Genetical and Biochemical Studies on the Red and Yellow Eye Pigments of *Drosophila melanogaster*¹⁾.

Toshifumi TAIRA

National Institute of Genetics, Misima

Received October 14, 1960

Introduction

Since Beadle and Ephrussi (1936) had reported their pioneering experiments, many papers on the formation of eye pigments of *Drosophila melanogaster* have been published. The eye color mutants can be conveniently used for revealing the relationship between genes and biochemical processes. In the eyes of a wild-type strain, there are two kinds of eye pigments, red and brown. Experiments with several mutants showed that the tryptophan metabolism has an intimate relation to the formation of the brown pigment. However, the biochemical process concerning the formation of red pigment has not been clarified.

A report on fluorescent substances examined chromatographically was published by Hadorn and Mitchell (1951). It gave an impulse to further studies on the formation of the red eye pigment. The fluorescent substances have been chemically identified with some pteridine derivatives by Nawa and Taira (1954), Forrest and Mitchell (1954 and '55) and Viscontini *et al* (1955), and their close relations to the formation of the red eye pigment have been suggested by Hadorn and Mitchell (1951), Nawa and Taira (1954), Forrest and Mitchell (1955), and Hadorn and his collaborators (1956). Viscontini *et al* (1957) found actually a pteridine derivative in the hydrolysates of the red eye pigment. This was a very important finding, although the chemical structure of any of the red pigments has not yet been determined. Recently, Nawa definitely determined the structure of sepiapterin, and the present author reported on the important role of pteridine reductase in the pteridine metabolism of *Drosophila*.

The object of the investigation of the writer was to find out, how do the genes concerned with the formation of red and yellow pigments control the pteridine metabolic system.

Material and methods

About thirty co-isogenic strains of *D. melanogaster* having one, two or three eye-color mutant genes were used for this experiment. It is well known that the quantities

1) Contribution from National Institute of Genetics, Japan, No. 308. The abbreviations used are: AHP, 2-amino-4-hydroxypteridine; BP, biopterin; IXP, isoxanthopterin; RP, red pigment; SP, sepiapterin.

of both eye pigments and pteridines produced in the eye tissues are strongly influenced by physiological conditions.

About one hundred eggs per culture vial were laid by several pairs of flies. All culture vials were kept at 25°C. Five days old flies were used for this experiment, because the amounts of eye pigments and of pteridines become almost constant within 3 to 5 days after emergence.

The eye pigments were extracted from 50 heads *sans* proboscis for 45 hours in a dark room conditioned at 30°C, with 4 ml of 30% acidic ethanol (pH, 2.0) for red and yellow eye pigments and with 4 ml of 0.27 N HCl-methanol for the brown pigment, according to the "double extraction" method of Ephrussi and Herold (1944). The extracts were immediately submitted to photometrical measurement. The optical densities were measured at the wave length of 420 $m\mu$ for yellow, 450 $m\mu$ for brown, and 480 $m\mu$ for red pigments (see Fig. 1). Then, photolysed red and yellow pigments were measured again at the respective wave lengths. The extinction ($E_{1cm}^{1\%}$) of sepiapterin, the main component of yellow pigment, with 0.1 N HCl was 340 at 410 $m\mu$, and that of drosopterin, the main component of red pigment, with 0.1 N HCl was

Fig. 1. Absorption spectrums of three eye pigments extracted with 30% acidic ethanol (pH 2.0) for red and yellow, and with 0.27 N HCl-methanol for brown: —•—, red; ---x---, yellow; ---o---, brown.

713 at 475 $m\mu$. By use of both values, the amounts of extracted red and yellow eye pigments from each mutant strain could be estimated. The relative amounts of red and yellow pigments were expressed in micrograms per millilitre per 100 milligrams of wet weight of the whole body, and that of the brown pigment was expressed using E_{450} instead of micrograms. Since this was unfavorable for females because of the considerable fluctuation in their body weight, the comparison of mutant strains was

performed with males. The mutant *w*, lacking any eye pigment, was used as a blank. The amount of yellow pigment was computed by deducting the value of the blank from the difference between the optical densities before and after 10 minute photolysis. The amount of red pigment was also computed by deducting the value of the blank from E_{480} after such photolysis, although it was scarcely changed. The amounts of yellow pigment in *se* flies and of red pigment in wild-type flies (Ore-R) were used as the respective standards.

The amounts of pteridines were measured as follows: 5 days old 30 male flies were dissected into heads and bodies, and both batches were homogenized with 0.15 ml of 30% acidic ethanol. After the addition of 0.02 ml of conc. ammonia, the mixtures were centrifuged. 0.05 ml of each supernatant was paper-chromatographed (Toyo-roshi, # 51, 40 × 40 cm) by using as solvents, n-propanol and 1% aqueous ammonia (2:1) for the first dimension, and 5% aqueous acetic acid for the secondary dimension (see Fig. 2). Then, the separated pteridines were cut off and eluted with 5 ml of 0.02 N NaOH. The relative amounts of pteridines in both heads and bodies of each strain were estimated from the relative intensities of fluorescence compared with the standard solution, 0.5 μ g of synthesized isoxanthopterin in 1 ml of 0.02 N NaOH. The amount of sepiapterin was estimated as the quantity of its photo-decomposed pteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid, because of its high instability in alkali solution. Xanthopterin-like substance (XP) on two-dimensional paper chromatogram was considered to be an artifact, because it was converted by xanthine oxidase into purplish blue fluorescent substance, which was not leucopterin. The preliminary results showed that the recovery of isoxanthopterin from two-dimensional paper chromatogram amounted to over 80% of the original amount.

