DEVELOPMENT OF EYE COLORS IN DROSOPHILA: FAT BODIES AND MALPIGHIAN TUBES IN RELATION TO DIFFUSIBLE SUBSTANCES

G. W. BEADLE[1]
Harvard University, Cambridge, Massachusetts

Received July 6, 1937

IT HAS been shown that diffusible substances are involved in the differentiation of certain eye colors in *Drosophila melanogaster* (EPHRUSSI and BEADLE 1937a; BEADLE and EPHRUSSI 1937). Fat bodies are concerned with the production of one of these substances (v^+ substance); two substances (v^+ and cn^+ substances) can be obtained from the Malpighian tubes of flies of certain genotypes. Preliminary experiments having to do with fat bodies and Malpighian tubes have been reported (BEADLE 1937a). It is the purpose of this paper to report more extensive studies of the relation of fat bodies and Malpighian tubes to eye color development. In particular, tests of fat bodies and Malpighian tubes of various eye color mutant types are reported. Certain experiments have been directed toward determining whether Malpighian tubes produce the substances obtainable from them or, on the other hand, whether these substances are produced in other parts of the body and are merely taken up by Malpighian tubes. Certain results bear on the question whether flies of certain constitutions, cinnabar for example, are characterized by complete absence of certain diffusible substances, cn^+ substance in the case of the cinnabar fly, or merely by what might be called sub-threshold concentrations.

MATERIAL AND METHODS

In the experiments reported here, most of the known eye color mutants were used. A list of these will be found in BEADLE and EPHRUSSI (1936a). In addition to the mutants listed in the above paper, certain experiments involve the mutants bright (*bri*), mahogany (*mah*), and suppressor of vermilion (*su-v*). An Oregon-r wild type stock was used.

All experimental animals were grown at a temperature of approximately 25°C.

The method of transplanting fat bodies and Malpighian tubes used was essentially that described by EPHRUSSI and BEADLE (1936). In the case of fat bodies, relatively large-bore, long-shaft pipettes were used. Unless otherwise designated, animals were taken for operations shortly before puparium-formation, that is, about three days after hatching from the eggs.

[1] Now at Stanford University, California.

Vermilion brown and cinnabar brown larvae were used in testing for the presence of, or production of, active substances, the former for v^+ substance, the latter for cn^+ substance. Results were recorded in a roughly quantitative way as described briefly by THIMANN and BEADLE (1937). In the arbitrary scale of color intensities used, 0 represents no effect and 5 represents the maximum possible effect or an eye color not distinguishable from that of brown flies of comparable age. It should be emphasized that the eye color values recorded are not necessarily strictly comparable unless specifically stated to be so. A color-value of, for example, 3 recorded at one time may not represent exactly the same modification as the same numerical value recorded at a different time. This difficulty could be largely avoided if a series of standard stocks with eye colors of various degrees of intermediacy between cinnabar brown (practically white and lighter than vermilion brown) and brown were used. Such a series of "standards" has not yet been developed.

Extracts of Malpighian tubes, referred to as "Ringer-extracts," were made by dissecting larvae, usually five at a time, and then transferring the tubes to known volumes of Ringer's solution in capillary tubes. After each set of five dissections the capillary tube was heated by holding it for a short time in boiling water. Finally the Malpighian tubes were thrown to the bottom by centrifuging and the clear extract drawn off with the micro-pipette used for injection. The number of larvae into which the total volume of extract was put was recorded; the volume of extract injected per larva is therefore approximately known. For injecting fluid, pencil-point micro-pipettes were used. With proper precautions, little liquid need be lost, though it is never possible to be absolutely certain that all of the injected liquid remains in the recipient.

Since preliminary experiments had indicated little or no difference between male and female donors of fat body or Malpighian tube transplants, no attempt was made to obtain the four possible sex-combinations in each series. In some cases larvae of one sex only were selected as donors, in other experiments donors were selected at random with respect to sex. In the preparation of extracts of various kinds, male and female donors were used indiscriminately. In all tables, under the heading "number of individuals" the order of recording of recipients is: female, male, and total.

FAT BODIES AND v^+ SUBSTANCE

If a portion of the fat body of a wild-type larva is transplanted to a vermilion brown larva, the latter develops an eye color strongly modified in the direction of brown (BEADLE 1937a). It has been shown that essentially the same result is obtained when such transplants are made about forty hours before puparium-formation as when they are made shortly

before puparium-formation. Fat bodies from male and female donors are equally effective in bringing about this modification. The simultaneous transplantation of an ovary seems to make no difference. Various sections of the fat body are effective; there is no indication of regional differences.

Tests of fat bodies of various eye color mutants

Tests of fat bodies of the various eye color mutants were made by transplanting the greater part of one lateral fat body to vermilion brown larvae. The results of such tests are summarized in table 1. Positive tests were obtained in all tests except those of fat bodies of vermilion and suppressor of vermilion, vermilion. It is seen that the mean numerical values of the tests vary considerably. This may be the result of differences in amount of v^+ substance produced by different fat bodies, but this is not at all certain for several reasons. In the first place, it is difficult to be sure that the amount of fat body transplanted is the same in different tests. Secondly, there is no way of being certain that the entire piece transplanted remains in the recipient. Lastly, there is a certain variability in scoring the various tests. In two instances (wild and *pr*), two series of tests are given in table 1 for a single type of fat body. In these cases, the tests were made at different times and the results are not strictly comparable because of possible differences in scoring. In both pairs of tests, there are rather wide differences. In the first test of purple, a very low value was recorded (0.9). In order to see whether or not this particular low value was of significance, tests for purple were repeated and at the same time tests of wild type and cinnabar brown fat bodies were tested. Care was taken to transplant as nearly the same amounts of fat body in all of these tests and at eclosion the recipients were compared carefully. These tests, indicated in the footnote to table 1, gave values very close to one another, 1.9, 1.8 and 1.9 respectively. In these cases, then, there is no indication of significant differences in the amount of v^+ substance produced.

Attempts to extract v^+ substance from fat bodies

Since it it known that v^+ substance is water-soluble (KHOUVINE, EPHRUSSI and HARNLY 1936; THIMANN and BEADLE 1937) and can readily be extracted from Malpighian tubes with hot Ringer's solution (BEADLE 1937a), attempts were made to extract this substance from fat bodies. Fat bodies were removed, placed in capillary tubes in Ringer's solution and broken up by heating and crushing. After centrifugation, a somewhat turbulent supernatant liquid was obtained. Injected into vermilion brown larvae, this gave negative results. Several extraction experiments are summarized in table 2. These unsuccessful attempts to extract v^+ substance from fat bodies indicate that v^+ substance is not produced

until after puparium formation. It is possible, of course, that the extraction method was inadequate, but this seems rather unlikely since it is known to work for Malphigian tubes. Another possibility is that the substance is released immediately on formation and consequently never reaches

TABLE 1

Effects of fat body transplants of various genotypes on the eye color of vermilion brown hosts.

