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Observations on the Regulation of Uricase Activity During Development of *Drosophila melanogaster*

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To elucidate the mechanisms involved in the regulation of uricase activity in Drosophila melanogaster, *a comparative analysis of the patterns of uricase activity during development was undertaken for the wild type,* Ore-R, *and the mutants* ry^2 *and* ma-l. *Uricase activity in* ry^2 *and* ma-l, *unlike that in* Ore-R, *increased rapidly following emergence of the adult. This study indicates that uricase in* Drosophila, *in contrast to that in several microorganisms, is not induced by uric acid, since* ry^2 *and* ma-l *with no detectable uric acid have higher activity than the wild type.*

INTRODUCTION

The regulation of uricase activity has been studied in higher organisms and microorganisms. In higher organisms, the mechanisms responsible for changes in uricase activity during development remain obscure (Barrett, 1964; Brown, 1938; Nelson, 1964). In microorganisms, uricase appears to be induced by uric acid (Scazzocchio and Darlington, 1968; Watanabe and Fukumoto, 1970).

In the present study, the wild-type pattern of uricase activity in the pupa and adult of *Drosophila melanogaster* was compared with the patterns of uricase activity in the mutants ry^2 and *ma-l*. These comparisons were undertaken as the initial step to elucidate the mechanisms involved in the regulation

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of uricase activity in *Drosophila*. For this purpose, the mutants ry^2 and *ma-l* were of particular interest, since both lack detectable levels of uric acid due to the absence of xanthine dehydrogenase (Glassman and Mitchell, 1959*a*; Morita, 1958; Glassman, 1965).

MATERIALS AND METHODS

Genetic Stocks

From electrophoretic studies, the *rosy* locus (*ry*: 3–52) appears to be the structural gene for xanthine dehydrogenase (Yen and Glassman, 1965). The biochemical basis for the absence of xanthine dehydrogenase, pyridoxal oxidase, and aldehyde oxidase in *maroon-like* (*ma-l*: 1–64.8) remains unexplained (Glassman, 1965; Glassman *et al.*, 1968; Collins and Glassman, 1969; Courtright, 1967). Stocks of *Drosophila* were provided by Dr. T. M. Rizki and Dr. I Oster.

Media

Adult and larval *Drosophila* were raised at 25 C on standard cornmeal–molasses–yeast food and cream of wheat food, respectively.

Enzyme Preparation

Homogenates were prepared by grinding whole flies or individual tissues from adults in ice-cold 0.25 M sucrose, *p*H 7.5. Either 20 xanthine dehydrogenase–deficient or 50 wild-type individuals were homogenized, filtered through cheesecloth to remove chitin, and adjusted to a final volume of 2.0 ml. The protein concentrations were approximately 1.2 mg/ml and 2.7 mg/ml, respectively, for 1-day-old adults (Lowry *et al.*, 1951).

Determination of Uricase Activity

Uricase (E.C. 1.7.3.3) was assayed by the spectrophotometric method described by Kalckar (1947) and Praetorius (1948) and modified by Tuskuda *et al.* (1968). To each sample were added the following reagents: 0.2 ml of 0.125 M boric acid (*p*H 9.0), 1.0 ml of 6.0×10^{-4} M EDTA, 0.2 ml of homogenate, and 0.2 ml of 5.26×10^{-4} M sodium urate. Control experiments omitted either sodium urate or homogenate until the end of the 90 min incubation period. After incubation, the reaction was stopped with 1.0 ml of ice-cold 10% trichloroacetic acid. Samples were then centrifuged for 20 min at $1470 \times g$. The difference in optical density at 292 nm between control and experimental indicated the level of uricase activity, since allantoin, the reaction product, does not absorb light at 292 nm.

Uricase activity was found to be linear with respect to time and concentration of protein for 210 min or until a change in optical density exceeded 0.30. The specific activity of uricase was defined as the change in optical density at 292 nm in 90 min per individual. The kinetics of uricase activity were measured for *Ore-R*, ry^2, and *ma-l*. K_m values were calculated by the method of Lineweaver and Burke.

Determination of Catalase Activity

Catalase (E.C. 1.11.1.6) was assayed according to the method of Baudhuin *et al.* (1964).