Experimental results

1. *Eye pigments and pteridines of single mutant strains.* The relative amounts of red and yellow eye pigments extracted from the mutant strains are shown in Table 1. The eyes of both *w* and *bw* strains have neither yellow nor red pigments. From the presented data, it is clear that each mutant gene shows a definite degree of inhibition of

Table 1. Relative amounts of red and yellow eye pigments extracted from male heads of single mutant strains.

Strains	Red pigment		Yellow pigment	
	μ g/100 mg	(%)	μ g/100 mg	(%)
Ore-R	122.5 $\pm$ 7.7	100.0	9.7 $\pm$ 2.4	5.2
<i>st</i>	127.1 $\pm$ 2.9	103.8	4.2 $\pm$ 1.0	3.2
<i>cn</i>	122.9 $\pm$ 3.2	100.3	8.0 $\pm$ 0.4	6.6
<i>v</i>	98.5 $\pm$ 5.6	80.4	5.2 $\pm$ 1.4	4.3
<i>ca</i>	19.1 $\pm$ 0.6	15.6	7.9 $\pm$ 0.1	6.5
<i>ry</i>	57.0 $\pm$ 2.5	46.5	8.5 $\pm$ 0.2	7.0
<i>se</i>	4.3 $\pm$ 1.1	3.5	121.3 $\pm$ 6.8	100.0
<i>cl</i>	29.2 $\pm$ 1.3	23.8	54.0 $\pm$ 2.3	44.5
<i>Hn^{r-1}</i>	61.8 $\pm$ 2.0	50.4	19.0 $\pm$ 1.6	15.7
<i>Hn^{r-3}</i>	26.7 $\pm$ 1.2	21.0	25.4 $\pm$ 2.5	20.9
<i>bw</i>	0	0	0	0
<i>w</i>	0	0	0	0

eye pigment formation. For example, *v* strain has about 80% of the red eye pigment of Ore-R (wild-type strain), *ry* and *ca* strains have about 45 and 15% respectively, and those three strains as well as *cn*, *st* and Ore-R strains have a small amount of yellow eye pigment. On the other hand, *Hn^{r-1}* and *cl* strains have about 50 and 23% red eye pigments and they have also 15 and 45% yellow eye pigment of *se* strain. *Hn^{r-3}* strain has about both 20% red and yellow eye pigments, and *se* strain has a

Fig. 2. Chromatographic pattern of pteridines in *Drosophila melanogaster*. Or-I: isodrosopterin, Or-II: drosopterin, R: neodrosopterin, XP: xanthopter-like substance, YP₂: isosepiapterin.

Table 2. Relative amounts of pteridines in male heads and bodies estimated from two-dimensional paper chromatogram. ($\mu\text{g}/100 \text{ mg}$ wet weight)

Strains	Heads (Eyes)				Bodies (Testes)				
	SP	BP	AHP	Total	SP	BP	AHP	IXP	Total
Ore-R	1.0	2.5	1.1	4.6	0.5	0.5	0.4	12.4	13.8
<i>cn</i>	1.0	2.1	1.0	4.1	0.6	0.5	0.4	8.8	10.3
<i>v</i>	0.9	2.4	1.4	4.7	0.7	0.6	0.8	9.8	11.9
<i>ca</i>	1.7	3.0	1.8	6.5	0.6	0.5	0.5	10.8	12.4
<i>ry</i>	1.6	4.2	2.4	8.2	0.4	2.2	8.2	0	10.8
<i>se</i>	39.3	7.9	11.9	59.1	0.6	0.6	0.7	8.8	10.7
<i>cl</i>	18.2	8.4	8.5	35.1	0.6	0.8	0.7	9.8	11.9
<i>Hn^{r-1}</i>	5.5	5.5	3.4	13.4	0.7	1.4	0.3	14.1	16.5
<i>Hn^{r-3}</i>	8.1	8.3	0.2	16.6	4.4	4.0	0.1	2.5	11.0
<i>bw</i>	0	0	0	0	0	0	0	0(0.4)+0	
<i>w</i>	0	0	0	0	0	0	0	0(0.2)+0	

†) Values in brackets show the contents of young adults

trace of red eye pigment besides a large amount of yellow eye pigment. The bulk of yellow pigment consisted of Viscontini and Möhlmann's sepiapterin (1959; SP).

