INACTIVATION OF POLIOMYELITIS VIRUS IN VITRO BY CRYSTALLINE VITAMIN C (ASCORBIC ACID)*

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Inactivation of the virus of poliomyelitis by various chemicals has formed the subject of investigation for many authors. The sum total of this work has demonstrated that this virus is peculiarly susceptible to the action of certain oxidizing agents (hydrogen peroxide, potassium permanganate, etc.), while at the same time exhibiting marked resistance against such general protoplasmic poisons as phenol.

Valuable as such studies are in that they aid in an understanding of the nature of the etiological agent, they are obviously of but little significance for the problem as to what chemical forces, if any, are engaged in the inactivation of the virus in the body of the normally insusceptible host or the recovered individual. An attempt to approach this question in a rational manner was made by studying the distribution of physiological poliocidal substances throughout the body. This led to the discovery that virus-neutralizing substances were present, not only in serum but in human tears (1), placenta (2) and pregnancy urine (3), as well as in adrenal extracts containing either the medullary (adrenalin) or the cortical (cortin) hormone (4). In the course of this work it was observed that the same endocrine secretions (adrenalin, cortin) (4) as well as certain antitoxic sera (5) were frequently capable of inactivating not only poliomyelitis virus but also diphtheria toxin. When it was furthermore found that an important constituent of the adrenal gland, *i.e.* vitamin C or ascorbic acid, possessed the power of inactivating diphtheria toxin *in vitro* and *in vivo* in extraordinarily small amounts (6), it became an im-

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portant problem to determine whether or not poliomyelitis virus is vulnerable, in a like manner, to the injurious effect of this substance.

This paper presents experiments bearing on this point.

EXPERIMENTAL

A total of 30 *rhesus* monkeys were injected intracerebrally with mixtures of a constant dose of virus (Aycock passage strain), *i.e.* 0.1 cc. of the supernatant of a centrifuged 10 per cent poliomyelitis cord suspension, and graded doses of vitamin C.[1] In addition, 5 monkeys received the same amount of virus mixed with saline or distilled water, for control purposes. The doses of vitamin C varied from as much as 100 mg. to as little as 0.05 mg. These quantities were obtained by progressive dilutions with distilled water of a freshly prepared 5 per cent solution of vitamin C, the respective doses being always contained in a volume of 1 cc.

In order to guard against a possible inactivation of the virus by the strong acid reaction of concentrated vitamin C solutions, the mother solution was adjusted at pH 6.6 to 6.8 by the addition of $\frac{N}{10}$ sodium hydroxide, immediately before making the dilutions for the various test doses. According to Amoss (7) the virus remains viable at least 48 hours at hydrogen ion concentrations ranging from pH 5.0 to 8.2. In Howitt's (8) experiments it was not destroyed by acidification to pH 4.4. The virus-vitamin C mixtures were incubated for $1\frac{1}{2}$ hours at 37°C., placed in the ice box overnight and injected the following morning intracerebrally into individual monkeys. The same technique was used in preparing the control mixtures. The injected monkeys were carefully observed for 1 month and symptoms recorded. The results obtained in this experiment are given in Table I.

As can be seen from Table I, there is a definite, fairly wide range, within which inactivation of poliomyelitis virus may be obtained by vitamin C. Absolute regularity of the phenomenon, however, is evidently limited to a narrower zone. In our experiments, the optimal quantities seem to lie between 10 and 5 mg., larger and smaller doses failing to protect with the same consistency. With the diminution of the dose below 1 mg. a gradual loss of inactivating power is clearly indicated, no neutralization whatsoever occurring with the smallest dose tested, namely 0.05 mg. An increase of the dose above 50 mg. was evidently not feasible because of the toxicity of larger amounts.

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

TABLE I

Inactivation of Poliomyelitis Virus in Vitro by Crystalline Vitamin C (Ascorbic Acid)

Monkey No.	Dose of virus	Amount of vitamin C	Result
	cc.	*mg.*	
Q18	0.1	100	Died 24 hrs.
Q19	0.1	100	" 24 "
O67	0.1	50	Complete paralysis, 11 days
O87	0.1	50	No paralysis
Q77	0.1	50	" "
Q82	0.1	50	" " (?)
O88	0.1	10	No paralysis
Q20	0.1	10	" "
Q75	0.1	10	" "
Q76	0.1	10	" "
Q21	0.1	5	" "
Q22	0.1	5	" "
Q78	0.1	5	" "
Q79	0.1	5	" "
O98	0.1	1	Complete paralysis, 11 days
Q23	0.1	1	No paralysis
Q85	0.1	1	" "
Q86	0.1	1	" "
Q25	0.1	0.2	Partial paralysis, 17 days
O86	0.1	0.2	No paralysis
Q66	0.1	0.2	" "
Q80	0.1	0.2	" "
Q57	0.1	0.1	Complete paralysis, 13 days
Q67	0.1	0.1	Partial paralysis, 10 days
Q56	0.1	0.1	No paralysis
Q81	0.1	0.1	" "
Q58	0.1	0.05	Complete paralysis, 19 days
Q60	0.1	0.05	" " 5 "
Q68	0.1	0.05	" " 9 "
Q83	0.1	0.05	" " 13 "
Q62*	0.1	—	" " 7 "
O90*	0.1	—	" " 7 "
Q26*	0.1	—	Partial " 9 "
Q54*	0.1	—	Complete " 12 "
Q84*	0.1	—	" " 9 "

* Control monkeys receiving virus mixed with either saline or distilled water.

Monkeys surviving without paralysis were reinfected 1 month later. All developed typical paralysis, except Q82 which died on the 15th day without symptoms of poliomyelitis.

DISCUSSION

The experimental data described in this report are interesting in several ways. First, they show that extraordinarily small amounts of vitamin C are capable of rendering non-infectious multiple paralytic doses of poliomyelitis virus. Second, they reveal a remarkable similarity in the quantitative aspects of this inactivation when compared with the neutralization of diphtheria toxin by vitamin C. The fact that two such heterogenous substances as diphtheria toxin and poliomyelitis virus, one a lifeless poison and the other a presumably living agent, should prove to be susceptible to the inactivating effect of no less than three highly reducing substances, *i.e.* adrenalin, cortin and vitamin C, all of which are present in the adrenal gland, seems to us particularly worthy of emphasis since it may serve to provide a common basis for a biochemical explanation of their destruction. The third, and undoubtedly most important, point of interest is to be found in the fact that vitamin C in the reduced form, as employed in these tests, is a normal constituent of various animal and human tissues, particularly adrenal and brain, and occurs also in a reversibly oxidized state in the blood (9). If the quantitative assays published in the literature are acceptable, the amounts of vitamin C normally present in the central nervous system are well within reach of the range found to be neutralizing in this paper. Further investigations will have to show whether the neutralization phenomenon observed when certain normal human and animal sera are brought into contact with poliomyelitis virus, is in any way influenced by the presence of vitamin C in the serum; also, whether the natural resistance of certain species of animals to intracerebral injection of this virus shows any correlation with the concentration of vitamin C in the brain or cord. Obviously, similar implications may be invoked for an explanation of the varying susceptibility of man to natural infection with poliomyelitis.

Experiments are now under way to determine whether vitamin C possesses any prophylactic or therapeutic properties in the treatment or prevention of experimental poliomyelitis.

SUMMARY AND CONCLUSIONS

The experimental evidence presented in this paper shows that multiple paralytic doses of poliomyelitis virus, when mixed with very small amounts of crystalline vitamin C (ascorbic acid), are rendered non-infectious as determined by intracerebral injection of such mixtures into *rhesus* monkeys.

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