STUDIES OF EYE PIGMENTS OF DROSOPHILA

II. EFFECT OF TEMPERATURE ON THE RED AND BROWN PIGMENTS IN THE MUTANT BLOOD (w^{bl})[1]

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Received July 7, 1944

THE red and brown eye pigments of *Drosophila melanogaster* are different in their properties and developmental history. The formation of the brown pigment depends on diffusible substances derived from tryptophane; that of the red pigment apparently does not. Hence, at least the final stages of the reaction chains leading to the formation of these pigments follow different paths. However, some mutant genes inhibit to various degrees the development of both pigments, and this suggests the existence of a common step in the two sequences of reactions. The nature of this common step is unknown. Its understanding would further clarify the mechanism of pigment formation and of its genetic control.

One might expect that a study of the two pigments in a mutant whose phenotype is affected by environmental conditions would throw some light on their relationship. The mutant blood (w^{bl}—a member of the series of multiple alleles at the white locus) shows such a dependence on temperature. Its eye color is pale brownish yellow at 30°C, and deep reddish purple at 17°C, with intermediates at temperatures between these two extremes. The primary purpose of the work reported here was to (1) determine the period of maximum sensitivity to temperature for each of the pigment components, and (2) describe in quantitative terms their variation with temperature. In the course of the investigation the question arose as to whether the changes with temperature are purely quantitative, and this has led us to (3) examine the absorption spectra of the pigments contained in the eyes of flies raised at different temperatures.

MATERIAL AND METHODS

The desirability of extracting pigment from flies having only one of the components has been pointed out in the first paper of this series dealing with the techniques of pigment extraction and measurement (EPHRUSSI and HEROLD 1944). Prior to this investigation two double recessive stocks were therefore prepared: blood scarlet ($w^{bl};st$) and blood brown ($w^{bl};bw$), the first of these serving for the extraction of red, the second for the extraction of brown pigment. In only one case a pure w^{bl} stock, containing both pigment components, was used.

Unless otherwise stated, the techniques of raising the flies and of pigment extraction used in this work are those described in the above mentioned paper. Owing to the reduction of pigment content in the flies studied here as com-

[1] Work supported by a grant from the PENROSE FUND of the AMERICAN PHILOSOPHICAL SOCIETY.

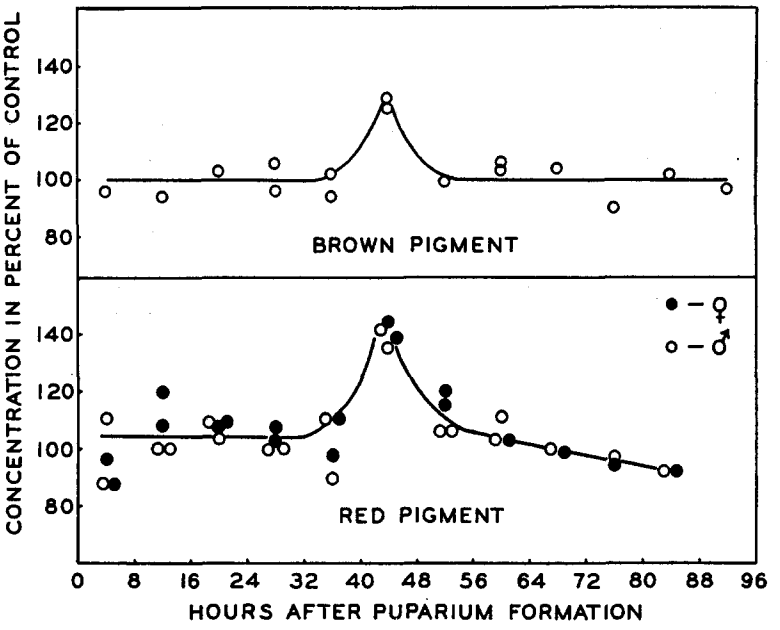

FIG. 1.—Relative concentration of pigment extracted from w^{bl};*bw* (above) and w^{bl};*st* (below) flies subjected to cold treatment at various stages of pupal development (Pulfrich, 470 mμ).

pared with that of pure *st* and *bw* stocks, the number of heads extracted was increased in the different experiments so as to give pigment concentration most favorable for the photometric measurements. The measurements were made with a Pulfrich photometer at 470 mμ. Spectral absorption curves were obtained with a Coleman spectrophotometer.

