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Effect of Diphtheria Toxin Upon Vitamin C in Adrenals of Guinea Pigs.

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Interest in the effect of seasonal variations in the diet of guinea pigs upon their susceptibility to bacterial toxins led to an extensive study¹ of the metabolism of vitamin A in these animals. The results indicated the participation of some other factor or factors in the increased variations in susceptibility which occur during the winter months. Published reports^{2, 3} directed our attention to vitamin C.

The guinea pigs used in the experiments were bred by this laboratory and weighed from 230 to 280 gm. They had been fed the routine winter diet: alfalfa hay, oats, barley, commercial rabbit pellets, cabbage, carrots and mangels, and sodium chloride and water *ad libitum*. During May, occasional supplements of grass were fed.

The adrenals of 10 guinea pigs injected early in December with a uniform lethal dose of diphtheria toxin were examined for their vitamin C content by the method of Bessey and King.⁴ One of 10 similar animals which had received one-half the dose of toxin, and one uninjected control were destroyed as each in the first group died. The average vitamin C content of the adrenals of the guinea pigs which had died from the effects of diphtheria toxin was reduced to less than 15% of that of the control animals. The change was much less in the adrenals of the 10 animals that received half the lethal dose of toxin. The average amount of vitamin C was 85.5% of that of the normal controls. In fact, the adrenals of the 5 which were destroyed approximately 48 hours after injection showed an increase in the vitamin C content, and it was not until 24 hours later that a diminution became apparent.

Although the slightly higher vitamin C content of the adrenals of the animals in the second group at the end of 48 hours might have been due to errors of random sampling, the possibility that the toxin, when not given in overwhelming doses, stimulated the ad-

¹ Torrance, C. C., *Am. J. Hyg.*, 1936, **23**, 74.

² Harde, E., *C. r. Acad., d. sc.*, 1934, **199**, 618.

³ Jungeblut, C. W., and Zwemer, R. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1229.

⁴ Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

renals to mobilize ascorbic acid seemed worth investigating. Accordingly, the following May, a group of animals were injected with one-third of the lethal dose of toxin. Five additional uninjected guinea pigs of the same weight, sex, and dietary history were used as controls. Four of the test animals were destroyed 24 hours after injection. There were marked subcutaneous edema and congestion at the site of injection but no gross changes in the adrenals. Chemical examination of these organs indicated that the average vitamin C content was 60% greater than that of the controls. A

TABLE I.
Vitamin C Content of Adrenals of Guinea Pigs Which Had Received One-third of a Lethal Dose of Diphtheria Toxin and Were Sacrificed at Various Intervals.

No. of animals	Survival time, hrs.	Vitamin C content of adrenals			Deviation of average from normal %	Gross physical reactions to toxin	
		Max. mg.	Min. mg.	Aver. mg.		Site of injection	Adrenals
5	controls	.070	.012	.032±.006	0		
4	24	.077	.030	.051±.005	+61.2	marked edema	normal
4	48	.057	.047	.052±.002	+63.4	very marked edema	slight congestion
5	58	.069	.028	.052±.005	+63.8	" " "	" "
4	72	.064	.030	.048±.004	+51.2	" " "	congestion
4	144	.150	.081	.117±.009	+269.5	necrosis	normal

comparison of test animals sacrificed 48 and 58 hours after injection indicated a still further, although slight, increase in this factor. At 72 hours, however, the vitamin C had declined but had not reached the level present in the control group. The 4 remaining test animals were killed on the sixth day. There was a reparative reaction around a central area of necrosis at the site of injection, and the adrenals presented a normal appearance. The average vitamin C content of the adrenals was 270% more than that of the control guinea pigs at the beginning of the experiment.

These results were duplicated when guinea pigs injected intradermally with 3 skin-test doses of diphtheria toxin were killed at daily intervals and their adrenals titrated for vitamin C.