VITAMIN C THERAPY AND PROPHYLAXIS IN EXPERIMENTAL POLIOMYELITIS*

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In previous experiments we have shown that multiple paralytic doses of poliomyelitis virus, when mixed with very small amounts of crystalline vitamin C (*l*-ascorbic acid), are rendered non-infectious as determined by intracerebral injection of such mixtures into *rhesus* monkeys (1). What made this phenomenon particularly interesting was the existence of a rather narrow quantitative range within which vitamin C seemed to be most effective, the optimal doses lying between 10 mg. and 1 mg. A similar zone was previously described by us in the inactivation of diphtheria toxin by vitamin C (2).

These observations naturally raised the question whether or not the injection of vitamin C into monkeys suffering from experimental poliomyelitis might be followed by any alteration in the severity of the infection. The experimental trial of this idea has led to encouraging results which have already been published in preliminary form (3). To summarize briefly: It was found that a small group of 4 monkeys which had received daily injections of 5 mg. of vitamin C, beginning with the day of the infection, survived without showing any symptoms of paralysis. In another group of 16 animals which had been treated with somewhat larger doses (50 to 100 mg.), 11 developed typical paralysis, 3 showed an atypical form of the disease (onset of paralysis delayed over 2 weeks), and 2 remained free from any paralytic symptoms. Finally, another group of 9 animals which had received very large doses of vitamin C (100 to 700 mg.) all succumbed to the disease in a typical manner. 10 control monkeys, injected

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intracerebrally like the treated animals with 0.1 cc. of a 10 per cent virus suspension, but left without treatment, all developed typical paralysis within from 6 to 11 days.

The limited data which formed the basis of this preliminary report naturally precluded the drawing of any definite conclusions. They seemed to suggest, however, that vitamin C when administered in the proper dose may possess distinct therapeutic properties in experimental poliomyelitis. It became necessary, therefore, to continue this line of work and to amplify our original observations with a larger number of tests. At the same time, it seemed advisable to investigate whether prophylaxis with vitamin C afforded any protection against subsequent inoculation with poliomyelitis virus. It is the object of this paper to present in detail the new experimental data obtained in the continued study of this problem, together with those reported previously in preliminary form.

EXPERIMENTAL WORK

The observations reported in this paper bear on a total of nearly 100 *rhesus* monkeys treated in various ways with vitamin C before or after intracerebral infection with poliomyelitis virus and about half that number of control animals which accompanied these tests. For the sake of clarity in presentation, these data will be arranged in three sections: the first dealing with therapeutic experiments; the second with prophylactic experiments; and the third describing experimental work of somewhat different nature, but generally related to the main problem under investigation.

1. Therapeutic Experiments

Rhesus monkeys weighing from 2000 to 3000 gm. were inoculated intracerebrally with three different amounts of poliomyelitis virus (Aycock strain), *i.e.*, 0.1 cc., 0.05 cc. and 0.01 cc. of a 10 per cent virus suspension. These animals were then treated for a period of 2 weeks with daily injections of vitamin C[1] of various dosage. 11 different series were run. In 9 of these, treatment was uniformly begun on the day of inoculation; in the last 2 series, the initial injection was delayed in some animals until 48 or 72 hours, respectively, after the day of infection.

[1] We are greatly indebted to Merck and Co., Rahway, New Jersey, for placing at our disposal a generous supply of cebione, their crystalline natural vitamin C preparation, in ampoules.

The doses of vitamin C covered a range from 700 mg. to 5 mg. and were mostly administered by the subcutaneous route. Control monkeys, which were infected intracerebrally at the same time with the corresponding dose of virus, accompanied each series. With the larger doses of virus we have satisfied ourselves, as a rule, with 2 controls for each series; when smaller doses of virus were employed, the number of controls was usually increased, in some cases to approximately equal the number of treated animals. All animals were carefully observed for 1 month and symptoms noted. In case of paralysis or death, an autopsy was made and the diagnosis confirmed by histological examination of the spinal cord. Surviving animals were reinoculated at the end of 1 month. The protocols of these individual series are given in Table I. A summary in which all animals are grouped according to the size of the infecting dose will be found in Table II, while Table III lists the results obtained in accordance with the amount of vitamin C injected.