IMPLANT‡	NUMBER OF INDIVIDUALS			NUMBER OF NEGATIVE TESTS	EYE COLOR OF HOSTS, MEAN AND RANGE
	♀	♂	Total		
wild	7	3	10	0	3.5 (3.0–4.0)
wild	3	1	4	0	1.8*(0.3–2.7)
bo	4	5	9	0	2.5 (2.0–3.7)
bri	1	1	2	0	3.2 (2.7–3.7)
bw	5	11	16	0	2.2 (1.0–3.0)
car	2	5	7	0	3.0 (2.7–3.7)
cd	4	6	10	0	2.4 (0.7–4.0)
cl	6	8	14	0	3.0 (1.7–4.0)
cm	3	5	8	1	1.9 (1.0–3.0)
g^2	6	2	8	0	4.0 (3.3–5.0)
Hn^r	1	4	5	1	2.4 (2.3–2.7)
lt	1	2	3	0	4.2 (4.0–4.3)
ma	8	6	14	0	3.7 (3.3–4.0)
mah	3	2	5	0	2.3 (2.3)
p^p	4	4	8	0	2.4 (1.3–4.0)
p^d	5	1	6	0	1.4 (1.0–2.3)
p^n	7	2	9	0	2.8 (2.3–3.3)
pr	3	7	10	1	0.9 (0.3–1.7)
pr	5	3	8	0	1.9*(1.3–2.7)
ras^2	3	5	8	0	2.5 (1.0–3.3)
rb	3	6	9	0	3.2 (1.3–4.0)
se	6	5	11	0	2.8 (2.0–3.3)
sed	5	6	11	0	2.2 (1.3–3.3)
sf^2	9	4	13	0	1.6 (0.7–2.3)
st	3	3	6	0	3.1 (2.0–3.7)
v	3	5	8	8	0.0 (0.0)
w	2	2	4	1	2.1 (2.0–2.3)
cn bw	2	2	4	0	1.8*(0.7–2.3)
su-v v	6	4	10	10	0.0 (0.0)

‡ Results of *ca* and *cn* transplants published in a previous paper (BEADLE 1937a).

* The indicated values for wild, *pr*, and *cn bw* are comparable in that the transplants were made at the same time and the hosts directly compared with one another.

a concentration in the fat body cell sufficiently high to give a test on extraction; this does not seem very probable.

FAT BODIES AND cn^+ SUBSTANCE

In contrast to results obtained in tests for v^+ substance, wild type fat bodies transplanted to cinnabar brown flies produce no modification of the

eye color (BEADLE 1937a). Evidently fat bodies do not produce *cn*$^+$ substance. In order to see whether this inability of the fat body to produce *cn*$^+$ substance might possibly be a peculiarity of certain genetic types,

TABLE 2

Test of Ringer-extracts of larval fat bodies for effects on the eye color of vermilion brown and cinnabar brown recipients.

DONORS	RECIPIENTS	NUMBER SETS FAT BODIES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	NUMBER OF INDIVIDUALS			EYE COLOR RECIPIENTS
					♀	♂	Total	
wild	*v bw*	?	?	?	11	7	18	0.0
wild	*v bw*	30	0.03	33	8	9	17	0.0
wild	*cn bw*	?	?	?	5	10	15	0.0
wild	*cn bw*	30	0.03	33	3	9	12	0.0
bri	*v bw*	30	0.02	11	4	6	10	0.0

tests were made of fat bodies of ten additional eye color mutants (claret was previously tested, BEADLE 1937a). All tests were clearly negative (table 3). Ringer-extracts of wild type fat bodies likewise give negative

TABLE 3

Tests for effects of fat body transplants of various genotypes on the eye color of cinnabar brown hosts.

IMPLANT‡	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS	IMPLANT	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS
	♀	♂	Total			♀	♂	Total	
bo	3	1	4	0.0	Hn^r	0	1	1	0.0
bw	3	4	7	0.0	*lt**	6	3	9	0.0
cl	2	4	6	0.0	p^p	4	3	7	0.0
cm	2	3	5	0.0	*rb*	4	6	10	0.0
g^2	5	4	9	0.0	*w*	3	6	9	0.0

‡ Results of wild type and *ca* transplants published in a previous paper (BEADLE 1937a).
* Donors male

results in tests for *cn*$^+$ substance (table 2). It is believed that a sufficiently large sample of genetic types have been tested to indicate rather strongly that *cn*$^+$ substance is never produced by fat bodies.

MALPIGHIAN TUBES AND DIFFUSIBLE SUBSTANCES

As shown by transplants made in the late larval stage, wild type Malpighian tubes produce, or at least release, both *v*$^+$ and *cn*$^+$ substances (BEADLE 1937a). A general survey of the release of these substances by Malpighian tubes of the various eye color mutants has been made and is presented below.

Comparison of different regions of Malpighian tubes

In *Drosophila melanogaster* there are four Malpighian tubes, an anterior and a posterior pair. Judging from general observations made during dissections of larvae of various genetic stocks, the size of the Malpighian tubes relative to larval size varies in larvae of different genetic constitutions. In some stocks the tubes are long and slender while in others they are relatively shorter and thicker. Presumably these relations are genetically controlled. Measurements of the posterior tubes of larvae of the sepia stock taken shortly before puparium formation showed that these tubes were about 3.2 mm long and about 0.4 mm in diameter at the mid-region. The anterior tubes are generally about 1.2 to 1.5 times the length of the posterior tubes. In diameter both anterior and posterior tubes are largest at their bases; both gradually taper toward their distal ends, the posterior tubes to the end and the anterior tubes to a point lying approximately in the mid-section. The anterior tubes of wild type larvae are obviously differentiated into two regions. The proximal regions are similar to the corresponding regions of the posterior tubes. The distal regions of the anterior tubes, one third to one half the total length, are white as contrasted with the yellow proximal regions, are generally more or less swollen, apparently relatively thin-walled, and have their lumens filled with white material—calcium carbonate according to the literature.

A large number of comparisons have been made of the release of v^+ and cn^+ substances by anterior and posterior tubes. In practically all tests recorded in tables 5 and 6, the two pairs were kept separate. The preliminary comparisons indicated a somewhat greater effect of the anterior pair (BEADLE 1937a), but examination of a large number of comparable tests showed little or no difference. In tabulating the data, therefore, the two pairs are listed together.

Comparisons of the yellow proximal regions of the anterior pair of tubes with the white distal regions of the same tubes in tests for both v^+ and cn^+ substances are summarized in table 4. In this table the tests within a given group, indicated by spacing, are strictly comparable. In these, as in all tests of Malpighian tubes made, presence of the implant at eclosion was determined by dissection. It is evident that the greater part, probably all, of the activity in both tests is confined to the yellow proximal region.

Tests of separate regions of the posterior pair of tubes are given in table 4. Tests for cn^+ substance only were made. The proximal and distal halves have approximately equal activities. Approximate separation into proximal one-tenth and distal nine-tenths show, as expected from the above, a large difference. There is, then, no indication of regional differences in activity in the posterior pair of tubes.

Malpighian tubes of various mutants and v^+ substance

The effects of Malpighian tube implants of various genetic types on the eye color of vermilion brown hosts are indicated in the tests summarized in table 5. In tests of Malpighian tubes two sources of variability, difficult to control in tests of fat bodies, are avoided. In the first place, entire Malpighian tubes can be transplanted and it is therefore possible to be reasonably certain that different tests are approximately comparable in so far

TABLE 4

Comparison of the effects of different regions of wild type Malpighian tubes on the eye color of vermilion brown and cinnabar brown hosts. Female donors.

