Reaction of Carotene with Nitrite Solutions

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Nitrite solutions of low pH, which would include any chemical changes in nitrite resulting from acidic conditions, are shown to cause destruction of carotene. Vitamin A alcohol is also readily destroyed, while β-apo-8′- and β-apo-12′-carotenal are more resistant to attack by these solutions. Since carotene is a major source of vitamin A for cattle, it may be destroyed by nitrite before absorption and conversion can take place. The recent apparent increase in vitamin A requirement for cattle fed forages high in nitrate may conceivably be explained on this basis.

In 1945, Wilson (*9*) demonstrated the disappearance of the yellow color from carotene solutions when they were shaken with nitrous acid. He had earlier reviewed the literature relating to the occurence of nitrite or nitrous acid in nature (*8*). He also determined the nitrate content of the expressed sap of a number of genera and species of plants, finding amounts ranging from a trace to 10,000 p.p.m. (*10*). Flynn *et al.* (*3*) found high levels of nitrate in corn plants, particularly after nitrogen fertilization and drouth conditions.

Using artificial rumens, Barnett and Bowman (*1*) have shown that nitrate can be reduced to nitrite by rumen liquor. Lewis (*6*) found nitrite accumulation in vivo under certain conditions of nitrate feeding. Case (*2*) has discussed the deleterious effects of both of these substances on animals, and investigation by O'Dell *et al.* (*7*) showed that dietary nitrite enhanced vitamin A deficiency in rats and reduced vitamin A storage when carotene was fed. Garner (*4*) reported an increase in the vitamin A requirement of cattle when nitrate-containing rations were being fed.

The following in vitro experiments were run to investigate further the effect of both nitrite and hydrogen ion concentration on the destruction of carotene, since nitrate has been found nondestructive at pH 1 to 7 by this laboratory.

Procedure

A Latin square experimental design was used in these experiments. The variables involved were the molar ratio of nitrite to carotenoid pigment and the pH of the solution. In the first series of experiments, nitrite to carotene ratios were varied from 1:1 to 100:1 and were used at each pH from 1 through 7. In the second series of experiments, β-carotene, β-apo-8′-carotenal, β-apo-12′-carotenal, and vitamin A alcohol were subjected to similar conditions.

Test tubes measuring 13 × 100 mm. were used in these experiments. The total volume per tube was 7 ml.—2 ml. of stock carotenoid solution, 2 ml. of the appropriate sulfuric acid solution, and 3 ml. of an appropriate dilution of the stock KNO_2 solution.

The reagents were mixed at room temperature, then incubated 4 hours at 37° C. The absorbance was determined by the use of a Beckman Model DU spectrophotometer. The blank was composed of the original reagent mixture with double-distilled water replacing the KNO_2 portion of the solution. β-carotene, β-apo-8′-carotenal, and β-apo-12′-carotenal were read at 450 mμ while vitamin A alcohol was read at 325 mμ.

Stock Solutions. Potassium nitrite stock solution was prepared to contain 126.48 μg. of KNO_2 per ml. After drying overnight, 7.925 grams of KNO_2 were weighed into a 500-ml. volumetric flask, and made to volume with double-distilled water. An aliquot of 7.98 ml. of this solution was diluted to 1 ltier to give the final desired concentration (126.48 μg. per ml. or 148.8 × 10^{-8} moles per ml.).

The carotenoid (or vitamin A alcohol) stock solutions were prepared as an aqueous dispersion with 1% Tween 80 (Atlas Powder Co., Wilmington, Del.). Crystalline β-carotene (Nutritional Biochemicals Corp.), β-apo-8′-carotenal, β-apo-12′-carotenal (Hoffmann–La Roche, Inc.), and vitamin A alcohol (Distillation Products Industries) were used. Each solution was prepared to contain a final concentration of 3.72 × 10^{-5} moles of dissolved reactant per ml. The crystalline product (4 mg. of β-carotene, 3.1 mg. of β-apo-8′-carotenal, 3.2 mg. of β-apo-12′-carotenal, 2.1 mg. of vitamin A alcohol) was dissolved in approximately 40 ml. of absolute ethanol by warming. The ethanolic solution was added slowly, with mixing, to 100 ml. of warm 1% Tween 80 solution. Additional 1% Tween 80 solution was used to bring the solution to volume in a 200 ml. volumetric flask.

A series of sulfuric acid solutions was prepared, ranging in pH from 0.5 to 5.5. Fresh, double-distilled water was used for the solution of pH 7. The pH of the sulfuric acid solutions was checked with a pH meter and adjusted when necessary. When diluted to the final concentration in the reaction, these solutions provided a calculated pH of 1 through 7.