THE TEMPERATURE EFFECTIVE PERIOD

EPHRUSSI and AUGER (1938) have shown that the pigmentation of the eyes of the mutant w^{bl} is not affected by temperature changes during larval development. The pupa is sensitive to these changes apparently at all stages, but the sensitivity has a peak in the second quarter of pupal development. However, the work of EPHRUSSI and AUGER did not take into account the existence in the eyes of Drosophila of two distinct pigment components and hence provides no answer to the question raised here as to the comparative behavior of the two pigments with respect to temperature.

Our experiments were conducted as follows. Large numbers of well-fed females were allowed to lay eggs over a 20-hour period at 25°C. Larvae were collected over a four-hour period and raised under "standard conditions" at 25° until pupation. Pupation occurs at 25° on the fourth day after hatching of the larva from the egg. At that time the bottles were inspected every hour and only pupae with white puparia were collected, this color indicating that the age of the pupae does not exceed one hour from puparium formation. The pupae were placed in vials, on filter paper moistened with Ringer solution.

For each eight hour period of the four days of pupal life a different group of pupae was subjected to 17°C. Before and after this "cold treatment" the pupae remained at 25°. The controls received no cold treatment.

Just prior to emergence, the pupae were transferred to vials containing the standard food. The imagoes were "aged" for four days prior to extraction. In the $w^{bl};st$ experiments heads of males and females were extracted separately. Only male heads were extracted in the $w^{bl};bw$ experiments.

TABLE 1

Effect of cold treatment at different developmental stages of w^{bl}; st. The treatment period is given in hours from puparium formation. N = number of heads extracted. E = extinction calculated per 10 heads in 1 cc of solvent. % = pigment content calculated in per cent of control. (Pulfrich, 470 mμ).

TREATMENT PERIOD	♂♂ N	♂♂ VOLUME (IN CC) OF SOLVENT	♂♂ E	♂♂ %	♀♀ N	♀♀ VOLUME (IN CC) OF SOLVENT	♀♀ E	♀♀ %
			Experiment 1					
0–8	77	3	0.066	88	98	3	0.067	88
8–16	100	3	0.075	100	94	3	0.090	120
16–24	100	3	0.083	110	67	3	0.081	108
24–32	100	3	0.075	100	73	3	0.078	104
32–40	102	3	0.068	90	80	3	0.084	112
40–48	110	3	0.102	136	100	3	0.107	143
48–56	101	3	0.080	106	58	3	0.090	120
56–64	83	3	0.077	103	114	3	0.077	103
64–72	118	3	0.074	100	77	3	0.075	100
72–80	100	3	0.073	98	34	2	0.072	96
80–88	64	3	0.069	93	101	3	0.069	93
Control	106	3	0.074	100	148	3	0.075	100
			Experiment 2					
0–8	95	3	0.069	111	95	3	0.060	97
8–16	101	3	0.062	100	46	2	0.067	108
16–24	92	3	0.065	105	66	3	0.068	109
24–32	104	3	0.062	100	80	3	0.066	106
32–40	92	3	0.069	111	104	3	0.061	98
40–48	122	3	0.088	142	57	3	0.088	142
48–56	77	3	0.066	106	65	3	0.072	116
56–64	97	3	0.069	111	—	—	—	—
Control	94	3	0.062	100	68	3	0.062	100

The results of four experiments are summarized in tables 1 and 2 and figure 1. In the latter the relative concentrations are given in percentage of the pigment content of untreated control flies, and the experimental points are placed in the middle of the eight hour treatment period.

It can be seen from these results that both pigments show a considerable increase during the period extending from 40 to 48 hours counted from puparium formation. Minor increases of pigment content caused by the short cold treat-

TABLE 2

Effect of cold treatment at different developmental stages of w^{bl};bw. The treatment period is given in hours from puparium formation. N=number of heads extracted. E=extinction calculated per 10 heads in 1 cc of solvent. %=pigment content calculated in per cent of control. (Pulfrich, 470 mμ).

