vored the AR/CH heterokaryotype, and made the homokaryotypes subvital; the back-selection favored an AR chromosome which happened to be lethal in double dose, and resulted in a population which consisted of CH/CH homokaryotypes and AR/CH heterokaryotypes, but lacked AR/AR entirely.

Summary.—Using a classification maze constructed by Professor Jerry Hirsch, we have selected populations of *Drosophila pseudoobscura*, for a positive and for a negative geotactic behavior. The three initial populations were respectively monomorphic for AR, monomorphic for CH, and polymorphic for AR and CH gene arrangements in their third chromosomes. All the initial populations were about neutral to gravity, yet all responded both to plus and to minus selection. The plus selection favored AR chromosomes, while the negative selection favored the AR/CH heterokaryotype. Relaxation of the selection resulted in a partial relapse towards the original state. Reversal of the selection gave an almost complete return to the original state. In the polymorphic AR/CH population the back-selection favored an AR chromosome which was lethal in double dose.

* The work reported in this article has been carried out under Contract No. AT-(30-1)-1151 of the U.S. Atomic Energy Commission with the Columbia University, New York.

¹ Carson, H. L., Cold Spring Harbor Symposia on Quantitative Biology, vol. 23 (1958), pp. 291–306.

² Carson, H. L., Cold Spring Harbor Symposia on Quantitative Biology, vol. 24 (1959), pp. 87–105.

³ Hirsch, J., in *Roots of Behavior*, ed. E. L. Bliss (New York: Harper, 1962), pp. 3–23.

⁴ Hirsch, J., and L. Erlenmeyer-Kimling, *Science*, **134**, 835–836 (1961).

⁵ Erlenmeyer-Kimling, L., and J. Hirsch, *Science*, **134**, 1068–1069 (1961).

⁶ Dobzhansky, Th., and O. Pavlovsky, these PROCEEDINGS, **41**, 289–295 (1955).

⁷ Dobzhansky, Th., and O. Pavlovsky, these PROCEEDINGS, **46**, 41–47 (1960).

⁸ Lerner, I. M., *Genetic Homeostasis* (New York: John Wiley & Sons, 1954).

⁹ Spiess, E. B., and B. Langer, *Evolution*, **15**, 535–544 (1961).

MATERNAL EFFECT OF MA-1+ ON XANTHINE DEHYDROGENASE OF DROSOPHILA MELANOGASTER, II. XANTHINE DEHYDROGENASE ACTIVITY DURING DEVELOPMENT*

BY EDWARD GLASSMAN† AND JANICE MCLEAN

DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF NORTH CAROLINA

Communicated by Bentley Glass, July 24, 1962

At least two loci are now known which control xanthine dehydrogenase in *Drosophila melanogaster*.¹ One of these, maroon-like eye-color (*ma-l*), is located on the X-chromosomes; the other, rosy eye-color (*ry*), is on the 3rd chromosome.² The mutants show many similarities. Both have a reddish-brown eye color due to a partial deficiency of the red eye pigment, and both lack detectable amounts of the enzyme, xanthine dehydrogenase.¹ As a result of this deficiency, *ma-l* and *ry* mutants accumulate the enzyme substrates (hypoxanthine and 2-amino-4-hydroxypteridine) and show no trace of the products (uric acid and isoxanthopterin) formed from these compounds.³ Xanthine dehydrogenase was partially purified from wild-type flies, and no activity could be detected in extracts of the mutants.⁴

These two mutants differ in various ways. Among these differences is the maternal effect of $ma-l^+$ on $ma-l$ in which the $ma-l$ progeny from $ma-l/ma-l^+$ females have wild-type eye pigmentation; this phenomenon does not occur in the case of ry^+ and ry .⁵ Biochemical studies have shown that in addition to increased amounts of red eye pigments, maternally affected $ma-l$ adult flies have traces of isoxanthopterin and uric acid (the enzyme reaction products) as well as traces of xanthine dehydrogenase itself. This effect is probably due to a maternal substance which the $ma-l^+/ma-l$ females pass through the egg and which is converted by their $ma-l$ progeny into xanthine dehydrogenase activity. The maternal substance is probably not xanthine dehydrogenase, since females which are homozygous for ry and which are therefore deficient in the enzyme can still have maternally affected $ma-l$ progeny.⁵ The maternal substance must therefore be a component of the enzyme-forming system or of the enzyme-regulating system. This experiment also shows that ry flies, though lacking xanthine dehydrogenase, can make the maternal substance which is deficient in $ma-l$ flies. These considerations lead to the schemes depicted in Figure 1.⁵

FIG. 1.—Possible relationships between $ma-l^+$ and ry^+ functions.

