THE INFLUENCE OF "FOLIC ACID" DEFICIENCY IN MACACA MULATTA ON SUSCEPTIBILITY TO EXPERIMENTAL POLIOMYELITIS[1]

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Received for publication April 4, 1946

For several years studies have been in progress in this laboratory on the relation of nutrition to resistance to virus infections of the central nervous system. The majority of the reports have involved experiments with mice, but a few have been with monkeys (Clark *et al.* 1945). In the case of the monkey, which requires certain known and unknown components of liver, it has been difficult to produce an uncomplicated deficiency. Thiamine deficiency has been studied by using sulfited liver extract which served as a source of unknown factors. Other work here has shown that a "folic acid" deficiency developed rapidly when our basal synthetic diet was fed along with the synthetic vitamins (Waisman and Elvehjem, 1943). We have therefore studied the possible relation of this deficiency to resistance to experimental poliomyelitis.

EXPERIMENTAL

The methods of handling and feeding the animals have been described previously (Waisman *et al.*, 1943). The basal diet ("folic acid" deficient), consisting of sucrose 73 parts, purified casein 18, mineral salts 4, cod liver oil 3, and corn oil 2, was fed *ad libitum*; and adequate quantities of ascorbic acid, thiamine, riboflavin, nicotinic acid, pyridoxine, calcium pantothenate, choline chloride, *p*-aminobenzoic acid, inositol, and biotin were given daily. The norite eluate concentrate of Hutchings *et al.* (1941) prepared from solubilized liver powder (fraction L)[2] was the source of "folic acid." All these concentrates were assayed by microbiological methods, using *Streptococcus faecalis* R, and were found to contain from 46 to 85 per cent of the original activity of the liver powder. In many instances the animals which were offered this "folic acid" supplement refused to consume it, because of their general weakness and lack of interest in food. In these cases, the vitamin supplement was thickened by adding some basal diet and the whole administered to the monkey by a stomach tube. This process was continued oftentimes for 10 days before the animals were willing to drink the supplement.

Optimum diets contained 3 to 5 per cent of liver extract powder;[2] these were prepared by adding the liver product (3 to 5 per cent) at the expense of the 95 parts of the mixture of dry ingredients.

[1] These studies were aided by a grant from the National Foundation for Infantile Paralysis, Incorporated.

[2] Donated by Wilson and Co., Chicago, Illinois.

The procedure developed for the production of "folic acid" deficiency consisted in placing the animals on the basal diet with the known crystalline vitamins until the first constant drop in weight was observed (about 2 months). They were then given the amount of the norite eluate concentrate that would just maintain the weight as determined experimentally. In most cases, however, the animals showed an increased appetite which reflected itself in definite weight gains within a week, and, at this point, the "folic acid" concentrate was discontinued. The animals were then allowed only the basal ration until nutritional failure again resulted. The acute deficiency was considered to be the syndrome following the first or second drop in weight, whereas a state of chronic deficiency was said to exist following 3 or more weight drops necessitating the administration of "folic acid" each time to maintain the animals. Blood studies throughout the periods of depletion have demonstrated the typical progressive leucopenia and the correction of this condition by feeding "folic acid" concentrates.

Since many factors, such as previous nutritional history, genetic variability, age and weight at the start of the experiment, the duration of the experiment, and the amount of "folic acid" fed, influence the response of the individual animal to a "folic acid" concentrate, it was impossible to obtain many animals at a comparable nutritional state at the time of the administration of the virus, even though the animals were placed on experiment at the same time. This was particularly true in the case of the short-term experiments (series 2, 3, 4); for in most cases the first constant drop in weight of the animals occurred after 7 weeks on the deficient ration, and if, as was the case in these series, the virus inoculation was performed during the eighth week, some animals of the group would not yet have shown nutritional failure.

The MV strain of poliomyelitis was used throughout. The supernatant of a 5 per cent infected monkey cord suspension, after overnight sedimentation in an ice chest, was used directly for all intranasal inoculations. These insufflations were made by dropping approximately 1 ml of the virus suspension into each nostril on 3 successive occasions within 24 hours, the first administration being preceded by irrigation of the nasal passages with a M/10 phosphate buffer solution at about pH 5 (Schultz and Gebhardt, 1934). Intracerebral injections were made in the conventional manner under ether anesthesia using as inoculum 1-ml dilutions of this 5 per cent cord suspension, as indicated. The monkeys were observed at least twice daily for signs of illness; the recorded time for the first signs of definite flaccid paralysis was calculated from the third intranasal insufflation. Necropsies were performed on all dead or ether-sacrificed monkeys, and histological studies were made. Bacteriological studies were made whenever indicated, especially in the dysenteries which often complicate "folic acid" deficiency in this animal (Waisman and Elvehjem, 1943). In such cases *Shigella paradysenteriae* was isolated from the stools (table 1). Examination of the intestinal mucosa at autopsy revealed lesions consistent with the diagnosis.