IMPLANT	HOST	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
Yellow proximal regions of anterior pair	*v bw*	3	4	7	4.0 (3.3–4.7)
White distal regions of anterior pair	*v bw*	2	4	6	0.0
Entire posterior pair	*v bw*	1	3	4	4.0 (4.0)
Yellow proximal regions of anterior pair	*v bw*	3	1	4	3.3 (3.3)
White distal regions of anterior pair	*v bw*	2	3	5	0.0
Yellow proximal regions of anterior pair	*cn bw*	4	6	10	3.1 (1.3–4.0)
White distal regions of anterior pair	*cn bw*	5	4	9	0.0
Proximal one-half of posterior pair	*cn bw*	6	5	11	1.8 (0.7–2.7)
Distal one-half of posterior pair	*cn bw*	5	3	8	2.0 (0.7–3.0)
Proximal one-tenth of posterior pair	*cn bw*	1	5	6	0.8 (0.3–1.3)
Distal nine-tenths of posterior pair	*cn bw*	6	3	9	2.6 (0.7–3.7)

as the amount of material transplanted is concerned. Secondly, implants can be recovered in the adult flies and all individuals on which the transplantation operation was not completely successful can be discarded. It is clear from table 5 that, notwithstanding these precautions, there is a good deal of variability in different tests. Some of this undoubtedly results from differences in evaluating color-grades in tests made at different times. There remain, however, differences which cannot be accounted for on this basis. These will be considered in a later section of this paper.

Malpighian tubes of various mutants and cn^+ substance

Tests, comparable to those for v^+ substance given above, for cn^+ substance are presented in table 6. Here again there are differences that cannot be accounted for by differences in evaluation of color-grades. The only negative test for cn^+ substance recorded is that of tubes of cinnabar larvae; this of course is in agreement with our previous interpretation of the relation of diffusible substance to eye color differentiation.

TABLE 5

Effects of Malpighian tube transplants of various genotypes on the eye color of vermilion brown hosts. Anterior and posterior tubes are listed together.

IMPLANT‡	SEX OF DONOR	NUMBER OF TUBES TRANSPLANTED	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
			♀	♂	Total	
wild	♀, ♂	2	11	6	17	3.2 (2.0–4.0)
bo	♀	2	5	5	10	3.5 (3.0–3.7)
bw	♀	2	3	6	9	2.8 (2.2–3.2)
bw	♂	2	3	7	10	2.0 (0.7–2.7)
car	♀	2	3	5	8	1.1 (0.7–2.0)
car	♀	2	6	8	14	2.0 (0.7–2.7)
cd	♀	2	8	3	11	3.2 (2.7–3.7)
cl	♀	2	6	7	13	2.4 (0.7–3.7)
cm	♀	2	2	3	5	2.6 (2.0–3.0)
cn	♀	4	6	2	8	2.5 (2.3–3.0)
g^2	♀, ♂	2	4	5	9	1.1 (0.7–1.7)
g^2	♀	2	5	0	5	1.0 (0.3–1.7)
Hn^r	♀	2	6	10	16	2.4 (1.0–3.3)
lt	♀	2	7	7	14	0.0 (?)
ma	♀	2	7	6	13	2.6 (1.0–3.7)
mah	♀	2	4	7	11	3.5 (3.0–4.3)
p^p	♀	2	6	3	9	0.0 (?)
pd	♀	2	8	7	15	2.7 (2.0–3.3)
pn^2	♀	2	6	5	11	2.5 (2.0–3.7)
pr	♀	2	4	7	11	2.4 (1.0–3.0)
ras^2	♀	2	7	3	10	3.1 (2.0–4.3)
ras^2	♂	2	4	5	9	3.7 (2.0–4.3)
rb	♀	2	4	5	9	2.8 (2.0–3.0)
se	♀	2	3	6	9	2.9 (1.3–4.0)
sed	♀	2	10	2	12	1.9 (0.3–3.0)
sf^2	♀	2	7	5	12	3.2 (2.0–4.3)
st	♀	2	7	6	13	0.0
st	♀	2	9	6	15	0.0
st	♀	4	4	2	6	0.0
v	♀	2	10	10	20	0.0
w	♀	2	6	2	8	0.0 (?)
su-v v	♀	2	6	3	9	0.0 (?)
su-v v	♀	4	4	3	7	0.3 (0.0–0.4)

‡ Tests of wild type (additional), *bri*, *ca*, and *cn* (2 tubes) published in a previous paper (BEADLE 1937a).

Relation between Malpighian tube color and release of substances

As previously pointed out (BEADLE 1937a), there appears to be a general relation between intensity of pigmentation of Malpighian tubes and their effects, when transplanted, on the eye color of vermilion brown and cinnabar brown hosts.

TABLE 6

Effects of Malpighian tube transplants of various genotypes on the eye color of cinnabar brown hosts. Two tubes, anterior or posterior, transplanted in each test.

IMPLANT*	SEX OF DONOR	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
wild	♀	2	5	7	4.2 (3.2–4.8)
bo	♀	3	10	13	3.3 (2.0–4.0)
bri	♀	4	8	12	0.8 (0.0–1.3)
bw	♀	4	7	11	2.4 (1.0–3.3)
car	♀	4	8	12	1.5 (1.0–2.3)
car	♀	4	4	8	1.0 (0.7–1.3)
cd	♂	6	4	10	3.0 (1.0–4.0)
cl	♀	10	4	14	2.6 (1.0–4.0)
cm	♀	3	5	8	2.7 (1.7–3.3)
cn	♀	5	7	12	0.0
g^2	♀, ♂	6	1	7	0.9 (0.7–1.0)
g^2	♀	3	3	6	0.4 (0.3–1.0)
Hn^r	♀, ♂	7	10	17	2.2 (1.0–3.0)
mah	♀	6	5	11	3.1 (1.0–4.0)
p^p	♀	6	4	10	0.6 (0.0–1.0)
p^d	♀	1	4	5	2.8 (2.7–3.0)
pn^2	♀	5	11	16	2.4 (1.0–3.7)
pr	♀	7	6	13	2.3 (1.7–2.7)
ras^2	♀	5	8	13	3.5 (0.7–4.7)
ras^2	♀	8	6	14	2.4 (1.0–3.3)
rb	♀	4	4	8	2.4 (1.8–3.0)
se	♀	7	2	9	2.3 (0.7–3.3)
sed	♀	7	4	11	2.1 (0.7–3.3)
sf^2	♀	5	5	10	2.9 (2.3–3.7)
st	♀	1	7	8	0.6 (0.3–0.7)
st	♀	9	8	17	0.3 (0.1–0.6)
v	♀	3	3	6	0.4 (0.3–0.7)
v	♀	3	3	6	0.4 (0.3–0.7)
su-v v	♀	8	7	15	0.4 (0.0–2.3)

* Tests of wild type (additional), *ca*, *lt*, *ma*, and *w* published in previous paper (BEADLE 1937).

The eye color mutants can be grouped, according to larval Malpighian tube color, as follows (see also BEADLE 1937b): bright yellow (approximately the same as wild type), wild type, *bo*, *cd*, Hn^r, *mah*, *pd*, pn^2, *se*, *sed*, and sf^2; pale yellow, *bri*, *bw*, *car*, *cn*, *ma*, *pr*, *st*, and *v;* very pale yellow, color largely confined to base of tubes, *cm*, g^2, *rb;* white, *ca*, *lt*, p^p, and *w*. Examinations of the tests of tubes of the first group (bright yellow) show that they give relatively strong effects, generally color values of two or more (tables 5 and 6). Whether the differences recorded within this group have any real significance is not known—there can be a reasonable doubt.

