

A FURTHER CONTRIBUTION TO VITAMIN C THERAPY IN EXPERIMENTAL POLIOMYELITIS*

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In previous publications experimental evidence has been adduced to show that vitamin C (*l*-ascorbic acid) may play an important part in enhancing resistance to poliomyelitic infection in the monkey. Not only could we demonstrate that ascorbic acid possesses powerful antiviral properties *in vitro* (1), but the administration of this substance to monkeys infected intracerebrally with the Aycock strain of poliomyelitis virus was followed by a milder infection than was observed in untreated control animals (2, 3). However, the fact could not be ignored that marked variations occurred in individual response, owing presumably to dissimilar degrees of utilization of the vitamin (4). Less favorable figures were obtained in another group of monkeys that had received synthetic ascorbic acid.

In the further course of this work an attempt was made to study the effect of vitamin C administration on experimental poliomyelitis induced by nasal instillation of the virus (RMV strain). What appeared to be encouraging results were obtained in two small series; however, because of the small number of animals and uncertainties in the technique of nasal instillation we refrained from drawing any conclusions from these preliminary observations. Meanwhile Sabin (5) has published data to show that in his tests vitamin C, either natural or synthetic, failed to exert any demonstrable effect on the course of experimental poliomyelitis induced by nasal instillation of the RMV strain of virus.

Since the very beginning of this investigation it had been realized that the measure of therapeutic success attainable by vitamin C administration depended upon certain variables the operation of which was not clearly understood. Most important among these was the kind and dosage of vitamin C, optimal results being obtained only with optimal doses of natural vitamin C. While the reasons for the apparent differential activity of the

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natural and synthetic product still remain obscure, it is of interest to note that Elmby and Warburg (6) reported similar discrepancies in antiscorbutic efficiency; moreover, Klodt and Stieb (7) have recently drawn attention to the different stability of the two preparations when exposed to oxidative agencies. Next in importance appeared to be the size of the infecting dose of virus which had to stand in a certain relation to the amount of ascorbic acid. Analogous examples of such quantitative interdependence in chemotherapeutic experiments between virus and drug have since been furnished by the action of prontosil album on lymphogranuloma infection in mice (Baer, 8) and the effect of tryptaflavin on experimental psittacosis (Mauer, 9).

Apart from such considerations, the experimental work, at this stage, left us with certain definite questions that called for further investigation. Obviously, there was a need, first, for determining whether the more virulent and more invasive RMV strain of virus was equally susceptible to inactivation *in vitro* by vitamin C as was the Aycok strain. In the second place, it became necessary to examine systematically the possibilities of influencing favorably by vitamin C administration the course of poliomyelitic infection, induced either by introducing large amounts of this highly virulent strain into the nasal portal of entry or by injecting small and carefully gauged doses of this virus by the intracerebral route. It is the purpose of this communication to present further results obtained in continued tests along the lines indicated above.

Methods

The RMV strain of virus was used exclusively in this work. *Rhesus* monkeys weighing from 2000 to 3000 gm. were infected either intranasally or intracerebrally.

All nasal infections were accomplished by instilling 1 cc. of a centrifuged 5 per cent suspension of virus into each nostril and repeating this procedure 4 hours later. It was soon recognized that, unless carefully controlled for details of technique, intranasal instillation may produce widely differing results in the infected animal. When virus suspensions were prepared from cords not older than 2 weeks and, following light centrifugation, the thick supernatant fluid, with tissue shreds, was squirted into the nostrils by pressure from a pipette armed with a rubber bulb, forcing the liquid forth and back repeatedly, practically all of the infected monkeys developed within 5 to 7 days a rapidly progressing typical paralysis which terminated in complete prostration of the animal. However, when suspensions were made from cords in glycerin for about 4 weeks, and a thin opalescent fluid, obtained after prolonged centrifugation, was simply allowed to drip into the nostrils, without exerting any pressure, a variable percentage of the infected animals developed complete or partial paralysis of the extremities, the remainder showing various degrees of facial paresis, often transitory, with weakness of the limbs, or no distinct paralytic symptoms at all. Monkeys that failed to develop characteristic paralysis of the extremities, but showed weakness and facial involvement, passed through a well marked fever cycle which lasted several days before the temperature returned to

normal, indicating that such episodes should be interpreted as "abortive takes" rather than as "missed cases." Both methods of nasal infection were used in our work; in the protocols the respective modes of instillation are designated either as "pressure method" or as "droplet method."

Concerning the intracerebral infection, it is sufficient to state that the technique was uniform throughout and identical with that previously followed, *i.e.* amounts of virus ranging from 1 cc. of a 1:100 to 1 cc. of a 1:200 dilution (0.01 cc. to 0.005 cc.) of the supernatant of a centrifuged 5 per cent cord suspension were injected into the frontal lobe of the brain.