It has also been shown that only $ma-l$ flies which emerge in the first six days of hatching are maternally affected; following this time, the adults which emerge have the typical $ma-l$ mutant eye-color. This is due to a change in the food and not to depletion of the maternal substance in aging females, since females transferred periodically to new food had maternally affected progeny in each successive bottle.⁵ The exact environmental cause for this effect has not been established.

This paper is concerned with an enzymatic analysis of these phenomena. We have been able to show that xanthine dehydrogenase is present in maternally affected $ma-l$ larvae but not in the eggs, indicating either an activation of or actual synthesis of this enzyme. In addition, it seems likely that the environmentally caused decline in the maternal effect can be correlated with the level of xanthine dehydrogenase activity in the developing flies.

Methods.—Mutants: The following mutant loci of *Drosophila melanogaster* were employed in this research: B = dominant Bar eye; Bx^3 = Beadex wing; ec = echinous eye; f = forked bristle; m = miniature wing; $ma-l$ = maroon-like eye color; $ma-l^{bz}$ = the bronzy allele of $ma-l$; $ma-l^+$ = the wild-type allele of $ma-l$; od = outstretched wing; ry = rosy eye color; ry^2 = the rosy² allele of ry ; ry^A = the rosy^A allele of ry ; ry^+ = the wild-type allele of ry ; st = scarlet eye color; v = vermilion eye color; y = yellow body color. The symbol $y f$ refers to an attached-X chromosome containing y , f and $ma-l^+$, while $M-5$ refers to the Muller-5 (base chromosome) which, in addition to the InS inversion which suppresses crossing-over, contains sc^{S1} B InS w^a sc^8 and $ma-l^+$. The wild-type strains employed were an Oregon-R stock obtained from The Johns Hopkins University and a Pacific stock obtained from the Institute of Animal Genetics, Edinburgh.

Fly culture: Flies were grown on a medium similar to that developed by E. B. Lewis.⁶ It contained 81.0% water, 1.2% agar, 5.0% dextrose, 2.5% sucrose,

8.3% corn meal, 2.3% dried yeast, 0.06% phosphoric acid, and 0.4% propionic acid. Generally, adults and third-instar larvae were obtained by putting 10 to 20 pairs of adults into a bottle and allowing them to lay eggs for 3 to 4 days. Larvae crawling on the sides of the bottle were used in the enzyme assay; adults were assayed within 2 hr of emergence. However, in the experiments involving the levels of xanthine dehydrogenase in various stages during development, all stages were obtained by collecting eggs and allowing the resulting larvae to develop in large vials under uncrowded conditions at 25°C.

Assay of xanthine dehydrogenase: The assay of xanthine dehydrogenase was accomplished by following the increase in fluorescence which occurs when 2-amino-4-hydroxypteridine is oxidized to isoxanthopterin,⁷ as applied to *Drosophila* by Glassman.⁸ A unit of enzyme activity is defined as that amount of enzyme which will produce a change of one unit on the meter scale of the fluorometer.⁸ This procedure is sensitive enough to assay the amount of xanthine dehydrogenase activity in a single fly. When assaying *ma-l* or maternally affected *ma-l* flies, 10 individuals were usually assayed at once. With wild type, usually 10 eggs or three individuals of more advanced stages were assayed together. The results are expressed as units of enzyme per individual or per 10 individuals.

Results and Discussion.—One difficulty with the single-fly assay developed several months after it was originally devised. A slight increase in fluorescence

FIG. 2.—The relation between xanthine dehydrogenase activity and the number of maternally affected *ma-l* larvae assayed.

The indicated number of larvae were homogenized together in 4.0 ml of 0.1 M Tris, pH 7.5, containing 10 mg of bovine albumin per ml. About 5 to 10 mg of charcoal were added, and after one hr, the charcoal was removed by centrifugation and filtration through sintered glass. One ml of the extract was assayed for xanthine dehydrogenase by the method described by Glassman.⁸

ence of xanthine dehydrogenase activity in these larvae. Paper chromatographic techniques similar to those employed by Forrest, Glassman, and Mitchell¹ have revealed the formation of isoxanthopterin in these extracts, but not in extracts of regular *ma-l* larvae.

