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Influence of Thiamine Deficiency in *Macaca mulatta* on Susceptibility to Experimental Poliomyelitis.*

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For several years, we have been studying the possible influence of dietary deficiencies on the susceptibility of monkeys and mice to experimental poliomyelitis. The results with Swiss mice maintained on thiamine-, pantothenic acid-, and riboflavin-deficient diets have already been published.[1,2,3] Since little was known of the exact nutritional requirements of the rhesus monkey, preliminary studies with highly purified diets were necessary[4] before the experiments with the virus could be undertaken. The earlier thiamine studies[4] gave sufficient basis for approximate thiamine requirements of these animals; the more exact daily needs for this vitamin were determined individually for the 12 animals in Series 3.

Experimental. The methods used in the nutritional care of the animals have been described in a previous publication.[4] The basal thiamine-deficient diet consisted of the following parts per 100: sucrose 73, purified vitamin-free casein† 18, mineral salts 4, corn oil 2, and cod liver oil 3. Each animal received a daily vitamin supplement which included riboflavin 1 mg, nicotinic acid 5 mg, calcium pantothenate 3 mg, pyridoxine 1 mg, choline chloride 50 mg, *i*-inositol 100 mg, para-aminobenzoic acid 100 mg, and a bisulfite-treated liver extract (Wilson) solution equivalent to 2.5 g of original material. In Series 3, monkeys 131, 133, 134, and in Series 4, monkeys 148, 149, 151, 152, and 153 received in addition to the thiamine-deficient diet No. 1, an added pyruvate level, 20 μg of a commercial concentrate of biotin or of crystalline biotin (methyl ester), and "folic acid" equivalent to 5 g of liver fraction L.[5] The thiamine dosage for each animal was selected so that a gradual decline was produced.

The MV strain of poliomyelitis virus was used throughout. A 5% suspension of cord was centrifuged lightly, and the supernatant employed either by intracerebral injection in the first series, or by intranasal in the others. In Series 3 and 4, the first administration of the virus was preceded by irrigation of the nasal passages with a phosphate buffer at about pH 5.[6] Then, approximately 1 ml of the virus was dropped into each nostril on 3 successive occasions within 24 hours. The monkeys were observed at least twice daily for signs of poliomyelitis and/or acute polyneuritis; in some instances a careful differentiation between these conditions was essential. The signs of mild thiamine deficiency in the monkey were cessation of normal growth, anorexia, apathy, and an unkempt appearance; while those of frank polyneuritis were tremors, ptosis, muscular weakness, cachexia, and a temporary spastic paralysis of the limbs. These signs could be readily alleviated either by tube feeding or by intra-peritoneal inoculation with adequate quantities of the vitamin.

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1 Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Infect. Dis.*, 1944, **74**, 41.

2 Lichstein, H. C., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 3.

3 Rasmussen, A. F., Jr., Waisman, H. A., and Lichstein, H. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 92.

4 Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F., *J. Nutrition*, 1943, **26**, 205; Waisman, H. A., and McCall, K. B., *Arch. Biochem.*, 1944, **4**, 265.

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5 Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, 1943, **26**, 361.

6 Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

The signs of poliomyelitis were excitability, fine tremors, incoordination, ataxia, weakness of different muscle groups followed by flaccid paralysis; with this MV strain of virus, the cases commonly terminated with quadriplegia and death of the animal. The recorded time for the first signs of definite flaccid paralysis was calculated from the third intranasal insufflation. In all cases necropsies were performed on the dead or ether-sacrificed monkeys; histological studies were made in almost all instances. Whenever indicated, as in complicating infectious diseases, bacteriological studies were carried out to aid in the diagnosis.

In Series 1 (Table I), a small preliminary study, only 4 monkeys were employed, 2 of which were maintained on an optimum diet and 2 on a thiamine-deficient diet. All monkeys, regardless of diet, succumbed to typical clinical poliomyelitis and were sacrificed after quadriplegia.

A total of 7 thiamine-deficient monkeys were used in Series 2 (Table I). In order to maintain the animals, each one received 100-275 μg thiamine in one or two intraperitoneal injections at some time after inoculation of the virus. Although 4 of these animals never showed clinical signs of the infection, conclusions on this basis are difficult, because these monkeys were acutely deficient in thiamine, and died of polyneuritis within the average incubation period of the poliomyelitis infection. These 4 non-paralytic animals died 4, 7, 9, and 11 days, respectively, after the third intranasal insufflation of the virus suspension. The cord sections of 3 of these 4 monkeys, numbers 66, 69, 42, present lesions typical of poliomyelitis, while in the fourth monkey, No. 72, the lesions are less definitive, but are consistent with a diagnosis of early asymptomatic poliomyelitis.

The 3 remaining monkeys showed typical clinical signs of the infection and typical pathology. Because of the premature death of animals from the acute thiamine deficiency in this series, it was deemed advisable to establish the daily minimum thiamine requirements of each monkey more exactly, so that they might survive throughout the period necessary for establishing the virus disease.