Two different preparations of vitamin C were used, *i.e.* either synthetic crystalline *l*-ascorbic acid (Merck) or crystalline *l*-ascorbic acid extracted from natural sources (Merck).¹ The natural product was of the same batches that had been employed in our previous work; however, this substance, in the course of time, apparently had undergone profound chemical alteration (oxidation ?) as suggested by a yellow discoloration of the originally snow-white powder. Unfortunately, no natural vitamin C of reliable and uniform composition can be obtained, at present, in quantities from any of the chemical or pharmaceutical manufacturers.

The in Vitro Inactivation of the RMV Strain of Poliomyelitis Virus by Vitamin C and by Hydrogen Peroxide

In studying the power of chemical agents to inactivate the virus *in vitro*, the possibility must be excluded that inactivation, if and when it occurs, is not caused by acid destruction. According to previous observations (1), the virus of poliomyelitis remains active over a wide range of pH values, extending from about 4.4 to 8.2. In our earlier work, the ascorbic acid solutions, before being brought in contact with the virus, were adjusted to a pH of 6.6–6.8, which definitely eliminated the chance of acid inactivation. A lower pH value of 5.2–5.3 was chosen deliberately in the present experiments so as to provide more favorable conditions for protecting the ascorbic acid against irreversible oxidation. This necessitated a preliminary test in order to determine whether the virus, when exposed to a pH of 5.2–5.3 for a period of about 18 hours, would fully retain its virulence. The first experiment was carried out to answer this question.

Experiment 1.—Decreasing amounts of the supernatant of a 5 per cent centrifuged cord suspension (0.1 cc. undiluted; 0.1 cc. of a 1:2 dilution; 0.1 cc. of a 1:10 dilution) were combined with 1 cc. of Sørensen's phosphate buffer of pH 5.28. Control tubes containing the same amounts of virus with saline accompanied the experiment. The mixtures were held for 1½ hours at 37°C. and overnight in the ice box; the total volume was then injected intracerebrally into individual monkeys. The results are recorded in Table I.

¹ We are indebted to Merck and Company for placing at our disposal generous amounts of natural and synthetic vitamin C.

As can be seen from Table I, all monkeys developed complete paralysis within from 5 to 7 days, indicating that amounts of virus ranging from 0.1 cc. to 0.01 cc. had remained fully viable, under the conditions of this test, at a pH range from 5.2 to 5.8.

Experiment 2.—In this experiment a constant amount of virus, *i.e.* 0.1 cc. of a 1:10 dilution of the supernatant of a centrifuged 5 per cent cord suspension, was combined with decreasing doses of synthetic vitamin C, varying from 50 mg. to 0.5 mg. These doses were always contained in a volume of 1 cc. and were obtained by progressive dilution with distilled water of a freshly prepared 0.5 per cent mother solution of synthetic crystalline *l*-ascorbic acid which had been adjusted at pH 5.25 by the addition of suitable amounts of normal and N/10 sodium hydroxide. Control tubes containing the same

TABLE I
Effect of Phosphate Buffer pH 5.28 on the Viability of Virus

Monkey No.	Chemical and pH	Virus	Result
		cc.	
1	Phosphate buffer pH 5.28	0.1	Complete paralysis 6 days
2	" " " "	0.05	" " 7 "
3	" " " "	0.01	" " 7 "
4	" " " "	0.01	" " 5 "
5	Salt solution	0.1	" " 6 "
6	" "	0.05	" " 5 "
7	" "	0.01	" " 7 "

amount of virus, but mixed with acidulated distilled water at pH 5.2, accompanied this test. After incubation for 1½ hours at 37°C. and stay in the ice box overnight the mixtures were injected intracerebrally into individual monkeys. The pH values of these mixtures had previously been checked following incubation and, again, before injection by colorimetric and electrometric readings. The results are recorded in Table II.

It will be seen from Table II that all of the monkeys receiving the vitamin C-virus mixtures remained free from paralytic symptoms while both control monkeys injected with the virus-distilled water mixtures developed complete paralysis on the 7th and 8th day, respectively. This experiment therefore confirms our previous observation that small amounts of vitamin C suffice to inactivate multiple paralytic doses of poliomyelitis virus *in vitro*. It appears further that this phenomenon may be obtained with a highly virulent strain of virus (RMV strain) as well as with a strain of moderate virulence (Aycock strain), using synthetic ascorbic acid as well as ascorbic acid extracted from natural sources.

In view of McCormick's (10) suggestion that vitamin B deficiency may

be a possible factor in susceptibility to poliomyelitis, it seemed of interest to investigate whether the water-soluble vitamins of the B complex also possess virucidal power. In repeated tests it was found that neither B₁ (thiamin chloride) nor B₂ (riboflavin), when mixed with the virus under similar conditions, brought about inactivation of the infectious agent. Negative results were also obtained with nicotinic acid and with glutathione. It appears therefore that the inactivation of poliomyelitis virus

TABLE II
Inactivation of Virus in Vitro by Synthetic Ascorbic Acid

Monkey No.	Vitamin C	Virus	pH of mixture after incubation	pH of mixture before injection	Result
8	0.5	0.01	5.37	5.2	No paralysis
9	0.5	0.01	5.37	5.2	" "
10	1	0.01	5.19	5.28	" "
11	1	0.01	5.19	5.28	" "
12	5	0.01	5.13	5.4	" "
13	5	0.01	5.13	5.4	" "
14	25	0.01	5.1	5.32	" "
15	25	0.01	5.1	5.32	" "
16	50	0.01	5.1	5.23	" "
17	50	0.01	5.1	5.23	" "
18	Salt solution	0.01	5.4	5.6	Complete paralysis 8 days
19	" "	0.01	5.4	5.6	" " 7 "

by ascorbic acid, within the limits of our tests, exhibits a certain degree of chemical specificity.