Results and Discussion

Figure 1 shows the results of triplicate experiments on the destruction of β-carotene by nitrite. The pH of the solutions was not a factor in the destruction of carotene in the control series, while destruction by nitrite depended markedly on the pH of the system. The amount of destruction at pH 5 to 7 at any given nitrite to carotene ratio was much less than at lower pH values. The destruction of carotene was greatest at pH 1 to 3 with a tendency toward a plateau at pH 2 to 3. At high nitrite to carotene ratios, the amount of destruction between pH 2 and 3 tended to equal that at pH 1. An intermediate amount of destruction occurred at pH 4.

Previous work (*5*) on degradation of carotenoids would indicate that one possible pathway of degradation is through the apo-carotenals. Figure 2 shows the results of duplicate experiments comparing the destruction of β-carotene, β-apo-8′-carotenal, β-apo-

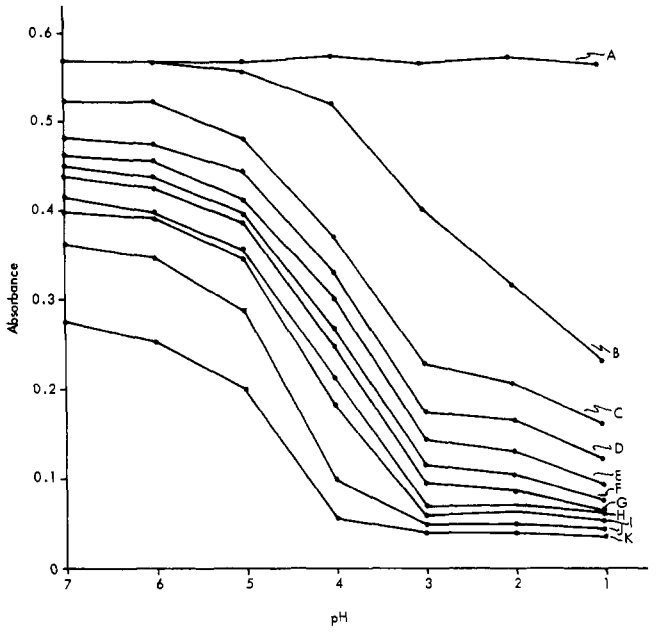

Figure 1. Destruction of β-carotene (4 hours at 37° C.) as a function of pH and nitrite concentration

Molar nitrite to carotene ratios: A-control, B-1:1, C-4:1, D-6:1, E-8:1, F-10:1, G-12:1, H-16:1, I-20:1, J-40:1, and K-100:1

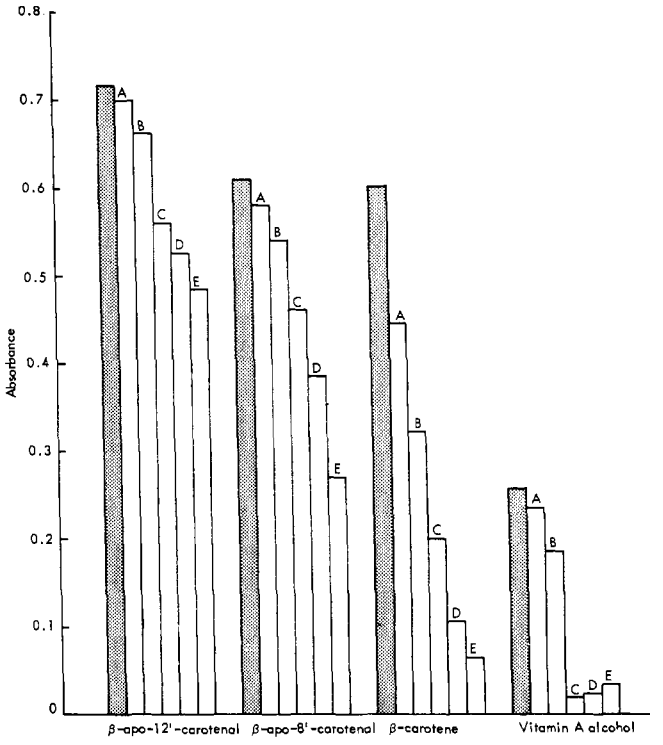

Figure 2. Comparison of the destruction of three provitamin A compounds and vitamin A alcohol by nitrite at pH 2

Molar nitrite to carotenoid ratios: solid bar-control, A-0.5:1, B-1:1, C-4:1, D-10:1, and E-20:1

12'-carotenal, and vitamin A alcohol. This figure compares results at pH 2 only. The two carotenals are more stable in the presence of nitrite than is β-carotene, β-apo-12'-carotenal being more stable than β-apo-8'-carotenal. Vitamin A alcohol was more susceptible to degradation by nitrite than the other three substances.

In silage, a pH of 4 is normal. Any nitrite formed at this pH would readily destroy carotene because of an extended reaction period and elevated temperatures. In the true stomach of animals (pH 2 to 3), the presence of nitrate along with dietary carotene could lead to a low vitamin A status. These data, although collected using pure compounds and relatively simple reaction mixtures, suggest a possible reason for the apparent increase in vitamin A requirement of cattle fed forages containing a high level of nitrate.

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