RESULTS

Kinetics of Uricase

Enzyme preparations from all genotypes had a single *p*H optimum of 9.0 and a temperature optimum at 26 C. The EDTA concentration and ionic strength of the buffer gave maximal activity for all strains. All 24-hr-old and 10-day-old *Ore-R*, *ma-l*, and ry^2 adults had K_m values within 2 SD of the mean K_m, 4.35×10^{-5} M.

Localization of Uricase

All the major tissues of males and females were separated and individually assayed. Uricase activity was detected only in the Malpighian tubules, an observation consistent with those made by Razet (1953) and Nelson (1964) for other Diptera.

Effect of Various Metabolites on Uricase Activity

Uricase activity was measured in the presence of the following metabolites: allantoin, xanthine, hypoxanthine, 2,6,8-trichloropurine, 2-amino-4-hydroxypteridine, and isoxanthopterin. All were inhibitory except allantoin (Table I).

Ontogeny of Uricase

Uricase activity was detected only in the larva and adult. No uricase activity was found in the puparium using dialyzed or undialyzed homogenates. This pattern of uricase activity is the same as that reported for *Musca domestica* and *Lucilia sericata* (Nelson, 1964; Brown, 1938).

Twenty-four hours after emergence, *ma-l* and ry^2 males and females had approximately 10–30 times the activity detected in the wild types, *Ore-R*,

Table I. Effect of Various Metabolites on Uricase Activity *in Vitro*

Reagent in assay	Concentration of reagent in assay (M)	Percent inhibition of uricase activity[a]
Allantoin	6.3×10^{-4}	5.8 (4)
Xanthine	8.2×10^{-5}	53.5 (4)
Hypoxanthine	9.2×10^{-5}	9.4 (3)
2, 6, 8-Trichloropurine	2.8×10^{-5}	67.5 (4)
Isoxanthopterin	4.2×10^{-5}	41.2 (3)
2-Amino-4-hydroxypteridine	4.1×10^{-5}	21.2 (2)

[a] The mean percent inhibition is followed by the number of independent determinations in parentheses.

Swedish-b, and *Canton-S* (Fig. 1 and Table II). The rapid increase of detectable uricase activity in *ma-l* and ry^2 adults was followed by a decline in uricase activity after 1–2 days.

Mixed Homogenates

Homogenates of different mutants and sexes were combined and assayed. Mixing experiments indicated that uricase activity was additive regardless of the ratios of protein. Pupal extracts did not inhibit uricase activity from adults.

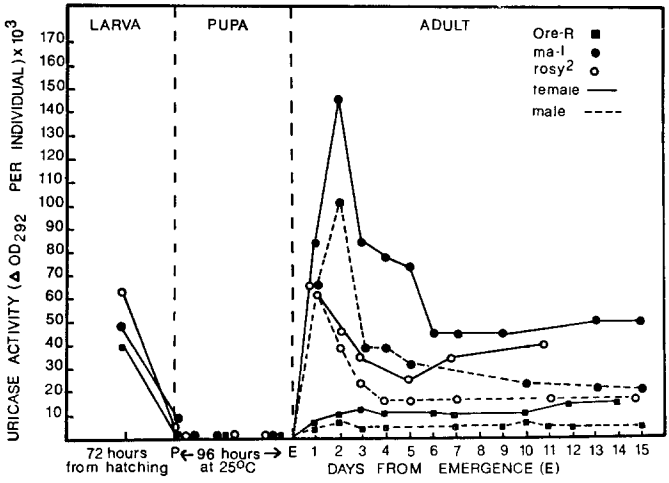

Fig. 1. The pattern of uricase activity in the late third instar larva, pupa, and adult.

