VITAMIN C AND TOXINS. PART IV. THE EFFECT OF TETANUS TOXIN ON VITAMIN C METABOLISM.

BY BAIDYANATH GHOSH.

The effect of the injection of 0·5 m.l.d. of tetanus toxin caused a decrease in true ascorbic acid value in the blood, liver, kidney and adrenal tissues of guinea-pigs. The urinary excretion of free ascorbic acid was lowered during toxic condition with simultaneous increase in combined ascorbic acid.

A comparative study was carried out for the estimation of combined ascorbic acid in the urine by the method of Scarborough and Stewart and that of Sen-Gupta and Guha.

Recently it has been observed (Ghosh, *J. Indian Chem. Soc.*, 1939, **16,** 241) that in guinea-pigs injection of sublethal doses of standardised diphtheria toxin caused an appreciable decrease of ascorbic acid content of blood, adrenal, liver and kidney. Similar decrease in the urinary excretion of free ascorbic acid was observed. Simultaneously, the amount of combined ascorbic acid also excreted in the urine during toxic condition was found to be always greater than that usually excreted by the same guinea pig in the normal state of health. The present investigation of the effect of tetanus toxin was undertaken in order to test the previous view that during the period of infection or disease, the blood, urine and tissues like adrenal, liver and kidney suffer a loss of ascorbic and that a part of this lost ascorbic acid is eliminated in the urine in a combined state. Recently, Kaiser and Slavin (*J. Pediat.*, 1938, **13,** 322) have shown a significant correlation between low blood ascorbic acid values and severe infection with hemolytic streptococci. Thus ascorbic acid seems to play an important part in the defence mechanism of the body as it is always affected during the period of infection or intoxication.

The method for the estimation of combined ascorbic acid in the urine which was described in the previous communication, was that of Scarborough and Stewart (*Biochem. J.*, 1937, **31,** 2231). In this paper the method described by Sen-Gupta and Guha (*J. Indian Chem. Soc.*, 1937, **14,** 95; Sen-Gupta and Guha, *Science and Culture*, 1938, **3,** 398; Guha and Sen-

Gupta, *Nature*, 1938, **141,** 974) is adopted for the estimation of combined ascorbic acid. A comparative study of these two methods has been carried out simultaneously with the same sample of urine and it is found that the method of Sen-Gupta and Guha can serve for the purpose and it can be done in a shorter interval of time.

EXPERIMENTAL

Guinea-pigs weighing between 250 and 350 *g.* were kept on a ration consisting of green grass and germinated gram. Five groups of guinea-pigs were taken, each group containing 5 animals. One group served as control while the animals of the other four groups were injected with 0·5 m.l.d. of standardised tetanus toxin. The blood was drawn out from the heart of guinea-pigs at intervals of 24, 48, 72 and 96 hours. The ascorbic acid content was determined from 2 c.c. of oxalated blood, after precipitation of proteins by trichloroacetic acid, by the usual titration with 2 : 6-dichlorophenol-indophenol (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935, **12,** 30). The results are given in Table I.

TABLE I.

Mg. of ascorbic acid per 100 c.c. of blood

No. of expt.	Normal	Hours after injection of 0·5 m.l.d. of tetanus toxin. 24	48	72	96
1	0·90	1·00	0·94	0·55	0·55
2	0·96	0·75	0·80	0·54	0·38
3	0·73	0·95	0·53	0·45	0·37
4	1·11	0·89	0·63	0·47	0·43
5	0·75	0·98	0·58	0·58	0·38
Mean	0·89	0·91	0·69	0·50	0·42

A progressive decrease in blood ascorbic acid was observed after 48 hours of toxin injection.

Similar experiments were carried out with other groups of guinea-pigs in order to determine the true ascorbic acid value of the adrenal, liver and kidney 24, 48, 72 and 96 hours after the injection of 0·5 m.l.d. of tetanus toxin (Table II). True ascorbic acid value was determined by the enzymic oxidation (with ascorbic acid oxidase) of an aliquot of the tissue filtrate after

the estimation of the total reducing substances present (Sen-Gupta and Guha, *Science and Culture*, 1938, **3,** 398; Guha and Sen-Gupta, *Nature*, 1938, **141,** 974).