Series 1 (table 1) consisted of 10 monkeys; 4 were maintained on the deficient diet until their condition was of a chronic nature, and 6 were fed the control diets. Following intranasal inoculation 5 of the 6 control animals showed clinical signs of poliomyelitis which progressed to quadriplegia, whereas none

TABLE 1

Influence of "folic acid" on susceptibility of M. mulatta to MV strain poliomyelitis virus

SERIES NO.	MONKEY NO., SEX	WT. (KG)	DIET	VIRUS CONC., ROUTE OF INOCULATION	FIRST SIGNS OF PARALYSIS (DAYS)	PATHOLOGY*	REMARKS
1	16 F	4.1	Optimum	5% I.N.	10	4+	Quadriplegia; approx. 3 mos. pregnant
	144 M	2.7	Optimum	5% I.N.	7	4+	Quadriplegia
	146 F	4.0	Optimum	5% I.N.	9	4+	Quadriplegia
	147	4.1	Optimum	5% I.N.	9	4+	Quadriplegia
	25 F	2.9	Optimum	5% I.N.	7	3+	Quadriplegia
	145	4.6	Optimum	5% I.N.		±	No clinical signs of polio; sacr. 35 days after inoc.
	62 F	3.8	Deficient	5% I.N.		1+	No clinical signs of polio; sacr. 35 days after inoc.
	141 M	2.2	Deficient	5% I.N.		1+	No signs of polio; sacr. 35 days after inoc.
	142 M	3.1	Deficient	5% I.N.		2+	No signs of polio; after 35 days placed on optimum diet; sacr. after 75 days
	143 F	2.5	Deficient	5% I.N.	See series 4		No signs of polio; placed on optimum diet.
2	13 M	5.4	Optimum	5% I.N.	6	3+	Quadriplegia
	105 M	3.0	Optimum	5% I.N.		3+	No frank paralysis; sl. tremors and weakness
	155 M	4.4	Deficient	5% I.N.	7	4+	Quadriplegia
	156 M	3.5	Deficient	5% I.N.	7	4+	Quadriplegia
	154 F	3.2	Deficient	5% I.N.	8	4+	Flaccid par. of both arms
	78 M	2.7	Deficient	5% I.N.		±	Died 4 days after inoc.; clinical dysentery
	159 M	2.4	Deficient	5% I.N.			Died 4 days after inoc.; extensive TB
3	104 M	4.7	Optimum	5% I.N.	6	4+	Quadriplegia
	89 F	3.8	Optimum	5% I.N.	7	4+	Quadriplegia
	162 M	2.7	Deficient	5% I.N.	9	4+	Quadriplegia
	163 F	2.6	Deficient	5% I.N.	7	4+	Quadriplegia
	171 M	2.7	Deficient	5% I.N.	7	4+	Quadriplegia
	172 M	3.0	Deficient	5% I.N.	6	4+	Quadriplegia
	175 F	2.7	Deficient	5% I.N.	8	4+	Quadriplegia
	176 F	4.9	Deficient	5% I.N.	8	4+	Quadriplegia
	177 F	2.2	Deficient	5% I.N.	9	3+	Quadriplegia
	178 M	2.8	Deficient	5% I.N.	7	4+	Quadriplegia
	179 F	4.4	Deficient	5% I.N.	7	3+	Quadriplegia
4	201 F	2.6	Optimum	1:20,000 I.Cer.	12	4+	Quadriplegia
	202	2.0	Optimum	1:20,000 I.Cer.	6	4+	Quadriplegia
	143 F	2.9	Optimum	1:20,000 I.Cer.	7	4+	Quadriplegia

TABLE 1—*Continued*

SERIES NO.	MONKEY NO., SEX	WT. (KG)	DIET	VIRUS CONC., ROUTE OF INOCULATION	FIRST SIGNS OF PARALYSIS (DAYS)	PATHOLOGY*	REMARKS
	185 M	2.7	Deficient	1:20,000 I.Cer.	10	3+	Quadriplegia
	192 M	2.1	Deficient	1:20,000 I.Cer.	9	4+	Quadriplegia
	194	1.9	Deficient	1:20,000 I.Cer.	6	3+	Quadriplegia
	196	2.0	Deficient	1:20,000 I.Cer.	11		Quadriplegia; remained alive 10 days foll. quadriplegia
	198	2.1	Deficient	1:20,000 I.Cer.	9	4+	Quadriplegia
	205 F	1.9	Deficient	1:20,000 I.Cer.		±	No clinical signs of polio; sacr. 35 days after inoc.
7	237 M	2.8	Optimum	5% I.N.	10	4+	Quadriplegia
	238 F	3.0	Optimum	5% I.N.	8	4+	Quadriplegia
	232 M	2.5	Deficient	5% I.N.	13	4+	Flaccid par. of both arms; alive and alert for 17 days foll. paralysis
	233 F	2.5	Deficient	5% I.N.		1+	No clinical signs of polio; sacr. 35 days after inoc.