A study of Table III, which considers only the effect of various dosages of vitamin C, without regard for the varying severity of the infection, leaves no doubt that large doses of vitamin C, *i.e.*, amounts ranging from 700 to 100 mg., had no influence whatsoever on the course of the disease. Thus, we find that all 10 monkeys which had been treated with such excessive amounts succumbed to the infection. On the other hand, animals treated with intermediate doses (50 to 10 mg.) occasionally remained free from paralytic symptoms or else developed paralysis only after prolonged incubation periods. This is illustrated by the fact that of a total of 19 monkeys in this category, 3 escaped the disease entirely and 3 came down with paralysis between 15 and 21 days, the remaining 13 animals developing typical poliomyelitis. More favorable results, however, were obtained with monkeys which had received 5 mg. of vitamin C. To wit, of a total of 33 animals treated with this particular dose, not less than 16 survived without showing any evidence of paralysis, 2 developed the disease after 15 and 16 days, respectively, while the remainder, *i.e.*, 15, succumbed to typical poliomyelitis.

These results must be evaluated, of course, in the light of the incidence of paralysis among the controls. If we consider the controls first *en bloc*, irrespective of differences in the size of the infecting dose, it appears that of a total of 38 control monkeys, 36 succumbed to the disease (34 developing typical poliomyelitis within less than 2 weeks incubation period and 2 after 17 and 16 days, respectively), while 2 failed to show any manifest symptoms of paralysis.

Directly comparable data may be obtained from a study of Table II in which all animals, treated and untreated, are listed according to the size of the infecting dose. It will be seen that of a total of 34 vitamin C-treated animals which had been infected with 0.1 cc. of virus, 25 developed typical and 3 atypical poliomyelitis, while 6 remained free from any paralytic symptoms. All 10 controls infected with the same dose of virus came down with typical poliomyelitis. Of 6 treated animals infected with half the amount of virus, *i.e.*, 0.05 cc., 1 escaped the disease, 2 developed atypical and 3 typical poliomyelitis. Again, all 7 controls infected with the same dose of virus developed the disease, 6 typically and

TABLE I

Vitamin C Therapy in Experimental Poliomyelitis

	Monkey	Dose of virus	Dosage of vitamin C	Result	Controls
		cc.	*mg.*		
Series 1	O83	0.1	500	Complete paralysis, 14 days	O69 Complete paralysis, 8 days
	O70	"	200	" " 8 "	O90 " " " "
Series 2	Q28	"	700	" " " "	
	Q29	"	"	" " " "	
	Q30	"	500	" " 5 "	
	Q31	"	"	" " 7 "	Q13 Complete paralysis, 8 days
	Q32	"	200	" " " "	Q26 Partial paralysis, 9 "
	Q33	"	50	Partial paralysis, 11 "	
	Q34	"	"	Complete paralysis, 5 "	
Series 3	Q48	"	500	" " 7 "	
	Q47	"	100	" " " "	
	Q46	"	50	" " 10 "	
	Q49	"	"	" " 8 "	Q54 Complete paralysis, 11 days
	Q50	"	"	" " 11 "	Q55 " " " "
	Q53	"	"	" " 21 "	Q90 " " 9 "
	Q51	"	25	" " 10 "	Q76 " " 11 "
	Q52	"	"	" " 7 "	
	Q45	"	"	No paralysis	
	Q78	"	"	Complete paralysis, 16 days	
Series 4	R13	"	"	" " 11 "	
	R15	"	10	" " 8 "	
	R16	"	"	" " 15 "	R12 Complete paralysis, 7 days
	R17	"	5	No paralysis	
	R18	"	"	" "	
Series 5	R19	"	25	" "	
	R20	"	10	Complete paralysis, 11 days	R25 Complete paralysis, 7 days
	R21	"	"	" " " "	R37 " " " "
	R22	"	5	No paralysis	
	R23	"	"	" "	
Series 6	S21	"	"	Complete paralysis, 7 days	S18 Partial paralysis, 8 days
	S25	"	"	" " 6 "	S20 Complete paralysis, 6 "
	S48	"	"	" " " "	R49 " " 14 "
	R51	"	"	" " 9 "	R50 " " 8 "
	R53	"	"	Partial paralysis, 8 "	R52 " " " "
		"			R60 " " 6 "
		"			R63 " " 9 "
		"			R3 " " 5 "