	EXPERIMENT 1				EXPERIMENT 2			
TREATMENT PERIOD	N	VOLUME (IN CC) OF SOLVENT	E	%	N	VOLUME (IN CC) OF SOLVENT	E	%
0–8	44	5	0.162	96	—	—	—	—
8–16	60	5	0.160	94	—	—	—	—
16–24	60	5	0.173	103	—	—	—	—
24–32	45	5	0.163	96	60	5	0.172	106
32–40	60	5	0.172	102	60	5	0.149	94
40–48	60	5	0.210	124	60	5	0.207	128
48–56	60	5	0.167	99	—	—	—	—
56–64	60	5	0.179	106	62	5	0.168	104
64–72	60	5	0.176	104	—	—	—	—
72–80	43	5	0.156	90	—	—	—	—
80–88	60	5	0.172	102	—	—	—	—
88–96	60	5	0.164	97	—	—	—	—
Control	60	5	0.169	100	64	5	0.162	100

ment during other periods of pupal development, if present, are obscured by the variations of the values obtained.

VARIATION OF PIGMENT CONTENT WITH TEMPERATURE

Since temperature affects the eye color of both w^{bl};*st* and w^{bl};*bw* flies only in the pupal stage, in the experiments to be described below larvae of the two stocks were raised at 25° until puparium formation. The pupae were placed in incubators at different temperatures: 17°, 20°, 25° and in some experiments 30°C. They remained at these temperatures until emergence of the flies, and the latter were "aged" at the same temperatures. The time allowed for ageing was five to six days at 25° and 30°, eight days at 20° and 12 days at 17° (on the assumption of a temperature coefficient = 3). Only male heads were extracted.

The results of four experiments are given in table 3. It can be seen that, in so far as absolute values are concerned, there are considerable differences between the two experiments on w^{bl};*st*, and on w^{bl};*bw*, whether the data are corrected by the weight of flies or not. These differences are in all probability due to differences in the media prepared at different times (see discussion in our first paper). However, the general character of the variation of pigment content with temperature, as measured here, is the same within each pair of experiments. Figure 2, representing the results of one of the experiments, shows that the variation with temperature of the brown pigment follows closely a straight line, while that of the red pigment follows a curve of a strikingly different shape. Both curves show an increase with decreasing temperature.

That the particular shape of the curve for red pigment is due to the presence

of the mutant gene w^{bl} is obvious from the comparison of the curve of figure 2 with that of figure 12 in our first paper. Additional evidence is provided by a single experiment where the red pigment was extracted from the mutant w^{bl}

