

Earlier it was found that the concentration dependent phenocopy effect of the xanthine dehydrogenase inhibitor 4-hydroxy-pyrazolo(3,4-d) pyrimidine (HPP) on drosopterins in cinnebar (cn) eyes decreases the amount of drosopterins slightly below the level of the rosy (ry) strain but never below 10% of the cn control value. We now asked the following: Is a low level drosopterin synthesis uncontrollable by the xanthine dehydrogenase metabolites? Or will these metabolites decrease the drosopterin synthesis further in other eye color mutants which normally have a rather small amount of drosopterins and a functional xanthine dehydrogenase? Therefore, HPP was fed to larvae of a control cn strain and of the following eye color mutants: claret (ca), orange (or66k), pink-peach (pp), raspberry (ras2), and rosy (ry2); all strains also contained cn in order to block the ommochrome synthesis and thus to facilitate the visual classification and the extraction of drosopterins in acidified ethanol. At various breeding temperatures (18°C, 22.5°C, 27°C), larvae were raised on control food and on HPP-food (0.005 M HPP), and drosopterins extracted from whole heads (1 head/.1 ml or for low values 2 heads/.1 ml), and the absorption at 485 mu determined in a Beckman microcuvette procedure. Our data demonstrate clearly that the HPP can further decrease the drosopterin formation in all mutant strains except ry, although to a different extent. Furthermore, HPP-feeding also causes the temperature dependent semi-lethality and delay in development which is so characteristic for the ry mutants and the HPP-caused phenocopy in cn;ry+ animals. The statistic evaluation of over 6 day old flies of the 22.5°C growth series shows highly significant differences (p<0.001) of the means of drosopterin quantities for control versus HPP-food for the genotypes cn;ca and cn;or66k and cn;pp and ras2;cn. However, there is always some residual drosopterin synthesis, although on different low levels for the different mutant strains.

Phenylalanine crystal implantation into pupae can increase the drosopterin synthesis in maroon-like and rosy eyes and in the HPP-caused rosy-like phenocopy of cn genotype, as published earlier. This suggested the following two working hypothesis: (A) Phenylalanine is involved in a control mechanism interacting with the xanthine dehydrogenase metabolites and, therefore, acts specific in the maroon-like and rosy mutant and the phenocopy. (B) Phenylalanine acts at a later, more general step in the drosopterin biosynthesis; in this case it should also increase the drosopterin formation in other eye color mutants which have an active xanthine dehydrogenase. The implantation of large phenylalanine crystals into abdomen of late pupae already forming drosopterins in their eyes or into 0-1 hr old flies resulted in a much better long-term survival compared to implantation in younger pupae. Obviously, a smaller increase is expected because 1/2 to 2/3 of the eye drosopterins are deposited before the onset of the experimental phenylalanine supply. Nevertheless, for cn;ca and cn;or66k and cn;pp an increased drosopterin synthesis to two- to three-fold amounts of the control value was found, which is almost as extensive as in the cn;ry2 flies used as a control in this experimental series. In contrast, the ras2;cn flies did not show a phenylalanine dependent increase of drosopterin synthesis, although in this mutant strain 3/4 of the normal drosopterin formation occurs after the eclosion of the flies and thus would be under the influence of the phenylalanine implant in the experimental series. These data suggest that phenylalanine interacts with some late step on the drosopterin pathway (hypothesis B), resulting in some mutants in a phenocopy distinctly different from the "normal" eye color phenotype.

These epigenetic metabolite control mechanisms thus drastically alter the eye color phenotype: (a) the inhibitor can decrease the drosopterin quantity to as low as 10% of "normal", and (b) the enhancer can cause a several-fold increase in drosopterin formation in various eye color mutants. These results are to be reported in detail elsewhere.