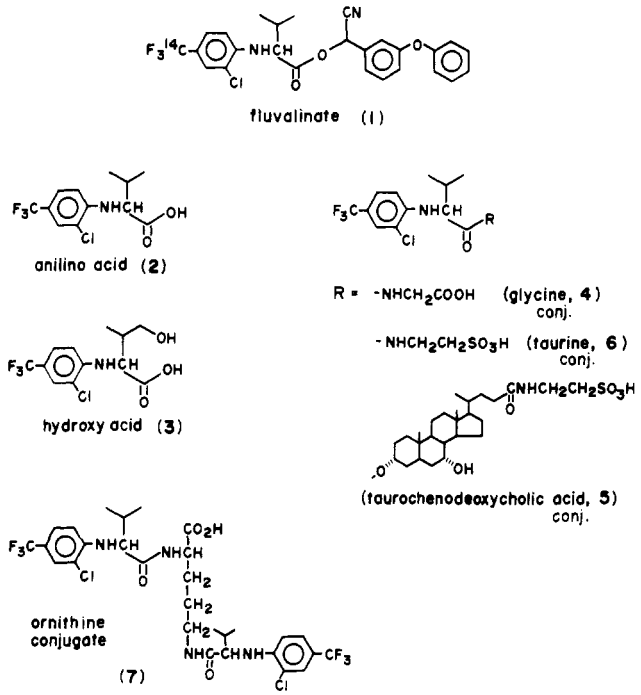

Figure 3. Structures of potential metabolites of fluvalinate from a laying hen.

little difference in formation of either diastereomer, it is likely that the observed unequal isomer ratio of the bile acid conjugate from egg yolk is a result of poor resolution and not differential occurrence of one isomer.

CONCLUSIONS

The rapid metabolism and excretion of radiolabel from chickens dosed with [*trifluoromethyl*-^{14}C]fluvalinate are probably indicative of the high metabolic rate and the high rate of food passage in chickens. Laying hens are known to excrete a meal of mash within 8 h (Hill, 1971). Fluvalinate itself is rapidly metabolized as evidenced by the low levels of parent compound in the excreta. The major products in excreta are the anilino acid (2), hydroxylated 2 (i.e., 3), and polar conjugates of 2 (adducts of taurine and taurochenodeoxycholic acid). The low levels of radiolabel in the eggs may be evidence for the lack of absorption of fluvalinate or its metabolites. The major products in egg yolks are fluvalinate, the anilino acid, and the taurochenodeoxycholic acid conjugate of the anilino acid. Low ^{14}C-labeled residues were found in all tissues examined, and analysis of fat and muscle indicated fluvalinate to be the major residue, but accompanied by some anilino acid and unknown polar radiolabel. The major metabolites from fluvalinate metabolism are summarized in Figure 3. There is no evidence for selective metabolism of any stereoisomers of fluvalinate.

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Reversibility of Thermal Degradation of Betacyanines under the Influence of Isoascorbic Acid

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Isoascorbic acid was effective in reversing the thermal degradation of betacyanines, the red pigments of beet. Heating an aqueous solution of red pigments cleaved the betacyanine molecule, causing the loss of red color. When isoascorbic acid was added either before or after heating, the red color of thermally degraded betacyanine in solution was restored almost completely after 24 h of storage in the dark at 25 °C. Isoascorbic acid did not produce a similar result with thermally degraded yellow betaxanthine pigments.

The red beet (*Beta vulgaris*) is a potential source of valuable water-soluble pigments, the so-called betalaines, for use as food colorants (von Elbe and Maing, 1973; von Elbe et al., 1974a,b; Pasch et al., 1975; Sapers and Hornstein, 1979). However, the instability of these natural colorants is a major obstacle to practical use (von Elbe et al., 1974b). Heat is one of the principal factors that causes loss of color through the degradation of betalaines. A

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previous investigation in this laboratory demonstrated that isoascorbic acid considerably improves the stability of red beet pigment. For aqueous solutions of beet pigments, the most useful isoascorbic acid concentration was found to be 0.1% (Bilyk et al., 1981). Isoascorbic acid, also known as erythorbic acid, is a stereoisomer of ascorbic acid and possesses significant antioxidant props (Esselen et al., 1945; Kadin and Osadca, 1959). The objective of the present study was to determine the effect of isoascorbic acid on thermally degraded betacyanines and betaxanthines, the two major classes of beet pigments.