Tubes from larvae of the second group give rather variable results. Cinnabar gives a medium test for v^+ substance, none for cn^+ substance, scarlet and vermilion tubes give negative results for v^+ substance and very weak tests for cn^+ substance. Tubes of the third group (very pale yellow with color confined to base of tubes) give variable results, garnet being relatively weak in both, carmine and ruby being relatively strong. The third group gives very weak tests for v^+ substance (close to 0) and somewhat stronger tests for cn^+ substance (see BEADLE 1937a for tests not recorded in tables 5 and 6).

It is seen from the above that the relation between Malpighian tube color and activity in tests for diffusible substances is in no sense an absolute one. Tubes as deeply pigmented as those of wild type all seem to be rather high in activity. Pale yellow tubes, however, appear to be almost as active in some cases. On the other hand, pale yellow tubes may have very little or no activity in the tests. White tubes have very little activity.

It is evident that some developmental relation exists between the pigment system in the eye and that in the Malpighian tubes. Just what this relation is, the available information does not tell us. It is not a simple qualitative relation between amount of one or both diffusible substances produced and the intensity of pigmentation of the Malpighian tubes. Further, there does not seem to be a linear relation between the amount of pigment in the eye and that in the Malpighian tubes.

Pigmentation in Malpighian tube transplants

In tests of Malpighian tubes for v^+ and cn^+ substances it was noted that in no case did the pigmentation of the tube implants appear to be modified by the host. In a further attempt to find out something as to the nature of pigment development in Malpighian tubes, transplants were made of tubes from all eye color mutants that differ from wild type in tube color, to wild type hosts. All operations were made in the late larval stage. The results are summarized in table 7. Tube color is apparently determined by the genetic constitution of the cells of the Malpighian tubes in all these cases. These results, considered in connection with experiments to be presented below, in which eye pigment and tube pigment extracts were injected, indicate that the yellow pigment normally present in larval tubes of certain genetic types is not taken up as such from the blood.

Do Malpighian tubes produce diffusible substances?

Since v^+ and cn^+ substances can be extracted from wild type Malpighian tubes in the late larval stage, a natural question is: are these substances produced by the cells of the Malpighian tubes, or do the tubes merely accumulate these substances from the blood? Various experiments have been made in an attempt to answer this and related questions.

Stages during which substances can be extracted from Malpighian tubes.—If it could be demonstrated that at a given stage Malpighian tubes contain no v^+ or cn^+ substance, it would be a simple matter to transplant the tubes at this stage and see whether they produce one or both substances subsequently. In an attempt to find such a stage of development, Ringer-extracts of wild type Malpighian tubes were made at various stages of larval development. The results of tests of such extracts are given in

TABLE 7

Pigmentation of Malpighian tubes of various genotypes, transplanted to wild type larval hosts three days after hatching. Under "number of individuals" female hosts, male hosts, and total number of individuals are given in the order listed. Two tubes (one pair) transplanted to each host, no distinction made between anterior and posterior pairs.

IMPLANT	COLOR OF LARVAL TUBES	SEX OF DONORS	NUMBER OF INDIVIDUALS			COLOR IMPLANTS AT ECLOSION
			♀	♂	Total	
bri	pale yellow	♀, ♂	9	5	14	pale yellow
bw	pale yellow	♀, ♂	8	4	12	pale yellow
ca	white	♀	8	6	14	white
car	pale yellow	♀	13	3	16	pale yellow
cm	pale yellow at base	♀, ♂	7	10	17	white
cn	pale yellow	♀, ♂	8	2	10	pale yellow
g^2	pale yellow at base	♀	5	5	10	white
lt	white	♀	9	3	12	white
ma	pale yellow	♀, ♂	10	5	15	pale yellow
p^p	white	♀	9	5	14	white
pr	pale yellow	♀, ♂	4	3	7	pale yellow
rb	pale yellow at base	♀, ♂	8	3	11	white
st	pale yellow	♀	7	2	9	pale yellow
v	pale yellow	♀	3	2	5	pale yellow
w	white	♀	6	5	11	white
cn bw	white	♀	8	4	12	white
v bw	white	♀	8	3	11	white

table 8. It is at once clear that both substances are present in the tubes at all stages of larval development tested; that is, at and following about twenty-four hours after hatching from the eggs. At this time the tubes are about one mm or less long and about 0.02 mm in diameter at their mid-regions. It would be rather difficult, but not impossible, to obtain extracts at earlier stages because of the small size of the tubes.

Accumulation of substances by Malpighian tubes.—In connection with the question of the production of substances by Malpighian tubes, it is important to know whether the tubes are capable of taking up v^+ and cn^+ substances from the blood if they are present. An obvious way to determine this is to add v^+ substance to the blood of vermilion larvae, the Malpighian tubes of which, of course, give negative results in tests for v^+ substance,

allow the larvae to live for a period of time and then make tests of the tubes. A similar test for the ability of cinnabar tubes to remove cn^+ substance from the blood is possible. In carrying out such experiments, rather concentrated Ringer-extracts of wild type Malpighian tubes were made. These extracts were injected into vermilion or cinnabar larvae (in some experiments vermilion brown and cinnabar brown larvae were used) at from four to twelve hours before puparium formation. Several hours after this injection the Malpighian tubes were removed from these original recipients and transplanted to vermilion brown or cinnabar brown larvae.

TABLE 8

Tests of Ringer extracts of wild type Malpighian tubes at different stages of larval development.

AGE DONORS (HRS. AFTER HATCHING)	NUMBER SETS OF TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS, MEAN AND RANGE
72±	40	?	40	*v bw*	10	7	17	3.1 (2.0–3.7)
72±	(same extract as above)			*cn bw*	7	10	17	3.2 (3.0–3.7)
48±	50	0.015	30	*v bw*	6	4	10	1.1 (0.7–1.7)
48±	(same extract as above)			*cn bw*	8	8	16	1.0 (0.7–1.3)
24±	60	0.005	6	*v bw*	1	1	2	3.3 (3.3)
24±	(same extract as above)			*cn bw*	4	0	4	2.6 (1.3–3.3)

(The experiments involving scarlet will be considered later). To avoid the possibility of transferring substances in solution in the blood, the tubes were rinsed twice in Ringer's solution. To be quite certain that substance in solution was not being transferred, Ringer's solution used in the first rinsing was tested. These rinsing-controls were negative (9 individuals in tests for v^+ substance and 8 in tests for cn^+ substance). The results of these experiments are summarized in table 9. It is quite clear that vermilion (or vermilion brown) tubes are capable of removing v^+ substance from the blood and that cinnabar (or cinnabar brown) tubes similarly remove cn^+ substance from the blood. Such experiments of course do not prove that v^+ and cn^+ substances normally found in the tubes is taken up from the blood.

In connection with experiments such as those described above, a note should be added regarding the effect of the extracts which contain the yellow pigment of the tubes on the color of the tubes of the original recipients. A very small amount of this yellow pigment does seem to be taken up, but it does not persist in the cells until eclosion. Thus, if a strong Malpighian tube extract is injected into vermilion brown larvae, which have

white tubes, the larvae appear quite yellow. If dissected after four or five hours, the Malpighian tubes are white or faintly yellow. At maturity, however, the tubes of such recipients are white; there appears to be no permanent change in color. This result agrees with the view, previously

TABLE 9

Measurements of the accumulation by Malpighian tubes of v^+ substance and cn^+ substance from larval blood. See text for details.