The chemical forces that lead to inactivation of the virus by vitamin C are not known. However, some light has been thrown on the possible mechanism of the reaction by the work of Lojkin (11) who studied in great detail various phases of the inactivation of crystalline tobacco mosaic virus by *l*-ascorbic acid. According to this author, the inactivation that occurs in vitamin C-virus systems is neither due to the reduced ascorbic acid nor to the irreversibly oxidized dehydroascorbic acid, but rather to the action of a specific intermediate product which is formed in the course of the catalytic auto-oxidation of ascorbic acid under the influence of copper ions; evidence is adduced to suggest that this product is a peroxide. Sim-

ilarly, Kligler and Guggenheim (12), in studying the detoxication of tetanus toxin by ascorbic acid, arrive at the conclusion that oxidation is an essential part of the reaction which serves to destroy both toxin and vitamin. It is generally accepted that poliomyelitis virus is peculiarly susceptible to the action of certain oxidizing agents, such as hydrogen peroxide, potassium permanganate, chlorine and ultraviolet light (13); quantitative data, however, on the virucidal effect of hydrogen peroxide are incomplete. This prompted us to determine more accurately the end point of antiviral efficiency of hydrogen peroxide on the strain of virus under investigation.

TABLE III
Inactivation of Virus in Vitro by Hydrogen Peroxide

Monkey No.	H ₂ O ₂ dilutions 0.5 cc.	Virus 0.5 cc.	Result
20	1:100	1:10 dilution (0.05)	No paralysis
21	1:250	" " "	Slight paresis 10 days
22	1:500	" " "	Complete paralysis 8 days
23	1:100	1:50 dilution (0.01)	No paralysis
24	1:500	" " "	" "
25	1:1000	" " "	" "
26	1:2500	" " "	" "
27	1:5000	" " "	Slight paresis 12 days
28	Control	1:10 dilution (0.05)	Complete paralysis 7 days
29	"	" " "	" " 8 "
30	"	1:50 dilution (0.01)	" " 9 "

Experiment 3.—Equal parts, *i.e.* 0.5 cc. of the supernatant of a 5 per cent centrifuged cord suspension, diluted 1:10 or 1:50, and 0.5 cc. of progressive dilutions of hydrogen peroxide (superoxol Merck = about 30 per cent H₂O₂ by weight) were combined and the mixtures held for 1½ hours at 37°C. Control tubes containing mixtures of virus and distilled water were set up at the same time. After stay in the ice box overnight these mixtures were then injected intracerebrally into individual monkeys. The results are given in Table III.

It will be gathered from Table III that whereas a 1:2500 dilution of hydrogen peroxide is sufficient to bring about complete inactivation of 0.01 cc. of virus, an amount 25 times stronger, to wit a 1:100 dilution of hydrogen peroxide, is necessary to insure complete inactivation of an amount of virus 5 times larger, *i.e.* 0.05 cc. While the virus of poliomyelitis is therefore readily inactivated by hydrogen peroxide, no simple linear

correlation seems to exist between the amounts of peroxide and the amounts of virus that must react so as to produce complete inactivation of the infectious agent. As a matter of fact, a concentration of hydrogen peroxide fivefold that required by the law of multiple proportions was necessary in our tests to compensate for a corresponding increase in the dose of virus.

The Effect of Vitamin C Administration in Monkeys Infected Nasally with the RMV Strain of Virus

A total of six different series were run. Each series comprised a number of animals that had received daily subcutaneous injections of either natural or synthetic vitamin C, beginning with the day of infection, and an adequate number of untreated controls. A wide range of vitamin C dosage was covered, including much larger amounts than had been used in our previous work. This was done, partly with the view of augmenting the chances for a therapeutic effect against the more virulent strain of virus and partly in the hope of being able to compensate for any possible inferior effectiveness of the synthetic product. The ascorbic acid solutions were adjusted to a pH of between 5 and 6 shortly before administration to avoid any tissue ulceration at the site of injection. In three of these six series the pressure method of intranasal instillation was used and in three the droplet method, both methods having been described in detail elsewhere in this paper. The protocols of the individual series and a summary of the results obtained with the two different methods of nasal infection are presented in Table IV.

