FURTHER OBSERVATIONS ON VITAMIN C THERAPY IN EXPERIMENTAL POLIOMYELITIS*

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(Received for publication, June 29, 1937)

We have previously presented data to show that the parenteral administration of natural vitamin C during the incubation period of poliomyelitis in experimentally infected monkeys is followed by a distinct change in the severity of the disease (1). This was indicated by the fact that among a total of 62 C-treated animals 19 survived without paralysis and 43 succumbed to the disease, while of a total of 38 untreated control animals only 2 failed to develop paralysis and 36 succumbed to the disease. It appeared therefore that about 30 per cent of all monkeys which had received injections of ascorbic acid escaped paralysis as contrasted with approximately 5 per cent of non-paralytic survivors among the controls, a margin sufficiently large to admit of little possibility that the observed difference in severity of the disease could have been accidental. However, in view of the fact that the virus failed to produce paralysis in 100 per cent of the controls, suggesting that variations in natural resistance do occur among normal monkeys, it was considered advisable to repeat these experiments once more with a larger number of animals. There was also the need for further investigation of some essential details which had hitherto been studied only imperfectly. Most important of these was the question as to the effectiveness of C treatment at various intervals following infection and the establishment of an optimum dosage of ascorbic acid. It remained also to be determined whether or not synthetic vitamin C possessed resistance-enhancing properties similar to that of the natural preparation.

* Aided by grants from The Rockefeller Foundation and the President's Birthday Ball Commission for Infantile Paralysis Research.

Methods

The present communication deals with observations based on a total of 282 intracerebrally infected monkeys, treated at various intervals with various doses of vitamin C. Of these, 181 had received natural vitamin C and 101 either one of two different synthetic compounds. A total of 98 control monkeys, which were infected intracerebrally with corresponding amounts of virus and remained untreated, accompanied these tests. There is also included a group of 30 animals, infected by intranasal instillation of virus and treated with natural vitamin C, and 15 similarly infected controls. The virus used for the intracerebral tests was the Aycock strain which had been employed in our previous work. For the nasal infection the Rockefeller mixed virus was used.[1]

The general technique followed closely that outlined in detail in a previous paper (1). *Rhesus* monkeys weighing from 2000 to 3000 gm. were injected intracerebrally with amounts of virus (Aycock strain) ranging from 0.01 to 0.1 cc. (1 cc. of a 1:100 to 1:10 dilution) of a 10 per cent suspension. The intranasal infection was accomplished by instilling 1 cc. of a 5 per cent suspension of virus (R. M. V.) into each nostril and repeating this procedure 4 hours later. The treated animals received daily subcutaneous injections of vitamin C, the dosage varying from 5 to 100 mg., for a period of 2 weeks. Three different preparations of crystalline vitamin C were used in these tests. The first was a product extracted from natural sources (Merck), the second and third were synthetic preparations manufactured by either Merck and Company or Hoffmann-La Roche. Although all three preparations gave the identical reduction values for *l*-ascorbic acid with the indophenol reagent, they looked slightly different as far as the texture and color of the individual batches were concerned. The natural product, moreover, was said to contain approximately 2.5 per cent of unidentified impurities absent in either of the synthetic preparations.[2]

A total of twelve different series were run, ten of which represented intracerebral and two intranasal tests. The number of treated animals in each series varied from 10 to 49, the number of accompanying controls from 5 to 20. In some of these series different C products were used side by side in order to secure more strictly comparable data. The time at which vitamin C was first administered covered a range extending from the 1st to the 5th day of the infection, *i.e.*, until shortly before the onset of paralytic symptoms. All animals were carefully observed and a daily temperature record maintained for a period of from 3 to 4 weeks. In case of paralysis or death, an autopsy was made and the diagnosis confirmed by histological examination of the cord. Some of the surviving animals

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[1] This virus was kindly given to us by Dr. Albert B. Sabin of The Rockefeller Institute.

[2] We are indebted to Merck and Company for their cooperation in placing at our disposal generous amounts of their vitamin C preparations.