TABLE I—*Concluded*

	Monkey	Dose of virus	Dosage of vitamin C	Result	Controls
		cc.	*mg.*		
Series 7	R89	0.05	5	Complete paralysis, 8 days	R95 Complete paralysis 8 days
	S5	"	"	" " 9 "	R91 " " 7 "
		"			R98 " " 11 "
		"			R54 " " 17 "
Series 8	S78	"	25	Complete paralysis, 9 days	S67 " " 12 "
	S75	"	5	" " 16 "	S68 " " " "
	S88	"	"	No paralysis	S94 " " 11 "
	S89	"	"	Partial paralysis, 15 days	
Series 9	S90	0.01	100	Complete paralysis, 9 "	
	S91	"	50	" " 10 "	S69 " " 7 days
	S79	"	25	No paralysis	S70 " " " "
	S76	"	5	" "	
Series 10	S95	"	"	Complete paralysis, 8 days	T1 Complete paralysis, 16 days
	S96	"	"	No paralysis	T2 " " 7 "
	S97	"	"	" "	T3 " " 8 "
	S98*	"	"	Complete paralysis, 9 days	T5 " " 7 "
	S99*	"	"	" " 8 "	T6 " " 9 "
	S100*	"	"	" " 9 "	T7 No paralysis
		"			T9 Complete paralysis, 7 days
Series 11	T18	"	"	Complete paralysis, 9 days	
	T19	"	"	" " 8 "	
	T20	"	"	No paralysis	
	T21	"	"	" "	
	T22	"	"	" "	
	T23	"	"	" "	T15 No paralysis
	T25**	"	"	" "	T16 Complete paralysis, 9 days
	T26**	"	"	" "	T17 " " " "
	T27**	"	"	" "	
	T28**	"	"	Complete paralysis, 9 days	
	T29**	"	"	No paralysis	
	T30**	"	"	Complete paralysis, 9 days	

All treated animals received daily injections of vitamin C of the indicated dosage for a period of 2 weeks, or until the onset of paralysis. Treatment was begun on the day of infection, excepting animals marked ** in which the first injection was not given until 48 hours after infection, and animals marked * in which the first injection was not given until 72 hours after infection.

1 after 17 days. More significant results were obtained with monkeys which had been infected with the smallest dose of virus. Of 22 vitamin C-treated animals,

TABLE II

Effect of Various Doses of Vitamin C in Experimental Poliomyelitis with Respect to Variations in the Size of the Infecting Dose of Virus

Treated Animals

Dose of virus	Dosage of vitamin C	Number of monkeys	No paralysis	Atypical paralysis	Typical paralysis
cc.	*mg.*				
0.1	700–100	9	0	0	9
	50– 10	16	2	3	11
	5	9	4	0	5
		34	6	3	25
0.05	700–100	0	0	0	0
	50– 10	1	0	0	1
	5	5	1	2	2
		6	1	2	3
0.01	700–100	1	0	0	1
	50– 10	2	1	0	1
	5	19	11	0	8
		22	12	0	10
		62	19	5	38

Untreated Control Animals

Dose of virus	Number of monkeys	No paralysis	Atypical paralysis	Typical paralysis
cc.				
0.1	19	0	0	19
0.05	7	0	1	6
0.01	12	2	1	9
	38	2	2	34

Atypical paralysis = onset of paralysis later than 2 weeks following infection.
Typical paralysis = onset of paralysis within 2 weeks following infection.

which had been infected with 0.01 cc. of virus, 12 remained free from any paralysis, while 10 came down with typical poliomyelitis. In contrast herewith, we find among 12 control animals infected with the same amount of virus, 10 which suc-

cumbed to the disease (9 typically and 1 after 16 days) and only 2 that failed to show any paralysis.

While it can readily be appreciated that the chances for a therapeutic effect must improve with a reduction in the size of the infecting dose, we believe that even animals infected with the larger amounts of virus show evidence of having benefited from the treatment, provided the dosage of vitamin C is kept within optimal limits. Thus, we have among a group of 14 monkeys infected with 0.1 cc. or 0.05 cc. of virus, which were treated with 5 mg. of vitamin C beginning with the day of the inoculation, no less than 5 survivors without paralysis, whereas all 26 controls infected with the same amounts of virus developed the disease. In a similar way, considerably better figures are obtained for monkeys which had been infected with the smallest amount of virus, *i.e.*, 0.01 cc., if we exclude animals treated with the larger doses and consider only those which had received 5 mg. of vitamin C. It will be found that of 10 such animals, in which treatment was begun on the day of infection, 7 escaped the disease. Even under adverse conditions, when treatment was delayed until 48 or 72 hours after infection, this method of treatment has apparently saved 4 of 9 monkeys from paralysis. When left without treatment, only 2 of 12 controls inoculated with the same dose of virus, *i.e.*, 0.01 cc., remained free from paralysis.