TABLE II.

A. *Adrenal.*

Mg. of ascorbic acid per g. of adrenal.

Hours after 0·5 m.l.d. of tetanus toxin injection.

No. of expt.	Normal	24	48	72	96
1	0·432	0·366	0·352	0·295	0·276
2	0·407	0·357	0·430	0·258	0·312
3	0·380	0·370	0·430	0·273	0·300
4	0·453	0·505	0·478	0·367	0·206
5	0·452	0·500	0·475	0·476	0·330
Mean	0·428	0·419	0·441	0·333	0·284

B. *Liver.*

Mg. of ascorbic acid per 5 g. of liver.

Hours after 0·5 m.l.d. of tetanus toxin injection.

No. of expt.	Normal	24	48	72	96
1	0·870	0·866	0·838	0·650	0·452
2	0·637	0·706	0·510	0·567	0·450
3	0·694	0·591	0·584	0·589	0·608
4	0·577	0·529	0·670	0·390	0·562
5	0·690	0·890	0·593	0·644	0·526
Mean	0·696	0·711	0·639	0·568	0·519

C. *Kidney.*

Mg. of ascorbic acid per g. of kidney.

No. of expt.	Normal	Hours after 0·5 m.l.d. of tetanus toxin injection. 24	48	72	96
1	0·136	0·116	0·115	0·111	0·073
2	0·093	0·086	0·077	0·090	0·087
3	0·060	0·102	0·077	0·104	0·096
4	0·087	0·064	0·084	0·064	0·076
5	0·112	0·094	0·072	0·066	0·053
Mean	0·097	0·092	0·084	0·087	0·077

The amount of combined ascorbic acid in the urine excreted by a guinea-pig during normal and toxic conditions was determined by the method of Scarborough and Stewart (*loc. cit.*) as previously described by Ghosh (*loc. cit.*). A comparative study of the values obtained by the method of Scarborough and Stewart (*loc. cit.*) and that of Guha and Sen-Gupta (*Nature,* 1938, **131,** 974; Sen-Gupta and Guha, *loc. cit.*) was made from the same sample of urine (*vide* Table III). An aliquot (20 c.c.) of the total acidified urine (50 c.c.), obtained after the removal of the thiosulphate by barium acetate method, was taken in a conical flask (100 c.c.) into which H_2S was allowed to bubble for 5 minutes. Then the flask, while H_2S was being made to bubble, was immersed in a boiling water-bath and the contents were heated for 15 minutes after which the flask was placed in a cold water-bath. When the flask was cold bubbling of H_2S was stopped and H_2S was then removed by a current of CO_2 or coal gas (previously bubbled through dichromate and sulphuric acid mixture*). The urine after the removal of H_2S was made up to the original volume (20 c.c.) by the addition of distilled water and an aliquot of it was titrated with the dye. The remaining portion was allowed to undergo enzymic oxidation in order to estimate the true ascorbic acid value. The values of ascorbic acid content (both free and combined) of the urine measured by this method of Sen-Gupta and Guha are given in Table IV. The ascorbic acid content (both free and combined) of the urine was estimated for three or four consecutive days before and after the injection of tetanus toxin into the same guinea-pig. The results are given in Table III.

* It has been found that coal gas, when purified by bubbling through chromic acid mixture, can be used to remove H_2S completely without affecting the ascorbic acid in the solution.

TABLE III.

Mg. of ascorbic acid excreted in the urine per animal during 24 hours.