TABLE I

Vitamin C Therapy in Experimental Poliomyelitis

Series	Date	Amount of virus	Vitamin C	Time of first administration of vitamin C	Dose	Number of animals	Results		
							Typical paralysis	Atypical paralysis*	No paralysis
	1936	*cc.*		*day of infection*	*mg.*				
1	Oct. 17	0.02	Natural (Merck)	1st	5	6	2	1	3
		"	" "	1st	25	3	1	0	2
		"	" "	2nd	5	6	3	0	3
		"	" "	2nd	25	3	2	0	1
		"	" "	3rd	5	6	0	2	4
		"	" "	3rd	25	3	0	0	3
		"	—	—	—	9	5	1	3
2	Nov. 17	0.01	Natural (Merck)	1st	5	5	4	0	1
		"	" "	1st	25	3	3	0	0
		"	" "	2nd	5	6	3	1	2
		"	" "	2nd	25	3	1	0	2
		"	" "	3rd	5	6	2	0	4
		"	" "	3rd	25	3	1	0	2
		"	—	—	—	8	7	0	1
3	Dec. 7	0.1	Synthetic (Merck)	1st	5	5	3	2	0
		"	" "	3rd	5	6	3	2	1
		"	" "	4th	5	5	4	1	0
		"	" "	5th	5	6	4	2	0
		"	—	—	—	12	12	0	0
	1937								
4	Jan. 16	0.05	Synthetic (Merck)	1st	5	10	10	0	0
		"	" "	3rd	5	10	8	1	1
		"	" "	5th	5	10	8	1	1
		"	" "	5th	50	10	8	1	1
		"	—	—	—	20	20	0	0
5	Feb. 4	"	Natural (Merck)	1st	5	5	4	1	0
		"	" "	1st	25	5	3	2	0
		"	" "	3rd	5	5	3	0	2
		"	" "	3rd	25	4	1	1	2
		"	" "	5th	5	5	3	2	0
		"	" "	5th	25	5	3	1	1

* Incubation period over 14 days.

TABLE I—*Concluded*

Series	Date	Amount of virus	Vitamin C	Time of first administration of vitamin C	Dose	Number of animals	Results		
							Typical paralysis	Atypical paralysis*	No paralysis
	1937	*cc.*		*day of infection*	*mg.*				
5	Feb. 4	0.05	Natural (Merck)	5th	50	5	1	2	2
	"	"	" "	5th	100	5	3	0	2
	"	"	Synthetic (Hoffmann-La Roche)	1st	5	5	2	1	2
	"	"	" "	1st	25	5	5	0	0
	"	"	—	—	—	11	11	0	0
6	Feb. 25	"	Natural (Merck)	5th	25	5	4	0	1
	"	"	" "	5th	50	5	3	1	1
	"	"	Synthetic (Hoffmann-La Roche)	3rd	5	5	3	0	2
	"	"	" "	3rd	25	5	4	0	1
	"	"	—	—	—	8	8	0	0
7	Mar. 22	"	Synthetic (Hoffmann-La Roche)	5th	5	5	3	2	0
	"	"	" "	5th	25	5	4	0	1
	"	"	" "	5th	50	5	4	0	1
	"	"	" "	5th	100	4	3	1	0
	"	"	—	—	—	5	5	0	0
8	Mar. 23	0.02	Natural (Merck)	3rd	5	10	6	1	3
	"	"	" "	3rd	25	10	8	0	2
	"	"	—	—	—	10	9	1	0
9	Apr. 26	0.05	Natural (Merck)	1st	5	15	12	0	3
	"	"	" "	1st	25	9	6	0	3
	"	"	" "	2nd	25	10	6	1	3
	"	"	" "	3rd	25	6	5	0	1
	"	"	" "	5th	5	4	3	0	1
	"	"	" "	5th	100	5	4	0	1
	"	"	—	—	—	10	9	0	1
10	May 24	"	Natural (Merck)	2nd	25	10	7	0	3
	"	"	—	—	—	5	5	0	0

were sacrificed, beginning with the 24th day, and their tissues examined for vitamin C content, others were kept until the termination of 1 to 1½ months.[3]

[3] The results of the vitamin C titrations will be found in another paper (2).

The protocols of the individual series are given in Table I. A summary in which all intracerebrally infected animals are grouped together according to the kind and amount of vitamin C used will be found in Table II, while Table III lists the results obtained with treatment begun on different days of the infection. Table IV brings together the results obtained in the two intranasal series.