Table II. Comparison of Uricase Activity in 1-Day-Old *ma-l* and ry^2 Heterozygotes to that in Homozygotes and Wild Types

Age[a] (hr)	P_1 genotype		F_1 genotype		Uricase activity in F_1[b]	
	Female	Male	Female	Male	Males	Females
23±3	*Ore-R*	*ma-l*	*ma-l*/+	—	—	20.0±9.9 (2)
22±3	*ma-l*	*Ore-R*	*ma-l*/+	—	—	19.9±3.8 (3)
24±3	*Ore-R*	ry^2	ry^2/+	ry^2/+	6.5±0.5 (3)	9.3±4.7 (3)
	Homozygotes					
24±3	*Ore-R*	*Ore-R*			2.4±0.9 (7)	8.5±2.9 (9)
24±3	*Swedish-b*	*Swedish-b*			4.3±0.6 (4)	5.8±1.4 (3)
24±3	*Canton-S*	*Canton-S*			5.3±1.0 (2)	4.9±0.4 (2)
24±3	*ma-l*	*ma-l*			68.9±7.4 (7)	83.8±3.7 (5)
24±3	ry^2	ry^2			63.1±8.8 (8)	68.8±11.9 (9)

[a] Age in hours from emergence of adults from the puparium.
[b] Mean values are followed by standard deviations and the number of independent determinations in parentheses.

Uricase Activity in Starved Flies

Uricase activity was measured in 24-hr-old adults starved from emergence. There was no significant difference in the amount of uricase activity in *ma-l* adults with and without food. However, *Ore-R* females required food for their slow increase in uricase following emergence (Table III).

Uricase Activity in Heterozygotes

Uricase activity in adults heterozygous for *ma-l* and ry^2 was measured to determine whether or not high or low uricase was dominant or recessive.

Table III. Effect of Starving Adults for 24 hr Following Emergence on Uricase Activity

Genotype	Sex[a]	Age (hr)	Uricase activity in flies given: Food[b]	Uricase activity in flies given: No food[b]	Ratio of the weight of flies no food/food
Ore-R	F	25±3	8.7± 2.4 (4)	1.8± 0.5 (4)	0.74 (4)
Ore-R	M	25±3	2.0 (1)	2.0 (1)	0.88 (1)
Ore-R	M+F	25±3	6.0± 3.0 (5)	1.6± 1.1 (5)	0.77 (5)
ma-l	F	27±2	94.0±17.3 (3)	92.9±15.0 (3)	0.72 (3)
ma-l	M	25±3	66.9± 9.7 (3)	69.3± 9.9 (3)	0.94 (3)
ma-l	M+F	27±2	69.7±11.1 (3)	67.8±17.6 (3)	—

[a] M, male; F, female.
[b] Mean values are followed by the standard deviations and the number of independent determinations.

The *ma-l* heterozygotes have intermediate uricase activity (Table II). The ry^2 female heterozygotes have uricase activity nearly identical to that of *Ore-R*, while ry^2 male heterozygotes are only slightly higher. Thus the ry^2 allele appears to be recessive to the wild type with respect to uricase activity. All singly assayed ry^2 flies from crosses of ry^2 heterozygotes had high uricase activity, while the wild type and heterozygotes all had low uricase activity. This eliminates the possibility that high uricase activity is associated with an autosomal recessive other than the *ry* locus or one closely linked to the *ry* locus.

Uricase Activity in Maternally Affected *ma-l* Flies

Homozygous *ma-l* females or hemizygous *ma-l* males which are obtained from heterozygous *ma-l* females have wild-type eye pigmentation as a result of a maternal effect (Glassman and Mitchell 1959*b*; Glassman and McLean, 1962). Since maternally affected *ma-l* adults resemble the normal phenotype with respect to eye color, the question arises as to whether uricase activity also regresses towards the wild type. Maternally affected male and female adults had high uricase activity which resembled that of *ma-l* and not *Ore-R* (Table IV).

Catalase Activity

Both catalase and uricase appear to be associated with the same organelle, the microbody, in both vertebrates and invertebrates (Hruban and Rechcigl, 1969; Locke and McMahon, 1971). Catalase and uricase activity might be coordinately controlled. However, there was no significant difference in the

Table IV. Test for Maternal Effect in 1-Day-Old *ma-l* Female and Male Adults

Maternal genotype	Genotype of progeny	Sex	Age (hr)	Uricase activity of progeny[b]
M5/*ma-l*[a]	*ma-l*/*ma-l*	F	28	116.8± 8.2 (5)
M5/*ma-l*	*ma-l*/	M	28	107.0±15.8 (5)
ma-l/*ma-l*	*ma-l*/*ma-l*	F	24	83.8± 3.7 (5)[c]
ma-l/*ma-l*	*ma-l*/	M	24	68.9± 7.4 (7)[c]
Ore-R	*Ore-R*	F	24	8.5± 2.9 (9)[c]
Ore-R	*Ore-R*	M	24	2.4± 0.9 (7)[c]

[a] The M5 stock has the following composition: $In(1)sc^{s1L}sc^{8R}+s$, $sc^{s1}sc^{8}w^{a}B$.