The finding of assayable xanthine dehydrogenase activity in maternally af-

units per minute was observed when the *ma-l* and *ry* mutants were assayed, whereas previously no change had been detected. No increase in the rate of change was observed when additional flies were homogenized together, an observation indicating that this phenomenon was not due to enzyme activity of any type in these mutants (Fig. 2). Yet this rise in fluorescence depended upon some component of the flies. We eventually ascertained that this was due to an interaction between the albumin⁸ used to stabilize enzyme activity and a factor found in boiled *Drosophila* extracts. It can be seen in Figure 2 that the small rate of increase in fluorescence does not become larger when the numbers of third-instar *ma-l* larvae are increased. On the other hand, there is a linear increase in the fluorescence units per min when more maternally affected *m ma-l* larvae are used; this indicates the presence

TABLE 1

XANTHINE DEHYDROGENASE ACTIVITY IN REGULAR AND MATERNALLY AFFECTED *ma-l* LARVAE

In all cases, the units of enzyme activity represent an average of assays on 3 different groups of 10 third-instar larvae which were homogenized together as described in Figure 1.

Parents		Male Progeny		Female Progeny	
Female	Male	Genotype	Enzyme activity	Genotype	Enzyme activity
<i>y ec v ma-l</i>	<i>y ec v ma-l</i>	<i>y ec v ma-l</i>	0.27	<i>y ec v ma-l</i>	0.28
<i>y f:=</i>	<i>y ec v ma-l</i>	<i>y ec v ma-l</i>	2.4*	<i>y f:=</i>	35.5†
<i>y ec v ma-l</i>	<i>M-5</i>	<i>y ec v ma-l</i>	0.23	<i>y ec v ma-l</i>	26.1†
				<i>M-5</i>	
<i>y ec v ma-l</i>	<i>y ec v ma-l</i>	<i>y ec v ma-l</i>	2.2*	<i>y ec v ma-l*</i>	2.1*
<i>M-5</i>				<i>y ec v ma-l</i>	
				<i>y ec v ma-l</i>	26.8†
				<i>M-5</i>	

* Enzyme activity indicative of maternally affected *ma-l* progeny.

† Enzyme activity indicative of *ma-l* *.

affected *ma-l* larvae led us to examine the levels of this enzyme in the various types of maternally affected and regular *ma-l* larvae. The data are presented in Table 1, where it can be seen that *ma-l* progeny which are maternally affected show significant amounts of xanthine dehydrogenase activity, whereas *ma-l* progeny which are derived from homozygous *y ec v ma-l* mothers exhibit the "zero" level of activity. These results led to an attempt to ascertain directly whether xanthine dehydrogenase activity increases during the development of maternally affected flies. These data are presented in Figure 3. It should be noted that the ordinate for the wild-type strains records the enzyme activity *per individual*, while that for the maternally affected *ma-l* reflects the enzyme activity *per 10 individuals*. Thus, the activity in maternally affected *ma-l* larvae approximates only 10 per cent of the activity in wild type.

Several interesting points are apparent. In spite of the fact that little or no enzyme activity can be detected in up to 10 eggs (which constitute a mixture of *y f:=* and maternally affected *m ma-l*), enzyme does appear in the maternally affected *ma-l* first-instar larvae. This small amount slowly declines during development. This indicates either activation, complementation, or synthesis *de novo* of xanthine dehydrogenase during early development of maternally affected *ma-l* flies. Although the wild-type stocks also had little or no activity in the egg, the amount constantly increases to high levels throughout development except in early pupae, where a significant decrease in xanthine dehydrogenase activity occurs. Ursprung⁹ has reported a similar decrease. The slight decrease observed for 2-day-old adults may not be real.

Another significant point in Figure 3 is the difference between the *m ma-l* and *ma-l (H)* stock. Both the *ma-l (H)* and the *m ma-l* strain were independently derived from the *ma-l/y f:=* stock in the *Drosophila* collection at the California Institute of Technology by E. Hadorn⁶ and the senior author, respectively. Although they are presumably the same *ma-l* allele, the stocks differ markedly. Whereas "maternally affected" *m ma-l* flies emerging *after* the sixth day from the initial date of eclosion do not exhibit the maternal effect, maternally affected *ma-l (H)* flies emerging up to 14 days after the initial date of eclosion still exhibit the maternal effect (E. Hadorn, unpublished). One must wait 17 days before the decline of the maternal effect in *ma-l (H)*. A partial explanation for these data can

be seen in Figure 3. The *ma-l* (*H*) stock has higher xanthine dehydrogenase activity when maternally affected. Thus, the maternal effect is manifest in these flies even when adverse environmental forces are operating.

