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Isolation, Characterization, and Quantitation of Isoxanthopterin from Drosophila melanogaster Strains Wild Type and White Apricot

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ABSTRACT

Wild type *Drosophila melanogasters'* eyes contain all six of the pteridines that help make up the color of the eye. Not all mutant strains' metabolic pathways function in a way that they can produce all of the pteridines like the wild type. The pigment isoxanthopterin absorbs light in the ultra violet spectrum; this pigment allows *Drosophila melanogaster* to see fluorescing pigments that humans are unable to, such as in some flowers. It is also responsible for the red color that the majority of strains *of Drosophila melanogaster* have, even if in small quantities. After decapitating the wild type and the mutant strain white apricot, they were crushed the flies' heads, which are mostly made up of the eyes, onto separate pieces of chromatography paper, using a 1:1 ratio of ammonium hydroxide and 2-propanol as a solvent, and allowed the isoxanthopterin to separate from the other pigments present, the pigments were then extracted from the paper. After analysis of the pigment using a UV-visible spectrophotometer, a standard curve was created in order to quantitate the amount of pigment found in each of the two, wild type and the mutant white apricot, for. The amounts of isoxanthopterin in the two varieties were similar revealing that wild type and white apricot mutants both must contain a xanthine dehydrogenase enzyme that catalyzes a number of reactions in the metabolic pathways in *Drosophila melanogaster*. The wild type average in the chromatogram using ten *Drosophila melanogaster* was 0.5820 ug/head while the white apricot value was 0.8163. The values for the chromatograms using 30 or more heads for the wild type and white apricot were 0.2029 and 0.2036 respectively. The reaction pertinent to this study was the formation of isoxanthopterin from 2-amino-4-hydroxypteridine and xanthopterin. This enzyme has shown to be imperative in the formation of isoxanthopterin. Mutant strains of *Drosophila melanogaster* whose eyes do not contain isoxanthopterin lack the ability to see in the blue-violet fluorescent spectrum and lack a strong red color in the eyes.

Keywords: *Catalyze, chromatography, Drosophila melanogaster, enzyme, isoxanthopterin, metabolic pathways, mutants, pteridines, xanthine dehydrogenase.*

INTRODUCTION

The eyes of some *Drosophila melanogaster* contain fluorescent pteridines that were separated by paper chromatography and characterized using UV visible spectrophotometer. Comparing the pigments found in the different colored eyes of *Drosophila melanogaster* and the presence or lack thereof, allowed for analyzes the spectrums at which wild and mutant strains *of Drosophila melanogaster* can absorb through their eyes. These biochemical analyses can be instructive in evolutionary and genetic studies. Pteridines in *Dropsophila melanogaster* have been researched by Rasmussen and Scossirolli (1954), Rasmussen (1954, 1955), Hubby and Throckmorton (1960), and Throckmorton (1962). These authors have analyzed the presence of pteridines in specific organs in *Drosophila melanogaster* as well as similar species but have found that these pigments are present in all wild eyes species similar to *Drosophila melanogaster*. In the mutant strains however, research has shown that there is a relationship between the quantities of pteridines in the internal organs and pigments found in the eyes. (Hardon and Mitchell, 1951 and Hardon 1958) Hardon and Mitchell studied the pigment presence and quantity of the pteridine pigments in different stages of the life cycle in *Drosophila melanogaster* and also compared the pigment differences in sex.

Using the heads of *Drosophila melanogaster* with different eye colors, the pigments were separated and characterized. Using this information and previous research, assumptions were made as to what enzymes or compounds are absent in the metabolic pathways that cause pteridines absence in the mutant strains. If isoxanthopterin is present that means that a xanthine dehydrogenase must be present to catalyze reactions necessary to form isoxanthopterin within the metabolic pathways (Forrest et al, 1961). *Drosophila melanogaster* that lack isoxanthopterin lack the ability to see in the violet fluorescent spectrum and also lack a strong red color in the eyes. By determining the presence and quantity of isoxanthopterin in the wild type and white apricot mutant strains of *Drosophila melanogaster* we can potentially answer behavioral and metabolic questions pertaining to possibly the most scientifically studied organism ever.