Relative amounts of pteridines in heads and bodies of single mutant strains were estimated from two-dimensional paper chromatograms and the results are summarized

in Table 2. The pteridines in heads were found to be concentrated in the eye tissues. Four strains, *se*, *cl*, Hn^{r-1} and Hn^{r-3} , contained a great deal of sepiapterin as well as a large amount of blue fluorescent pteridine. Therefore, they are called by the author "sepiapterinic strains". The largest part of the blue fluorescent pteridine consisted of two main components, viz. biopterin (BP) and 2-amino-4-hydroxypteridine (AHP), which could be separated by two-dimensional paper chromatography. A great difference in the chemotype (chemical expression of a genotype) between *Henna*-recessive alleles, Hn^{r-1} and Hn^{r-3} , was found, although the sepia eye color of the allelic mutants was phenotypically very similar. In Hn^{r-3} eyes the amounts of SP and BP were larger than in Hn^{r-1} eyes, but as to the amount of AHP the relation was reversed. In *se* eyes, SP, BP and AHP occurred in largest amounts compared with those of other mutants, *cl*, Hn^{r-3} and Hn^{r-1} . They were gradually decreasing in the given order. These results correspond closely with the increasing amounts of red pigment in their eyes, as shown in Table 1. These findings may be due to a difference in the inhibition mechanism of red pigment formation. The amount of isoxanthopterin (*IXP*) which has been considered by the author as an endproduct of pteridine metabolism, did not change in flies older than 3 days. Therefore, it is almost the same in the males of any strain except *ry* and Hn^{r-3} . It was observed that both strains, *w* and *bw*, had no trace of *IXP* at 5 days age, but had a small amount of *IXP* only at stages ranging from late pupae to young adults. *IXP* in Hn^{r-3} bodies was very scarce despite the normal level of activity of pterine dehydrogenase (Taira and Nawa, 1957). It was only 20% of that of Ore-R. However, much of SP and BP was accumulated in its bodies. Such a phenomenon was not observed in its allelic mutant, Hn^{r-1} . There was no *IXP* in *ry* bodies. It was noticeable that the amounts of both BP and AHP in *ry* eyes were larger than those of Ore-R, and that they had more BP than AHP, but in the bodies the relation was reversed.

The main difference between the sexes was found in the amount of pteridine in the bodies. The amount of pteridine in male bodies was much larger than in female bodies. According to the chromatographical analysis of several organs prepared by the author, it was actually found that most of the body pteridines were contained in the testes.

2. *Eye pigments and pteridines of double and triple mutant strains.* Several kinds of eye colors in double or triple mutant strains are summarized in Table 3. The phenotypes in respect to eye color are classified into four groups: (1) A-group — All strains homozygous for *w* gene had white eyes, the appearance of eye color being completely inhibited. (2) B-group — The *bw* gene inhibited completely the formation of red and yellow eye pigments, and when it was combined with *v*, *cn* or *st* gene in homozygous state, the eyes became colorless. The genic action of *bw* was also epistatic to other genes such as *ca*, *se*, *cl*, Hn^{r-1} , Hn^{r-3} and *ry*. (3) C-group — The eyes of this group showed all sepia color. They accumulated a large amount of yellow pigment in the eyes, and had a small amount of brown pigment, although the formation of the latter was inhibited by *v*, *cn* or *st* gene. The reason why a trace of brown pigment appears will be discussed later. (4) D-group — The eye color of this group was light red, due

to a small amount of red pigment.

Table 3. Eye colors of double and triple mutant strains.

	Genotype	Eye color		Genotype	Eye color
A	<i>w v</i>	white	B	<i>v; bw</i>	colorless
	<i>w; cn</i>	white		<i>cn bw</i>	colorless
	<i>w; ca</i>	white		<i>v; cn bw</i>	colorless
	<i>w; se</i>	white		<i>bw; st</i>	colorless
	<i>w; cl</i>	white		<i>bw; ca</i>	brown
	<i>w; Hn^{r-3}</i>	white		<i>bw; se</i>	brown
	<i>w; ry</i>	white		<i>bw cl</i>	brown
	<i>w; bw</i>	white		<i>bw; Hn^{r-1}</i>	brown
C	<i>v; se</i>	sepia		<i>bw; Hn^{r-3}</i>	brown
	<i>v; cl</i>	sepia		<i>bw; ry</i>	brown
	<i>v; Hn^{r-1}</i>	sepia	D	<i>v; ry</i>	light red
	<i>v; Hn^{r-3}</i>	sepia		<i>cn; ry</i>	light red
	<i>v; cn; se</i>	sepia		<i>v; cn; ry</i>	light red
	<i>cn; se</i>	sepia		<i>v; ca</i>	light red
	<i>cn; Hn^{r-3}</i>	sepia		<i>cn; ca</i>	light red
	<i>cl; ca</i>	sepia		<i>v; ca ry</i>	light red
	<i>se ry</i>	sepia		<i>cn; ca ry</i>	light red
	<i>v; se ry</i>	sepia		<i>ca ry</i>	(young adult) light red

The relative amounts of red and yellow eye pigments extracted from double or triple mutant strains are represented in Table 4. Mutant genes *v*, *cn* and *st* connected with tryptophan metabolism did not affect the formation of red and yellow

Table 4. Relative amounts of red and yellow eye pigments extracted from male heads of double and triple mutants.