NUMBER EXPERIMENT	SOURCE OF EXTRACT	NUMBER SETS TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS MEAN AND RANGE
1953	wild	75	0.035	33	*v bw*	3	8	11	3.1 (2.3–3.3)
1954	wild	(same extract as above)			*cn bw*	4	1	5	3.0 (2.7–3.3)
	(*v bw* Malpighian tubes taken from larvae of exp. 1953, 5½ hours after injection; transplanted to *v bw* larvae)					8	1	9	0.6 (0.3–1.3)
	(*cn bw* Malpighian tubes taken from larvae of exp. 1954, 6 hours after injection; transplanted to *cn bw* larvae)					3	0	3	0.0
1967	wild	50	0.02	27	*v*	6	4	10	Intermediate
	(*v* Malpighian tubes taken from larvae of exp. 1967, 2½ hours after injection; transplanted to *v bw* larvae)					10	3	13	0.9 (0.7–1.3)
1976	wild	80	0.02	22	*cn bw*	4	5	9	4.4 (4.3–5.0)
	(*cn bw* Malpighian tubes taken from larvae of exp. 1976, 2¾ hours after injection; transplanted to *cn bw* larvae)					7	5	12	0.5 (0.1–1.0)
1989	wild	50	0.02	21	*cn*	4	3	7	Intermediate
	(*cn* Malpighian tubes taken from larvae of exp. 1989, 2¾ hours after injection; transplanted to *cn bw* larvae)					3	10	13	1.0 (0.4–1.3)
2009	wild	50	0.015	15	*st*	.....			.....
	(*st* Malpighian tubes taken from larvae of exp. 2009, 4 hours after injection; transplanted to *v bw* larvae)					6	6	12	0.0
2023	wild	50	0.01	11	*st*	.....			.....
	(*st* Malpighian tubes taken from larvae of exp. 2023, 2¾ hours after injection; transplanted to *v bw* larvae)					6	7	13	0.0

expressed, that the yellow pigment normally found in wild type larval tubes is not taken up as such from the blood.

Are v^+ and cn^+ substances normally present in larval blood?—Knowing that Malpighian tubes can take up the two diffusible substances from the blood, it becomes important to know whether these substances are normally present in larval blood. The work of EPHRUSSI, CLANCY and BEADLE (1936), and BEADLE, CLANCY and EPHRUSSI (1937) indicates that v^+ sub-

stance is not present in the blood of wild type larvae. A similar result was obtained in tests of larval body fluids for cn^+ substance (HARNLY and EPHRUSSI 1937). It has been shown, however, that both of these diffusible substances can be extracted from wild type larval Malpighian tubes. Since it has been shown by THIMANN and BEADLE (1937) that these substances are inactivated when pupae are crushed in air, presumably by enzymic oxidation, the question is raised as to whether the negative tests obtained in direct transfers of larval fluid are the result of absence of the active substance in the blood or merely of its inactivation in air. Extracts of whole larvae are of course useless for answering this question since it is known that larval Malpighian tubes contain the active substances. Direct transfers of larval fluid are difficult to make without exposing large surfaces of the fluid to air. Additional tests have been made for both substances, but they have invariably given negative results. Direct transfers of fluid removed in a nitrogen chamber gave negative results for v^+ substance, but the tests were unsatisfactory because of technical difficulties. Larval fluid obtained by crushing larvae under nitrogen and centrifuging through an asbestos and glass wool filter have given positive results (in *v bw*, 6 individuals gave a mean value of 1.1; in *cn bw*, 13 tests gave a mean value of 1.4), but the active substances may have all come from the Malpighian tubes.

With the knowledge that vermilion and cinnabar Malpighian tubes are able to take up v^+ and cn^+ substances when these are added to the blood, it was possible to devise an experiment that would answer the question under consideration. Malpighian tubes from vermilion larvae were transplanted to wild type larvae about twenty-four hours prior to puparium formation. Shortly before puparium formation, the vermilion tube implants (dorsal or ventral pairs) were removed and re-implanted in vermilion brown hosts. In two experiments of this type, made at different times, the following results were obtained:

HOST	IMPLANT RECOVERED	NUMBER OF INDIVIDUALS	EYE COLOR OF HOSTS, MEAN AND RANGE
v bw	2 tubes	2, 0; 2	1.5 (1.0–2.0)
	1–1½ tubes	2, 5; 7	0.6 (0.1–1.3)
v bw	2 tubes	3, 2; 5	0.6 (0.1–1.3)

Similar results were obtained when the vermilion tubes were removed from the original hosts shortly after puparium formation.

It is evident, since vermilion tubes invariably give negative tests when transplanted directly to vermilion brown larvae (table 5), that the tubes grown for about twenty-four hours in wild type larvae had removed v^+ substance from the blood. It does not necessarily follow, of course, that in

taking up v^+ substance from larval blood, Malpighian tubes play an entirely passive role. By the nature of the test for it, it is obvious that v^+ substance may actually consist of two or more developmentally related substances. It is entirely conceivable that Malpighian tubes may take up one or more substances and release one or more different substances concerned in the test-reactions. If, however, v^+ substance is defined as anything that will produce the characteristic change in a test animal of appropriate genotype—and this is the only definition that we can at present make use of— it does follow that the negative results obtained in previous tests of larval fluid were the result of inactivation or, less probably, were obtained because the concentrations present were too low to give measurable tests.

Similar tests for the presence of cn^+ substance in larval blood are of course possible but they have not been made.

Attempts to exhaust Malpighian tubes of diffusible substances.—Various methods have been used in an attempt to exhaust living Malpighian tubes of v^+ and cn^+ substances with a view to seeing whether they could subsequently produce these substances.

Tubes of adult wild type flies, taken shortly after eclosion, transplanted to late larvae, give positive tests for both substances. In such tests, using females as donors, the following results were obtained:

HOST	NUMBER OF INDIVIDUALS	EYE COLOR OF HOSTS, MEAN AND RANGE
v bw	5, 7; 12	2.4 (2.0–3.7)
cn bw	5, 5; 10	0.5 (0.3–0.7)

In order to see whether the substances had been exhausted from adult tubes, extractions were made in hot Ringer's solution. From such extracts the following tests were obtained:

NUMBER SETS TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NO. OF INDIVID-UALS	EYE COLOR RECIPIENTS, MEAN AND RANGE
43	0.02	19	*v bw*	5, 1; 6	0.9 (0.3–1.3)
(same extract as above)			*cn bw*	5, 6; 11	1.0 (1.0–1.3)

Taken at face value, these results show that v^+ substance, if not cn^+ substance, is produced by the adult tubes after transplantion. In the injection tests an extract of about 8.5 single tubes per larva gave a test in *v bw* of 0.9, whereas two tubes (one pair) transplanted gave a test for v^+ substance of 2.4. In spite of this large difference in the activity of the transplanted tubes as compared with the extract, some doubt can be entertained as to the validity of the indicated conclusion; for one thing, such a conclusion is based on the assumption that a quantitative extraction was made.