FIG. 3.—Xanthine dehydrogenase activity in various stages of development. *Main figure:* The main part of Figure 3 represents the units of xanthine dehydrogenase activity during development in two wild-type strains. The various bars from left to right at each time represent Oregon-R females, Oregon-R males, Pacific females, and Pacific males, respectively. The enzyme activity, which is expressed per individual, was determined by assaying 3 different extracts containing 10 individuals (eggs) or 3 individuals (all other stages), as described in Figure 1. *Insert:* This part of Figure 3 depicts the units of activity of xanthine dehydrogenase found in various stages during the development of maternally affected *ma-l* strains. The abscissa is the same as the main part of the figure; the ordinate depicts the enzyme activity per 10 individuals. This was determined by assaying 3 different groups of 10 individuals homogenized together as described in Figure 1.

Interesting data were obtained when the maternally affected *ma-l* larvae from *y f: = ;st ry¹* mothers were assayed for the enzyme. It should be remembered that these female parents do not have xanthine dehydrogenase activity. The data presented in Table 2 indicate that their maternally affected *ma-l* progeny do indeed contain xanthine dehydrogenase. Furthermore, it will be noted that the average xanthine dehydrogenase activity found in maternally affected *ma-l* progeny derived from *y f: =* mothers is the same as those derived from *y f: = ;st ry¹* mothers. If the schemes in Figure 1 approximate the correct situation, one might have expected that the *y f: = ;st ry¹* mothers would accumulate the maternal substance more than the *y f: =* mothers, so that the maternally affected flies from *y f: = ;st ry¹* parents would have more enzyme. This did not occur, a result probably indicating either that the maternal substance cannot accumulate or that the amount of the maternal substance which can enter the egg is limited or that the schemes are wrong. Further research on first-instar larvae, however, may yet reveal differences between the maternally affected *ma-l* progeny from the two types of females. It is also of interest that no dosage effect of *ry/+* versus *+/+* was observed in the two types of maternally affected *ma-l* (i. e., *ma-l;ry⁺/ry⁺* vs. *ma-l;ry/ry⁺*). Many workers^{4, 10} have reported that *ry/+* flies have less activity than *+/+* flies. No effect was seen here. This could be due to the fact that no dosage phenomenon occurs in larvae or, more likely, that the amount of enzyme formed in these larvae is

TABLE 2
XANTHINE DEHYDROGENASE ACTIVITY IN MATERNALLY AFFECTED *ma-l* LARVAE FROM *ry* AND NON-*ry* MOTHERS

The units of enzyme activity were determined by assaying extracts of the three groups of 10 third-instar larvae which were homogenized together, as described in Figure 1.

Crosses		Enzyme activity in ten larvae	Male Progeny (3rd instar larvae)	
Female parent	Male parent		Mean	Average mean
<i>p ft</i> : =; <i>st ry</i> ¹	<i>m ma-l</i>	3.0	2.6	3.35
		2.3		
		2.5		
"	<i>ma-l</i>	3.2	3.5	
		3.2		
		4.1		
"	<i>v od ma-l</i> ^{b2}	4.1	4.1	
		4.4		
		3.8		
"	<i>v f Bx</i> ³ <i>ma-l</i>	2.6	3.2	
		3.4		
		3.6		
<i>y f</i> : =	<i>m ma-l</i>	3.0	3.3	3.37
		2.9		
		3.2		
"	<i>ma-l</i>	2.7	2.8	
		3.3		
		2.5		
"	<i>v od ma-l</i> ^{b2}	2.9	2.9	
		2.8		
		2.9		
"	<i>v f Bx</i> ³ <i>ma-l</i>	3.8	4.7	
		4.7		
		5.7		

so slight that it does not exceed the lowered synthetic capabilities of the *ry*/⁺ combination.

Conclusion.—The nature of the maternal substance is not known. Two lines of evidence indicate that it is not xanthine dehydrogenase. First, *y f*: =; *st ry*¹ females, which lack detectable amounts of xanthine dehydrogenase, have *ma-l* progeny which have significant amounts of this enzyme, and second, an increase in xanthine dehydrogenase activity occurs in early development of maternally affected *ma-l* larvae. That *y f*: =; *st ry*¹ females can have maternally affected *ma-l* progeny could be due to the fact that an inhibited enzyme is present in *ry* flies and that this inhibition is released in the *ma-l* offspring. However, the fact that the enzyme does increase in maternally affected *ma-l* flies from *ry*⁺ females makes it seem likely that either activation, complementation, or synthesis *de novo* of xanthine dehydrogenase is taking place.