Strains	Red pigment		Yellow pigment	
	μg/100 mg	(%)	μg/100 mg	(%)
Standard :				
{Ore-R	122.5±7.7	100.0	—	—
<i>se</i>	—	—	121.3±6.8	100.0
<i>se st</i>	1.7±0.1	1.4	119.2±1.8	98.3
<i>cn; se</i>	4.2±0.8	3.4	119.0±1.3	98.1
<i>v; se</i>	4.1±0.6	3.3	109.6±2.8	90.4
<i>v; cl</i>	36.9±0.9	30.1	50.5±2.5	41.6
<i>v; Hn^{r-1}</i>	60.6±2.5	49.5	15.6±1.1	12.9
<i>v; Hn^{r-3}</i>	25.9±1.3	21.1	26.8±7.2	22.1
<i>cn; Hn^{r-3}</i>	27.7±3.6	22.6	30.3±1.9	25.0
<i>cn; ry</i>	23.8±1.2	19.4**	7.2±1.7	5.9
<i>v; ry</i>	21.9±1.9	17.9**	7.2±1.1	5.9
<i>ca ry</i>	11.4±1.2	9.3*	4.2±0.7	3.5
<i>se ry</i>	0.5±0.1	0.4*	97.2±8.6	80.1**
<i>v; se ry</i>	1.0±0.2	0.8*	67.7±6.3	55.8**
<i>v; ca</i>	11.8±0.6	9.6*	6.8±1.3	5.6
<i>cn; ca</i>	18.6±1.0	15.2	7.7±1.4	6.3
<i>cl; ca</i>	18.8±0.5	15.3	72.1±8.0	59.4*
<i>v; bw, cn; bw & bw; st</i>		0		0
<i>bw; ca, bw; se & bw; ry</i>		0		0

**) Striking change in amount.

pigments, but the amount of the latter in *v; se* strain was markedly decreased. The eyes of young adults of *v; se*, *cn; se* and *se st* strains were phenotypically yellowish, but the adults over 6 hours old after emergence became indistinguishable from the original

se strain. Amounts of both red and yellow pigments in *v*; Hn^{-1} and *v*; Hn^{-3} eyes did not decrease as compared with the respective original strains. The amounts of red pigment in *v*; *ry* and *cn*; *ry* eyes were much less than in *ry* eyes, and that of *v*; *ca* eyes was also decreased, but the amount in *cn*; *ca* eyes did not decrease and was almost the same as in *ca* strain. On the other hand, the amounts of red pigment of *ca ry* and *se ry* strains were less than those of *ca* and of *se* strains. The amount of yellow pigment in *se ry* was about 80% of that of *se* eyes. The quantity of red pigment in *v*; *se ry* eyes was close to that of *se ry* eyes and that of yellow pigment was much less than in *se ry* eyes amounting to 50% of that found in *se* strain. The observation of different amounts of red and yellow pigments in *ry*, *v*; *ry*, *se ry* and *v*; *se ry* eyes indicates a specific action of *ry* gene. The genic action of *cl*; *ca* on the amounts of eye pigments is very interesting. The amount of red pigment in this double mutant strain was nearly the same as in *ca* strain and that of yellow pigment was close to that of *cl* strain. At the lowest level, *bw* gene inhibited completely both red and yellow pigments, as mentioned above.

The red eye pigment was separated paper-chromatographically into three main components, drosopterin, isodrosopterin and neodrosopterin, according to Viscontini's designations (Viscontini *et al*, 1957). The results are shown in Table 5. The reduction of red pigment in both *cn*; *ry* and *v*; *ry* eyes was considered not to depend on the lack of those components but to be due to their proportional decrease.

Table 5. Relative amounts of three components of red eye pigment.†

Strains	Orange-I‡		Orange-II		Red	
	μg/100mg	(%)	μg/100mg	(%)	μg/100mg	(%)
Ore-R	28.1	100.0	49.2	100.0	12.5	100.0
<i>cn</i>	26.5	94.5	54.4	110.5	13.3	106.7
<i>v</i>	18.8	67.0	34.8	70.6	9.3	74.2
<i>ry</i>	16.1	57.5	25.8	52.4	8.1	65.1
<i>cn</i> ; <i>ry</i>	4.5	16.1	7.9	15.9	2.1	16.8
<i>v</i> ; <i>ry</i>	5.6	20.0	9.3	18.8	1.8	14.6

†) Estimation from one-dimensional paper chromatogram by using as solvent methyl-ethylketone, propionic acid and water (75 : 25 : 35).

‡) Orange-I, Orange-II and Red respectively corresponding to Viscontini's Isodrosopterin, Drosopterin and Neodrosopterin (Viscontini *et al*, 1957).