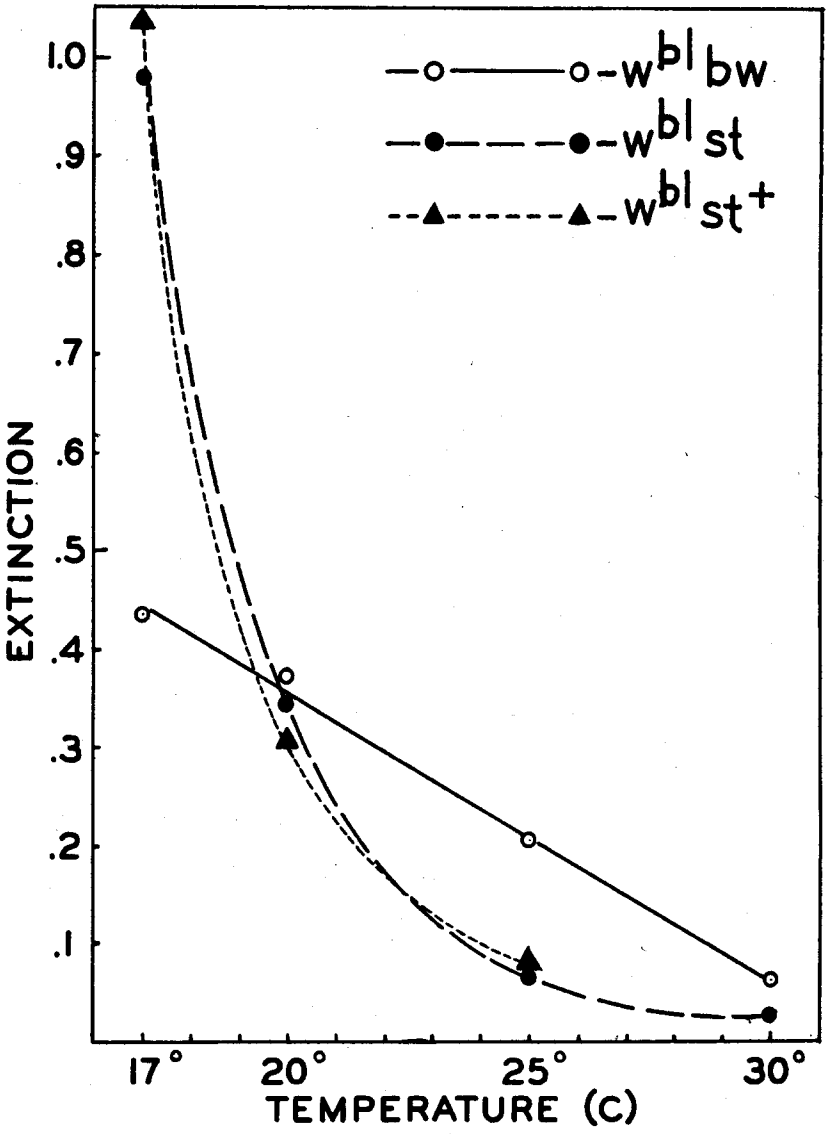

FIG. 2.—Light absorption by extracts from w^{bl};*bw* (brown pigment), w^{bl};*st*, and w^{bl} (red pigment) flies raised at different temperatures (Pulfrich, 470 mμ).

(homozygous for the wild type allele of *st*). The results of this experiment are given at the bottom of table 3 and by the third curve of figure 2. This curve is similar to that given by w^{bl};*st*.

Whether the variations of the brown pigment in w^{bl};*bw* are different from

those found in *bw* within the same temperature range is more difficult to judge. Figure 12 of our first paper shows that the pigment content in the mutant *bw* also decreases somewhat with increasing temperature and that this variation is probably linear. Our data are not numerous enough for the calculation of regression coefficients, but a comparison of the absolute values shows that the variation with temperature of the extinction per head and per degree C is greater in w^{bl};*bw* than in *bw*. However, it will be seen from the considerations presented in the next section that some doubt is cast on the value of quantitative comparisons.

ABSORPTION SPECTRA OF THE EXTRACTS

The data discussed in the preceding section and illustrated by figure 2 reflect the picture of quantitative changes in pigment content only so long as it can be

TABLE 3

Light absorption by extracts of eyes of w^{bl};bw, w^{bl};st and w^{bl} ♂♂ raised at different temperatures. T = temperature. E = extinction calculated per 10 heads in 1 cc of solvent. Wt = average weight of one male in mgs. $E_{corr.}$ = extinction corrected by weight. Aver. = average extinction of different samples corrected as above. N = number of heads extracted. (Pulfrich, 470 mμ).

GENOTYPE	EXPERIMENT 1						EXPERIMENT 2				
	T	*N*	*E*	WT.	$E_{corr.}$	AVER.	*N*	*E*	WT.	$E_{corr.}$	AVER.
w^{bl};*bw*	30	81	0.052	0.846	0.061	0.061					
"	25	50	0.192	0.905	0.212		56	0.162	1.004	0.161	0.160
"	25	39	0.182	0.878	0.207	0.207	55	0.160	1.004	0.159	
"	25	39	0.174	0.878	0.198						
"	20	58	0.318	0.883	0.360	0.371	45	0.287	0.925	0.310	0.315
"	20	62	0.337	0.884	0.381		46	0.296	0.925	0.320	
"	17	45	0.348	0.820	0.424		45	0.369	0.940	0.393	0.391
"	17	50	0.377	0.861	0.438	0.436	39	0.365	0.940	0.388	
"	17	45	0.385	0.861	0.447						
w^{bl};*st*	30	118	0.0264	0.963	0.0274	0.0274	173	0.0208	0.925	0.0225	
"	25	168	0.0672	0.976	0.0689	0.065	262	0.056	0.992	0.0565	
"	25	168	0.0584	0.956	0.0611						
"	20	70	0.3528	1.019	0.3462	0.345	141	0.2744	0.990	0.277	
"	20	70	0.3332	0.968	0.3442						
"	17	64	0.868	1.004	0.865	0.980	118	0.752	0.919	0.818	
"	17	63	0.924	0.844	1.095						
w^{bl}	25	145	0.080	0.986	0.081						
"	20	90	0.322	1.035	0.311						
"	17	115	0.925	0.895	1.034						