Whether the data are taken as a whole or are interpreted according to gradations in the size of the infecting dose, it appears that treatment with 5 mg. of vitamin C has reduced the incidence of paralysis roughly by one-half as compared with untreated controls. Thus, we have among a total of 33 monkeys which were treated with this dose, 51.5 per cent (17) that developed paralysis against 94.7 per cent (36) among a total of 38 controls. Similarly, among 19 of these treated animals, which had been infected with 0.01 cc. of virus, the incidence of paralysis stands at 42.1 per cent (8) as compared with 83.3 per cent (10) among 12 corresponding controls. While the above figures would seem to be definitely significant, we do not wish to overemphasize their importance, because of the limited number of controls inoculated with the smallest dose of virus. Moreover, a study of the individual series makes it clear that distinct limitations are set in the extent and regularity of the therapeutic effect by experimental factors that are beyond control. All attempts to improve upon these results by other modifications in the form of treatment—*i.e.*, further reduction in the dosage of ascorbic acid to 1 mg. and less; gradual increases from day to day; intraspinal injection of this substance, or combination of vitamin C administration with the injection

of cortin—have been without avail. It is possible that a greater percentage of treated animals might survive after intranasal instillation of the virus, but this method of infection would automatically increase the number of survivors among the controls without necessarily strengthening the statistical significance of the results.

It is a common experience that the infectivity of passage strains of virus may fluctuate considerably from time to time (4). But even at times of maximum virulence an occasional monkey may survive without paralysis following intracerebral injection of an amount of virus which is capable of producing prostrating paralysis or death in all other animals in that particular series. Obviously, such irregularities occur more frequently with the smaller doses of virus al-

TABLE III

Comparison of the Effect of Various Dosages of Vitamin C

Dosage of vitamin C	Number of animals	No paralysis	Atypical paralysis	Typical paralysis
mg.				
700–100	10	0	0	10
50– 10	19	3	3	13
5	33	16	2	15
	62	19	5	38

Explanation for atypical and typical paralysis see in Table II.

though they are not at all uncommon with animals given massive doses. As regards the particular strain of virus employed in this work, we have observed over a period of about 7 years among several hundred control animals between 5 and 10 per cent that have failed to develop paralysis upon intracerebral injection of doses of virus ranging from 1 cc. to 0.01 cc. of a 10 per cent virus suspension. Moreover, when paralysis occurred, it was usually so severe that the animal became completely prostrated. Repeated titrations of our strain during the year when these experiments were under way indicate that the minimum paralytic dose of virus at that time was well below 0.01 cc. (see Table IV). The reasons for the occasional survival without paralysis of control animals injected intracerebrally with multiples of the minimum paralytic dose of virus are not clear. They

are just as obscure as the more pronounced variations in the susceptibility of monkeys observed with methods of infection other than intracerebral injection of the virus (intranasal, subcutaneous, intradermal) or the widespread insusceptibility among other animal species, including man. Suffice it to say that technical errors can usually be eliminated and that such animals evidently possess an exceptionally high degree of natural resistance. This is also suggested by the fact that the resistant animals often refuse to develop

TABLE IV

Titrations of Infectivity of Aycock Strain of Poliomyelitis Virus during 1935 (Intracerebral Injection)

Monkey	10% virus suspension	Result
	cc.	
N83	0.1	Complete paralysis, 7 days
N84	"	" " 8 "
N85	"	" " 5 "
N86	0.05	" " 9 "
N87	"	" " 6 "
N100	"	" " 5 "
O1	0.01	" " " "
O2	0.005	" " " "
O3	0.001	Questionable paralysis
O20	0.1	Complete paralysis 7 days
O21	"	" " 5 "
O22	0.01	" " 9 "
O23	"	Partial paralysis, 17 "

the disease on reinfection. In order to determine to what extent such refractory animals may have been present among our treated animals and controls, we have reinoculated all surviving animals at the end of the 1 month period of observation.

It appears from Table V that of 15 treated animals which had escaped paralysis and which had not died of intercurrent disease before 1 month had elapsed, all but 1 developed typical poliomyelitis upon intracerebral reinjection with 0.1 cc. or 0.01 cc. of virus. Of the 2 surviving controls, on the other hand, 1 remained refractory while

the other developed typical poliomyelitis. This result, in our opinion, suggests that practically all of our treated animals were potentially susceptible to the virus and that, if any escaped paralysis during the first infection, the escape may well have been due to the vitamin C treatment. It also serves to illustrate that a non-paralyzing infection