On the assumption that the chances of exhausting Malpighian tubes for a given substance would be increased if the tubes were grown in a host deficient for the substance concerned, wild type tubes were transplanted to vermilion brown larvae and allowed to remain until eclosion of the hosts. The implants were then removed and tested in two ways, by extraction and by again transplanting to vermilion brown larvae. The extraction experiments gave results as follows:

NUMBER OF PAIRS TUBES EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION OF RECIPIENTS	NUMBER OF INDIVIDUALS	EYE COLOR RECIPIENTS MEAN AND RANGE
43	0.015	27	*v bw*	7, 4; 11	1.1 (0.7–1.7)
(same extract as above)			*cn bw*	6, 8; 14	0.9 (0.7–1.0)

Evidently these tubes contained small amounts of both substances. From tests made by transplanting to vermilion brown larvae, only two individuals were obtained. These gave a mean color value of 0.8 (0.7–1.0). Here there is no clear indication of production of v^+ substance after the second transplantation.

TABLE 10

Attempts to exhaust wild type Malpighian tubes of v^+ and cn^+ substances by extraction with Ringer's solution at 25°C. See text for details.

NUMBER SETS TUBES EXTRACTED	RINGER'S USED (CC)	TREATMENT	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR OF RECIPIENTS, MEAN AND RANGE
30	0.02	Extracted with cold Ringer's for 65 to 120 minutes	17	*v bw*	3	2	5	3.2 (3.0–3.3)
				cn bw	4	6	10	2.8 (2.0–3.3)
30	0.02	Soaked in cold Ringer's for 60 to 90 min., extracted with hot Ringer's	19	*v bw*	5	1	6	1.4 (0.7–2.0)
				cn bw	7	2	9	1.9 (1.3–2.3)
30	0.02	Soaked in cold Ringer's for 75 to 115 min., rinsed, extracted with hot Ringer's	22	*v bw*	4	6	10	2.1 (0.7–2.3)
				cn bw	8	1	9	2.1 (1.3–2.7)

It is known that both v^+ and cn^+ substances diffuse from wild type Malpighian tubes if these are allowed to stand in Ringer's solution at room temperature for a short time (BEADLE 1937a). A series of experiments was made in which tubes were partially extracted in Ringer's solution at 25°C and then extracted with hot Ringer's solution or transplanted to test lar-

vae. Tests of cold (25°C) Ringer-extracts of tubes from wild type larvae approaching puparium formation are summarized in table 10. Relatively strong tests are recorded for both substances. In order to find what proportion of the two substances were extracted by this treatment, experiments were made in which tubes were allowed to stand in Ringer's solution for time intervals of the order of one to two hours. The Ringer's solution was pipetted off, and hot Ringer-extracts were made as usual. Comparisons of the two types of extracts show that a good deal more than half of the diffusible substance had gone into the cold Ringer's solution in from one to two hours (table 10). The results of transplantation tests of tubes allowed to stand in Ringer's solution at 25°C for various intervals of time (table 11) show no convincing evidence of a decrease in their activity. For intervals longer than about 90 minutes, entire implants were not recovered at emergence; fragments only were recovered and in many cases nothing was found on dissection.

TABLE 11

Transplantation of wild type Malpighian tubes from late larvae after standing in Ringer's solution at 25°C. Comparable data grouped by horizontal spacing.

TIME IN RINGER'S SOLUTION (MIN.)	HOST	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS, MEAN AND RANGE
		♀	♂	Total	
0	*cn bw*	3	1	4	2.8 (1.7–3.0)
35	*cn bw*	3	2	5	2.8 (2.7–3.0)
55	*cn bw*	3	1	4	2.0 (1.0–3.0)
90	*cn bw*	0	1	1	3.0
0	*cn bw*	10	3	13	3.0 (2.3–4.0)
145–150	*cn bw*	7	9	16*	2.6 (2.0–3.3)
65–110	*v bw*	2	6	8*	2.5 (1.7–3.7)
90–120	*v bw*	3	0	3*	1.8 (1.3–2.0)

* Intact implants not recovered; generally small pieces or nothing found at eclosion.

From the numerical values obtained in the transplantation and extraction tests just considered, one might conclude that tubes partially extracted with cold Ringer's solution produce additional substances after transplantation. On the strength of these experiments alone, however, the evidence cannot be considered entirely convincing.

Production of cn⁺ substance by vermilion Malpighian tubes.—According to the interpretation previously given of the relation between v^+ substance and cn^+ substance vermilion flies should be deficient in cn^+ substance.

Table 6 shows that vermilion Malpighian tubes transplanted to cinnabar brown larvae, modify the eye color slightly indicating production or release of cn^+ substance. This can be interpreted in the same way as we have previously interpreted the formation of cn^+ substance by vermilion eye-implants, that is, production of cn^+ substance by vermilion Malpighian tubes when grown in the presence of v^+ substance. If a vermilion fly is totally deficient in both substances, as opposed to having sub-threshold concentrations, and if the above interpretation is correct, extracts of vermilion Malpighian tubes should contain no cn^+ substance. Actually it is found that such extracts are negative in tests for cn^+ substance (table 12). We can now invert the argument in the following way: Extracts of

TABLE 12

Tests of concentrated extracts of Malpighian tubes of vermilion and cinnabar larvae.

DONORS	NUMBER SETS TUBES EXTRACTED	RINGER'S USED (cc)	NUMBER LARVAE INJECTED	CONCENTRATION RATIO	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE-COLOR RECIPIENTS, MEAN AND RANGE
v	36	?	19	1.9	*v bw*	2	2	4	0.0
v	(Same extract as above)			1.9	*cn bw*	10	5	15	0.0
v	32	0.01	11	2.9	*v bw*	5	6	11	0.0
v	45	0.01	13	3.5	*cn bw*	8	4	12	0.0
v	100	0.012	11	9.1	*v bw*	5	1	6	0.0 (?)*
v	(Same extract as above)			9.1	*cn bw*	5	0	5	0.0
v	235	0.03	21	11.2	*v bw*	10	1	11	0.0
v	80	0.01	8	10.0	*cn bw*	1	2	3	0.0†
cn	60	0.01	16	3.8	*v bw*	0	3	3	1.1 (0.3–2.0)
cn	(Same extract as above)			3.8	*cn bw*	7	3	10	0.0
cn	80	0.01	9	8.9	*v bw*	1	0	1	3.0
cn	(Same extract as above)			8.9	*cn bw*	4	1	5	0.0

* Only three comparable controls available for females; male clearly negative.

† Injections not entirely satisfactory; some fluid lost.

larval vermilion tubes contain no cn^+ substance, at least not in measurable quantities. Such tubes, transplanted to cinnabar brown larvae, modify the eye color, showing that cn^+ substance has been supplied to the eyes by the Malpighian tubes. It follows, therefore, that vermilion Malpighian tubes, in the presence of v^+ substance supplied by the cinnabar brown host, have produced cn^+ substance.

The evidence considered above indicates that Malpighian tubes do produce v^+ and cn^+ substances, at least under certain conditions. Whether they also take up appreciable amounts of these substances from the blood in the larval stage is another question that cannot be answered at present. If they do, there presumably must be an undiscovered source of these sub-

stances in the larvae for there is evidence that fat bodies produce v^+ substance only after puparium formation and it seems improbable that eye tissue can produce appreciable amounts of the two substances in larval stages. The question must remain open until additional experimental evidence is at hand.