[b] Mean values are followed by the standard deviations and the number of independent observations in parentheses.

[c] These values are taken from Fig. 1 and are presented for comparison.

catalase activity in whole homogenates or isolated Malpighian tubules of 48-hr-old *ma-l* as compared to *Ore-R*.

DISCUSSION

If uricase activity in *Drosophila* were induced by uric acid, there would be no or little uricase activity in ry^2 and *ma-l*, since these two mutants have no detectable uric acid (Mitchell *et al.*, 1959; Morita, 1958). Both *ma-l* and ry^2 had high uricase activity following emergence, indicating that uric acid is not an inducer of uricase in *Drosophila*. The high uricase activity in *ma-l* and ry^2 (Fig. 1) may be due to a metabolite which accumulates in the pupa of *ma-l* and ry^2 and is only capable of activating or inducing uricase upon emergence of the adult. This molecule might be xanthine, hypoxanthine, one of their immediate precursors, or possibly one of the pteridines which accumulate in ry^2 and *ma-l* but not in *Ore-R* (Mitchell *et al.*, 1959; Morita, 1958; Hadorn, 1958; Rizki and Rizki, 1962; Hubby and Forrest, 1960; Forrest *et al.*, 1961).

The results of the starvation experiment were consistent with the hypothesis that an inducer or activator of uricase activity is stored in the pupa. No factor in the food was necessary for the rapid increase in uricase activity in *ma-l*, but standard food was necessary for the slow increase in activity in *Ore-R* females (Table III). Auf der Maur (1961) reported that the increase in uricase activity between the fourth and fifteenth day of adult life in *Ore-R* and *white*, a sex-linked mutant, was dependent on a metabolite found in the standard food but not in food containing only sugars.

Since ry^2 and *ma-l* from homozygous ry^2 and *ma-l* parents, respectively, are also deficient in red pigment, the high or low uricase activity may be correlated with the amount of eye pigmentation. However, maternally affected *ma-l* males and females have a wild-type complement of eye pigmentation and show the high uricase phenotype. Maternally affected *ma-l* adults have only 2 to 5% of the wild-type amount of xanthine dehydrogenase activity at the time of emergence (interpolated from Fig. 3 of Glassman and McLean, 1962). A mutant, *white*, with no eye pigmentation has only slightly greater uricase activity than *Ore-R* (Friedman, 1971; Auf der Maur, 1961). Therefore, the high uricase phenotype appears to be strongly correlated with low xanthine dehydrogenase activity and not eye pigmentation.

Finally, are the changes in uricase activity during development of *Drosophila* a reflection of regulation by inhibitors and activators of a latent pupal uricase and/or the number of enzyme molecules? No definitive evidence for activation or inhibition was obtained. First, the kinetics of uricase activity from ry^2 and *ma-l* were essentially identical to that of the wild type. Second,

homogenates having high uricase activity showed no influence on the uricase activity from homogenates having low or no activity. Third, hypoxanthine, xanthine, and 2-amino-4-hydroxypteridine, which *ma-l* and ry^2 accumulate, were inhibitors of uricase *in vitro*. Isoxanthopterin, absent from ry^2 and *ma-l*, also inhibited uricase activity and might therefore be an explanation for the reduced levels of uricase activity in *Ore-R* as compared to *ma-l* and ry^2.

Thus uric acid cannot be considered the inducer of uricase activity in *Drosophila*, since the mutants ry^2 and *ma-l* in the absence of uric acid have uricase activity higher than the wild type does. Uricase activity in *Drosophila* and perhaps in other higher organisms is regulated differently than in some microorganisms in which uric acid is the inducer of uricase. It is possible that one or more of the purine precursors of uric acid or a pteridine which accumulates in the pupa of ry^2 and *ma-l* influences uricase activity in *D. melanogaster*.

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