TABLE V

Reinfection of Surviving Monkeys, Treated and Untreated

Monkey	Type of animal	Dose of virus	Result	Controls
		cc.		
R17	Treated	0.1	Complete paralysis, 7 days	S18 Partial paralysis, 8 days
R19	"	"	Partial paralysis, 11 "	S20 Complete paralysis, 6 "
R22	"	"	Complete paralysis, 6 "	R3 " " 5 "
R23	"	"	" " 9 "	R49 " " 14 "
		"		R50 " " 8 "
		"		R52 " " " "
		"		R60 " " 6 "
		"		R63 " " 9 "
S76	Treated	0.01	Complete paralysis, 10 days	
S79	"	"	" " 11 "	
S96	"	"	" " 8 "	T12 Complete paralysis, 8 days
S97	"	"	" " 7 "	
T7	Untreated	"	No paralysis	
T20	Treated	"	Complete paralysis, 5 days	
T21	"	"	Partial paralysis, 11 "	
T23	"	"	Complete paralysis, 7 "	
T25	"	"	Partial paralysis, 11 "	T31 Complete paralysis, 8 days
T26	"	"	Complete paralysis, 9 "	
T27	"	"	No paralysis	
T29	"	"	Partial paralysis, 11 days	
T15	Untreated	"	Complete paralysis, 9 "	

with poliomyelitis virus rarely if ever is followed by immunity in the monkey, a fact which we have amply demonstrated in a previous paper (5).

2. Prophylactic Experiments

In view of the fact that therapeutic injection of vitamin C in proper doses seemed to have a favorable effect on the course of the established disease, we have

next investigated whether a prophylactic administration of this substance afforded any protection against subsequent inoculation. To achieve this end, two different methods were followed. A total of 16 monkeys were prepared by daily injections of vitamin C of various dosage (25 mg. to 500 mg.) for a period of 1 or 2 weeks and then injected intracerebrally with 0.1 cc. of virus; at the same time, 8 control animals were injected intracerebrally with the identical dose of virus. A second group of 10 monkeys were given daily, for a period of 2 weeks, approximately 150 mg. of vitamin C in their food. This amount represents merely a rough estimate since the eating habits of monkeys make it impossible to obtain control over the amount actually ingested. At the end of the 2 weeks period, these monkeys were injected intracerebrally with 0.01 cc. of virus; 7 control animals injected with the same dose of virus accompanying this test. The results of these two experiments appear in Table VI.

As may be seen from Table VI, we have in the first experiment only one monkey which survived without paralysis, the remaining 15 animals all developing the disease like the corresponding 8 controls. In the second experiment (feeding) we find among 10 prepared animals again one survivor without paralysis and 2 additional animals that developed the disease after prolonged incubation periods (18 and 19 days, respectively). This result, however, is offset by the fact that of 7 corresponding control animals, one survived without paralysis and another one developed the disease only on the 15th day. We conclude, therefore, under the conditions of the test, that prophylaxis with vitamin C was without any significant effect on the course of subsequent infection with poliomyelitis virus.

3. Miscellaneous Experiments

In this section we have grouped together some miscellaneous experiments which are not strictly concerned with vitamin C prophylaxis or therapy but which are nevertheless intimately related to the main problem under investigation.

The first question we were interested in was whether the apparent therapeutic effect of vitamin C could be ascribed to any direct action of the ascorbic acid on the virus. An answer to this question could be found by determining whether or not vitamin C, when injected intracerebrally in a single dose at the site of infection, was capable of preventing the disease. Accordingly, a group of 4 monkeys was injected intracerebrally with 5 mg. and 10 mg. of vitamin C, respectively. After 2 hours had elapsed, a dose of 0.05 cc. of virus was injected through the same puncture in the skull into the brain. Another group of 4 monkeys was treated in the same way, except that here the virus injection preceded the injection of the drug by 2 hours. All 8 experimental animals developed paralysis as did all of the 9 controls which had been infected with the same dose of virus. It follows from this experiment that vitamin C, although seemingly virucidal *in vitro*, fails to accomplish a local sterilizing effect in the central nervous system, under the conditions of our test.

