THE INFLUENCE OF DIETARY INTAKE OF ASCORBIC ACID AND PROTEIN ON BOUND ASCORBIC ACID IN GUINEA[1] PIGS

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Bound ascorbic acid (BAA) has been reported to occur in animal tissues and fluids and important functions have been postulated for this form of ascorbic acid (AA). Holtz and Walter (1940) suggested that BAA was made up of AA combined with a specific carrier protein and constituted a rather stable storage form of the vitamin. Sumerwell and Sealock (1951) have suggested that BAA may have an enzyme function.

The BAA content of liver and spleen was reported to be increased by a protein free diet (Kratinova, 1950) and decreased by vitamin C deficiency (Dayton et al., 1956). The objective of this study was to investigate more thoroughly relationships which might exist between dietary intake of AA and protein, and BAA levels in blood serum or liver.

Two guinea pig feeding trials were conducted using two different basal rations. In the first trial, semi-purified rations similar to that described by Reid et al. (1953) containing 17.5 or 30 per cent protein (casein) but lacking AA, were fed to two groups of animals. Two other groups received the same ra-

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tions but also received a supplement of 3 mg. of AA per day in a gelatin capsule. After an initial adjustment period of one week, during which time all animals received a 3 mg. per day AA supplement, the animals were fed the designated rations ad libitum for a period of three weeks.

Only two groups of animals were used in the second trial and a commercial rabbit pellet ration served as the basal ration. One group received no supplement and the other 3 mg. of AA per day. In other respects the two trials were alike.

Blood samples were obtained from the toe of each animal each week, and in the second trial the livers were removed and analyzed at termination. The method of Sumerwell and Sealock (1952) as modified by Lewis et al. (1960) was used for determination of BAA in blood serum and liver. The 2,4-dintrophenylhydrozone method of Lowry et al. (1947) was slightly modified for AA determinations.

Table I shows the BAA values obtained for the blood serum of the guinea pigs at the end of the first trial. Animals on the AA deficient rations became severely scorbutic in 14 days. However, BAA values were not depressed.

The results of the second trial, in which the basal ration was a commercial rabbit pellet, are shown in Table II. Again, no significant differences were found between the BAA levels, in either blood serum or liver tissue, of severely scorbutic and healthy animals. These results are not in accord with those of Sumerwell and Sealock (1952), Dayton et al. (1956) or Kratinova (1950) All of the above workers reported a decrease in BAA as the animals became depleted of AA.

The results of this study might lead one to assume that BAA levels in the animal are not a function of dietary intake of AA or protein. However, such a conclusion should be reached with reservations. Although the BAA value ob-

Table I

RESULTS OF GUINEA PIG TRIAL I

No. of animals	Protein in ration	AA added to diet	Weight changes*	Serum BAA*
	per cent	mg./animal/	gm./day	mg./100 ml.
10	30	3.0	3.0	0.16±0.01
10	30	0.0	- 4.1	0.19±0.01
9	17.5	3.0	0.9	0.16±0.02
6	17.5	0.0	- 4.4	0.18±0.01

* Average values for samples taken at the end of the third week.

Table II

RESULTS OF GUINEA PIG TRIAL II

Animal No.	AA Supplement	Body* weight changes	Serum AA**	Serum BAA**	Liver AA**	Liver BAA**
	mg./animal	gm./day				
1	0.0	-13.4	0.00	0.26	0.43	0.47
2	0.0	- 3.3	0.00	0.24	0.47	0.38
3	0.0	- 24.1	0.00	0.25	0.58	0.48
4	0.0	- 2.7	0.00	0.24	0.41	- -
5	0.0	- 21.4	0.00	0.24	0.39	0.47
Average		-13.0	0.00	0.25±.01	0.46±.07	0.45±.05
6	3.0	+13.0	0.13	0.22	4.12	0.44
7	3.0	+13.0	0.15	0.23	4.94	0.43
8	3.0	+ 6.0	0.11	0.21	3.51	0.41
9	3.0	+18.7	0.11	0.23	4.29	0.48
10	3.0	+ 6.1	0.10	0.22	3.66	0.44
Average		+11.4	0.12+.02	0.22+.01	4.11+.18	0.44+.02

* Weight changes during the final week of the trial.

** Values obtained at the termination of the trial expressed in mg./100 ml. serum or mg./100 g. liver.

tained by the analytical method employed are highly reproducible, the values are subject to considerable error resulting from interfering substances (Lewis

et al., 1960). Consequently, these results represent, at best only relative values. Similar reservations should be attached to the results of the investigations of Hastings and Spencer (1952) and Dayton et al. (1956) in which similar methods were employed. Unfortunately, it appears that no better analytical method is available for BAA in animal tissues and fluids.

In summary, variations in ascorbic acid or protein intake of guinea pigs was not shown to influence bound ascorbic acid levels in the blood serum or liver of guinea pigs when a 2, 4-dinitrophenylhydrazine procedure was used for bound ascorbic acid assay. This analytical method gave reproducible results but was not sufficiently specific.

REFERENCES

Dayton, P. G., Reichenthal, J. and Burns, J. J., Proc. Soc. Exptl. Biol. Med. 91, 326 (1956).

Hastings, W. H. and Spencer, C. F., J. Marine Res. 11, 241 (1952).

Holtz, P. and Walter, H., Klin. Wochschr. 19, 136 (1940), Z. Physiol. Chem. 263, 187 (1940).

Kratinova, K. R., Ukrain. Biokhim. Zhur. 22, 425 (1950).

Lewis, W. R., Chiang, J. L., and Gross, S., Arch. Biochem. Biophys. (In press).

Reid, M. E. and Briggs, G. M., J. Nut. 51, 341 (1953).

Sumerwell, W. N. and Sealock, R. R., J. Biol. Chem. 196, 753 (1952).

Lowry, O. H., Lopez, J. A. and Bessy, O. A., J. Biol. Chem. 160, 609 (1945).