RESULTS

A study of Table I shows that in every one of the ten series, irrespective of the size of the infecting dose of virus, a definite though variable number of C-treated animals escaped paralysis.[4] Similar survivals among accompanying untreated controls occurred only in three series, two of the latter representing animals which had been infected with the smallest amount of virus, *i.e.*, 0.02 and 0.01 cc., respectively. While there can therefore be no doubt that the absence of paralysis among C-treated animals was clearly significant in at least seven series in which all corresponding controls succumbed to the disease, the percentage of non-paralytic survivors in the three remaining series was considerably higher among the treated animals than among the controls. Thus, in series 1 we have surviving without paralysis 16 (59.2 per cent) of 27 treated animals against 3 (33.3 per cent) of 9 controls, in series 2, 11 (42.3 per cent) of 26 treated animals against 1 (12.5 per cent) of 8 controls, and in series 9, 12 (24.4 per cent) of 49 treated animals against 1 (10 per cent) of 10 controls. Obviously, the lighter infection favored the occurrence of non-paralytic survivors among both treated and untreated animals alike, but to a dissimilar extent.

Important differences in the efficacy of the natural and synthetic vitamin C preparations are revealed by Table II. Among a total of 181 infected monkeys, which had been treated with natural vitamin C, 58 (32 per cent) survived without paralysis, 16 (8.8 per cent) developed an atypical attack of poliomyelitis (incubation period longer than 2 weeks), and 107 (59.1 per cent) succumbed to the disease in typical fashion. On the other hand, among a total of 101 in-

[4] It should be pointed out that a majority of the non-paralytic survivors pass through a distinct fever cycle during the infection, resembling an abortive attack of poliomyelitis, and that there are very few animals in which no rise in temperature is noted at any time.

fected monkeys which had been treated with synthetic vitamin C (Hoffmann-La Roche or Merck), 11 (10.8 per cent) survived without paralysis, 14 (13.8 per cent) showed an atypical attack of poliomyelitis, and 76 (75.2 per cent) succumbed to the disease in a typical manner. The above figures should be compared with the results obtained with control animals infected with the same amounts of virus which remained untreated. Of a total of 98 controls 5 (5.1 per cent) failed to show any paralysis, 2 (2 per cent) developed poliomyelitis after a

TABLE II

Comparison of the Effect of Natural and Synthetic Vitamin C in Experimental Poliomyelitis

Preparation of vitamin C	Dose	Number of animals	Result		
			Typical paralysis (incubation 1 to 14 days)	Atypical paralysis (incubation over 14 days)	No paralysis
	mg.				
Natural (Merck)	100	10	7 (70 per cent)	0 (0 per cent)	3 (30 per cent)
	50	10	4 (40 " ")	3 (30 " ")	3 (30 " ")
	25	82	51 (62.1 " ")	5 (6 " ")	26 (31.7 " ")
	5	79	45 (56.9 " ")	8 (10.1 " ")	26 (32.9 " ")
		181	107 (59.1 per cent)	16 (8.8 per cent)	58 (32 per cent)
Synthetic (Merck or Hoffmann-La Roche)	100	4	3 (75 per cent)	1 (25 per cent)	0 (0 per cent)
	50	15	12 (80 " ")	1 (6.6 " ")	2 (13.3 " ")
	25	15	13 (86.6 " ")	0 (0 " ")	2 (13.3 " ")
	5	67	48 (71.6 " ")	12 (17.6 " ")	7 (10.4 " ")
		101	76 (75.2 per cent)	14 (13.8 per cent)	11 (10.8 per cent)
Controls (untreated)	—	98	91 (92.8 per cent)	2 (2 per cent)	5 (5.1 per cent)

prolonged incubation period, and 91 (92.8 per cent) succumbed to typical paralysis.

