., *J. Infect. Dis.*, 1939, **65**, 214.

9 Toomey, J. A., personal communication.

10 Guest, G. M., and Rapoport, S., *Physiol. Rev.*, 1941, **21**, 410.

have recently reported that monkeys which had been kept indoors for a number of months on a diet obviously deficient in vitamin D exhibited chemical and roentgen evidence of rickets and osteoporosis. Therefore, in the first tests we used 4 rhesus monkeys which had been out of doors at the dealer's farm for a number of weeks (April, 1940) and then several weeks in our own animal quarters, where they received a diet of whole milk, oranges, bananas, bread and peanuts. Roentgenograms were made of the long bones and the serum calcium and inorganic and organic, acid-soluble phosphorus in the blood were determined to indicate the status of vitamin D nutrition. The significance of the concentrations of organic acid-soluble P in the blood cells of monkeys, in relation to their intake of vitamin D and seasonal exposure to sunlight, has been discussed in a recent review.[10] It has been shown that in these and other animals the concentration of organic acid-soluble P in the blood cells constitutes an index of the state of the labile phosphorus reserves of the body and of phosphorus metabolism as it is influenced by vitamin D.[10, 11] The chemical data on monkeys 3, 5, 7, and 8 listed in Table I are representative of the range of values determined in the bloods

TABLE I.
Development of Poliomyelitis Following Intrasciatic Injection of Virus in Relation to Vitamin D Nutrition.

	Ca and P in blood before inoculation				
Rhesus monkey No.	Ca mg/100 cc	Inorganic P mg/100 cc whole blood	Organic P mg/100 cc cells	Roentgenograms of long bones	Result of intrasciatic inoculation
3	11.0	5.1	54.6	Normal	F_3 Par. 4 Prostr. 6†
5	12.6	4.4	49.5	"	F_3 " 4 Dead 5
7	11.0	6.2	55.5	"	F_3 " 3 " 5
8	11.8	4.9	61.4	"	F_3 " 3 Prostr. 4
3-39	—	(2.2) 7.8*	(37.6) 85.3*	"	F_3 " 3 " 5
3-41	—	(4.4) 6.4*	(46.8) 61.9*	"	F_4 " 4 " 5
3-40	—	3.1	47.6	Marked rickets and scurvy	F_5 " 5 " 7
3-42	—	2.9	50.9	Slight rickets and scurvy	F_4 " 4 " 6

* The initial P values are in parenthesis, while those marked with the asterisk were determined on the 12th day of continuous daily administration of 5,000 U.S.P. units of vitamin D or 2 days before inoculation of virus.

†F_3 Par. 4, Prostr. 6—fever 3rd day, paralysis 4th day, and prostrate 6th day after inoculation.

[11] Rapoport, S., and Guest, G. M., *J. Biol. Chem.*, 1938, **126**, 749.

of normal monkeys and the roentgenograms of their long bones were normal. Each of these monkeys received an inoculation of 1 cc of a 10%, lightly centrifuged suspension of freshly passaged M.V. virus into the right sciatic nerve; in accordance with the suggestion first made by Fairbrother and Hurst[2] the nerve fibers were traumatized with the needle for the purpose of exposing some of the axis cylinders. The 4 animals promptly developed typical paralytic poliomyelitis with no indication that the absence of vitamin D deficiency in any way influenced the course of the infection.

For the next tests 4 apparently healthy rhesus monkeys were selected at random from a group of animals just arrived (November, 1940) from India. In 2 of these (3-40 and 3-42) roentgenograms of the long bones showed evidence of varying severity of rickets and scurvy. The concentration of inorganic P in the whole blood of 3 (3-39, 3-40, 3-42) was low and the concentration of organic acid-soluble P in the cells of one (3-39) was remarkably low. The borderline low normal concentrations of organic acid-soluble P in the cells of 2 (3-40 and 3-42) with moderately low concentrations of inorganic P, were considered to be compatible with mild rickets either in the course of development or of healing. Monkeys 3-39 and 3-41 were given 5,000 units of crystalline vitamin D in propylene glycol (drisdol, 20 drops) by mouth daily for 2 weeks before inoculation of the virus. On the 12th day the concentrations of inorganic P in the whole blood and of organic acid-soluble P in the cells were found to be greatly increased. Such increases in concentration of organic acid-soluble P to above normal have been found in the blood cells of rachitic rats after the administration of healing doses of vitamin D.[11] When virus was similarly injected into the right sciatic nerve of these 4 monkeys they all promptly developed poliomyelitis, and the course of the disease in the 2 vitamin D-deficient animals (3-40 and 3-42) did not differ significantly from that in monkeys without this deficiency.

Discussion. The results of this study indicate that vitamin D deficiency is not a necessary prerequisite for the spread of poliomyelitis virus along peripheral nerves. It may be of interest to note that Harrison and Woolpert[8] as well as Toomey[9] used glycerinated M.V. virus in their unsuccessful intrasciatic infection experiments while we used freshly passaged virus. A lower concentration of virus in the inoculum may, therefore, have been a factor in their experiments. Dr. Toomey has recently informed us that when he used our strain of virus and our procedure of intrasciatic inoculation, his monkeys regularly developed poliomyelitis regardless of whether or not they were deficient in vitamin D.

Summary. Poliomyelitis developed in all of 8 rhesus monkeys following the injection of freshly passaged "M.V." virus into their sciatic nerves. In 6 of these monkeys roentgenologic examinations of the long bones and determinations of inorganic and organic acid-soluble phosphorus in the blood just before inoculation gave no evidence of vitamin D deficiency. Indications of varying degrees of this deficiency were present in the other two animals.

There was no evidence that the capacity of poliomyelitis virus to invade the central nervous system along peripheral nerves depended on vitamin D deficiency.

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Simultaneous Inoculation of Variola and Vaccinia Viruses in Embryonated Eggs.

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In connection with some observations on the stability of variola virus it became of interest to determine the behavior of a small amount of vaccinia virus in the presence of a large amount of variola virus on inoculation in embryonated eggs. The relation of the ensuing findings to the problem of mutability as it pertains to variola virus will be considered elsewhere. The results are reported here simply as one example of the activity of 2 infective agents inoculated simultaneously in fertile eggs, a phase of that method on which there appears to be little data.

Two bacteriologically sterile strains of variola virus (Minnesota and Chinese) which had been carried through 202 and 38 transfers, respectively, in embryonated eggs were used, together with a single strain of vaccinia virus (New York City Board of Health) maintained by occasional egg transfer. The 2 variola strains react identically in 10-day fertile hen's eggs following implantation on the retracted chorioallantoic membrane. By the 3rd day a 10% membrane suspension of either strain produces a thickened confluent area at the site of inoculation but does not affect the embryo. The strain of vaccinia virus behaves very differently, and the 2 virus species may generally be identified by inspection. Eggs inoculated with this strain of vaccinia show a much thinner area of reaction in