EXPERIMENTAL SECTION

Sample Preparation. Beet juice powder (0.38% betanine) was prepared by lyophilizing fresh beet juice according to the procedure previously described (Bilyk, 1979). Purification was accomplished by a thin-layer chromatographic method (Bilyk, 1981), yielding a purified betacyanine fraction containing 5.73% betacyanine, calculated as betanine, and a purified betaxanthine fraction containing 1.22% betaxanthine, calculated as vulgaxanthine I. For determination of the effect of heating and subsequent storage on beet pigments, weighed amounts of the beet pigment fractions, equivalent when diluted to 200 mL to 11.4 ppm of betanine (0.3% w/v beet juice powder or 0.02% w/v purified betacyanine) or to 14.7 ppm of vulgaxanthine I (0.12% w/v purified betaxanthine), were dissolved in 1–2 mL of distilled water. These concentrated pigment solutions were transferred quantitatively through one neck of a 500-mL two-neck round-bottom flask to 198–199 mL of distilled water, with refluxing at 100 °C under a N_2 blanket to heat degrade the pigments in the absence of air. Samples of the solutions were withdrawn after 3, 6, 9, and 12 min and cooled immediately to room temperature. Isoascorbic acid (0.1% w/v) was added to the beet pigment solutions either before or after heating and cooling and to unheated controls, prepared by dissolving pigment fractions in cold water, and the pH was adjusted with 0.1 N NaOH to 5. The absorbance of pigment solutions was measured against a distilled water blank with a Perkin-Elmer 552 recording UV–visible spectrophotometer between 350 and 650 nm. The pigment solutions to be placed in storage were sterilized by filtration through disposable filters (Falcon 0.22-μm membrane filters, Becton-Dickinson) under vacuum and pipetted aseptically with sterile disposable plastic syringes (Plastipac, Becton-Dickinson) into sterile test tubes (Vacutainer, Becton-Dickinson). Samples prepared with and without isoascorbic acid were stored for 24 h in darkness at 25 °C after which time spectrophotometric measurements were made. All data points represent the average of three measurements.

RESULTS AND DISCUSSION

The beet juice powder contains a mixture of red and yellow pigments. The red betanine pigment displays an absorption maximum in the region of 535–540 nm while vulgaxanthine I (yellow), the major betaxanthine in beets, has a maximum at 476–478 nm (Nilsson, 1970). When these pigments are heated at 100 °C, they undergo thermal degradation as a function of time. Heating presumably splits the betacyanine molecule into betalamic acid and cyclodopa (Saguy et al., 1978). The visible spectra of heated and unheated beet pigments are illustrated in Figure 1. Spectral analysis shows a progressive decrease of the betacyanine (538 nm) and betaxanthine (478 nm) bands and a progressive increase of a band at 430 nm attributed to betalamic acid. The same phenomenon occurred with purified betacyanines. However, in that case, the betalamic acid bands were much smaller than those

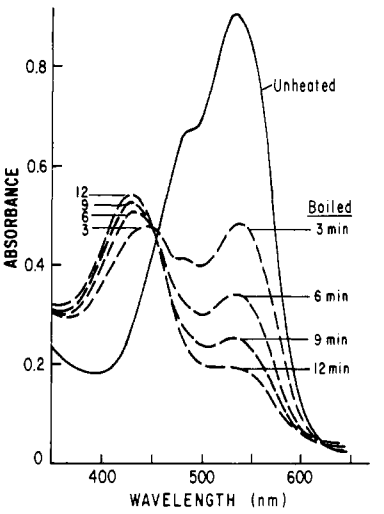

Figure 1. Spectral evidence of pigment degradation in aqueous solutions of beet juice powder, as a function of boiling time.

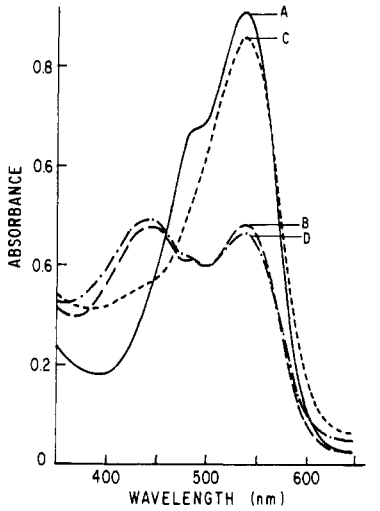

Figure 2. Regeneration of red pigments in beet juice powder solution containing isoascorbic acid, after 3 min of heating at 100 °C and storage for 24 h in dark at 25 °C. (A) Unheated control; (B) after heating; (C) after storage (isoascorbic acid present); (D) after storage (without isoascorbic acid).

obtained with the unfractionated beet juice powder, since only the betacyanines were present. The beet juice powder produced an additional amount of betalamic acid from the degraded betaxanthines contained therein.