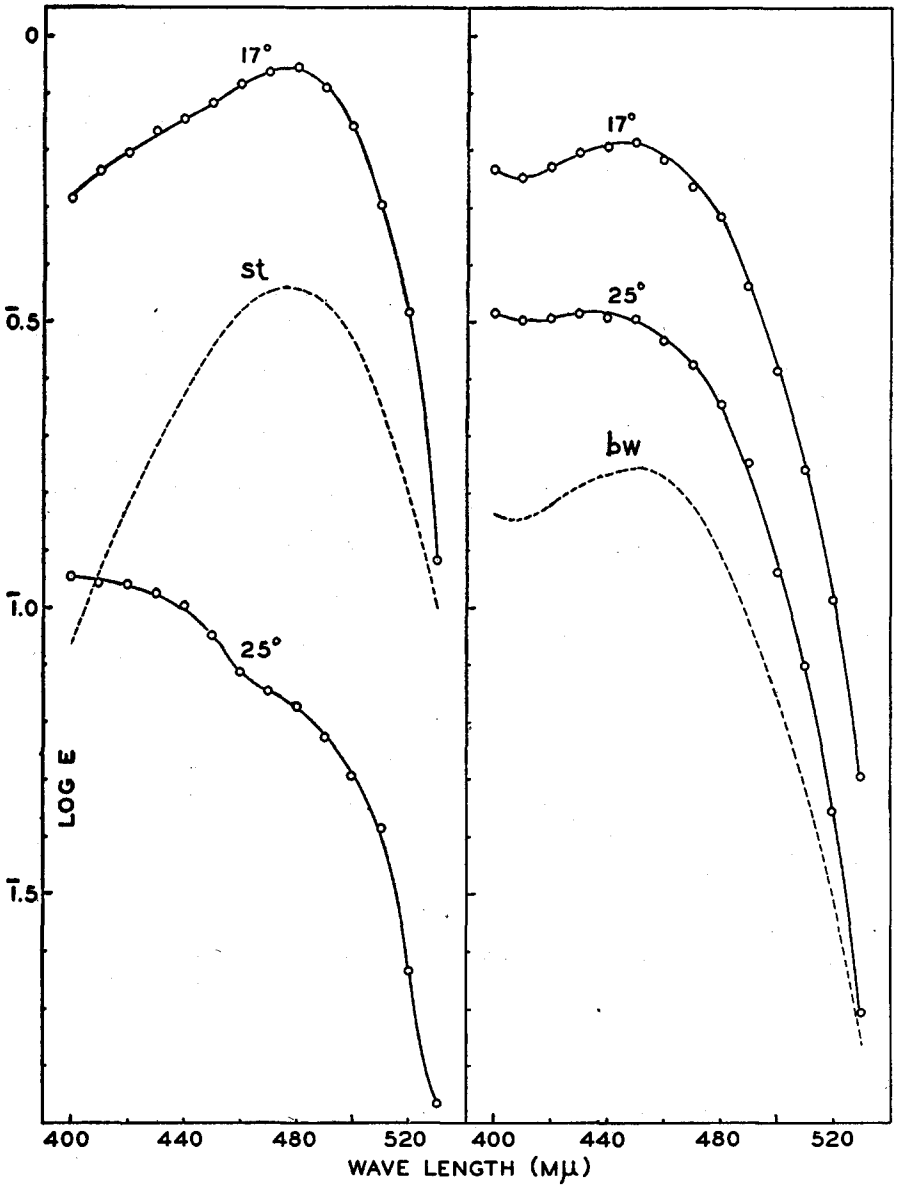

FIG. 3.—Absorption curves of extracts from w^{bl};*bw* (right) and w^{bl};*st* (left) flies, raised at 17° and 25°C. Broken lines—absorption curves of extracts from *st* and *bw* eyes (Coleman).

assumed that there is no change in the quality of the pigments extracted. Two experiments were performed in order to test whether this was actually the case, and the results of one of these are given in figure 3, where *log E* is plotted against wave length. Under these conditions similar pigments give parallel curves independently of concentration differences.