The presence of xanthine dehydrogenase activity in *ma-l* larvae derived from *y f*: =; *st ry*¹ females indicates a type of complementation *in vivo* in which the product of the *ma-l*⁺ gene in the maternal parent reacts with the product of the *ry*⁺ gene in the progeny to produce active xanthine dehydrogenase. This suggests that mixing extracts of the *ma-l* and *ry* mutants with each other might produce the same result. This has recently been accomplished;¹¹ thus, there is probably no protein synthesis *de novo* associated with the *ma-l* locus in maternally affected *ma-l*.

Summary.—The mutant *ma-l* usually lacks detectable amounts of xanthine dehydrogenase. However, third-instar *ma-l* larvae derived from *ma-l*⁺ females

have between 5 and 10 per cent of the amounts of xanthine dehydrogenase activity found in wild-type larvae; younger larvae have higher relative amounts; eggs have no detectable activity. This indicates that the enzyme activity increases in early maternally affected *ma-l* larvae and that the maternal substance is not the enzyme itself. The fact that *ry* females, which also lack detectable amounts of xanthine dehydrogenase, can have maternally affected *ma-l* progeny indicates a type of complementation *in vivo* between the *ry* and *ma-l* mutants. This phenomenon has its counterpart in the occurrence of complementation *in vitro* between these two nonallelic mutants.¹¹

* This work was supported by a grant (RG-8202) from the National Institutes of Health. For the first paper in this series, see reference 5.

† Senior Research Fellow of the National Institute of Health (GSF-14,911).

¹ Forest, H. S., E. Glassman, and H. K. Mitchell, *Science*, **124**, 725 (1956).

² Bridges, C. B., and K. S. Brehme, *The Mutants of Drosophila melanogaster*, Carnegie Institution of Washington, Publication 552 (Washington, D. C., 1944).

³ Hadorn, E., and I. Schwink, *Nature*, **177**, 940 (1956); Mitchell, H. K., E. Glassman, and E. Hadorn, *Science*, **129**, 268 (1958).

⁴ Glassman, E., and H. K. Mitchell, *Genetics*, **44**, 153 (1959).

⁵ Glassman, E., and H. K. Mitchell, *Genetics*, **44**, 547 (1959).

⁶ Personal communication.

⁷ Burch, H. B., O. H. Lowry, A. M. Padilla, and A. M. Combs, *J. Biol. Chem.*, **223**, 29 (1956).

⁸ Glassman, E., *Science*, in press (1962).

⁹ Ursprung, H., *Experientia*, **17**, 232 (1961).

¹⁰ Hubby, J. L., and H. S. Forest, *Genetics*, **45**, 211 (1960), Glassman, E., J. D. Karam, E. C. Keller, Jr., submitted to *Genetics* (1962).

¹¹ Glassman, E., these PROCEEDINGS, **48**, 1491 (1962).

HEMOGLOBIN INHERITANCE IN INBRED MOUSE STRAINS, II. GENETIC STUDIES*

BY JOHN J. HUTTON, JOHN BISHOP,† RICHARD SCHWEET, AND
ELIZABETH S. RUSSELL

UNIVERSITY OF KENTUCKY COLLEGE OF MEDICINE, LEXINGTON, AND ROSCOE B. JACKSON MEMORIAL
LABORATORY, BAR HARBOR, MAINE

Communicated by Sewall Wright, August 27, 1962

The principal purpose of this communication on the genetics of variations in mouse hemoglobins is presentation of information concerning the linkage relations of genes controlling the structure of the α - and β -chains. The possibility of genetic manipulation and test matings, combined with the ready availability of mice from highly inbred strains with known differences in hemoglobin structure, suggests that genetic studies of mouse hemoglobins may be extremely useful in providing a model for the understanding of human hemoglobin inheritance.

Although inheritance of variations in human hemoglobin structure is currently a topic of great interest in medical genetics,¹⁻³ information concerning the linkage relations of genes controlling the structure of human α -, β -, and δ -chains is necessarily limited to a very few favorable pedigrees segregating for abnormalities of different chains.⁴⁻⁷ The α - and β -chains of mouse hemoglobin show considerable