MATERIALS AND METHODS

Wild type and white apricot mutant strains were

ordered from Carolina Biological Supply Company and raised on Carolina Biological Supply Company's formula 4-24 instant Drosophila medium blue in a incubator at approximately 25°C. The *Drosophila melanogaster* were anaesthitized using Carolina fly nap and the heads were removed using a sharp scalpel under a Bauch and Lomb 0.7X – 3X dissecting microscope until a minimum of 10 heads was reached. The heads were then carefully put into a line approximately 2cm above the bottom of Whatman number 1 chromatography paper and crushed and smeared with a glass rod directly onto the chromatography paper. The papers were then placed into a 100ml graduated cylinder that was covered with Pechiney Plastic Packaging parafilm "M" laboratory film and wrapped in tin foil to keep out light. The solvent used was a 1:1 ratio of 2-propanol and ammonium hydroxide and each run took anywhere from six to nine hours. After the solvent had nearly reached the top of the paper, the papers were removed and wrapped in tin foil till extraction. The papers were removed from the foil in a dark room and allowed to dry for approximately 15 min. Then using a UL 977C ultra-violet light set to longwave (365nm) to see the violet isoxanthopterin band. This band was cut out in its entirety and then cut up into smaller pieces in the bottom of a test tube. The pieces were then washed twice, first with 2ml of solvent then again with 1ml of solvent measured with a Gilson 1000ul adjustable pipette. These solutions were then wrapped in foil and capped to prevent outside light and evaporation from effecting volume and concentration. A standard solution of 100,200ug/L was then made up and the standard solution was used to calculate the percent extracted for my samples. The standard solutions were applied to the chromatography paper using a Gilson1000ul adjustable pipet and put one drop on at a time and allowed to dry before the next drop was applied to keep the starting point of the solution on the paper as small and concentrated as possible. From here the extraction process was the same as the other samples.

These solutions were then analyzed by a Varian Cary Win UV-visible light spectrophotometer version 2.00 and software with the "scan" application to find the wavelength of the peaks and absorbance values. Using this data calculated a range for my isoxanthopterin standards. The stock solution was made up to be 100,200ug/L using ≥97.5% pure isoxanthopterin obtained from Sigma-Aldrich. Using this stock solution a set of standards were made, the concentrations were as follows: 250ug/L, 500ug/L, 1000ug/L, 2000ug/L, 4000ug/L, and 8000ug/L and were made up into 25ml volumetric flasks and a Gilson 1000ul adjustable pipette. Using these standards and the Cary WinUV "concentration" application a standard curve was set up at a wavelength of 223.9nm, (the wavelength of the standards' peak), and calculated the concentration of the samples, and mg of isoxanthopterin per head, corrected by the percent extraction found from the standard paper chromatography test.

This process was repeated with 10 to 39 *Drosophila melanogaster* heads per chromatogram of wild and white apricot strains to show the reproducibility of the experiment.

RESULTS

The R_f values for the paper chromatography had a mean of 0.448±0.10 with a 95% confidence interval and a standard deviation of 0.08245.

Using the absorptions from the samples the concentration of each was calculated in accordance with the calibration curve, which contained 6 standards ranging from 250ug/L to 8000ug/L and had a correlation coefficient of 0.99731, and then the average amount was found, in ug, of isoxanthopterin in each of the *Drosophila melanogasters'* heads.

