A MUTANT AFFECTING PTERIDINE METABOLISM IN *DROSOPHILA MELANOGASTER*¹

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THE pteridine compounds that accumulate in the eyes and testis sheath of *Drosophila* have been the subject of many reports. Their chemistry and structure have been studied principally by FORREST (FORREST and MITCHELL 1955; FORREST, HATFIELD and VAN BAALEN 1959) and by VISCONTINI (VISCONTINI 1958). The assembly of quantitative data on the effect of mutant strains on the accumulation of these compounds was initiated by HADORN and MITCHELL (1951). But the biochemical basis of these differences has been resolved in only two mutants. In maroon-like and rosy the lack of xanthine dehydrogenase activity results in the absence of the major testis compound isoxanthopterin (FORREST, GLASSMAN and MITCHELL 1956). Studies of other mutants causing qualitative and quantitative changes in the pteridine accumulations have failed to reveal the metabolic lesion responsible for the disturbance (HADORN 1956, 1958).

Extensive studies on the accumulation of these compounds in other *Drosophila* species have been made from the point of view of relating changes in metabolism with evolution (HUBBY and THROCKMORTON 1960; THROCKMORTON and MAGALHAES 1962) or correlating the pteridine accumulation with taxonomic findings (BUZZATI-TRAVERSO and RECHNITZER 1953; RASMUSSEN 1954). From the first kind of study it has clearly been shown that changes with evolution have only affected the compounds present in the testes. This kind of organ specific change has not been found in mutants studied thus far. Furthermore, it is clear that patterns of change in testis accumulation of pteridines are related to phylogenetic sequences.

This report concerns a recessive mutant, *lix* (little isoxanthopterin), recently recovered in *Drosophila melanogaster*. Evidence suggests that a metabolic step prior to the biosynthesis of 2-amino-4-hydroxypteridine is affected in the mutant. The phenotype is organ specific in that only pteridine accumulation in the testis sheath is changed. Two other features are striking: the accumulation in the testes of a blue fluorescent compound unique to the mutant, and easily detectable amounts of the red drosopterin pigments in this organ. The presence of red pigments and of elevated amounts of yellow fluorescing compounds in the testes is a characteristic of primitive members of the genus. The end result of the action of the wild allele of this mutant is small amounts of pigments and large amounts of isoxanthopterin in the testes. Such action is characteristic of highly derived

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species. It is suggested that this allele is a new acquisition of the derived forms within this genus.

MATERIALS AND METHODS

The mutant *lix* was isolated from a stock culture of Oregon-R (Ore-R) by A. Y. HESSLER (1960). Its detection was possible after chromatographic analysis of testes squashes. Isoxanthopterin is present in only small amounts compared to wild type. Crossover data and other characteristics of the mutant will be reported below. Ore-R and *rosy*² (*ry*²) were the other strains used in this study. A double mutant *lix;ry*² was derived in the conventional manner. Flies were raised at 25° ± 1° on yeast-agar-sucrose medium (CARPENTER 1950).

Quantitative fluorometry, chromatographic procedures, and xanthine dehydrogenase assays with hypoxanthine as substrate have been described previously (HUBBY and FORREST 1960). The method of GLASSMAN and MITCHELL (1959) was employed for assaying xanthine dehydrogenase with 2-amino-4-hydroxypteridine as substrate. Hypoxanthine was determined with commercial xanthine oxidase; uric acid was determined with commercial uricase (PLESNER and KALCKAR 1956). Both commercial preparations were obtained from Nutritional Biochemical Corporation. Identification of all fluorescent compounds was made by chromatographic comparison with authentic samples. Isoxanthopterin, compound A, biopterin and 2-amino-4-hydroxypteridine were kindly supplied by H. S. FORREST.

RESULTS AND DISCUSSION

The only visible phenotype of *lix* is a dark yellow-orange testis sheath, but this character is not dependable for classification. For crossover studies, the individuals were chromatographed to determine the presence or absence of the *lix* allele after phenotypic classification of the marker genes was made. These results positioned *lix* on the X chromosome at 21.4 ±. The mutant is sex-limited and shows no maternal effect.

