

VITAMIN C AND TOXINS. PART II. THE EFFECT OF THE ADMINISTRATION OF VITAMIN C TO GUINEA-PIGS INJECTED WITH DIPHTHERIA AND TETANUS TOXINS.

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The effect of the injection and ingestion of ascorbic acid before and after injection with diphtheria and tetanus toxins is investigated and the question of immunity by vitamin C administration is discussed.

In the preceding communication (*J. Indian Chem. Soc.*, 1938, 15, 438) we have described the effect of vitamin C and other reducing substances on diphtheria and tetanus toxins *in vitro*. It has been shown that the inactivating effect of ascorbic acid in diphtheria toxin is not solely a p_H effect and is to be attributed at least partially to the action of the vitamin itself. This property of vitamin C led us to carry out certain experiments *in vivo* in relation to vitamin C and diphtheria and tetanus intoxication. The effect of the injection of the vitamin immediately before and after the injections of the toxins into guinea-pigs was studied. The effect of the injection of massive doses of the vitamin some time after the injection of toxins was also investigated. Further the protective action if any, of feeding or injecting with the vitamin for a period preceding the injection of the toxins was sought to be explored. The experiments were undertaken both as a sequel to our *in vitro* experiments and also in view of the claims of Greenwald and Harde (*Proc. Soc. Expt. Biol. Med.*, 1935, 32, 1157) that ingestion of the vitamin confers protection on the guinea-pigs against subsequent injection of diphtheria toxin. King and Menten (*J. Nutrition*, 1935, 10, 129, 141) have, of course, shown that in vitamin C deficiency guinea-pigs are more susceptible to infection but Jungeblut (*J. Immunol.*, 1937, 33, 203) has found that ingestion of the vitamin does not confer complete protection against the effect of the injection of tetanus toxin. The subject obviously requires further investigation.

EXPERIMENTAL.

The diphtheria and tetanus toxins were standardised and their m.l.d.'s determined. Guinea-pigs weighing between 250 and 300 g. were taken as experimental animals. In the following experiments guinea-pigs injected with the toxin alone were always kept as controls and these died

in course of 3-5 days. Toxins were diluted with saline solution. Likewise, the reducing substances were dissolved in saline.

Injection of Ascorbic Acid immediately before and after Injection of the Toxins.

One m. l. d. of the toxin was injected, and ascorbic acid (100 mg.) in saline (1 c.c.) was injected within one minute after the injection of the toxin, and on a site remote from that of the toxin injection. There appears to be some protective action (Table I). Another reducing substance, cysteine, was injected in a similar manner, which however, unlike ascorbic acid, failed to protect the animals. Hydrochloric acid in saline (1 c.c. p_H 2.0) which was used as a control for the acidity of the ascorbic acid solution, when similarly administered, gave negative results. Neutralised ascorbic acid in saline at p_H 6.8 has so far failed to check the effect of toxin injection as was also observed in *in vitro* experiments (Ghosh and Guha, *loc. cit.*). The results are shown in Table I.

TABLE I.

Substances injected after 1 m. l. d. of toxin had been injected.	(a) Diphtheria toxin.			(b) Tetanus toxin.		
	Treated.	Survived.	Died.	Treated.	Survived.	Died.
Ascorbic acid (100 mg.)	8	6	2*	9	4	5
Cysteine (100 mg.)	5	None	5	5	None	5
Neutralised ascorbic acid (100 mg.)	4	„	4	4	—	4
Hydrochloric acid	5	1	4	5	—	5
Toxin (m. l. d.) alone.	6	None	6	6	—	6

* Of these, one died after 12 days and the other after 16 days.

Injection of 100 mg. of ascorbic acid a minute before the toxin injection gave the following results (Table II), which again indicate a somewhat favourable effect of the injection of ascorbic acid.

TABLE II.

Toxin.	Number of animals		
	Treated.	Survived.	Died
Diphtheria	5	2	3
Tetanus	4	2	2

Delayed Injection of Massive Doses of Ascorbic Acid.

A delay in the injection of ascorbic acid (100 mg.) even half an hour after the toxin injection failed, however, to protect the animals. Repeated daily injections of ascorbic acid in massive doses for 3 to 4 days after the injection of the toxins also failed to save the animals from death (Table III).

TABLE III.

Toxin.	Period after which ascorbic acid was injected.	Amount of ascorbic acid injected daily.	No. of animals	
			Treated.	Died
A. Diphtheria	$\frac{1}{2}$ hour	100 mg.	3	3
	1 hour	100	2	2
	„	250	2	2
	1 day	350	5	5
	„	500	3	3
	„	1000	2	2
	2 days	300-500	3	3
B. Tetanus	1 day	350-750	4	4

In the cases of animals injected with diphtheria toxin subsequent injection after one day of 250 mg. of cysteine and of hydroquinone into two animals failed to exert any protective action. In the case of hydroquinone the animal died within 1 hour with shivering and convulsions. The cysteine-treated animal also succumbed.