TABLE VI

Attempts at Vitamin C Prophylaxis in Experimental Poliomyelitis

	Monkey	Preparation	Dosage of vitamin C	Dose of virus	Result	Controls
			mg.	*cc.*		
Experiment 1	O63	1 wk. injection	200	0.1	Complete paralysis, 9 days	O62 Complete paralysis, 7 days
	O74	" " "	50	"	Partial paralysis, 11 "	O90 " " 8 "
	O75	" " "	"	"	Complete paralysis, 9 "	O69 " " " "
	Q36	" " "	25	"	" " " "	
	Q37	" " "	"	"	" " 7 "	
	Q39	" " "	50	"	" " 13 "	Q54 Complete paralysis, 11 days
	Q40	" " "	"	"	" " 11 "	Q55 " " " "
	Q41	" " "	100	"	Partial paralysis, " "	Q90 " " 9 "
	Q42	" " "	"	"	" " 12 "	Q76 " " 11 "
	Q44	" " "	500	"	Complete paralysis, 7 "	
	Q69	2 wks. "	100	"	" " 10 "	
	Q70	" " "	"	"	" " 8 "	
	Q71	" " "	50	"	No paralysis	Q87 Partial paralysis, 16 days
	Q72	" " "	"	"	Complete paralysis, 7 days	
	Q73	" " "	25	"	" " 8 "	
	Q74	" " "	"	"	" " " "	
Experiment 2	S30	2 wks. feeding	150	0.01	Partial paralysis, 18 "	T1 Complete paralysis, 16 days
	S31	" " "	"	"	Complete paralysis, 7 "	T2 " " 7 "
	S32	" " "	"	"	" " 11 "	T3 " " 8 "
	S33	" " "	"	"	" " 7 "	T5 " " 7 "
	S34	" " "	"	"	No paralysis	T6 " " 9 "
	S36	" " "	"	"	Complete paralysis, 19 days	T7 No paralysis
	S37	" " "	"	"	Partial paralysis, 9 "	T9 Complete paralysis, 7 days
	S39	" " "	"	"	Complete paralysis, 10 "	
	S40	" " "	"	"	" " 14 "	
	S41	" " "	"	"	" " 9 "	

Experiments were next carried out to determine whether the addition of vitamin C to normal, non-neutralizing monkey serum would render the serum virucidal. While such "vitaminized" sera have occasionally brought about neutralization of the virus in mixture tests, irregular and confusing results were obtained on repetition with different doses of vitamin C. This makes it difficult to delimit clearly a neutralizing zone that might be compared with the range of natural vitamin C content of serum. Again, we have been unable up to the present to determine that the serum of normal monkeys, which had received large doses of vitamin C, acquires the property of neutralizing the virus as the result of such preparation. It seems as if inactivation of the virus by vitamin C in the presence of serum is by no means as regular and clear cut a phenomenon as its inactivation by the same substance in aqueous solution. This phase of the work is being continued.

We should finally mention in passing a short set of experiments designed to test the specificity of the apparent therapeutic effect of vitamin C. This could best be done by determining whether the use of other vitamins had any beneficial effect on the course of the disease. 4 monkeys were injected intracerebrally with 0.1 cc. of virus. 2 were given daily injections of 100 mg. of vitamin A (in the form of crystalline carotene), the 2 other animals receiving instead daily injections of 1 gm. of vitamin B (in the form of powdered yeast). All 4 monkeys developed typical poliomyelitis as did 4 accompanying controls. Apparently therefore, under the conditions of our test, vitamins A and B were without therapeutic effect in experimental poliomyelitis.

DISCUSSION

For a number of years we have contended that success in the control of infantile paralysis must depend upon the accumulation of more precise knowledge regarding the mechanism of natural protection against this disease (6). Once it is clearly understood why the majority of children and an even greater number of adults fail to develop paralysis upon exposure to the infectious agent, or—and this is expressing the same thought perhaps more trenchantly—why certain exceptional children are so highly susceptible that first contact with the virus leads to invasion of the central nervous sytem, it might be possible to apply the same principles to the therapy and prophylaxis of the disease.

Protection in poliomyelitis occurs in the form of two basic types of resistance which we believe to differ fundamentally from each other. The first is found in the solid immunity which is acquired after recovery from a paralyzing attack, a form of protection essentially due to a refractory state of the nerve tissue, which may or may not be accompanied by the presence of circulating antibodies. This protection, specific in character, nearly absolute in intensity and permanent in duration, in our opinion follows only after contact of the susceptible nerve cells with living virus, its development depending upon the production of actual lesions. The second, and as it seems to us more important type of protection, is represented by the resistance of naturally insusceptible individuals to this extraordinarily selective disease. Such resistance is expressed by complete insusceptibility of certain animal species, by the varying susceptibility of different human races, or in individual variations within the same race. The extensity and intensity of this non-specific resistance is characteristically conditioned by innate and environmental factors, such as heredity, sex, age, season and locality.