Table 1: Statistical Data Analysis of Extractions

Type	# of Heads	Abs.	Conc. ug/L	ug/head
Wild 1	10	0.3984	2036	0.6108
Wild 2	10	0.3632	1844	0.5532
W. Apricot	10	0.5238	2721	0.8163
Wild	31	0.4550	2345	0.2269
Wild	33	0.3912	1996	0.1788
W. Apricot	39	0.5102	2647	0.2036

The statistical analyses on the values from the chromatograms containing ten wild type *Drosophila melanogaster* heads and the values from the chromatograms containing 31 and 33 wild type *Drosophila melanogaster* heads separately due to the fact that there were discrepancies in the ratios between the two.

The standard deviation of the ug/head of the chromatograms containing ten heads was 0.4101 and the mean was 6.314. The standard error of this value was 0.2902. The standard deviation of the ug/head of the chromatograms containing 31 and 33 heads was 1.584 and the mean was 6.47. The standard error of this value was 1.120.

The percent extraction correction was not used due to the fact that the data from the standards from the chromatograms was inconclusive as to what a correct percent extraction value would equal.

DISCUSSION

The values found for the confidence intervals from

the R_f values of the chromatograms are relatively high. This could be due to the differing rates of evaporation of the two compounds used in the solvent causing the ratio of 2-propanol and ammonium hydroxide to be ever changing. I also believe that the white apricot chromatograms could have had different R_f values due to the other pigments present in the wild type chromatograms that are not present in the white apricot mutant strain. The average R_f value for the white apricot chromatogram was 0.58 while the average R_f value for the wild type was 0.415. Despite this relatively large difference, when looking at the ultra-violet and visible spectrum (210nm to 800nm) scans of these two chromatogram bands I found that throughout the entire scanned spectrum they are nearly identical leading me to believe that they are in fact the same molecule despite differing R_f values.

Since I did not have multiple white apricot samples with a similar number of heads to run in the ultra-vilolet visible spectrophotometer, I could not do statistical analyses on them. I did however do statistical analyses on the two wild type chromatograms containing ten heads and the chromatograms containing 31 and 33 heads separately. This data also lacks precision which is hard to find the source of with the limited amount of data I had available.

The numbers for the chromatograms containing 31 and 33 heads have significantly lower ug/head values than the chromatograms containing ten heads. I believe this flaw is not in my technique but a flaw in this particular experimental design. I do not think it is necessarily set up for finding quantitative values. If trying to simply find the presence of a pigment, these methods and materials are an accurate and efficient means of doing so.

Possibilities of error could be in the extraction of the pigment from the chromatogram paper. The bands containing the pigment isoxanthopterin were cut out of the paper and then were cut into small pieces and placed in a test tube to which two separate washings were carried out with a pipette by adding the solvent to the test tube, stirring and decanting the liquid off. If the concentration of isoxantopterin was higher, such as in the 31 and 33 head samples, this type of washing could cause more isoxanthopterin to remain in the test tube containing the paper and not quantitatively been removed. This would account for the different ug/L values found in the chromatograms containg ten heads and the ones containing 31 and 33 heads.

Also small number of samples and the age of the *Drosophila melanogaster* could have played a role in the relatively high error in values. While I did not find any evidence of age being a factor in other scientific research, in looking at the *Drosophila melanogasters* through the microscope, it was evident that the younger looking samples' eyes did have more of a visible red color than the older looking specimens. This type of error would most likely been eliminated if more data had been collected.

In conclusion, despite the error in this experimental design, I do trust the values found in this research enough to conclude that the white apricot mutant strain and the wild type do both contain the pigment isoxanthopterin. This also proves the presence of a xanthine dehyrogenase enzyme needed to catalyze reactions in the metabolic pathways of *Drosophila melanogaster* in order to create the pigment isoxanthopterin (Forrest et al, 1961). I also believe that the white apricot mutant strain does, in fact, contain more of the isoxantopterin pigment than the wild type as my figures do show.

I would like to encourage others to attempt to make improvements to this experimental design or adjust the number of *Drosophila melanogaster* heads used in an attempt to obtain quantitative data of the amounts of isoxanthopterin in the eyes of *Drosophila melanogaster strains.*

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