* Stages of poliomyelitis lesions in *Macaca mulatta* as used in table 1:

± 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.

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.

of the animals on the deficient ration (nos. 62, 141, 142, and 143) ever showed clinical signs of the infection. After the 35-day observation period following inoculation, 2 of the deficient animals (nos. 62 and 141) were sacrificed, and histological studies of the central nervous system sections from these showed definite, although slight, lesions (1+) of poliomyelitis. Monkeys nos. 142 and 143 were placed on the control ration 35 days following inoculation, and one of them (no. 142) was subsequently sacrificed after 40 days on this diet. Histological studies of cord sections showed more marked lesions (2+) of poliomyelitis than did animals nos. 62 and 141. Monkey 143 was subsequently reinoculated (intracerebral route) as a control in series 4 after 166 days on the control diet; it succumbed to typical clinical poliomyelitis. It is manifestly impossible on the basis of this one animal (no. 143) to determine whether the lack of resistance to the infection following the second inoculation has any immunological basis or whether the change to an optimum diet was the determining factor.

The potency of the virus suspension employed is further demonstrated by the fact that, in addition to the 10 animals in this series, 5 animals in another experiment on a high pyruvate diet (Waisman and McCall, 1944) were inoculated with the same virus at the same time and by the same route; 4 of the 5 animals succumbed to the infection.

Series 2 consisted of 5 deficient animals and 2 controls. The intranasal inoculation was made 8 weeks after the animals were placed on the deficient diet; consequently the deficiency was considered acute, since the first constant drop in weight of all the deficient animals began at or after the time of inoculation. All of the controls and 3 of the 5 deficient animals showed clinical signs of the infection, whereas the remaining 2 deficient animals died on the fourth day following inoculation. At autopsy it was found that one of these two animals died of dysentery and the other animal had an extensive miliary tuberculosis. The results of this series do not show any increased resistance on the part of the deficient animals.

A third series was conducted by intranasal inoculation of 2 control and 9 acutely deficient animals. All the animals, regardless of diet, succumbed to typical clinical poliomyelitis and were sacrificed after quadriplegia.

In the fourth series 6 acutely deficient and 2 control animals were employed. The virus was administered by the intracerebral route, using the dilution indicated. All the control monkeys and all the deficients except no. 205 showed clinical signs of the infection. Monkey no. 205 showed no clinical signs of infection and was sacrificed 35 days following inoculation. Histological studies on central nervous system sections of this animal showed "indeterminate" ($\pm$) lesions, suggesting the possibility of abortive poliomyelitis. It is interesting to note that one of the deficient animals (no. 196) was able to remain alert and survive without any special care for 10 days after quadriplegia was first noted.

Series 5 and 6 (15 monkeys) are not included in the table because the dilution of virus employed in series 5 (1:200,000) was too great, and very few animals succumbed to the disease. Series 6 was composed of the surviving animals of series 5, thus making the results difficult to interpret.

Series 7 was set up in an attempt to reproduce the conditions of series 1. Two animals were fed the control diet, and 4 were placed on the basal diet. The difficulty of maintaining monkeys in a chronic state of "folic acid" deficiency over long periods of time was again well brought out by this experiment. A critical weight loss, if not immediately recognized as such, resulted in complicating dysentery and death of the animal in 48 hours even though "folic acid" therapy was immediately instituted. At the time of the intranasal inoculation, therefore, only 2 of the deficient animals were still alive (nos. 232 and 233).

Both controls became paralyzed to the extent of quadriplegia (in 8 to 10 days), whereas only one (no. 232) of the deficient monkeys showed any clinical signs of the infection. This one showed paralysis of the right arm on the thirteenth day and paralysis of the left arm on the following day, and was alive and alert for 17 days after onset of the paralysis; however, microscopic examination of CNS sections showed overwhelming typical lesions (4+). The other deficient monkey (no. 233) showed no clinical signs following inoculation and was sacrificed on the thirty-fifth day; micropathology was 1+. The results of this series are in accord with those of series 1 in that the animals in a chronic state of "folic acid" deficiency demonstrate increased resistance to experimental poliomyelitis.

CONCLUSIONS

The results of these experiments indicate that the rhesus monkey, when deficient in "folic acid," exhibits an increased resistance to poliomyelitis virus when the deficiency is of a chronic nature, but not when the deficiency is of the acute type.

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