Two opposing theories have been invoked to explain the mechanism of this natural resistance, particularly in the case of man. The first, supported mainly by indirect epidemiological deduction, interprets the phenomenon as due to well nigh universal immunizing contact with the virus, progressing with the advance of age. It is assumed to be brought about by strictly subclinical immunization or by manifest but abortive attacks. According to the second theory, poliomyelitis is essentially a developmental disorder of youth, and protection against the disease is chiefly due to physiological factors. The fundamental difference in these two concepts is perhaps more clearly illustrated by their implications. The immunization theory regards all human beings as primarily susceptible and attributes protection exclusively to acquired specific immunity; hence, cases must develop either in individuals that have escaped such immunizing contact or else in persons that are less immunizable. The so called maturation theory, on the other hand, holds that man, under natural conditions of infection, is normally insusceptible and can carry the virus with impunity. Production of the disease which occurs only in comparatively few individuals, in spite of widespread chances for infection,

must therefore be brought about by some transient physiological abnormality or deficiency, hormonal or nutritional in character.

It has been our conviction that the known facts agree better with the theory of physiological resistance than with the assumption of an almost universal latent immunization. The reasons for this belief are founded briefly (*a*) on the inability to demonstrate clearly a process of subclinical immunization in either the monkey or man; (*b*) the lack of evidence that virucidal function of the serum and bodily resistance to infection in man or animals are necessarily correlated with previous exposure to the virus, and (*c*) direct experimental data which indicate that poliocidal substances found in normal human or animal tissues, serum or other body fluids are probably not of antibody character but resemble agents of vitamin-like or hormonal nature (7).

We have reviewed this controversy since on its determination depends any reasonable approach to treatment and prevention of poliomyelitis. Specific means of therapy through convalescent or immune serum having failed, experimentally as well as clinically, attention has concentrated lately on specific prophylaxis. Two methods of active immunization have been proposed, one involving the use of killed, the other the use of live virus. As might have been predicted, both have been given up. It appears that immunization with killed virus, although engendering antibody formation, falls short of providing cellular protection which is the prominent characteristic of true immunity in poliomyelitis (8). The use of live virus, on the other hand, even though attenuated, involves too great a risk in human beings. The chances, moreover, are that naturally insusceptible individuals will destroy the virus without deriving any more benefit from it than from casual contact, and that susceptible individuals may develop the disease (9). It is inevitable, therefore, that logical prophylaxis and treatment of infantile paralysis must center around attempts to imitate the mechanism of natural defense by enhancing normally functioning non-specific agencies, or by correcting the existing physiological deficiency by providing the missing nutritional or hormonal elements in adequate amounts and proportions. It seems from the results of our work that this might possibly be accomplished by supplying the infected individual with an optimum amount of vitamin C.

The assumption that vitamin C is an important factor of non-specific defense in infantile paralysis is suggested not only by the fact that ascorbic acid behaves like an unusually potent virucidal agent *in vitro*, but is also supported by certain other considerations. Thus, vitamin C is found in all normal body fluids which possess poliocidal properties (serum, tears, placenta, pregnancy urine, adrenal extracts). Next to the adrenal glands, it is present most abundantly in the central nervous system (10). During pregnancy and lactation its concentration in the body seems to be sharply increased, the placenta and milk representing the principal sources of exogenous supply for the embryo and newborn during prenatal and postnatal life (11). It is assumed by some investigators (12) that infants during the first few months after birth are capable of synthesizing this substance, like the rat, but that this capacity is lost or greatly diminished at the time of the physiological involution of the adrenal cortex, which occurs towards the end of the 1st year. These two points in the development of the child coincide characteristically with the maxima and minima of susceptibility to poliomyelitis.[2] Little is known about the actual demand for vitamin C, the extent of its assimilation and rate of excretion in health and disease and under varying environmental conditions. Suffice it to say, that although the precise relationship between the glands of internal secretion and vitamin metabolism is as yet not well understood, it is becoming increasingly clear that their operation is intimately interlocked since fluctuations in the supply of the latter lead to dysfunction of the former. Even though fully developed *C avitaminosis*, long recognized as the classical cause of scurvy, is but rarely observed today under normal living conditions, recent experience has shown that examples of minor deficiencies, or *C hypovitaminoses*, are by no means infrequent (13). The so called prescorbutic state, for instance, is not uncommonly found in actively growing children whose vitamin C requirements are known to exceed greatly those of adults for the maintenance of an adequate level. That this level may change considerably from time to time, possibly through changes in the thyroid gland (14), is suggested by seasonal fluctuations in the content of vitamin C in cows' milk (15) and in the rate of

[2] A similar phenomenon may be observed in diphtheria.

its excretion in man through the urine, as determined by tolerance tests (16). A serious disturbance of vitamin C storage is furthermore indicated during poliomyelitic infection by the occurrence of severe lesions in the adrenal gland (17) and an actual loss of reducing substances in this organ (18).[3] To this must be added the pathognomonic frequency in infantile paralysis of gastrointestinal disorders—so often encountered in children suffering from a low grade C hypovitaminosis —which in turn lead to a further impoverishment of the vitamin C reserves of the body; this may be due, in part at least, to the vitamin-splitting properties of certain intestinal strains of *B. coli*, as suggested by the work of Stepp and Schroeder (21). A vicious circle might thus be formed which would lower resistance in certain individuals at certain times to a critical point and permit systemic invasion of the ubiquitous virus, thus serving to reconcile the constitutional peculiarities of the poliomyelitic child (22) with the epidemiological vagaries of the disease.