It is clear from an inspection of Table IV that in three series in which an infection of maximum severity was produced by flooding the nasal portal of entry with huge amounts of virus, vitamin C administration, irrespective of the kind and amount of ascorbic acid used, failed singularly to exert any influence on the course of the disease. Thus, with the exception of one non-paralyzed survivor among a total of 50 treated animals and one surviving animal with facial paralysis among a total of 20 controls, all monkeys developed complete and prostrating paralysis within about one week's incubation period.

Somewhat different results were obtained in the three series with a less drastic mode of infection. Not only was the disease produced by the droplet method of instillation much less severe than that induced by the pressure method, but the symptoms became progressively lighter from series to series in both groups of animals, treated and untreated. Undoubtedly, the droplet instillation created conditions under which only minute amounts of virus reached the olfactory area, allowing for a variable number of ani-

mals to develop typical paralysis of the extremities while the remainder showed either slight transitory involvement of the central nervous system or escaped paralysis entirely. As one would expect, the margin of differ-

TABLE IV
Effect of Vitamin C Treatment in Monkeys Infected Intranasally

Series	Monkeys	Method of nasal instillation	Daily dosage of vitamin C (natural or synthetic)	Result		
				Complete paralysis	Partial paralysis	No paralysis
I	C-treated 30	Pressure	mg. 10-100	29	0	1
	Controls 10	"	—	10	0	0
II	C-treated 15	"	150-500	15	0	0
	Controls 5	"	—	5	0	0
III	C-treated 5	"	500	5	0	0
	Controls 5	"	—	4	1*	0
I-III (total)	C-treated 50	"		49 (98%)	0 (0%)	1 (2%)
	Controls 20	"		19 (95%)	1 (5%)	0 (0%)
IV	C-treated 28	Droplet	100-300	20	0	8
	Controls 5	"	—	4	1*	0
V	C-treated 18	"	100-300	2	1	15
	Controls 10	"	—	2	5†	3
VI	C-treated 10	"	150-400	0	0	10
	Controls 5	"	—	1	2*	2
IV-VI (total)	C-treated 56	"		22 (39.3%)	1 (1.8%)	33 (59%)
	Controls 20	"		7 (35%)	8 (40%)	5 (25%)

* Animals marked thus showed only facial paralysis with weakness of limbs.

† Of the 5 animals in this group, 1 monkey developed only facial paralysis with weakness of limbs, the remaining four showing distinct partial paralysis of the extremities.

ence between the severity of the experimental infection in the treated animals and the controls, though favoring the treated animals consistently in every series, became more pronounced with the lighter infections. This may simply mean that the chances increased for the conversion of abortive infections into the complete suppression of paralytic symptoms although the comparatively small number of experimental animals makes a proper interpretation of such borderline results very difficult. When the data

for the last three series are taken as a whole, it will be found that among a total of 56 C-treated animals there were 23 (41 per cent) that developed complete or partial paralysis of the extremities while 33 (59 per cent) showed no paralytic symptoms whatsoever; among a total of 20 controls, on the other hand, there were 11 (55 per cent) that developed complete or partial paralysis of the extremities, 4 (20 per cent) that showed facial paresis with weakness of the limbs and 5 (25 per cent) that escaped paralysis altogether. The percentage of monkeys developing severe paralysis was therefore hardly significantly reduced in the treated group as compared with the non-treated group (41 per cent against 55 per cent); however, it is clear that the percentage of monkeys that remained objectively free from any paralytic symptoms was about twice as high in the treated animals as in the untreated controls (59 per cent against 25 per cent).

The Effect of Vitamin C Administration in Monkeys Infected Intracerebrally with the RMV Strain of Virus

In view of the limited success obtained in the experiments listed above in which C administration was begun on the day of infection, it was thought advisable to prepare monkeys by daily injections of large doses of ascorbic acid over a period of 2 weeks and then to continue treatment from the day of infection with smaller amounts. A total of five different series were run, each series comprising a variable number of C-treated animals and an adequate number of controls that remained untreated. The infecting dose of virus varied from 0.01 cc. to 0.005 cc., injected intracerebrally. Only synthetic vitamin C was used in these tests. The results have been brought together in Table V.

It appears from Table V that all 20 control animals, without exception, developed paralysis of various degrees within the first 2 weeks following infection; among a total of 30 C-treated animals, on the other hand, 22 showed typical paralysis, 3 developed the disease after definitely prolonged incubation periods (15 to 18 days), and 5 remained entirely free from paralytic symptoms. As might have been predicted, the greater number of non-paralytic survivors was observed in the group of monkeys infected with the smaller amount of virus, *i.e.* 0.005 cc. Taking the data as a whole it follows, therefore, that the percentage of monkeys developing prostrating paralysis was only slightly lower in the treated group than in the control group (66 per cent against 75 per cent); however it is evident that C treatment served to change appreciably the proportion between partially paralyzed animals and those that remained objectively free from any paralytic symptoms. In other words, whereas none of the controls failed to exhibit

some degree of paralysis, fully 16 per cent of the treated animals showed no evidence whatsoever of any paralytic involvement. This difference is further paralleled by the regular occurrence of a definite fever cycle in all of the partially paralyzed controls as contrasted with the completely afebrile course in the non-paralytic treated animals (see Chart 1).