The Effect of Previous Ingestion and Injection of Vitamin C.

Greenwald and Harde (*loc. cit.*) claimed that the ingestion of 10-20 mg. of ascorbic acid increased the resistance of guinea pigs to 1 m. l. d. of toxin after the 1st or 2nd day of feeding. We fed guinea-pigs daily 50 mg. of ascorbic acid for six days and on the 7th day 1 m. l. d. of the toxin was injected. Practically all the animals died within three days like the controls. Almost similar negative results were obtained when the animals were injected with 20 mg. of ascorbic acid daily for 6 days before toxin injection (Table IV). It seems that if some resistance is conferred on the animals, it can only be slight.

TABLE IV.

Toxin used (1 m.l.d.).	Number of animals			Toxin used (1 m.l.d.).	Number of animals		
	Treated.	Survived.	Died.		Treated.	Survived.	Died.
Diphtheria	6	None	6	Diphtheria	6	2	4
Tetanus	6	1	5	Tetanus	6	None	6

*Does the Previous Injection of Toxin-vitamin C Mixture increase
Resistance against the Toxins?*

That guinea-pigs which have survived for 5-7 weeks the injection of the toxin-vitamin mixture may develop some resistance against further injection of 2 m. l. d's of the toxin was claimed by Greenwald and Harde (*loc. cit.*). Negative results under similar circumstances were, however, obtained by us when 2 m. l. d's of the diphtheria toxin were injected. In the cases where 1 m. l. d. of the diphtheria toxin was used the majority of animals survived.

TABLE V.

Period in weeks between toxin- vitamin injection and fresh toxin injection.	Dose.	Number of animals		
		Treated.	Survived.	Died.
5	2 m. l. d's of diphtheria toxin.	4	None	4
5	1 m. l. d. of diphtheria toxin.	4	3	1

Another set of experiments was carried out to investigate whether three consecutive injections of the toxin-vitamin mixture previous to the injection of 2 m. l. d's of the diphtheria toxin can produce any immunity. No increased resistance or immunity was observed. In this case 1 m. l. d. of the diphtheria toxin mixed with 25 mg. of ascorbic acid was injected into guinea-pigs every fourth day; after 3 such toxin-vitamin injections 2 m. l. d's of the diphtheria toxin were injected into the animals, All the four animals, so treated, died within 4 days.

DISCUSSION.

Although *in vitro* experiments show that vitamin C may have some specific inactivating effect on diphtheria and tetanus toxins (Ghosh and Guha, *loc. cit.*), the *in vivo* experiments do not favour the view that the vitamin confers any definite protection against diphtheria and tetanus toxins. The action of the toxins is apparently so quick and powerful that the possible action of the vitamin, which is seen when the vitamin is mixed with the toxins prior to injection, is nullified when the vitamin and the toxins are separately injected. There is some protective action shown by the vitamin if injected immediately before and after injection of the toxins and this cannot be due to local neutralisation of the toxins by the vitamin, as they are injected in different sites. It seems possible that in these cases the temporary rise in the acidity of the blood following ascorbic acid injection may be responsible for the detoxication of the toxins, which are very susceptible to the lowering of p_H . The fact that previous injection and ingestion of vitamin C during a fairly long period before toxin injection confers hardly any protection shows that the vitamin cannot be looked upon as a direct antitoxic agent. The previous injection of the vitamin-toxin mixtures also did not appear to increase the resistance of the guinea-pigs to these toxins. King and Menten (*loc. cit.*) have shown, however, that guinea-pigs deficient in vitamin C are more susceptible to diphtheria toxin. Abbasy, Hill and Harris (*Lancet*, 1936, *ii*, 1413) have also observed that rheumatism and tuberculosis cause depletion of vitamin C reserves of the body, which is apparently a result of the increased metabolism of vitamin C. It would seem, therefore, that although vitamin C may not be a direct antitoxic agent, "its deficiency would likely make an organism more susceptible to certain infections and aggravate some diseased conditions. Normally, therefore, vitamin C in liberal quantities should form part of the food as an indirect anti-infective agent. Further observations from this laboratory on the powerful inhibitory effect of vitamin C on the growth of certain bacteria *in vitro* also indicate the possibility that normal consumption of vitamin C may be helpful in guarding against bacterial infections. Further work in this line is, however, in progress."

CONCLUSION.

(i) Injection of ascorbic acid immediately before and after injection of 1 m. l. d. of the diphtheria and tetanus toxins appears to have some protective action on guinea-pigs,

(2) A delay in the injection of ascorbic acid failed to save the animals, even when massive doses of the vitamin were administered.

(3) Previous administration of ascorbic acid either orally or by injection failed to confer any definite protection against the toxin.

(4) Previous injection with the toxin-vitamin mixture also failed to confer any protection against diphtheria toxin injected subcutaneously.

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