It is therefore evident that the percentage of non-paralytic survivors following treatment with natural vitamin C was about six times as large as that of the untreated controls. In the animals treated with synthetic vitamin C this percentage was only twice that of the controls. In both treated groups the percentage of animals developing atypical paralysis was approximately the same,

between 5 and 6 times as large as that observed among controls. However the difference between the two treated groups is again clearly demonstrated by the fact that treatment with natural vitamin C had reduced the incidence of typical paralysis by about one-third as compared with the controls (from 92.8 to 59.1 per cent), while the re-

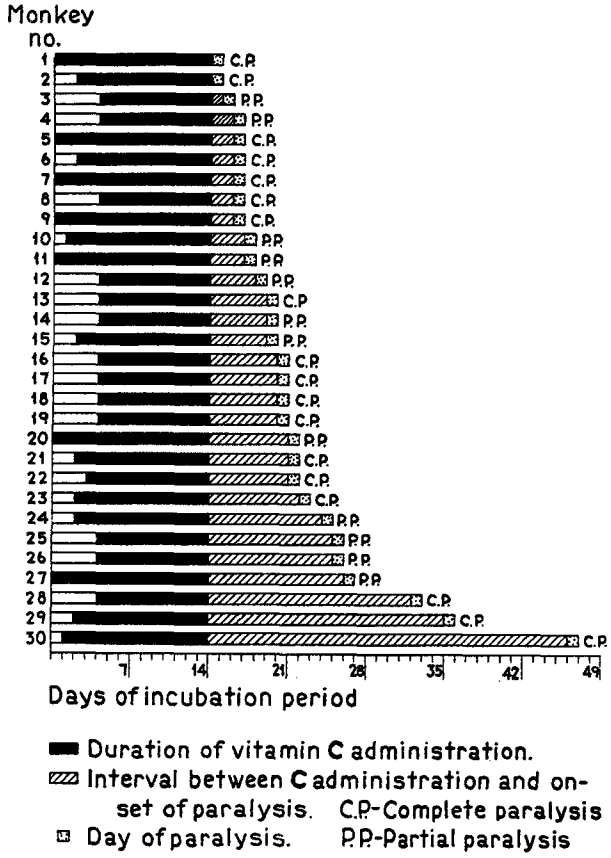

CHART 1. Delayed paralysis in C-treated monkeys.

duction for the two synthetic preparations combined was less than one-fifth (from 92.8 to 75.2 per cent).

Detailed data concerning the delayed onset of paralysis in a total of 30 C-treated monkeys are given in Chart 1. It will be noted that the incubation period in these animals extended from a minimum of

15 days to a maximum of 47 days. Although these animals carried live virus in the central nervous system for long periods of time, there was no immunizing effect from such a carrier state as indicated by the fact that a majority developed eventually severe prostrating paralysis.

The figures given in Table II, even though listed according to dosage of vitamin C, do not lend themselves readily to a fair evaluation of the relative efficacy of different amounts of vitamin C since treatment with such doses was begun on various days of the disease. Caution should therefore be used in their interpretation. As they stand, however, there is very little difference in the percentage of non-paralytic survivors among the several groups treated with different amounts of natural vitamin C, the figures running from 30 per cent to 32.9 per cent. A similar uniformity may be observed in the results obtained following treatment with synthetic vitamin C, except that the figures for all doses are consistently lower, ranging from 0 per cent in a small group of 4 animals treated with 100 mg. to between 10.4 per cent and 13.3 per cent for the remaining groups.

A detailed analysis of our data according to the kind of vitamin C used, dosage employed and day of first administration is given in Table III. It becomes at once apparent that the number of animals in some of the subdivisions, particularly with the synthetic preparations, is too small to allow of a fair interpretation. However it would seem that the administration of 5 or 25 mg. of natural vitamin C, begun on the day of infection, produces about the same result, *i.e.*, between 22.5 per cent and 25 per cent of the animals surviving without paralysis. When the first administration of vitamin C is delayed until the 2nd and 3rd day of the infection, considerably higher percentages of non-paralytic survivors are observed, especially among the animals injected with 5 mg. Thus it would appear that between 41.6 per cent and 48 per cent of the animals treated with 5 mg. and between 34.6 per cent and 38.4 per cent of those treated with 25 mg. of vitamin C escape paralysis at that time. We are rather inclined to the belief that the seemingly better figures are more apparent than real and do not indicate a real improvement of C treatment at the later stages of the disease. It should be remembered that a great portion of the animals listed in these two categories were taken from series 1 and 2 in which very small doses of virus were used,

resulting in an abnormally high accumulation of non-paralytic survivors among both treated animals and controls. If an allowance were made for this fact and the figures brought into accord with those of the other categories, the percentage of non-paralytic survivors among animals treated at this time would not materially exceed 30 per cent to 35 per cent. However there would still remain a slight advantage in favor of the animals treated with 5 mg. over those treated with 25 mg. More conclusive results are obtained with animals in which vitamin C treatment was not begun until the 5th day of the disease. At this time distinctly larger doses of vitamin C are apparently required to obtain significant results. Thus the percentage of non-paralytic survivors increases progressively from 11.1 per cent (5 mg.) to 20 per cent (25 mg.), and reaches a maximum of 30 per cent when doses of 50 to 100 mg. are used.