TABLE V
Effect of Vitamin C Treatment in Monkeys Infected Intracerebrally

Type of animal	Number of animals	Dose of virus <i>cc.</i>	Dosage of vitamin C (synthetic)	Result			
				Typical paralysis (incubation period up to 14 days)		Atypical paralysis (incubation period over 14 days)	No paralysis
				Complete paralysis	Partial paralysis		
C-prepared	12	0.01	300 mg. daily for 14 days before infection, and continued after infection	11	0	0	1
Controls	7	0.01	—	7	0	0	0
C-prepared	18	0.005	300 mg. daily for 14 days before infection; 100 mg. daily after infection	9	2	3	4
Controls	13	0.005	—	8	5	0	0
C-prepared (total)	30			20 (66.6%)	2 (6.6%)	3 (10%)	5 (16.6%)
Controls (total)	20			15 (75%)	5 (25%)	0 (0%)	0 (0%)

In summarizing the experimental data, one would feel justified in concluding that, under the conditions of our tests, prophylactic and therapeutic administration of synthetic ascorbic acid has given evidence of being capable of modifying the course of the disease. However, it is clear that the results were not nearly as striking as previously reported with optimal doses of natural vitamin C in monkeys infected intracerebrally with the Aycoc strain of virus. As a matter of fact, in spite of using larger amounts of ascorbic acid and combining prophylactic preparation with therapeutic administration, the percentage of non-paralytic survivors in this study did not differ materially from the figure obtained in previous work with the synthetic compound (16 per cent against 10 per cent).

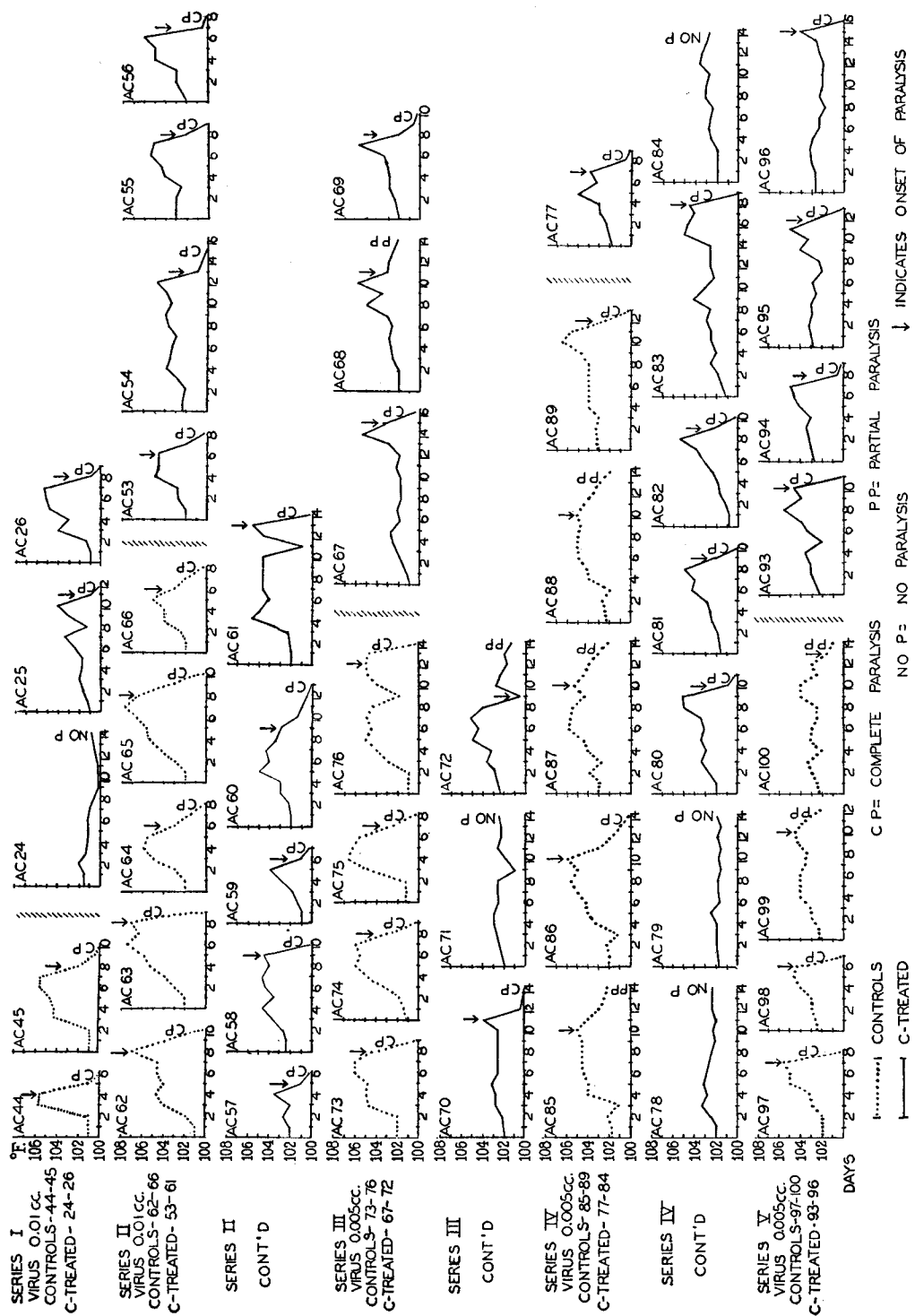


CHART 1. Effect of vitamin C administration in monkeys infected intracerebrally with the RMV strain of virus.