The large reduction in isoxanthopterin characteristic of *lix* prompted an investigation of xanthine dehydrogenase activity in extracts of this mutant. Results of these studies using both hypoxanthine and 2-amino-4-hydroxypteridine as substrates are presented in Table 1. These results do not show any evidence of involvement of this enzyme in the metabolic disturbance of this mutant. When crude extracts of *lix* were added to wild-type enzyme preparations, they showed no indication of an inhibitor of xanthine dehydrogenase. Further confirmation of these observations is presented in Table 1 where analyses of 96 hour Ore-R and *lix* pupae reveal equal levels of uric acid and the lack of hypoxanthine. Also presented in Table 1 are similar data for *ry*² and *lix;ry*². Note that in the presence of *ry*² no uric acid is found and large amounts of hypoxanthine accumulate.

Quantitative fluorometric analyses of heads and testes of Ore-R, *lix*, *ry*² and *lix;ry*² are reported in Table 2. It will be seen that the amounts of drosopterins, sepiapteridine and biopterin in the heads of *lix* are not significantly different from wild type. There is a slight but significant ($P < .01$) decrease in 2-amino-

TABLE 1

Enzymatic and purine content of various genotypes

Source	Xanthine dehydrogenase activity*		Purine content†	
	Hypoxanthine as substrate	2-amino-4- hydroxypteridine as substrate		
Oregon-R	.05 .06 .06	1.2	<0.02	2.3±.2
<i>lix</i>	.07 .09 .08	1.9	<0.02	2.4±.3
<i>rosy</i> ²	0	0	1.8±0.2	0
<i>lix;rosy</i> ²	0	0	1.9±0.3	0

* With hypoxanthine as substrate, activity is expressed as change in optical density at 340 mμ/min/mg protein under conditions described in HUBBY and FORREST 1960. With 2-amino-4-hydroxypteridine as substrate, the activity is expressed in arbitrary fluorometric units as change/min/mg protein as described in GLASSMAN and MITCHELL 1959. Lower centrifugal forces employed in preparation of these extracts result in lower specific activities than obtained previously (HUBBY and FORREST 1960).

† Single pupae from which imagoes were about to emerge were homogenized in appropriate buffers, boiled and centrifuged to remove protein. Next they were subjected to microdeterminations of the purines as described in PLESNER and KALCKAR 1956. Amount of purine is expressed in terms of the wet weight of whole pupae.

4-hydroxypteridine in the mutant. In the testes *lix* is characterized by sharply reduced amounts of isoxanthopterin. In this organ the amounts of sepiapteridine, compound A, riboflavin-like, and biopterin are elevated. By analysis of variance all compounds differ significantly from wild type at the .01 level except compound A, where the difference is significant only to the .05 level. The striking presence of a blue fluorescent compound called "lix substance" in this report is another characteristic of *lix*, and with age *lix* accumulates drosopterins pigments in the testis sheath. It is this accumulation of red pigments plus the elevated quantities of the three yellow substances that produce the darker color of the testis sheath in the mutant.

Comparison of the amounts of 2-amino-4-hydroxypteridine in *ry*² and in *lix;ry*² reveals that the accumulation of this compound in the double mutant is nearly suppressed. The quantity of "lix substance" in the double mutant is also reduced from the amount present in *lix*. Drosopterins accumulate in *lix;ry*² in amounts exceeding that of *lix* alone. The mutant *ry*² has a pale testis sheath. But this is quite variable and has been noticed by others. The amounts of the yellow pigments in the double mutant are higher than in *ry*², but again there is a great amount of variability.

The attempts to isolate and purify "lix substance" have been unsuccessful because the compound is unstable and is present in only small amounts. Attempts to transform "lix substance" enzymatically by wild-type extracts have been unsuccessful.

It has been suggested (HUBBY and THROCKMORTON 1960) that the biosynthetic pathway of the pteridines in *Drosophila* is a bipartite one. The sequence involving biopterin, sepiapteridine and compound A would lead to the drosopterins; the

TABLE 2
Quantitative fluorometry of the fluorescent compounds in the heads and isolated testes of various genotypes*

Source	Drosopterins	Sepiapteridine	2-amino-4-hydroxypteridine	Compound		Riboflavin-like	Isoxanthopterin	Lix substance
				Biopterin	Compound A			
Heads								
Oregon-R	31.2±1.2	35.2±1.3	22.4±0.7	12.5±0.8				
<i>lir</i>	33.0±0.9	37.3±2.3	16.0±1.1	12.9±1.2				
Testes								
Oregon-R	0	16.1±1.1	0	22.4±1.9	1.0±0.5	3.0±0.9	152.0±4.9	0
<i>lir</i>	4.5±0.9	32.5±2.0	0	31.6±1.0	2.8±0.4	20.2±0.7	12.8±1.0	18.0±0.3
<i>rosy</i> ²	0	4.4±1.0	106.0±12.5	5.6±1.0	0	2.8±0.4	0	0
<i>lir;rosy</i> ²	8.0±2.0	20.8±3.5	8.0±1.0	9.2±1.0	0	10.4±1.4	0	2.8±1.0