In Series 3 (Table I), 18 monkeys were employed, 3 of which were control animals on a natural diet consisting of skimmed milk, corn, carrots, potatoes, and whole wheat bread. Fifteen were placed on a thiamine-deficient diet, but 3 of these were fed in addition, 4 g of sodium pyruvate daily by mouth, by stomach tube, or by intraperitoneal inoculation. Blood pyruvic acid levels were determined on all monkeys except the 3 on a natural diet.[4] In addition to determining the susceptibility of thiamine-deficient monkeys, this series was set up to test the hypothesis that the increased level of pyruvic acid resulting from thiamine deficiency might play a role in resistance to poliomyelitis. A typical protocol for one monkey, No. 126, in this series is given in Table II. Of the 12 deficient monkeys, 2, No. 58 and No. 127, showed no definitive paralysis, but No. 58 died one day after signs of weakness were observed. Histological studies of central nervous system sections from No. 58 showed definite although slight lesions of poliomyelitis, while No. 127, which never showed any signs of poliomyelitis, was sacrificed 28 days after virus was administered, and showed lesions suggesting abortive poliomyelitis of the type we designate "indeterminate." The other 10 deficient animals showed frank flaccid paralysis of 2 or more limbs.

The 3 control animals on a natural diet were in poor condition, and were used only because of the war and consequent shortage of animals. One of these monkeys, No. 102, died shortly after the first intranasal instillation of the virus, while the other 2, although weakness was recorded for both animals, never showed any signs of poliomyelitis. Monkey No. 101 died 11 days after virus was given, from acute dysentery, while No. 100 was sacrificed 56 days following the start of the experiment. Microscopic study of central nervous system sections from these 2 animals revealed "indeterminate" lesions not definitely those of poliomyelitis but consistent with the suggestion of early damage by that virus. Since the monkeys maintained on a thiamine-deficient diet plus pyruvate were from the same shipment as the control animals, the cause of the resistance of 2 of these animals, No. 133 and No. 134, to poliomyelitis may be queried. It may have

TABLE I.

Influence of Thiamine Level and of Increased Pyruvate on Susceptibility of *M. mulatta* to MV Strain Poliomyelitis Virus.

Series No.	Monkey No. Sex	Wt, kg	Diet No.*	First signs of paralysis (days)	Pathology†	Remarks
I	36 F	2.3	1	8	3+	Quadriplegia
	37 F	2.4	1	10	No sections	"
	38 F	2.1	2	7	" "	"
	39 F	2.3	2	8	" "	"
II	40 M	2.7	1	8	4+	Quadriplegia; polyneuritis
	42 M	2.9	1		2+ cervical 1+ lumbar	Polyneuritis; died 7 days following inoc.; no paralysis
	51 M	2.8	1	7	No sections	Quadriplegia; polyneuritis
	66 M	3.3	1		1+	Polyneuritis; died 4 days following inoc.; no paralysis
	69 F	2.0	1		3+	Polyneuritis; died 9 days following inoc.; no paralysis
	70 F	2.7	1	7	4+	Polyneuritis; flaccid par. of one limb
	72 F	1.5	1		±	Polyneuritis; died 11 days following inoc.; no paralysis
III	27 M	4.8	1	9	3+	Polyneuritis; flaccid par. of 2 limbs
	28 F	3.6	1	6	4+	Quadriplegia
	33 F	4.3	1	18	4+	"
	52 F	2.7	1	9	3+	Flaccid par. one limb
	55 M	3.4	1	6	3+	Polyneuritis; quadriplegia
	58 M	3.6	1		1+	No definite par.; marked ataxia, tremors, weakness 8th day following inoc.
	75 M	3.3	1	7	3+	Polyneuritis; flaccid par. of 2 limbs
	93 F	2.8	1	12	4+	Quadriplegia
	107 M	2.4	1	13	1+	Hemiplegia
	108 M	2.9	1	6	3+	Quadriplegia
	126 F	2.2	1	12	4+	Quadriplegia; polyneuritis
	127 M	2.0	1		±	Sacrificed 28 days following inoc.; no signs
	100		3		±	Sacrificed 56 days following inoc.; irritable, weak 7 days
	101		3		±	General weakness, especially legs, 10 days following inoc.; died 11th day; clinical and bact. evidence of dysentery
	102		3			Died after first intranas. insufflation; dysentery
	131 M	2.4	4	7	4+	Quadriplegia
	133 F	2.0	4		±	Sacrificed 33 days following inoc.; no signs
	134 M	2.1	4		±	Sacrificed 29 days following inoc.; no signs
IV	16 F	1.9	2	10	4+	Quadriplegia
	144 M	2.7	2	7	4+	"
	146 F	4.0	2	9	4+	"
	147	4.1	2	9	4+	"
	25 F	2.0	2	7	3+	"
	145	4.6	2		±	Sacrificed 33 days following inoc.; no signs
	148	2.5	4	8	4+	Quadriplegia
	149 F	2.1	4	9	3+	Flaccid par. of 2 limbs
	151 M	2.5	4		±	General weakness, no signs of paralysis; died 22 days after inoc.
	152 M	3.4	4	8	3+	Flaccid par. of 2 limbs
	153 M	3.0	4	8	4+	Quadriplegia

* 1. Thiamine deficient diet; 2. Optimum diet (control animals); 3. Natural diet (control animals); 4. Modified thiamine-deficient diet plus 4 g sodium pyruvate per day.