POSSIBILITY OF SUB-THRESHOLD CONCENTRATIONS OF DIFFUSIBLE SUBSTANCES

In earlier papers on diffusible substances in relation to eye color development, the tacit assumption was made that a vermilion fly has no v^+ or cn^+ substance (BEADLE and EPHRUSSI 1936). Later it was pointed out (EPHRUSSI and BEADLE 1937a; BEADLE and EPHRUSSI 1937) that such an assumption is not justified. Extraction experiments provide a means of answering this question experimentally. If a vermilion fly differs from a wild type fly merely in having less v^+ and cn^+ substances, it should be possible to demonstrate the presence of these substances by using concentrated extracts. A similar test should be possible in determining whether a cinnabar fly contains any cn^+ substance. Such tests have been made by extracting large numbers of Malpighian tubes in small volumes of Ringer's solution; the results are summarized in table 12. Extracts of vermilion tubes give negative results in tests for both substances up to concentration-ratios of 11.2 (ratio of number of sets of tubes extracted to volume of extract injected per larva). Extracts of cinnabar tubes give positive tests for v^+ substance, but negative tests for cn^+ substance. The highest concentration-ratio investigated was 8.9. By means of such experiments, it is of course impossible to prove that the substance in question is absent; the concentration can always be assumed to be below the test threshold. For practical purposes, however, these experiments can be taken to favor our original assumption, that is, absence of both substances in vermilion and absence of cn^+ substance only in cinnabar.

TESTS FOR SUBSTANCES IN OTHER ORGANS

It is known that under certain conditions eye tissue is capable of producing and liberating v^+ and cn^+ substances (EPHRUSSI and BEADLE 1937b). Extracts of eyes (whole heads) of freshly emerged mature wild type flies made up in hot Ringer's solution give weak positive tests for both v^+ and cn^+ substances (table 13). Apparently somewhat more of both of these substances is available than is normally used by the eyes. This result is in agreement with the tests of extracts of Malpighian tubes of adult flies in showing the presence at this stage of small amounts of both substances. HARNLY and EPHRUSSI (1937), in experiments in which body fluid was injected, found traces of both substances in pupae just before and

at the time of eclosion. Extracts of scarlet eyes give results similar to those for wild type (table 3). These will be discussed in a later section.

TABLE 13

Tests of Ringer-extracts of adult eyes (heads) for v^+ and cn^+ substances.

DONORS	NUMBER HEADS EXTRACTED	RINGER'S USED (CC)	NUMBER LARVAE INJECTED	CONSTITUTION RECIPIENTS	NUMBER OF INDIVIDUALS ♀	♂	Total	EYE COLOR RECIPIENTS MEAN AND RANGE
wild	70	0.04	40	*v bw*	9	8	17	0.4 (0.2–0.8)
	(same extract as above)			*cn bw*	6	8	14	0.3 (0.2–0.4)
st	60	0.03	22	*v bw*	6	3	9	0.5 (0.3–0.6)
	(same extract as above)			*cn bw*	2	7	9	0.2 (0.1–0.3)

Extracts of adult eyes contain eye-pigment. Shortly after injection of such extracts, the Malpighian tubes of the recipients become bright reddish-orange in color. This pigment, taken up from the blood, largely disappears in the adult, but traces of it may remain. MORGAN (1930) has studied the uptake of eye-pigment by the Malpighian tubes following injury to the eye, feeding larvae crushed eyes, and following injection of eye-pigment into adult flies. The pigment accumulated by the tubes following injection of eye-pigment appears to be quite different from that normally found in the tube; it is quite red as compared with the yellow pigment characteristic of wild type Malpighian tubes.

Extracts of testes of adult wild type flies made up in hot Ringer's solution are very similar in color to extracts of Malpighian tubes. Tested for v^+ and cn^+ substance, the following results were obtained:

NO. PAIRS TESTES EXTRACTED	RINGER'S USED (CC)	NO. LARVAE INJECTED	CONSTITUTION RECIPIENTS	NO. OF INDIV.	EYE COLOR RECIPIENTS
55	0.02	24	*v bw*	8, 4; 12	0.0
	(same extract as above)		*cn bw*	3, 7; 10	0.0

Extracts of wild type *D. virilis* testes which are orange-red in color gave negative results in tests for the two substances made by injection into larvae of *D. melanogaster* (13 individuals in v^+ substance test, 10 in test for cn^+ substance).

THE SCARLET CHARACTER

Although scarlet flies are known to produce v^+ substance (BEADLE and EPHRUSSI 1936; see also tests of fat bodies, this paper), Malpighian tubes of scarlet larvae transplanted to vermilion brown have no effect on the eye color of the host. On the assumption that such tubes might take up

v^+ substance from the blood after its production by the fat bodies, tests were made by transplanting scarlet tubes at various stages of development. The results were negative at all stages of pupal development up to and including the adult stage (table 14). Evidently scarlet tubes do not

TABLE 14

Tests of scarlet Malpighian tubes taken at various stages of development after puparium formation and transplanted to vermilion brown larvae. Unless otherwise indicated, one pair tubes, anterior or posterior, transplanted in each test.

TIME AFTER PUPARIUM FORMATION (HRS.)	NUMBER OF INDIVIDUALS			EYE COLOR OF HOSTS
	♀	♂	Total	
0– 2.5	6	5	11	0.0
20 –21.5	6	0	6	0.0
47.5–55.8	9	6	15	0.0
66.2–74.8	8	6	14	0.0
66.8–75.5 (4 tubes)	3	4	7	0.0
Just before eclosion	3	4	7	0.0
Just after eclosion	8	2	10	0.0

take up v^+ substance from the blood. A further test of this was made by injecting concentrated extracts of wild type tubes into scarlet larvae some hours before puparium formation. Two to $4\frac{1}{2}$ hours later the tubes were removed from these scarlet larvae and transplanted to vermilion brown test-larvae. Again the results were negative (table 9), indicating that under these conditions scarlet tubes do not take up detectable quantities of v^+ substance. Tests were then made to see if scarlet tubes in some way inactivate v^+ substance. Fifty sets of wild type Malpighian tubes were extracted in 0.02 cc of Ringer's solution in the usual way. This extract was divided into two samples of equal volume. One sample was kept as a control. To the other, 25 sets of scarlet tubes were added (without heating), allowed to stand at 25°C for $2\frac{1}{4}$ hours, and then heated and centrifuged. Both extracts were then tested by injection into vermilion brown larvae. The results of these tests follow:

EXTRACT	NO. LARVAE INJECTED	NO. OF INDIVIDUALS	EYE COLOR RECIPIENTS, MEAN AND RANGE
st tubes added	9	3, 6; 9	4.2 (4.0–4.3)
control	12	5, 6; 11	4.1 (4.0–4.3)

Under the conditions of the experiment there was evidently little or no inactivation by the scarlet tubes.

Extracts of the eyes of scarlet adult flies show that v^+ substance is present (table 13).

The facts given above provide an interesting clue as to the possible nature of the scarlet character. Scarlet belongs to the vermilion-like group of eye colors (SCHULTZ, 1935); phenotypically it is indistinguishable from vermilion and cinnabar. WRIGHT (1932) has pointed out that a simple interpretation, in terms of physiological development, of the fact that the double recessive vermilion scarlet is not distinguishable from the single eye colors and of the similar interactions of vermilion and scarlet with brown (both double recessives with brown are practically white) follows the assumption that the wild type alleles of both vermilion and brown are concerned with different steps in a chain reaction necessary for the development of wild type eye color. We have evidence from transplantation experiments that the formation of v^+ substance may be regarded as one of the steps in such a chain reaction, the production of cn^+ substance another. In scarlet both substances are present and we can suppose that the break in the series of chain reactions is in the utilization of one or both of these substances in the eye. On this interpretation, the scarlet character, so far as the end result is concerned, is the same as vermilion or cinnabar; the difference lies in the manner in which the end result is brought about. The inability of scarlet Malpighian tubes to take up v^+ substance from the blood lends some support to this interpretation in that it shows that a mechanism similar to that required by the suggested hypothesis must exist in the cells of the Malpighian tubes.