The relative amounts of pteridines in heads and in bodies of double or triple mutant strains estimated from two-dimensional paper chromatograms are given in Table 6. In comparison with the results of Table 2, it may be assumed that the proportion of the three pteridines, SP, BP and AHP, in the eyes of all sepiapterinic strains is almost constant and shows little change in the double and triple mutants. The amounts of pteridines were not affected by genes related to tryptophan metabolism such as *v* except for a few cases. For instance, the amounts of SP in *v*; *se* and *v*; *cl* eyes decreased to about 70% of those in *se* and *cl* eyes. The quantities of SP in *v*; Hn^{-1} and *v*; Hn^{-3} eyes did not change. The amount of SP in *se ry* eyes was less than in *se* eyes but both BP and AHP increased. Amounts of all SP, BP and AHP, in *v*; *se ry* eyes were smaller

Table 6. Relative amounts of pteridines in male heads and bodies estimated from two-dimensional paper chromatogram. ($\mu\text{g}/100\text{mg}$ wet weight)

Strains	Heads (Eyes)				Bodies (Testes)				
	SP	BP	AHP	Total	SP	BP	AHP	IXP	Total
<i>v; Hn^{r-3}</i>	7.5	7.1	0.3	14.9	4.3	4.1	0.1	2.1	10.6
<i>v; Hn^{r-1}</i>	5.0	2.4	1.7	9.1	0.9	2.0	0.4	12.2	15.6
<i>v; cl</i>	13.4	6.6	6.8	26.8	0.8	1.8	0.6	11.3	14.5
<i>v; se</i>	28.1	7.3	8.8	44.2	1.1	2.1	0.6	10.1	13.9
<i>v; ca</i>	1.1	3.1	2.4	6.6	0.7	0.6	0.6	10.0	11.9
<i>v; ry</i>	2.1	4.5	2.8	9.4	0.4	1.2	6.9	0	8.5
<i>se ry</i>	27.8	10.6	15.6	54.0	0.9	1.9	7.2	0	10.0
<i>v; se ry</i>	21.7	7.6	8.4	37.7	0.9	2.0	7.2	0	10.1
<i>bw; se</i>	0	0	0	0	0	0	0	0	0
<i>bw; ca</i>	0	0	0	0	0	0	0	0	0
<i>bw; ry</i>	0	0	0	0	0	0	0	0	0

than in *se* and *se ry* eyes. This indicates again a specific action of *ry* gene. A striking positive correlation was observed between SP and BP of sepiapterinic strains, but it was not always detected between SP and AHP. Excluding *Hn^{r-3}* strain, however, some correlation between increase of AHP in eyes and decrease of red eye pigment was observed. As for pteridine content in strains homozygous for *ry* gene, IXP was not detected in their testes but more AHP was accumulated in testes than in eyes. Pteridines in double mutant strains homozygous for *bw* gene did not show on any chromatograms.

3. Brown pigment and the relation to other pigmentations. The relative amount of brown

Table 7. Relative amounts of brown eye pigment extracted from male heads of single, double and triple mutant strains.

Strains	$E_{450}/\text{ml/g}$	(%)
Ore-R	7.4	100.0
<i>v, cn & st</i>	0	0
<i>ca</i>	3.0	40.5
<i>bw</i>	5.6	75.7
<i>ry</i>	7.1	95.9
<i>se</i>	9.5	128.4
<i>cl</i>	10.7	144.6
<i>Hn^{r-1}</i>	10.3	139.2
<i>Hn^{r-3}</i>	9.8	132.4
<i>v; se</i>	1.3	17.6**
<i>v; cl</i>	1.1	14.9**
<i>v; Hn^{r-1}</i>	1.3	17.6**
<i>v; Hn^{r-3}</i>	2.1	18.4**
<i>cn; se</i>	1.6	21.6**
<i>cn; Hn^{r-3}</i>	1.5	20.3**
<i>se st</i>	1.7	23.0**
<i>v; ry & cn; ry</i>	0	0
<i>se ry</i>	9.7	131.1
<i>v; se ry</i>	0.9	12.2**
<i>ca ry</i>	1.1	14.9**
<i>v; ca & cn; ca</i>	0	0
<i>cl; ca</i>	3.3	44.6*
<i>bw; ca</i>	1.6	21.6**
<i>bw; se</i>	7.7	104.1*
<i>w</i>	0	0

pigment extracted with acidic methanol, after extraction of red and yellow pigments, is shown in Table 7, in which two groups are distinguished, one of single and the other of double and triple mutants. It is a well-known fact that tryptophan mutants such as *v, cn* and *st* strains have no brown pigment in eyes. The amount of brown pigment in *bw* eyes was about 75% of that of Ore-R, but the whole brown pigment could not be completely extracted from *bw* eyes either in single or double-triple mutants having the *bw* gene in homozygous state, and a trace always remained. The unextracted brown pigment was considered to be solidly bound by chromoprotein. The brown pigment of other strains was extracted completely. The amount of brown pigment in *ca* eyes was about 40% of that of Ore-R, and that of *ry* was nearly equal. However, the amounts of brown pigment of

sepiapterinic strains such as *se*, *cl*, Hn^{r-1} and Hn^{r-3} were much larger. Strains such as *v*; *ry*, *cn*; *ry*, *v*; *ca* and *cn*; *ca* had no brown pigment. On the other hand, the quantities of brown pigment of strains such as *v*; *se*, *v*; *cl*, *v*; Hn^{r-1} , *v*; Hn^{r-3} , *cn*; *se*, *cn*; Hn^{r-3} and *se st* were about 20% of that of Ore-R despite the fact that the formation of brown pigment was inhibited by the tryptophan mutant genes. This is the cause why their aged flies become phenotypically indistinguishable from the single sepiapterinic strains. The amount of brown pigment in *se ry* eyes was nearly equal to that of *se* eyes. In *v*; *se ry* eyes, a small amount of brown pigment was also detected. The quantity of brown pigment of *cl*; *ca* strain was almost the same as in *ca* strain, but those of *ca ry* and *bw*; *ca* strains were much smaller than in the single strains. The amount in *bw*; *se* strain was intermediate between those of *se* and *bw* strains. The tryptophan mutant genes greatly reduced the amount of brown pigment of the sepiapterinic strains and the residual amount may be due to brown pigment derived from another source.