Much less regular results are observed in animals treated with the two synthetic preparations, due, in part at least, to the small number of animals in some of the subdivisions. However, here again it would seem that better figures are obtained with animals treated on the 3rd day of the disease (about 20 per cent) than with those in which treatment was begun on the day of infection (0 to 5 per cent). While the percentage of non-paralytic survivors shows also a tendency to drop when treatment is delayed until the 5th day of the disease, there is no unmistakable correlation to the size of the dose employed.

In comparing the results obtained with natural and with synthetic vitamin C it can safely be stated that, irrespective of the interval that had elapsed since infection, the percentage of non-paralytic survivors among animals treated with natural vitamin C, dose for dose, is distinctly higher than that observed for animals which had received either of the two synthetic preparations. Good results can still be obtained with natural vitamin C until the 3rd day, and fair results, with adequate dosage, on the 5th day of the disease. Treatment with synthetic vitamin C, on the other hand, is so irregular, on the whole, and so inefficient that it is doubtful whether the data will bear critical examination, except for the suggestion of a slight reduction in the severity of the disease as compared with untreated controls.

We have run only two series in which the effect of natural vitamin

C was studied on the course of experimental poliomyelitis induced by nasal instillation of the virus. In all animals treatment was begun

TABLE III

Effect of Various Doses of Natural and Synthetic Vitamin C When Administered on Different Days of Infection

Preparation of vitamin C	Day of infection	Dose	Number of animals	Result		
				Typical paralysis (incubation 1 to 14 days)	Atypical paralysis (incubation over 14 days)	No paralysis
		mg.				
Natural (Merck)	1st	5	31	22	2	7 (22.5 per cent)
		25	20	13	2	5 (25 " ")
	2nd	5	12	6	1	5 (41.6 " ")
		25	26	16	1	9 (34.6 " ")
	3rd	5	27	11	3	13 (48 " ")
		25	26	15	1	10 (38.4 " ")
	5th	5	9	6	2	1 (11.1 " ")
		25	10	7	1	2 (20 " ")
		50	10	4	3	3 (30 " ")
		100	10	7	0	3 (30 " ")
			181	107 (59.1 per cent)	16 (8.8 per cent)	58 (32 per cent)
Synthetic (Merck or Hoffmann-La Roche)	1st	5	20	15	3	2 (5 per cent)
		25	5	5	0	0 (0 " ")
	3rd	5	21	14	3	4 (19 " ")
		25	5	4	0	1 (20 " ")
	4th and 5th	5	26	19	6	1 (3.8 " ")
		25	5	4	0	1 (20 " ")
		50	15	12	1	2 (13.3 " ")
		100	4	3	1	0 (0 " ")
			101	76 (75.2 per cent)	14 (13.8 per cent)	11 (10.8 per cent)
Controls (untreated)			98	91 (92.8 per cent)	2 (2 per cent)	5 (5.1 per cent)

on the day of infection and maintained, as usual, for a period of 2 weeks. The results are given in Table IV. It will be noted that all

10 animals injected with 5 mg. of vitamin C succumbed to the disease, as did 9 of 10 animals injected with 25 mg. On the other hand, there were 9 animals that escaped paralysis among a group of 10 monkeys which had received doses of 50 to 100 mg. of vitamin C. Since all of the 15 untreated controls which accompanied these tests developed paralysis, it would seem that very encouraging results may be obtained with vitamin C treatment in this type of infection, although much larger doses of ascorbic acid are evidently required than for intracerebral tests. However it is planned to continue this work on a larger scale before valid conclusions can be drawn.