No. of expt.	No. of days.	Free ascorbic acid.		Combined ascorbic acid in terms of ascorbic acid. Scarborough & Stewart.		Sen-Gupta & Guha.	
		Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection.
I	1	0·331	0·224	0·162	0·206	0·114	0·143
	2	0·280	0·148	0·108	0·269	0·085	0·259
	3	0·251	0·205	0·131	0·115	0·009	0·151
	4	0·280	0·214	0·160	0·174	0·070	0·176
	Mean	0·285	0·197	0·140	0·191	0·069	0·182
II	1	0·332	0·249	0·192	0·241	0·107	0·139
	2	0·332	0·201	0·210	0·240	0·112	0·196
	3	0·332	0·185	0·148	0·196	0·048	0·195
	4	0·332	0·206	0·210	0·283	0·049	0·251
	Mean	0·332	0·210	0·190	0·240	0·097	0·195
III	1	0·134	0·158	0·130	0·135	0·039	0·029
	2	0·202	0·132	0·123	0·075	0·038	0·077
	3	0·243	0·100	0·090	0·247	0·040	0·123
	4	0·218	0·100	0·043	0·300	0·010	0·094
	Mean	0·199	0·122	0·096	0·189	0·032	0·080
IV	1	0·131	0·162	0·232	0·215	0·016	0·043
	2	0·178	0·162	0·111	0·105	0·005	0·000
	3	0·246	0·125	0·054	0·250	0·037	0·086
	4	0·150	0·136	0·059	0·270	0·044	0·065
	Mean	0·176	0·146	0·114	0·210	0·025	0·048
V	1	0·134	0·131	0·091	0·233	0·000	0·087
	2	0·239	0·179	0·094	0·241	0·066	0·129
	3	0·170	0·158	0·074	0·248	0·024	0·097
	4	0·150	0·158	0·109	0·222	0·170	0·169
	Mean	0·173	0·156	0·092	0·236	0·065	0·095
VI	1	0·174	0·110	0·097	0·298	0·000	0·054
	2	0·158	0·162	0·030	0·109	0·016	0·163
	3	0·174	0·162	0·093	0·176	0·044	0·098
	Mean	0·168	0·144	0·067	0·194	0·020	0·105

Another set of experiments was carried out with guinea-pigs injected with 0·5 m. l. d of tetanus toxin and the results obtained by Sen-Gupta and Guha's method are shown in Table IV.

TABLE IV.

Injected with 0·5 m. l. d. of tetanus toxin.

Mg. of ascorbic acid excreted in the urine during 24 hours per guinea-pig.

No. of Expt.	No. of days.	Free ascorbic acid. Before toxin injection.	Free ascorbic acid. After toxin injection.	Combined ascorbic acid in terms of ascorbic acid (Sen-Gupta & Guha). Before toxin injection.	Combined ascorbic acid in terms of ascorbic acid (Sen-Gupta & Guha). After toxin injection.
	1	0·218	0·221	0·006	0·163
I	2	0·260	0·148	0·230	0·107
	3	0·270	0·120	0·120	0·190
	Mean	0·249	0·163	0·118	0·153
	1	0·273	0·347	0·049	0·143
II	2	0·350	0·290	0·139	0·170
	3	0·314	0·221	0·089	0·090
	4	0·347	0·221	0·143	0·091
	Mean	0·320	0·269	0·103	0·123
	1	0·252	0·185	0·038	0·175
III	2	0·286	0·215	0·164	0·093
	3	0·260	0·217	0·100	0·038
	4	0·280	0·221	0·070	0·191
	Mean	0·269	0·202	0·094	0·124
IV	1	0·270	0·228	0·050	0·041
	2	0·224	0·138	0·056	0·124
	3	0·225	0·257	0·098	0·150
	4	0·246	0·224	0·046	0·126
	Mean	0·241	0·211	0·062	0·110

DISCUSSION.

The injection of a sublethal dose of tetanus toxin into guinea-pigs has been found to produce a diminution of the free ascorbic acid content of blood, liver, kidney, adrenal and urine. At the same time urine in the infected condition appeared to contain a considerably increased amount of combined ascorbic acid, as estimated by the methods of Sen-Gupta and Guha (*loc. cit.*) and of Scarborough and Stewart (*loc. cit.*). By the latter method the values obtained are greater, as the treatment with H_2S is more prolonged. But in both methods, ascorbic acid oxidase was used and differences in the combined ascorbic acid content of the urine between the normal and toxic conditions were noticeable.

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DEPARTMENT OF APPLIED CHEMISTRY,
UNIVERSITY COLLEGE OF SCIENCE,
CALCUTTA.

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CONTENTS

PAGE