4. *Developmental change of pteridine content and excrements of male flies.* The frozen-dried materials of whole bodies at six developmental stages of three mutant strains, *cn*, *se* and Hn^{r-3} , and a wild strain (Ore-R) were used for this experiment. The materials were homogenized with 30% acidic ethanol and then several drops of conc. ammonia were added to the mixtures. The homogenates were centrifuged at $2,500 \times g$. The supernatants were chromatographed by two-dimensional method, and the separated pteridines were measured by the same procedure as described above. The results are represented in Fig. 3.

Sepiapterin (SP) was not detected in the third instar larvae, but it appeared at the prepupal stage of all strains. The amount of SP in sepiapterinic strains gradually increased in the course of development. Red pigment in *cn* and Ore-R increased suddenly after the later pupal stage instead of SP. Biopterin (BP) was already detectable at larval stage, and its amount gradually increased. The increase of BP was remarkable in the sepiapterinic strains. However, BP was constant in adults after 3 days following the emergence of the insect. 2-amino-4-hydroxypteridine (AHP) in Hn^{r-3} flies was scarcely detected

Fig. 3. Changes of pteridine contents and red pigment (circle; o) in the course of development.

throughout all stages, but isoxanthopterin (IXP), oxidized from AHP enzymatically, was already found in the majority of the strains. Therefore, it may be assumed that AHP was already contained in the third instar larvae. The amount of IXP increased in the course of development and reached the maximum in 3 days old male flies, but unexceptedly it decreased markedly only in *cn* male flies after 7 days. since emergence.

Excrements of these strains were collected from about 3,000 male flies, and pteridines in them were also analyzed chromatographically. There was so little of them that their identification were very difficult. Hence, it may be assumed that the flies scarcely excrete pteridines.

Discussion

The eye color mutants of *Drosophila melanogaster* are a very convenient material in studies of the relationships between genic action and its products. The eye pigments in a wild type strain consist of two main components, red and brown. The latter are metabolized from tryptophan, as reported by Beadle and Tatum (1941), Ephrussi (1942) and Kikkawa (1941 and '53). The former have not been clearly analyzed. Hadorn and Mitchell (1951) reported that the concentration of several fluorescent substances is characteristic of each eye color mutant and may affect the formation of red eye pigment. Nawa and Taira (1954) showed that the fluorescent substances represent several kinds of pteridines and that the yellow pigment of *se* strain coincides in chemical nature with Hirata's xanthopterin-B (Hirata *et al*, 1950).

The experimental results reported in this paper show a high correlation between the amounts of red and yellow eye pigments and those of pteridines. The pteridine metabolic system was found to be localized in the tissues of eyes and testes, although its physiological role has not been clarified as yet. A preliminary experiment of the author showed that all eye colors of *Drosophila* mutants show a definite proportion between the amounts of red and brown pigments. According to the amounts of eye pigments and pteridines, the eye color mutants are classified into the following groups: (1) Lack of both red or yellow eye pigments and pteridines; *w* and *bw* strains, (2) Reduced amounts of the two eye pigments and pteridines; *ca*, *ry* and *Hn^{r-3}* strains, (3) Reduced amount of either of the two eye pigments or pteridines; *v*, *cl* and *Hn^{r-1}* strains, (4) Normal amount of both eye pigments and pteridines; *cn*, *st*, *se* and Ore-R.

As shown in Table 1, the amount of red eye pigments of *v* strain was decreased to 80% of that of Ore-R. Clancy (1948) has obtained similar results, but Nolte (1954) has assumed that this finding might have depended on the smaller eye-size of *v* strain. The present author checked the eye-size carefully, but he could not detect such a difference. The smaller amount of red eye pigment of *v* strain, therefore, seems to be due to another cause, namely to an indirect effect of the accumulated tryptophan on some processes of red pigmentation. The tryptophan metabolism and the pteridine metabolism are considered to be independent from each other, but interactions suppressing the amount of red or yellow pigment were observed in the chemotypes (chemical expression of a genotype) of *v*; *ry* and *cn*; *ry* or *v*; *se ry*. According to the

data of Hadorn (1956) and Hadorn and Graf (1958), a transplantation of normal malpighian tubes into *ry* prepupae stimulated the formation of red pigment in *ry* eyes increasing its amount to the normal level. Then, the enzymatic system of pigment formation in *ry* eyes may be normal, only the diffusible precursor of red pigment (*ry*⁺ substance) being in much less concentration. The depressing effect of *ry* gene on the supply of the common precursor of pteridine is strongly influenced by other mutant genes, as shown in Tables 1 and 4. A metabolic pathway from sepiapterin to red pigment could be assumed from these results, but the conversion is very complicated.