TABLE IV

Effect of Natural Vitamin C in Experimental Poliomyelitis (Intranasal Infection)

Series	Dose of vitamin C	Number of animals	Result		
			Typical paralysis (incubation 1 to 14 days)	Atypical paralysis (incubation over 14 days)	No paralysis
	mg.				
11	5	10	10	0	0
	25	10	8	1	1
	—	10	10	0	0
12	50	5	1	0	4
	100	5	0	0	5
	—	5	5*	0	0

* One of the 5 controls listed in series 12 as developing typical paralysis showed no distinct paralysis of the extremities but a very marked facial paralysis.

It seems to us that the significance of results obtained in therapeutic studies, such as the one here reported, lies not so much in isolated observations of success, or lack of success, obtained in one or several small experimental series, but that their intrinsic value is considerably strengthened if such results can be duplicated in prolonged investigations. For this reason it is interesting to compare the data obtained in this study with those published in our previous report, even though the two studies differ substantially among themselves in important details such as dosage of vitamin C, time of ad-

ministration, and amount of virus used. It will be noted from Table V that the two sets of figures are in exceptionally good agreement. Thus the average percentage of intracerebrally infected monkeys which escape paralysis following treatment with natural vitamin C stands between 30.6 per cent and 32 per cent, while only between 5.1 per cent and 5.2 per cent of the untreated controls fail to develop paralytic symptoms. These data are now based on a total of 243 (181 plus 62) treated monkeys and 136 (38 plus 98) controls, leaving little doubt as to the accuracy of the observations. However, further detailed statistical analysis of the two sets of figures shows

TABLE V

Effect of Natural Vitamin C in Experimental Poliomyelitis
(Comparison of Data Obtained in This Study with Data Previously Published)

Experimental data	Type of animal	Number of animals	Result		
			Typical paralysis	Atypical paralysis	No paralysis
Present data...	C-treated	181	107 (59.1 per cent)	16 (8.8 per cent)	58 (32 per cent)
Previous data...	" "	62	38 (61.1 " ")	5 (8 " ")	19 (30.6 " ")
Present data...	Controls	98	91 (92.8 " ")	2 (2 " ")	5 (5.1 " ")
Previous data...	"	38	34 (89.4 " ")	2 (5.2 " ")	2 (5.2 " ")

that the percentage of animals treated with 5 mg. alone has not inconsiderably dropped from that reported heretofore. Accidental features, connected with the limited number of animals used, may have caused the first figure to be unduly high. There is also a possibility that different preparations of natural vitamin C may differ conspicuously in the degree of their purity or the extent to which other extraneous substances may modify the therapeutic effect.

DISCUSSION

The data presented in this paper, which are based on a number of experimental animals almost thrice as large as previously reported,

leave no doubt that the administration of natural vitamin C during the incubation period of experimental poliomyelitis is followed by a definite alteration in the severity of the disease. This is indicated by the fact that about six times as many treated animals escape paralysis (32 per cent) as do corresponding controls (5.1 per cent). Conversely, the incidence of typical paralysis stands at 59.1 per cent among the treated monkeys as compared with 92.8 per cent among untreated control animals. The given percentages are essentially identical with those previously published by us, *i.e.*, there has been neither a significant reduction nor a conspicuous increase, when the data are taken as a whole. These figures demonstrate clearly the possibilities as well as the limitations of therapy with natural vitamin C in experimental poliomyelitis.

Different results were obtained when synthetic vitamin C was used. In contrast to the above figures, we find only about twice as many non-paralytic survivors in animals treated with this substance (10.8 per cent) as among the controls, and the incidence of typical paralysis stands at 75.2 per cent in this treated group.

It would appear, therefore, that the measure of therapeutic success depends upon certain variables which, in order of significance, may be listed as follows: First, the kind of vitamin C; second, the dosage of vitamin C; third, the rapidity of the infection as determined by the amount of virus and type of strain; and fourth, the time of first administration of vitamin C. As a fifth factor, equally as important as the other four, we should mention differences in individual response from monkey to monkey which necessitate running large series of animals if significant results are to be obtained.

