Does the U. S. P. Vitamin A-deficient Diet Require Supplementation With Ascorbic Acid?

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It has been generally assumed that the rat does not require a dietary source of ascorbic acid. This assumption has been based on the fact that the rat does not develop the symptoms usually considered characteristic of ascorbic acid deficiency in the guinea pig when subsisting on diets definitely scorbutogenic to the latter animal. Furthermore, it has been found that the rat, while subsisting on many different diets, excretes in its urine considerably more ascorbic acid than it ingests, thereby indicating a probable endogenous source of the vitamin. It has been found in comparatively recent years that the ascorbic acid content of rat tissues and the amount excreted in the urine of the rat is influenced by the composition of the rat's diet with respect to constituents other than the specific vitamin. Certain compounds like carvone, when ingested by the rat, result in increased ascorbic acid production and storage, whereas other dietary conditions have produced the opposite effect.

More recent reports seem to indicate that perhaps a relationship exists between ascorbic acid metabolism and vitamin A utilization. Kimble and Gordon (*2*) found that ascorbic acid therapy resulted in increased vitamin A blood levels in human subjects without an increase in vitamin A intake. Since this was published, other investigators have reported abnormally low blood and tissue levels of ascorbic acid associated with vitamin A deficiency in the rat. Among these latter reports are that of Jonsson, Obel, and Sjoberg (*1*) and the more recent reports of Mayer and Krehl (*3*).

These observations seem to raise the question as to whether the data obtained by means of the usual biological assay for vitamin A, such as with the official U. S. P. technic, are actual measures of vitamin A potency or whether the data are vitiated by the complications arising from concomitant ascorbic acid deficiency. Since the question merited consideration, the following study was undertaken.

Eighty young rats from our breeding colony, ranging in age from 21 to 24 days, were placed in individual metal cages and fed liberal allowances of the U. S. P. vitamin A-deficient diet. One-half of the young rats received the unsupplemented vitamin A-deficient diet; the other half, the deficient diet after it had been enriched with ascorbic acid at the rate of 5 gm of the crystalline vitamin to 1 kg of the ration. The ascorbic acid was incorporated into the ration just before the ration was allocated to the test animals. Each rat was weighed at weekly intervals during the first 3 weeks of the depletion period and then daily until the end of the depletion period, which ranged from 28 to 34 days. When depleted, 32 rats from each dietary group (16 males and 16 females) were selected for the vitamin A feeding test. As a supplement to the vitamin A-deficient diet (with and without ascorbic acid), 10.5 U. S. P. units of vitamin A (New U. S. P. Vitamin A Reference Standard) were fed to each rat weekly for a 4-week test period. The vitamin A supplement was dissolved in Wesson oil in such a manner that 1 ml of the solution contained 15 U. S. P. units. The supplement was fed on Mondays, Wednesdays, and Fridays in the amounts of 0.2, 0.2, and 0.3 ml, respectively. During the supplementary feeding period all test animals were weighed biweekly. Accurate food consumption records were kept at all times and the animals were examined frequently for external symptoms of abnormal physical conditions.

TABLE 1

EFFECT OF SUPPLEMENTING THE U. S. P. VITAMIN A-DEFICIENT DIET WITH ASCORBIC ACID ON THE RESPONSE OF YOUNG RATS

Sex of test animals	No. of animals used	Avg. initial wt. (gm)	Wt. at end of depletion period (gm)	Avg. amount of vitamin A fed daily (U.S.P. units)	Avg. gain in wt. during: week 1 (gm)	week 2 (gm)	week 3 (gm)	week 4 (gm)	Total gain in wt. (gm)	Standard deviation
			U. S. P. Vitamin A-deficient Diet							
Males	16	44.2	133.8	1.5	7.6	6.9	9.5	6.4	30.4	6.8
Females	16	44.0	122.6	1.5	6.7	8.3	7.4	6.1	29.1	7.3
Avg.	both sexes	44.1	128.2	1.5	7.2	7.6	8.5	6.3	29.8	7.1
			U. S. P. Vitamin A-deficient Diet + Ascorbic Acid							
Males	16	43.8	133.3	1.5	6.9	6.8	9.3	7.1	30.1	7.5
Females	16	44.1	123.9	1.5	6.1	7.5	7.2	6.8	27.6	6.3
Avg.	both sexes	44.0	128.6	1.5	6.5	7.2	8.2	7.0	28.9	6.9

A summary of some of the results obtained is presented in Table 1. From these data it is apparent that no improved growth response, during either the depletion period or the subsequent vitamin A feeding period, resulted from supplementing the basal diet with crystalline ascorbic acid. Furthermore, examination of the test animals did not reveal that the ascorbic acid-fed rats were superior physically to those receiving the regular U. S. P.

vitamin A-deficient diet. The data indicate that no improvement in the results obtained in the usual biological assay for vitamin A was achieved by supplementing the basal diet with crystalline ascorbic acid. Somewhat different results might have been obtained, however, had the B-vitamins been furnished in the crystalline form, as in the studies of Mayer and Krehl (*3*), instead of in the form of dried brewers' yeast, as in this study.