It has seemed of interest to submit the cords of paralyzed monkeys to careful histological examination so as to compare the extent and type of the lesions in the control animals with those of monkeys which had received vitamin C, even though treatment had obviously failed to prevent paralysis. In the majority of the cases we found the pathological process in the cords of the C-treated animals to differ distinctly from that presented by untreated controls. This difference concerned more the type of cellular reaction than the extent of its involvement. While the controls showed uniformly severe nerve cell destruction, with fully developed polymorphonuclear invasion and neuronophagia, as well as intensive interstitial cellular response, the lesions in the ganglion cells of C-treated animals were characterized by a good deal of chromatolysis, but rarely was cell degeneration complete and the degree of microglial proliferation and lymphocytic infiltration appeared decidedly less marked. Moreover, the lining of the central canal was better preserved in the latter, with more regular spacing and less crowding of the ependymal cells. In addition, we have often observed neuronal shrinkage and open perineural spaces, resulting in pictures which recall the type of cellular reaction described by Altmann (14) in the central nervous system of dogs that had been injected with large amounts of ascorbic acid. It is not certain whether the latter reaction served, in part, to protect the nerve cells against more extensive damage from the virus; however to postulate an anti-edematous effect of ascorbic acid in the affected areas would not be inconsistent with the accepted physiological function of the vitamin.

The described differences will readily become apparent from a study of Figs. 1 to 10 which represent typical fields of cord sections, cut from similar levels, of either C-treated or control animals.

DISCUSSION

The results recorded in this paper serve to affirm and extend our previous observations on the effect of crystalline vitamin C on poliomyelitis virus. They clearly show that small amounts of ascorbic acid, either natural or synthetic, are capable of inactivating *in vitro* multiple paralytic doses of two different strains of this virus. This virucidal action of vitamin C, however, is by no means limited to poliomyelitis virus. Thus, several other animal and plant viruses, *i.e.* vaccinia virus, herpes virus, rabies virus, foot and mouth disease virus, tobacco mosaic virus, and even bacteriophage, are also inactivated by this substance (15), as are a number of bacterial toxins, including diphtheria, tetanus, dysentery, staphylococcus, and anaerobic toxins (16). On the other hand, such antiviral effects so

far have not been obtained with any vitamin other than ascorbic acid, excepting the possible action of vitamin D on poliomyelitis virus (Toomey, 17). Vitamin C, therefore, may truthfully be designated as the "antitoxic and antiviral" vitamin.

There are reasons for believing that the inactivation occurring in virus-vitamin C systems is brought about by oxidation through some peroxide. This assumption finds support in our observation that poliomyelitis virus, like tobacco mosaic virus (18), diphtheria toxin (19), and tetanus toxin (20), is highly vulnerable to the action of hydrogen peroxide. This exquisite susceptibility to oxidizing agents stands in marked contrast to the remarkable resistance of the same viruses and toxins to protoplasmic poisons, such as phenol for instance, and serves to emphasize the similarity that seems to exist between viruses, toxins, and enzymes, as a group, when comparing their reaction to certain chemicals with those of living agents of disease.

When we come to examine the experimental evidence that vitamin C may act as an antiviral agent in the infected animal, the data, at first sight, are not very encouraging. Thus, Holden and Molloy (21) failed to observe any therapeutic effect from the administration of ascorbic acid to rabbits infected with herpes virus; similarly, Langenbeck and Enderling (22) reported negative results with ascorbic acid treatment of guinea pigs suffering from experimentally produced foot and mouth disease. As far as poliomyelitis is concerned, it seems safe to state that, under certain restricted experimental conditions, suggestive evidence is on hand to show that vitamin C is capable of influencing favorably the course of the infection in monkeys. However, this effect is comparatively slight and limited by a number of factors many of which are not yet under experimental control. Most important among these is probably the size of the infecting dose of virus. When the intranasal route is chosen, infection with quantitatively graded doses is frankly impossible. Huge amounts of virus suspension must be forced up the nasal passages to ensure the occurrence of paralysis with any degree of regularity and it remains undetermined whether the olfactory neuron has made contact with one or with multiple paralytic doses. As a matter of fact, simple calculation of the amounts involved suggests that, even though most of the instilled fluid is swallowed, enough must remain to represent an actual infecting dose which is many times larger than the minimum amount of virus which will regularly produce paralysis upon intracerebral inoculation. It is therefore not surprising that vitamin C treatment of monkeys infected by the nasal route should have failed to produce any therapeutic effect in Sabin's (5) hands as it did in ours when