As shown in Table 2 and Fig. 3, there was a high correlation between the amount of sepiapterin and biopterin in eyes and in testes. On the basis of careful chemical analysis, Nawa (1960) has proposed for sepiapterin the following structure (I), although Forrest and Mitchell (1954) and Viscountini and Möhlmann (1959) independently proposed for it different chemical structures.

(I) Sepiapterin

(II) Biopterin

According to formula (I), sepiapterin is a structural isomer of biopterin (II). This finding is very important in the consideration of the pteridine metabolism. The writer (1960a) has reported that a pteridine reductase converts sepiapterin, namely dihydropterin, to tetrahydro-form and the latter pteridine is converted to 2-amino-4-hydroxypteridine (AHP) by autoxidation. Furthermore, he (1960 b) has found that a different activity of reductase is observed between *Henna*-recessive alleles. From the results mentioned above, the pteridine metabolic system of *Drosophila* may be visualized as follows :

†) Glassman, E. and Mitchell, H.K. (1959)

The total amount of pteridines was much larger in eyes than in testes. Every strain, except *ry* and *Hn*⁻³, has a small amount of isoxanthopterin (IXP) in the eyes.

The amount of IXP in eyes of the wild strain was almost the same as in *se* eyes which contain a great deal of AHP. This indicates that pterine dehydrogenase which converts AHP to IXP is contained in eye tissues as well as in testes but it is much less active in the former. Such a low activity of that enzyme may relate to some mechanism of red pigment formation from tetrahydropterin in the eyes. Thus, the only difference between pteridine metabolism in eyes and in testes is whether red pigment is formed or not.

Forrest *et al* (1959) have assumed that three components of red pigments could be converted from the corresponding yellow pigments, and Viscontini and Möhlmann (1959) also have proposed that the red pigments might be the oxidized form of sepiapterin and isosepiapterin. However, those assumptions do not agree with the results of our red pigment analysis. Such disagreement in red pigment analysis may be clarified by the correct determination of the structure of the red pigment.

Several investigators have pointed out that IXP is converted from AHP by pterine or xanthine dehydrogenase. Hadorn and Schwinck (1956) and Forrest *et al* (1956) reported independently that *ry* strain completely lacked IXP. Nawa *et al* (1958) and Glassman and Mitchell (1959) found that a deficiency of pterine or xanthine dehydrogenase was the cause. However, the reduction of red pigment in *ry* and *v*; *ry* eyes cannot be plausibly explained by those evidences.

Forrest and Mitchell (1955) predicted the conversion of uric acid into pteridine, but Taira and Nawa (1958) did not agree, because uric acid is insoluble in neutral solution and the process of the change in the amount of uric acid in the course of development is quite alike in *w* or *bw* strain lacking pteridines and other strains having much pteridines. Albert (1957) transformed purines into pteridines *in vitro*, but most of them have not been recognized *in vivo*. On the other hand, Forrest and Mitchell (1955) and Patterson *et al* (1956) independently synthesized biopterin, one of pteridines found in *Drosophila*, by condensation of pyrimidine derivatives and monosaccharides under certain conditions. Okubo (1958) observed the accumulation of N-acetylhydroxytyramine glucoside in the pupal extracts of *ca* strain, having a small amount of eye pigments. These evidences indicate that pteridine may be synthesized enzymatically with both metabolites of carbohydrates and nucleic acids *in vivo*.

Double mutants, such as *v*; *se*, *v*; *cl v*; *Hn^{r-1}*, *v*; *Hn^{r-3}*, *cn*; *se*, *cn*; *Hn^{r-3}* and *se st*, have a small amount of brown pigment despite the inhibition by tryptophan mutant genes, as shown in Table 7. From these facts, it might be assumed that there are two kinds of brown pigment, as suggested by Zentzschel (1958). A kind of brown pigment may be derived from quinone like substances.

Summary

Single and double or triple eye color mutants of *Drosophila melanogaster* were used for the experiments. The relative amounts of eye pigments, extracted from heads without proboscis by means of "double extraction" method, were estimated by photometrical measurement. Several kinds of pteridines, which were contained mainly

in eyes and testes, were separated by paper chromatography and their amounts were measured fluorometrically. Their relative amounts could be estimated by comparing them with each other.

The eye pigments of *Drosophila* are composed of three kinds, red, yellow and brown. Red and yellow pigments have been proved to be pteridine derivatives, and a metabolic pathway from yellow to red was suggested on the basis of experimental results. A metabolic relationship between several pteridines was indicated on the basis of the influence of various mutant genes on the amount of pteridines.

Since pteridines are located in eyes and in testes, a remarkable sexual difference was detected; their total amount in male bodies was about 20 times as large as in female bodies. The genic actions of both *w* and *bw* strains are assumed to block up the supply of the common pteridine precursor. The route of supply is more or less depressed by the action of *ca*, *ry* and *Hn^{r-3}* genes. The homozygous *Hn^{r-3}* fly was considered to have incomplete conversion of sepiapterin to tetrahydropterin, because of reduced activity of pterine reductase. The *cl* and *Hn^{r-1}* genes inhibit considerably the transformation of tetrahydropterin into red pigment, but the *se* gene blocks it completely. From all results, a scheme on pteridine metabolism of *Drosophila* was devised.