The figure clearly shows that neither the red nor the brown pigment extracts

from flies homozygous for the gene w^{bl} give absorption spectra identical with those obtained from the mutants *st* and *bw* respectively. Moreover, the shapes of the absorption curves undergo changes depending on the temperature at which the extracted flies were raised. The changes in shape of the curves are much more drastic in the case of the red pigment.

DISCUSSION

Although the red and brown pigments of the eye of Drosophila differ markedly in their known chemical and physiological characteristics, it has been suggested that they may be closely related in the unknown early phases of their formation. This suspicion is strengthened by the demonstration reported here that, in the temperature-sensitive mutant w^{bl}, maximal variations of both the red and the brown pigments are produced by temperature changes during the same developmental stage (within eight hours). This is particularly striking in view of the fact that (in the eyes of wild type) at 25°C the deposition of brown pigment begins forty-eight hours after puparium formation, while the red pigment does not appear until much later (about seventy hours).

The relationship between the two pigments could be thought of in terms of a competition of two chromogens for the same substrate, in a manner similar to that proposed by LAWRENCE and PRICE (1940) in the case of anthocyanins and anthoxanthins in plants. Evidence for the existence of a common substrate in the eye pigments of Drosophila has recently been reviewed by EPHRUSSI (1942). It may be recalled in this connection that MAINX (1938) has suggested that these pigments are bound, *in vivo*, to a protein carrier and that BECKER'S (1942) recent chemical study of several Ommatins (a pigment group which includes the brown pigment of Drosophila) has confirmed this view.

However, it has been shown in our first paper that the genes *st* and *bw*, in suppressing one of the pigments, do not increase the amount of the other. This lends no support to the idea of a competition at or after the stage of pigmentogenesis in which the genes *st* and *bw* are operative. A competition at an earlier stage is not excluded.

It was thought at the outset of this investigation that the study of the variations of the phenotype of w^{bl} under the influence of temperature might reveal a competitive relationship of this sort, that is the increase of the amount of one of the pigments at the expense of the other. The data reported above do not support this interpretation. Although precise quantitative comparisons are impossible on account of the changes of the spectra of both the red and brown pigment extracts, there is but little doubt that the quantity of both pigments varies in the same direction as a function of temperature. The immediate effect of temperature therefore must be on the total amount of pigment precursor (s), or on one of its constituent parts available for the formation of pigment, rather than on the shares of the precursor which will be converted to brown versus red pigment.

The fact that the mutant w^{bl} "sensitizes" to temperature both he red and brown pigment systems further emphasizes the idea of a common step in the two reaction chains. Conclusions concerning the rates of change in quantity of

the two pigments cannot be drawn on account of the observed modifications of the absorption spectra. The nature of these modifications will be discussed in the next paper of this series.

SUMMARY

The variation with temperature of the brown and red pigments in the eyes of the mutant w^{bl} was studied.

Cold treatment during different stages of pupal development shows that the period of greatest sensitivity, as revealed by the increase in the amount of both pigments, occurs between 40 and 48 hours after puparium formation. The concomitant variation of the two pigments suggests their close relationship.

The absolute amounts of both pigment components seem to increase at lower temperatures. The rates of change of the two pigments could not be ascertained, for the pigment extracts obtained from flies raised at different temperatures give different absorption spectra.

The existence of a common step in the chains of reactions leading to the formation of red and brown pigment is discussed in the light of these results.

ACKNOWLEDGMENTS

The authors wish to thank MESSRS. MAXWELL ABELMAN and IRVING YOUNG who performed most of the experiments on the temperature effective periods as members of a course "Problems in Genetics" given by the senior author.

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