the same severe method of intranasal instillation was employed. With a less drastic method of intranasal instillation, on the other hand, in which only threshold amounts of virus reach the olfactory fibers, the picture of the disease in control animals becomes so variable that the results do not lend themselves to unequivocal interpretation; but the impression is gained that treated animals stand a better chance of converting abortive attacks into an altogether non-paralytic course. Nevertheless, it has not been possible to duplicate the seemingly startling results obtained in two preliminary experimental series with nasal infection (3). The reasons for this discrepancy can only be conjectured; presumably a combination of factors, such as irregularities inherent in the mode of nasal instillation and fluctuations in virulence of the virus provided conditions under which vitamin C appeared to act more favorably on the course of the infection than can be substantiated by repeated tests.

When the same strain of virus is used to infect monkeys by the intracerebral route, a definite range of minimal doses can be found which results in the production of paralysis with a high degree of certainty. Control animals injected with such amounts (0.01 to 0.005 cc.) show but little individual difference in response to the virus as indicated by the uniformity of the preparalytic fever curve, the fixed incubation period, and the severe and generalized paralysis; with the smaller doses of virus, however, the extent of central nervous system involvement is somewhat variable as shown by the occasional recovery of a monkey following isolated paralysis in one or several limbs. This pattern of the experimental disease in control animals appears to be significantly changed in monkeys which have received vitamin C, the latter animals showing signs of individual variation in response which are not observed in untreated controls. This variation may either take the form of prolonged incubation periods, or lead to the evolution of a completely afebrile course without any objective paralytic symptoms; but there is no reduction in the extent and severity of paralysis, once vitamin C has failed to prevent its onset. It would rather seem as if the fate of the infected animal which receives vitamin C is determined by a critical equilibrium of several component factors which, in their totality, tend to interfere with virus propagation; hence, ascorbic acid may do nothing more than tip the balance in favor of a successful outcome by increasing the forces of natural resistance. Certainly, the therapeutic or preventive action of this substance is too limited, irregular, and complex to permit one to regard it in any sense as a true chemotherapeutic agent in experimental poliomyelitis. However, it is conceivable that its action may be complemented by the introduction of other physiological factors, which would

tend to augment its effect, such as metal ions, other vitamins and hormones, or immune serum; or, that ascorbic acid, because of its basic biochemical properties, may ultimately become the mother substance that would yield more powerful antiviral agents of true chemotherapeutic character.

One may ask with good reasons what bearing the foregoing experimental data have on the rationale of using ascorbic acid in the prophylaxis and therapy of the human disease. An answer is not easily provided since the factors which permit natural infection in the susceptible child are not reflected in the animal experiment. Certain it is, that the monkey does not exhibit that degree of susceptibility which characterizes human predisposition. The point that appears crucial for any prognostication is whether or not the selective human susceptibility to the disease is related fundamentally to C metabolism. With any convincing evidence to that effect we would seem to be on more hopeful ground. For there are many examples to show that vitamin administration will restore deficient individuals to normal levels of defense; but whether a superabundance of vitamin C can enhance adequate function to hypernormal efficiency is by no means certain (23). In this connection the extensive observations of Heaslip (24) on a recent epidemic of poliomyelitis in South Australia are of great interest. In estimating the urinary output of vitamin C, under a load test, a mean figure of 19.9 per cent excretion was obtained in 60 cases of poliomyelitis as contrasted with a mean figure of 44.3 per cent in 45 healthy contacts, suggesting some relationship between the degree of vitamin C saturation and the infectious and non-infectious state. A correlation was further shown to exist between the severity of the attack and the level of urinary excretion of the vitamin; moreover, comparing the severity of the disease and urinary excretion of vitamin C among urban and rural cases it was found that the distribution of cases corresponded with the facts collected in a previous nutritional survey on the state of nutrition in urban and rural populations. A study of the geographical spread of the epidemic in several Australian States finally suggested some relationship between the incidence of the disease, its age distribution, and the known facts regarding the nutritional state of the respective populations. On the basis of these findings Heaslip states that the associated phenomena remain unexplained unless the assumption is made that the vitamin deficiency preceded the disease and concludes "that a low level of vitamin C nutrition predisposes to infection and severity of attack."

SUMMARY AND CONCLUSIONS

1. Multiple paralytic doses of poliomyelitis virus (RMV strain), when brought together with small amounts of synthetic ascorbic acid *in vitro*,

are rendered non-infectious as determined by intracerebral injection of such mixtures into *rhesus* monkeys.

2. Vitamin C administration to monkeys infected intranasally with the RMV strain produces results which differ in accordance with the technique employed for nasal instillation. With an infection of maximum severity, induced by flooding the nasal portal of entry with large amounts of virus, vitamin C administration fails to exert any demonstrable influence on the course of the disease. With a less forceful method of droplet instillation, the picture of the disease in control animals becomes so variable that the results cannot be easily interpreted; but the available data suggest that vitamin C treatment may be a factor in converting abortive attacks into an altogether non-paralytic infection.