During thermal processing of beet products, a significant pigment loss takes place. However, the red pigments can be regenerated from the thermally degraded betacyanine during storage (von Elbe et al., 1981). In the present study with beet pigments, an effort was made to find out the relationship between pigment regeneration and the presence of isoascorbic acid, which is known to be a stabilizer of the red color of beets (Bilyk et al., 1981). The effects of adding 0.1% isoascorbic acid to heated beet pigments are illustrated in Figure 2. The spectrum denoted by A was obtained from the unheated beet juice powder solution (control) and contains betacyanines (538 nm) and also a small proportion of betaxanthines (478 nm). The solution was heated at 100 °C for 3 min (resulting in the destruction of about 50% of the original pigments) and a new spectrum taken, scan B. A new band appearing at 430 nm probably resulted from the formation of betalamic acid. Isoascorbic acid was then added to the above heated solution, which was then stored in the dark for 24 h at 25 °C. The spectral analysis was repeated, yielding scan C. The spectrum

Table I. Thermal Degradation of Beet Pigments

pigment	% pigment retention[a]					
			0.1% isoascorbic acid added			
			before boiling		after boiling	
	no additives					
	after 3-min boiling	same 24-h storage[b]	after 3-min boiling	same 24-h storage[b]	after 3-min boiling	same 24-h storage[b]
crude pigments in beet juice powder	52	50	56	98	52	90
red beet pigments (betacyanines) purified on TLC	40	32	47	88	40	76
yellow beet pigments (betaxanthines) purified on TLC	55	48	57	53	55	50

[a] Based on the absorbance at the absorption maxima, 538 nm for betacyanines and 478 nm for betaxanthines. [b] In the dark, 25 °C.

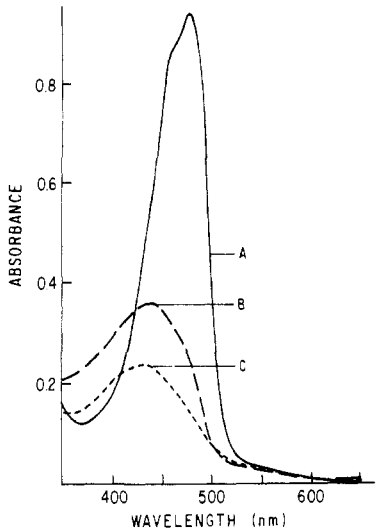

Figure 3. Thermal degradation of purified betaxanthine and the effect of storage in dark at 25 °C. (A) Unheated control; (B) after heating; (C) after storage (isoascorbic acid present).

clearly shows that the red pigment has re-formed (538-nm band), and the absorption band for betalamic acid (430 nm) has disappeared. A similar sample heated 3 min and stored 24 h in the dark at 25 °C without the addition of isoascorbic acid gave scan D. No regeneration process occurred in this sample. A comparable test was carried out by using purified betacyanine, and similar results were obtained. Degraded betaxanthine, the yellow pigment of beet, however, did not regenerate under the influence of isoascorbic acid when stored in the dark for 24 h at 25 °C. This is demonstrated in Figure 3. Scan A represents the solution of purified betaxanthine (478 nm). After 3 min of heating at 100 °C (scan B) this band disappeared almost completely, and a new band appeared which corresponds to betalamic acid (430 nm). Scan C was obtained from the heated sample after the addition of isoascorbic acid and storage for 24 h in the dark at 25 °C. It shows that no regeneration of betaxanthine took place in the presence of isoascorbic acid.

Similar tests were carried out with samples to which 0.1% of isoascorbic acid was added before heating. Pigment retention data obtained after heating at 100 °C for 3 min and 24 h of storage are recorded in Table I. The addition of isoascorbic acid to the pigment solutions prior to heating resulted in a small increase in pigment retention during heating. The addition of isoascorbic acid before heating also enhanced pigment regeneration compared to postheating addition. Purified betacyanines were more heat sensitive and regenerated to a lesser extent than the pigments in beet juice powder.

A mechanism of regeneration has been suggested in which a Schiff base equilibrium exists between betacyanine and its proposed products, cyclodopa and betalamic acid (von Elbe et al., 1981). The products are optically active cyclic amino acids. While isoascorbic acid and ascorbic acid are both stabilizers against pigment degradation, isoascorbic acid is about twice as effective, suggesting some involvement of their stereoconfigurations (Bilyk et al., 1981). Since betalamic acid contains the aldehyde and amine functionalities, it should be capable of dimerization and even higher degrees of polymerization in competition with the cyclodopa reaction. Conceivably, the ascorbic acid isomers and betalamic acid may form a labile imidimium ion intermediate through the former's lactone carbonyl that would preclude dimerization and allow competitive reaction with cyclodopa. Recognition of these possible steps provides a basis of future study to elaborate the mechanism and also to positively identify the degradation products.

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