Acknowledgment

The author wishes to express his thanks to Prof. H. Kikkawa for his invaluable advice and encouragement in the course of this work. Thanks are also due to Drs. C. Oshima and S. Nawa for their kindful suggestions. Furthermore, the writer is greatly indebted to Dr. F.A. Lilienfeld for her critical reading of the manuscript.

Literatures

- Albert, A. 1957. The transformation of purines into pteridines. *Biochem. J.* **65**: 124-127.
- Beadle, G. W. and Ephrussi, B. 1936. The differentiation of eye pigments in *Drosophila* as studies by transplantation. *Genetics* **21**: 225-247.
- Beadle, G. W. and Tatum, E.L. 1941. Experimental control of development and differentiation: Genetic control of developmental reactions. *Amer. Nat.* **75**: 107-116.
- Clancy, C.W. 1948. Comparative measurements of eye color pigment in *Drosophila melanogaster*. *Genetics* **33**: 606.
- Ephrussi, B. 1942. Analysis of eye color differentiation in *Drosophila*. *Cold Spr. Harb. Symp. quant. Biol.* **10**: 40-48.
- and Herold, J.L. 1944. Studies of eye pigments of *Drosophila*. I. Methods of extraction and quantitative estimation of the pigment components. *Genetics* **29**: 148-175.
- Forrest, H.S. and Mitchell, H.K. 1954. Pteridines from *Drosophila*. II. Structure of the yellow pigment. *J. Amer. Chem. Soc.* **76**: 5658-5662.
- & ——— 1955. *Ibid.* III. Isolation and identification of three more pteridines. *Ibid.* **77**: 4865-4869.
- , Glassman, E. and Mitchell, H.K. 1956. Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in *D. melanogaster*. *Science* **124**: 725-726.
- , Hatfield, E. and Van Baalen, C. 1959. Characterization of a second yellow compound from *Drosophila melanogaster*. *Nature* **183**: 1269-1270.
- Glassman, E. and Mitchell, H.K. 1959. Mutants of *Drosophila melanogaster* deficient in xanthine dehydrogenase. *Genetics* **44**: 153-162.

- Hadorn, E. 1959. Pattern of biochemical and developmental pleiotropy. Cold Spr. Harb. Symp. quant. Biol. **21** : 363-373.
- and Graf, G.E. 1958. Weitere Untersuchungen über den nicht-autonomen pterinstoffwechsel der Mutante *rosy* von *Drosophila melanogaster*. Zool. Anzeiger **160** : 231-243.
- and Schwinck, I. 1956. A mutant of *Drosophila* without isoxanthopterin which is non-autonomous for the red eye pigments, Nature **177** : 940-941.
- Kikkawa, H. 1941. Mechanism of pigment formation in *Bombyx* and *Drosophila*. Genetics **26** : 587-607.
- 1953. Biochemical genetics of *Bombyx mori* (Silkworm). Adv. Genet. **5** : 107-140.
- Nawa, S. 1960. The structure of the yellow pigment from *Drosophila*. Bull. Chem. Soc. Japan : in press.
- and Taira, T. 1954. Pterins found in silkworm and *Drosophila*. Proc. Jap. Acad. **30** : 632-636.
- , ——— and Sakaguchi, B. 1958. Pterine dehydrogenase found in *Drosophila melanogaster*. Proc. Jap. Acad. **34** : 115-119.
- Nolte, D.J. 1954. The eye pigmentary system of *Drosophila*. IV. The pigments of the vermilion group of mutants. J. Genet. **52** : 111-126.
- Okubo, S. 1958. Occurrence of N-acetylhydroxytyramine glucoside in *Drosophila melanogaster*. Med. J. Osaka Univ. **9** : 327-337.
- Patterson, E.L., Miltrey, E. and Stockstad, E.L.R. 1956. The synthesis of a pteridine required for the growth of *Crithidia fasciculata*. J. Amer. Chem. Soc. **78** : 5868-5871.
- Taira, T. 1960a. Enzymatic reduction of *Drosophila* yellow pigment. Nature : in press.
- Taira, T. 1960b. A biochemical study on allelism at *Henna-locus* in *Drosophila melanogaster*. Jap. J. Genet. **35** : in press.
- and Nawa, S. 1958. No direct metabolic relation between pteridine and uric acid, flavins or folic acid in *Drosophila melanogaster*. Jap. J. Genet. **33** : 42-45.
- Viscontini, M., Lesser, E., Karrer, P. and Hadorn, E. 1955. Fluoreszierende Stoffe aus *Drosophila melanogaster*. Helv. Chim. Acta **38** : 1222-1224.
- Hadorn, E. and Karrer, P. 1957. *Ibid.* : die roten augenfarbstoffe. Helv. Chim. Acta **40** : 579-585.
- and Möhlmann, E. 1959. *Ibid.* : die gelb fluoreszierende Stoffe : Sepiapterin und Isopiapterin. Helv. Chim. Acta **42** : 836-841.
- Zentzschel, B. 1958. Entwicklung und Lage der augenpigmente bei Verschiedenen *Drosophila* mutanten. Zeit. f. Vererb. **89** : 509-520.