The markedly superior therapeutic efficacy of natural vitamin C, as contrasted with the synthetic preparations, is surprising, since we found but little discrepancy in virucidal action between the two in *in vitro* experiments. It is realized that the assumption of a real difference between the two kinds of products will find little favor with the biochemist who considers them as identical, except for possible impurities in ascorbic acid extracted from natural sources. Similarly, clinical experience has proven that both preparations possess the same antiscorbutic properties in man and animals. However, recent data tend to show that the natural substrate of vitamin C

contains additional factors, absent in the chemically pure synthetic product, which may be present in variable amounts in natural preparations. We are alluding here to the so called P factor discovered by Szent-Györgyi (3) which exerts an antipurpuric effect and the so called J factor of von Euler and Malmberg (4) which apparently protects against pneumococcic infection in guinea pigs. Divergent results between synthetic and natural vitamin C have also been described by Havas and Gal (5) who found that the same concentration of ascorbic acid either stimulated or inhibited the growth of seedlings, depending upon whether the natural or synthetic product were employed. There is finally a possibility that mineral impurities of the natural product may serve as activators (6).

The problem of dosage is complicated by the fact that the largest amounts of ascorbic acid are not always necessarily optimal. This is clearly shown by a series of recent observations in widely differing fields all of which demonstrate that excessive amounts of vitamin C may bring about a reversal of the effect noticed with smaller doses (7). While our data are still too limited to make deductions certain, it would appear that during the first few days of the infection doses of 5 to 25 mg. produce an optimum therapeutic effect and that definitely larger doses, ranging from 50 to 100 mg., are required for treatment which is delayed until shortly before the expected onset of paralytic symptoms. For the same reason it can easily be understood that a faster progressing infection such as that induced by the R.M.V. strain responds only to doses of vitamin C definitely larger than those which are sufficient to halt the slower infection with the Aycock virus. The different dosage required for the two types of infections can certainly more easily be explained on that basis than by assuming differences in type of strain or mode of infection. The size of the effective dose of vitamin C therefore seems to be directly proportional to the speed of the infection and the stage at which the infectious process is advanced.

It is altogether impossible to say why the therapeutic effect should be so irregular, varying as it does, series for series, among different animals even though the average percentage of non-paralytic survivors remains fairly well fixed. The nature of this individual factor at present is wholly unknown. Obviously there are other conditions

which determine the extent to which vitamin C proves of benefit to the monkey. From the results of our vitamin C titrations of various monkey tissues it would seem possible that the protected animals may utilize vitamin C more effectively than the unprotected ones (2). Whether the degree of this utilization depends upon an unknown endocrine factor is an open question.

The mechanism by which vitamin C accomplishes its therapeutic effect still remains obscure. Obviously the simplest explanation would be to assume that ascorbic acid, not unlike a chemotherapeutic agent, inactivates poliomyelitis virus directly in the central nervous system, particularly since the latter can be shown to store this substance. Such inactivation would then be largely non-specific, since ascorbic acid, properly adjusted for pH, has proven capable of inactivating by direct contact *in vitro* every toxin and virus that has been investigated (8). However in spite of the frequent occurrence of such crude "antiviral" and "antitoxic" phenomena *in vitro*, therapeutic results with vitamin C have been obtained only in a minority of infectious diseases, among which tuberculosis (9) and diphtheria (10) are outstanding. This would suggest that vitamin C may conceivably act in a more specialized manner in certain types of tissue injury by enhancing the mechanism of natural defense. An example of such action is found in the observation that mice injected with vitamin C are protected against fatal doses of acetonitril (11), a poison to which they are seasonally more susceptible in the summer than in the winter (12). Evidence supporting the supposition that poliomyelitis virus is not destroyed directly by the vitamin *in vivo* comes from our observation that a considerable number of treated animals develop paralysis after greatly prolonged incubation periods, *i.e.*, at a time following discontinuance of C treatment when the artificially raised C levels have dropped again to their normal values (see Chart 1). In such cases the virus was clearly not killed but kept in check possibly as long as the cells maintained a high concentration of vitamin C. The irregularity of the therapeutic effect, so characteristic of all our experiments, would also plead in favor of a complex reaction, mediated through the cells, rather than for a direct virucidal action.