† Stages of poliomyelitis lesions in *Macaca mulatta* as used in Table I:

± Indeterminate: A few (4 to 6 or 8 per section) focal areas of microglial infiltration; most of

the ganglion cells appear normal; some, however, show chromatolysis with margination of Nissl substance and a few show more marked degeneration with satellitosis of microglial cells and lymphocytes; congestion of vessels with occasional slight hemorrhage. Only an occasional polymorphonuclear leucocyte observed in the sections; no meningitis; no perivascular round cell infiltration.

This picture is considered consistent with early asymptomatic poliomyelitis but is not pathognomonic; in this instance, since poliomyelitis virus was introduced, these lesions are probably due to that virus.[7]

1+ Slight perivascular round cell infiltration especially in medulla; larger amount of microglial infiltration than in the "indeterminate" category; extensive chromatolysis with margination of Nissl substance and eccentric nuclei; necrosis of few ganglion cells with invading microglial phagocytes and lymphocytes but very few polymorphonuclear leucocytes; usually slight hemorrhage.

Animals with this degree of damage have commonly shown tremors, ataxia, and weakness, but no paralysis.

2+ Typical lesions with perivascular cuffing, oedema, easily recognized necrosis of nerve cells with neuronophagia, composed of about equal numbers of microglial cells, lymphocytes, and polymorphonuclear leucocytes. Disappearance of an appreciable number of ganglion cells.

3+ Marked typical lesions with extensive nerve cell necrosis and neurono-phagocytosis; most of the invading cells involved in this process are polymorphonuclear leucocytes. Round cell meningitis.

4+ Overwhelming typical lesions with hardly a normal ganglion cell left at any level of the cord studied. Many polymorphonuclear leucocytes throughout.

TABLE II.

Typical protocol of a *M. mulatta* Monkey Maintained on a Thiamine-deficient Diet and Given MV Virus by the Intranasal Route (Monkey No. 126).

10/ 1/43	M-3 + 2½ g sulfited liver extr. per day.
10/15	First signs of thiamine deficiency.
	Minimum thiamine requirement established as 50 μg per day.
	Blood pyruvic acid determination after 36 hr starvation = 3.4 mg per 100 ml blood.
11/24	MV virus given intranas. 3 times during previous 24 hours. Temp. 103°F.
11/29	Blood pyruvic acid level = 3.02 mg per 100 ml blood.
11/30	Temp. 103.3°F.
12 /1	" 104.0 very irritable.
12/ 2	" 104.9 continued irritability.
12 /3	" 104.9 " "
12/ 4	" 104.2 " "
12/ 5	" 106.2 face hyperemic; marked irritability; slight incoordination of movements.
12/ 6	" 104.2 fine head tremors; flaccid paralysis of both arms.
12/ 7	" 100.2 ptosis; quadriplegia; marked head tremors
12/ 8	" 97.4 no significant change; respiration quite normal.
12/ 9	Found dead 8 a.m.
	Necropsy performed at 9 a.m. No gross changes observed.
	Micropathology: 4+. Extremely acute poliomyelitis; not a single normal nerve cell observed in the cord sections. Parenchymatous degeneration of all the viscera; marked hyperplasia of germinal centers of spleen.
	Conclusion: Marked acute poliomyelitis.

been due in part to the generally poor nutrition of the animals.

Series 4 (Table I) presents another attempt to determine whether with monkeys free from dysentery and with no complicating deficiencies, the pyruvate hypothesis has any validity. One of 5 monkeys, No. 151, fed a thiamine-deficient diet plus added pyruvate showed no sign of poliomyelitis infection but died 22 days after inoculation with interstitial pneumonia. One of the controls, No. 145, maintained on a complete diet also showed no signs of the disease and was sacrificed in 33 days. Both of these animals showed the "indeterminate" pathology as described in Table I.

Summary. The influence of thiamine deficiency on the susceptibility of *M. mulatta* to experimental poliomyelitis has been studied in 40 animals. The results indicate that this species, when deficient in thiamine, does not exhibit an increased resistance to poliomyelitis virus in contrast to the well-demonstrated resistance of Swiss mice similarly fed.[1,8]

[7] Harmon, P. H., Shaughnessy, H. J., and Gordon, F. B., *J. Prevent. Med.*, 1931, **5**, 139.

[8] Foster, C., Jones, J. H., Henle, W., and Dorfman, *J. Exp. Med.*, 1944, **79**, 221; 1944, **80**, 257.