* Fluorometry was performed with a Farrand Model A Fluorometer with a Corning No. 5860 primary filter. A Corning No. 3389 filter was used in conjunction with the following interference filters: Farrand 408 m μ (isoxanthopterin); Farrand 453 m μ (2-amino-4-hydroxypteridine, biopterin, and lix substance); Farrand 553 m μ (drosopterins, sepiapteridine, compound A, and riboflavin-like). Single heads were analyzed, 0.1 μ g/ml anthranilic acid was set at 5 units on the galvanometer for the estimation of drosopterins and sepiapteridine, and at 10 for estimating 2-amino-4-hydroxypteridine and biopterin. Ten pairs of testes were analyzed. Galvanometer settings were as follows: drosopterins, sepiapteridine, compound A, and riboflavin-like at 20; 2-amino-4-hydroxypteridine, biopterin and lix substance at 20; and isoxanthopterin at 10. The data for the two organs cannot be compared directly. Midway through this study it was discovered that anthranilic acid decays upon standing. In the study of the testes, fresh solutions were prepared daily. The data shown for the heads are consistent *inter se*.

other sequence, stemming from a common precursor to the above, would end in the conversion of 2-amino-4-hydroxypteridine to isoxanthopterin. Other schemes are tenable. The reduction of isoxanthopterin and the increment in the other compounds in the testis sheath of *lix* are in agreement with this suggestion but do not add greatly to the argument.

The marked reduction in the amount of 2-amino-4-hydroxypteridine in the double mutant *lix;ry*² as compared to the amounts present in *ry*² indicates the point of metabolic lesion in *lix* to lie on the pathway leading to the synthesis of that compound. The blue fluorescent "lix substance" may be an intermediate or a derivative of an intermediate leading to this compound; thus its presence in *lix* would be a reflection of the accumulation of a substrate at the point of enzymatic deficiency commonly found in biochemical mutants. Full confirmation of these ideas await characterization and metabolic reactions, if any, of this compound.

Of more general interest is the fact that the mutant *lix* mimics more primitive members of the genus *Drosophila* in its pattern of pteridine accumulation in the testis sheath. It will be recalled that primitive species of the subgenus *Sophophora*, such as members of the *obscura* group, have orange testes containing drosopterins and large amounts of the yellow pigments. In highly derived forms of the subgenus there is complete suppression of the drosopterins and marked or complete reduction of the yellow compounds in the testis sheath. *Drosophila melanogaster* and other members of the *melanogaster* group are intermediate in the completeness of this suppression, (HUBBY and THROCKMORTON 1960). Furthermore, data obtained in the previous work cited suggested that the genotypes controlling pteridine metabolism had been preserved through large sections of the genus. Though different mechanisms may have been employed in suppressing the accumulation of the compounds, it appeared that only a few basic changes had occurred. It was predicted that one of these was the reduction of all the colored compounds in the testis sheath resulting from an increased utilization of the shunt pathway via isoxanthopterin. The action of the wild allele of *lix* clearly does this in *melanogaster* in that it favors that part of the pathway leading to isoxanthopterin. This action limits the availability of material in the testis sheath for the synthesis of the yellow compounds and their final conversion to the drosopterins. Conversely the mutant allele of *lix*, by partially blocking one step leading to isoxanthopterin, favors the pathway involving the yellow and red pigments. The wild allele may be a metabolic acquisition which is characteristic of those highly derived *Drosophila* species lacking drosopterins and other pigments in their testis sheaths.

SUMMARY

The new mutant *lix* is described, and the evidence is presented which indicates a metabolic lesion in the mutant prior to the synthesis of 2-amino-4-hydroxypteridine. The mutant contains a blue fluorescent compound not previously found in *Drosophila melanogaster*. It is also characterized by low levels of isoxanthopterin and elevated amounts of three yellow compounds in the testis sheath.

Furthermore, there are drosopterins present in the testis sheath. These characteristics suggest a relation between the action of the wild allele of the mutant and the changes that have occurred in the evolution of pteridine metabolism in the genus.

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