It has been pointed out by competent workers in vitamin research

that the chemist has superseded the pharmacologist who must surely recognize the probability that substances of an intensive physiological activity may exercise therapeutic functions that may seem comparatively remote from their normal nutritional mode of action (13). And such phrases as "conditioned vitamin deficiencies" or "toxic avitaminoses" continue to appear in the literature devoted to this new and promising field. If we are to accept vitamin C, therefore, as a therapeutic agent on the basis of its established physiological action rather than of its normal rôle in nutrition, the question arises in what way the anti-infectious effect of ascorbic acid in poliomyelitis can be correlated with its biochemical activity. Since it is commonly suspected that one of the functions of vitamin C is to regulate the oxidation-reduction potential of cellular respiration (14), it becomes relatively easy to imagine that an increased supply of the vitamin, which has a tendency to diminish during the infection, serves to maintain the oxidation-reduction system of nerve cells at a level at which the oxylabile virus is restrained from intracellular propagation. While this suggestion is still entirely hypothetical, it is in harmony with the observations of Brodie and Wortis (15) who have demonstrated a diminution in the oxygen consumption rate of nerve tissue infected with poliomyelitis virus.

In the light of these remarks it will readily be understood why vitamin C may be useful as an auxiliary therapeutic agent in certain infectious diseases which have little in common with the classical symptoms of frank scurvy. As far as poliomyelitis is concerned, the rationale of such treatment, at present, is predicated only by the experimental data given in this paper. It would be considerably strengthened by the establishment of a causal relationship between susceptibility to the disease and faulty C metabolism. Such demonstration would have to come from clinical studies and from epidemiological observations involving a correlation between the extent of the so called prescorbutic state in different age groups, latitudes, and seasons, on the one hand, and the distribution of cases of poliomyelitis, on the other. It is not likely, however, that abnormally low C levels are summarily produced by a low intake of vitamin C, except in regions where the content of vitamin C in food is notoriously inadequate, such as in some rural districts of northern countries (16).

It would seem rather as if the C requirements of exceptional individuals at certain times are greater than can be met and that crises occur, precipitated by a combination of environmental factors such as light, fatigue, intestinal disturbances, and other unknown agencies, which may facilitate central nervous system invasion of the ubiquitous virus.

SUMMARY AND CONCLUSIONS

1. A group of 181 monkeys were infected intracerebrally with amounts of virus ranging from 0.01 to 0.05 cc. of a 10 per cent virus suspension. At different intervals following infection treatment of these animals was begun with daily injections of 5 to 100 mg. of natural vitamin C for a period of 2 weeks. Of 89 monkeys treated on the 1st or 2nd day of infection 26 (29.2 per cent) survived without showing any evidence of paralysis; of 53 monkeys treated on the 3rd day of the infection 23 (43.3 per cent) survived without showing any evidence of paralysis; of 39 monkeys treated on the 5th day of the infection 9 (23 per cent) survived without showing any evidence of paralysis.

2. A group of 101 monkeys were infected intracerebrally with amounts of virus ranging from 0.05 to 0.1 cc. of a 10 per cent virus suspension. At different intervals following infection treatment of these animals was begun with daily injections of 5 to 100 mg. of synthetic vitamin C for a period of 2 weeks. Of 25 monkeys treated on the 1st day of infection 2 (8 per cent) survived without showing any evidence of paralysis; of 26 monkeys treated on the 3rd day of the infection 5 (19.2 per cent) survived without showing any evidence of paralysis; of 50 monkeys treated on the 4th and 5th day of the infection 4 (8 per cent) survived without showing any evidence of paralysis.

3. The above two groups of treated animals were accompanied by a group of 98 control monkeys which were infected intracerebrally with the same amounts of virus and remained untreated. In this group there were 5 (5.1 per cent) animals which survived without showing any evidence of paralysis.

4. The figures, taken as a whole, show that among 181 monkeys treated with natural vitamin C 58 (32 per cent) survived without paralysis, and among 101 monkeys treated with synthetic vitamin C

11 (10.8 per cent) survived without paralysis. In comparing the percentage of non-paralytic survivors of the two treated groups with that of the untreated controls (5.1 per cent) it is found that about six times as many animals escaped paralysis following treatment with natural vitamin C as did the corresponding controls. In the group of animals treated with synthetic vitamin C, on the other hand, there were only about twice as many non-paralytic survivors as among the controls.

5. The results obtained in this investigation, as far as they are concerned with the therapeutic effect of natural vitamin C in experimental poliomyelitis, are in close agreement with the data previously published.

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