3. The administration of synthetic vitamin C to monkeys infected intracerebrally with small doses of the RMV strain gives results comparable to those previously obtained with this substance in monkeys infected intracerebrally with the Aycock strain of poliomyelitis virus.

4. The implications of the findings are discussed.

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EXPLANATION OF PLATE 33

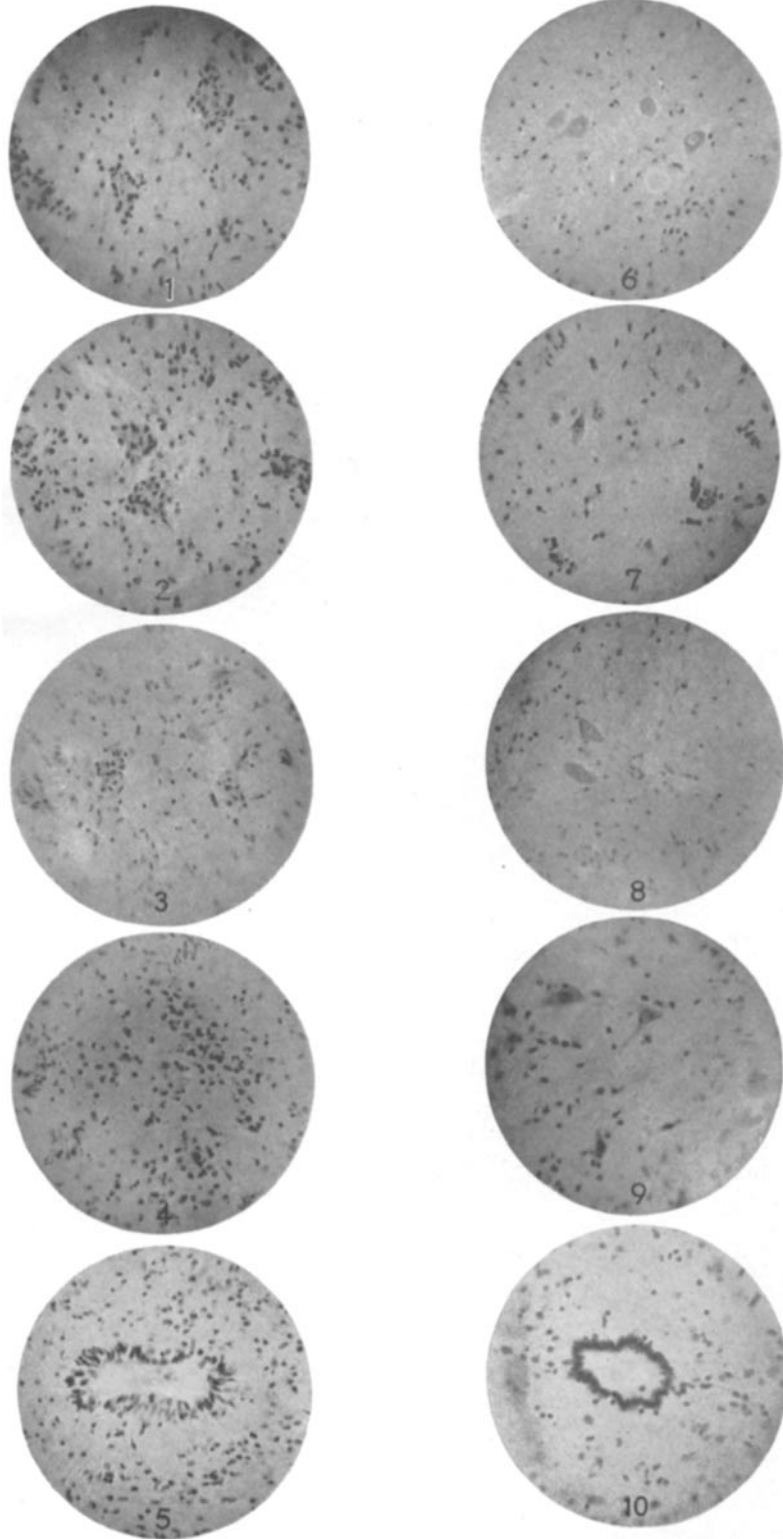
FIGS. 1 to 4. Sections of various levels of the cords of untreated paralyzed control monkeys.

FIG. 5. Central canal of a paralyzed control monkey.

FIGS. 6 to 9. Sections of various levels of the cords of C-treated paralyzed monkeys.

FIG. 10. Central canal of a C-treated paralyzed monkey.

Note the difference in the degeneration of nerve cells and the extent of the inflammatory reaction between untreated controls and C-treated animals; also the difference in the degree of edema shown by the alignment of the ependymal cells of the central canal in the two groups of animals.



(Jungeblut: Vitamin C therapy in poliomyelitis)