References

1. JONSSON, G., OBEL, A. L., and SJOBERG, K. *Z. Vit. Forsch.*, 1942, **12**, 300.
2. KIMBLE, MARIAN S., and GORDON, EDGAR S. *J. biol. Chem.*, 1939, **128**, lii.
3. MAYER, J., and KREHL, W. A. *Arch. Biochem.*, 1948, **16**, 313; *J. Nutrition*, 1948, **35**, 523.

The Effectiveness of Carbarsone in Rendering Young Mice Free of Intestinal Protozoa

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Carbarsone (*p*-carbamino-phenyl-arsonic acid) was used in an attempt to eradicate the intestinal protozoa of young mice needed for other work. These results are presented in the hope that they will be of value to others who are relying upon this arsenic derivative to obtain amoebae-free animals prior to infection with *Endamoeba histolytica*. All the mice used here were raised and maintained on an adequate diet prepared in the laboratory (*4*). Care was taken in the preparation of this diet to prevent contamination, and it was stored in a deep freezer until used. The drinking water provided was sterilized. The doses of the chemical used were suggested by the work of Reed, *et al.* (*5*) on monkeys and rabbits and that of Gabaldon (*2*) on rats. The doses, dissolved in alkaline aqueous solution, were forced into the stomach through a blunted, 18-gauge needle attached to a 1-cc tuberculin syringe. The cages containing the mice undergoing treatment were kept in a fume hood located in a room outside of the animal quarters. Before use, the hood was disinfected and made dust free by use of an air filter. In a further attempt to minimize the possibility of reinfection from outside sources, all cages were sterilized just prior to their use.

In the first experiment, 60 mice, 6 weeks old, were selected. These were divided according to sex into 6 treatment groups of 10 mice each. Each group was confined in a separate cage and after overnight fasting was given 1 through 6 mg of carbarsone daily, respectively, for 10 consecutive days. The average weight of the mice was 20 gm, so that the dosage for those given 1 mg was at the rate of 50 mg/kg (*5*). No mice were lost during treatment, and there were no visible signs of toxicity. A 7th group of 10 mice of the same age was included as untreated controls. These were not confined to the fume hood but were fed the same diet and were fasted for the same periods as the treated mice. As expected, there was loss of weight in all groups from the fasting, but this was regained soon after treatment. At intervals following treatment, one mouse from each of the 7 groups was sacrificed to determine, by repeated cecal examinations, the effectiveness of the treatment. The first checks were made 4 days post-treatment, and thereafter checks were made at 2-day intervals. In all cases, the control mice showed large numbers of flagellates, including *Giardia muris* (*3*) in about one-half, and 8 of the 10 had infections with an amoeba tentatively designated as *E. muris*. Of the mice given 1-, 2-, and 3-mg treatments of carbarsone, respectively, those killed 4–10 days after treatment showed no amoebae, but flagellates were found in decreasing numbers correlated with the increasing doses. The remainder of the mice, killed 12–22 days post-treatment, showed flagellates and/or amoebae. It was thus clear that carbarsone in these doses was not effective.

In the second experiment, the dosage of carbarsone was increased according to the recommendation of Gabaldon (*2*). Mice of the same age as above were divided according to sex into 3 groups of 10 each and received, after overnight fasting, 10, 20, and 30 mg, respectively, for 5 days. Three mice of the group treated with 20 mg and one of the group treated with 30 mg died soon after treatment. These deaths probably resulted from toxicity, but diarrhea and the nervous symptoms ascribed by Gabaldon (*1*) to such toxicity were not observed. It should be pointed out that the largest dosage used here (30 mg) was considerably less than that found by Gabaldon to be the minimal dose producing toxic symptoms in rats (4,000 mg/kg of body weight, or equivalent to 80 mg/20 gm of body weight). The 10 untreated controls showed about the same rate and intensity of protozoan infections as those above. For 20 days post-treatment, none of the treated mice showed protozoa, except for an occasional *G. muris*. However, those mice killed 22 or more days after treatment showed large numbers of *E. muris*.

While it is not possible to exclude reinfection by airborne amoebae cysts from wild mice, this seems extremely unlikely in view of the precautions mentioned above. It appears from these results, therefore, that carbarsone, as used, exerted an amoebastatic effect but did not eradicate *E. muris*. This could lead to error in interpreting results of experimental *E. histolytica* infections following such carbarsone treatment, since the active, unstained trophozoites of the two amoebae are quite similar in certain morphological details and in type of motility.

References

1. GABALDON, A. *Amer. J. Hyg.*, 1936, **23**, 122–131.
2. GABALDON, A. *Amer. J. trop. Med.*, 1936, **16**, 621–639.
3. HEGNER, R., ROOT, F. M., AUGUSTINE, D. L., and HUFF, C. G. *Parasitology*. New York: D. Appleton-Century, 1938. Chap. 1.
4. LARSH, J. E., JR., and THOMPSON, L. M. *Amer. J. Hyg.*, in press.
5. REED, A. C., ANDERSON, H. H., DAVID, N. A., and LEAKE, C. D. *J. A. M. A.*, 1932, **98**, 189–194.

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