There are, however, some contradictory experimental facts that must not be ignored. First, we have been unable to obtain neutralization of the virus *in vitro* with normal monkey brain in spite of its presumably high vitamin C content (23). The same is true for adrenal tissue, although its three principal physiological constituents, *i.e.*, adrenalin, cortin and ascorbic acid, in isolated form, have proved highly virucidal. Second, the concentration of vitamin C in the tissues is said to diminish with the advance of age (24), while the reverse is observed with respect to the neutralization phenomenon by serum. Third, vitamin C-deficient guinea pigs are still refractory to poliomyelitic infection (25). These questions need further elucidation as does the problem of how vitamin C accomplishes its apparent therapeutic effect in experimental poliomyelitis, whether through direct action on the virus, or by stimulating certain enzyme systems, or by changing cell permeability. It is noteworthy that ascorbic acid, unlike immune serum, fails to display any preventive effect in the monkey while there are reasons for believing that it may be effective in therapy. This would seem to indicate that the substance operates only when actually

[3] Again it should be pointed out that similar observations have been made in diphtheria (19) which seems to share certain aspects of its susceptibility problem with infantile paralysis (20).

needed, and that vitamin C upon injection into normal, non-deficient animals is not retained but rapidly eliminated. However, different results might be obtained in human prophylaxis, provided that further research reveals a causal relationship between disposition to infantile paralysis and faulty vitamin C metabolism.

SUMMARY AND CONCLUSIONS

1. A group of 34 monkeys were infected intracerebrally with 0.1 cc. of a 10 per cent virus suspension. Following infection, 9 animals were treated with daily injections of 700 to 100 mg., 16 with 50 to 10 mg. and 9 with 5 mg. of vitamin C for a period of 2 weeks. In the whole group there were 6 animals that survived without showing any evidence of paralysis. 2 of these had received 50 to 10 mg. while 4 had received 5 mg. All of 19 untreated control monkeys, infected simultaneously with the same amount of virus, developed paralysis.

2. Another group of 6 monkeys were infected intracerebrally with 0.05 cc. of virus. Following infection, one animal was treated in the same manner with 25 mg. and 5 with 5 mg. of vitamin C. In the whole group there was one animal that survived without showing any evidence of paralysis. This animal had received 5 mg. All of 7 untreated control monkeys, infected simultaneously with the same amount of virus, developed paralysis.

3. A third group of 22 monkeys were infected intracerebrally with 0.01 cc. of virus. Following infection, one animal was treated in the same manner with 100 mg., 2 with 50 to 10 mg., and 19 with 5 mg. of vitamin C. In the whole group there were 12 animals that survived without showing any evidence of paralysis. One of these had received 10 mg., while 11 had received 5 mg. Of 12 untreated control monkeys, infected simultaneously with the same amount of virus, 2 failed to show any paralytic symptoms and 10 developed paralysis.

4. A summary of the results obtained in all three groups shows: (*a*) that among a total of 62 treated monkeys, 19 survived without paralysis and 43 succumbed to the disease, while of a total of 38 untreated controls, only 2 failed to develop paralysis and 36 succumbed to the disease; (*b*) that treatment with large doses of vitamin C was without any beneficial effect (all 10 monkeys which had received 700

to 100 mg. developing paralysis), that the administration of intermediate doses was followed by occasional survival without paralysis of the treated animal (3 monkeys surviving of a total of 19 which had received 50 to 10 mg.), and that nearly one-half of the animals which had received small doses escaped the disease (16 monkeys surviving of a total of 33 which had received 5 mg.).

5. Attempts to protect monkeys against subsequent intracerebral infection by the prophylactic administration of vitamin C, either *per os* or parenterally, have produced negative results.

6. The pathogenesis of infantile paralysis is discussed in the light of the experimental findings and the possibility is suggested that vitamin C represents one of the deficiency factors in the susceptibility problem of the human disease.

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