Nickla, H.* Arizona State University, Tempe, Arizona. Riboflavin content in Malpighian tubes of D. melanogaster.

Larvae of many eye color mutants of D. melanogaster have less yellow pigment (YP) in their Malpighian tubes (MPT) than wild type larvae[1]. No mutants other than those influencing eye color have been found to alter the color of MPT[2]. Kikkawa[3] has suggested that the YP in MPT of D. melanogaster is 3-hydroxykynurenine, an intermediate in the synthesis of brown eye pigments[4], while Forrest and Mitchell[5] have implicated sepiapterin, an intermediate in the synthesis of red eye pigments[6]. However, YP in MPT of the American roach (Periplaneta americana), Tribolium confusum, and Ephestia kuhniella is mainly riboflavin[7,8,9]. In this communication, results are presented which suggest that the major component of YP in MPT of D. melanogaster is riboflavin.

The technique used for chromatographic separations followed that of Hadorn and Mitchell[10]. Ascending paper chromatography was carried out in the dark with n-propanol and 5% ammonia (2:1) as the solvent. Flies were reared on standard agar-cornmeal-brewer's yeast-molasses-sucrose-propionic acid medium.

For comparison with YP, sepiapterin was obtained from heads of sepia mutant flies by paper chromatography and was identified by its fluorescent color (yellow) and Rf value[10,11]. Following elution in 50% aqueous acetone[5], the visible portion of the absorption spectrum was determined (in 50% acetone) at pH values 1.8, 7.4, and 13.4. In alkaline solution, there is a maximum at 441nm; in neutral and acid solution, there is a maximum at 418nm. Riboflavin was also chromatographed and eluted in 50% aqueous acetone. At pH 6.1 the absorption maxima are 445nm and 375nm; 447nm and 375 nm at pH 8.9; and 450nm and 353nm at pH 12.0. Yellow pigment from MPT was separated chromatographically from abdomens of approximately 1,300 wild type (Urbana) female flies. These chromatograms, when viewed under ultraviolet light reveal a bright yellow spot with approximately the same Rf value as riboflavin and sepiapterin. Several mutants (light and clot), which have pale MPT upon visual examination, were chromatographed. In both cases there was a reduction in concentration of this yellow fluorescent spot indicating that it is responsible for the yellow color of MPT in wild type flies. Following elution, the visible portion of the absorption spectrum of YP was determined at pH values 7.6 and 13.3. The absorption spectra obtained at these pH values are similar, which suggests the absence of large amounts of sepiapterin. In addition, the portion of the spectrum from 430nm to 540 nm resembles that of riboflavin.

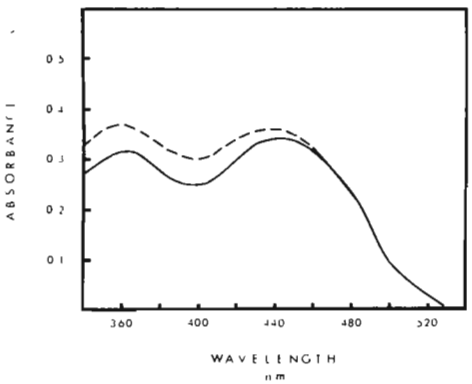

Figure 1. Absorption spectrum of riboflavin before (solid line) and after (broken line) treatment with bromine.

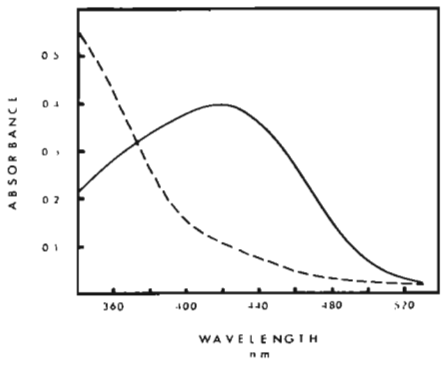

Figure 2. Absorption spectrum of sepiapterin before (solid line) and after (broken line) treatment with bromine.

Bromine causes immediate decolorization of sepiapterin producing two blue fluorescent compounds[5], without altering the characteristic yellow color of riboflavin[12]. Figures 1 and 2 present the absorption spectra of riboflavin and sepiapterin respectively before and after treatment with bromine (0.1ml of bromine/3ml of solution). Inspection of the sepiapterin

solution revealed a complete absence of yellow color after bromine treatment. Similar treatment of YP from MPT did not alter its absorption spectrum (Figure 3) and did not cause loss of color. The absence of the typical riboflavin spectrum (Figure 3) probably results from incomplete purification of YP as only single dimension chromatography was used.

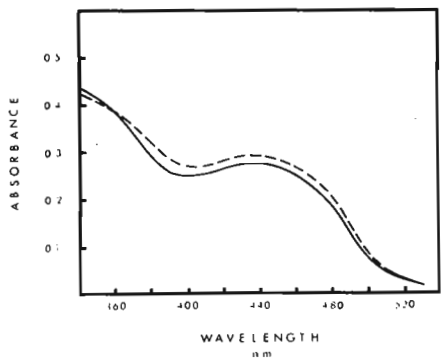

Figure 3. Absorption spectrum of the yellow pigment from the Malpighian tubes of wild type flies before (solid line) and after (broken line) treatment with bromine.

After bromine treatment, riboflavin, sepiapterin, and YP were chromatographed using two solvent systems and their Rf values were determined (Table 1). The fluorescent color was determined with an ultraviolet light. The absence of a blue fluorescent spot in bromine-treated YP strongly suggests that little, if any, sepiapterin is present in MPT. Since 3-hydroxykynurenine is an alpha amino acid, its presence can be determined by the ninhydrin test. Chromatograms (developed in n-propanol and 5% ammonia) containing kynurenine, sepiapterin, and YP from MPT were sprayed with a 0.2% (in acetone) solution of ninhydrin. Only kynurenine produced a positive ninhydrin reaction. Hadorn and Mitchell[10] also found that ninhydrin-positive materials are found only in trace amounts in chromatographed MPT.

Weber and Roberts[14] demonstrated that the primary site of riboflavin storage in Tribolium confusum is in the MPT. They concluded that the ability to store riboflavin in MPT is under control of a single gene whereas the amount stored is controlled by a small number of different genes. Sang[15] demonstrated that riboflavin is an essential dietary factor for normal development in D. melanogaster. The results presented in this communication support the hypothesis that riboflavin accumulates in MPT[13], and that the ability to absorb riboflavin from the diet and store it in MPT is intricately related to eye pigment metabolism in D. melanogaster. The nature of this relationship is under investigation.

Table 1. Mean Rf values of riboflavin, sepiapterin, and the yellow pigment from Malpighian tubes following treatment with bromine. Kynurenine not previously treated with bromine is also given.

Compound	Visible Color	Fluorescent Color	Rf* A	Rf* B
Riboflavin	Yellow	Yellow	.34	**.22**
Sepiapterin	Colorless	Blue	.16	.17
Yellow Pigment	Yellow	Yellow	.35	.23
Kynurenine	Yellow	Blue	.55	.36

*Solvents: (A) n-propanol and 1% ammonia (2:1), (B) n-butanol, acetic acid, and water (4:1:1).

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