SUPPRESSOR OF VERMILION

BEADLE and EPHRUSSI (1936b) concluded from various transplantation experiments that the recessive gene suppressor of vermilion (*su-v*), when combined with vermilion, somehow partially restores the reaction leading to the formation of v^+ substance. Tests of *su-v v* fat bodies for v^+ substance are negative (table 1) as are tests of pairs of Malpighian tubes of the same constitution (table 5). Four Malpighian tubes of a *su-v v* fly give a positive test for v^+ substance, but so weak that, by itself, the test would scarcely be convincing. To test the results previously obtained, two series of eye-transplants involving suppressor of vermilion were made. The results follow:

IMPLANT	HOST	NO. OF INDIVIDUALS	PHENOTYPE OF HOST	PHENOTYPE OF IMPLANT
su-v v	*v bw*	5, 5, 11, 0; 21	*v bw*	*su-v v*
v bw	*su-v v*	10, 0, 8, 2; 20	*su-v v*	Intermediate between *v bw* and *bw*

The first shows that a *su-v v* eye-implant does not release any appreciable quantity of v^+ substance; presumably the implant itself produces v^+ substance, but not enough to give completely wild type eye color. The second result shows that a *su-v v* fly produces some v^+ substance, but not enough

to bring about a complete change from vermilion brown to brown. This confirms the results reported in our earlier paper.

OCELLUS COLOR

Both vermilion brown and cinnabar brown flies have white ocelli. It was found (BEADLE 1937a) that when a strong modification of the eye color of a vermilion brown fly is brought about by supplying v^+ substance, the ocellus color is modified toward brown. The corresponding change in ocellus color in cinnabar brown flies was not obtained. The experiments reported here confirm this finding. Aside from noting these relations, no experiments have been especially directed toward a more thorough study of ocellus color.

DISCUSSION

The relation of the studies reported here to the results obtained by STURTEVANT (1932) in his investigations of mosaic individuals of *D. simulans* made up of vermilion and not-vermilion tissue are somewhat puzzling. STURTEVANT found a strong correlation between the type of pigment developed in vermilion eye-tissue and the constitution of the gonads. Where both gonads were not-vermilion and female, genetically vermilion eye tissue developed not-vermilion pigmentation. On the other hand, when the gonads were vermilion and male, vermilion eye-tissue developed the characteristic vermilion color. There were a few exceptions to this behavior. In previous transplantation work (EPHRUSSI and BEADLE 1935), we were unable to find any evidence of a direct relation between gonads and v^+ substance. In the studies reported above, the experiments involving ovaries give no indication that presence or absence of wild type ovary implants has any influence on the end result. The only interpretation that the writer can offer is that the relations found by STURTEVANT result from a relation in terms of cell lineage between gonads and the organs which produce v^+ substance. This would mean that in elimination gynandromorphs with female gonads, such as were studied by STURTEVANT, the probability would be high that a considerable portion of the tissue of the fat bodies and, presumably, also of the Malpighian tubes, would be female (not-vermilion). Correspondingly, in individuals with male gonads, the probability would be high that fat bodies and Malpighian tubes would be male (vermilion). On such an interpretation relation between gonads and eye-pigment differentiation in gynandromorphs would, of course, be indirect; this would agree with the results of transplantation studies. There appears to be little point in discussing the facts regarding embryological development in relation to the interpretation suggested above; the available knowledge of the cell-lineage relations of the organs concerned is insufficient.

SUMMARY

1. In general, the experiments reported were designed to determine where, when, and how the diffusible substances concerned in eye color development are produced.

2. Fat bodies of all eye color mutants studied, except those from vermilion larvae, are shown to produce v^+ substance following transplantation to appropriate test animals.

3. Extracts of fat bodies gave negative results in tests for v^+ substance; presumably they produce v^+ substance only after puparium formation.

4. Fat bodies do not produce cn^+ substance.

5. Malpighian tubes of wild type larvae release both v^+ and cn^+ substances following transplantation. Both substances can be extracted from the larval tubes with hot Ringer's solution.

6. The white distal regions of the anterior pair of Malpighian tubes were negative in all tests for the two substances.

7. Malpighian tubes from larvae differing in genetic constitution with respect to eye color genes may vary in the amount of v^+ and cn^+ substance released. There is a general, but not simple, relation between release of diffusible substances and depth of pigmentation of the Malpighian tubes.

8. Malpighian tube color is autonomous in all combinations studied when tubes are transplanted in late larval stages.

9. Both v^+ and cn^+ substances are present in the Malpighian tubes of wild type larvae at and following the earliest stage studied—about twenty-four hours after hatching from the eggs.

10. Larval Malpighian tubes normally not containing or producing a particular diffusible substance, can take up that substance if it is added to the larval blood; vermilion tubes can take up v^+ substance and cinnabar tubes can take up cn^+ substance.

11. By making use of the fact just mentioned it has been possible to demonstrate the presence of v^+ substance in the blood of wild type larvae during some part or all of a 24-hour interval just prior to puparium formation.

12. Various attempts were made to exhaust living Malpighian tubes of their diffusible substances; these attempts were partially successful.

13. The evidence favors the view that Malpighian tubes produce at least a part of the substances that can be obtained from them.

14. Concentration experiments failed to demonstrate the presence of either v^+ or cn^+ substance in vermilion Malpighian tubes or the presence of cn^+ substance in cinnabar tubes.

15. It is suggested that there is a block to the utilization of v^+ or cn^+ substance, or both, in scarlet flies.

16. Under the conditions of the experiments reported, ocellus color is autonomous in cinnabar, dependent in vermilion.

17. The results of the work on fat bodies and Malpighian tubes are discussed in relation to STURTEVANT'S studies of gynandromorphs in *D. simulans* in which vermilion eye color was concerned.

LITERATURE CITED

BEADLE, G. W., 1937a Development of eye colors in Drosophila: fat bodies and Malpighian tubes as sources of diffusible substances. Proc. Nat. Acad. Sci. **23**: 146–152.

1937b The inheritance of the color of Malpighian tubes in *Drosophila melanogaster*. Amer. Nat. **71**: 277–279.

BEADLE, G. W., CLANCY, C. W., AND EPHRUSSI, B., 1937 Development of eye colors in Drosophila: pupal transplants and the influence of body fluid on vermilion. Proc. Roy. Soc., B. **122**: 98–105.

BEADLE, G. W., AND EPHRUSSI, B., 1936a The differentiation of eye pigments in Drosophila as studied by transplantation. Genetics **21**: 225–247.

1936b Development of eye colors in Drosophila: transplantation experiments with suppressor of vermilion. Proc. Nat. Acad. Sci. **22**: 536–540.

1937 Development of eye colors in Drosophila: diffusible substances and their interrelations. Genetics **22**: 76–86.

EPHRUSSI, B., AND BEADLE, G. W., 1935 La transplantation des ovaires chez la Drosophile. Bull. Biol. Fr. Belg. **69**: 492–502.

1936 A technique of transplantation for Drosophila. Amer. Nat. **70**: 218–225.

1937a Développement des couleurs des yeux chez la Drosophile: revue des expériences de transplantation. Bull. Biol. Fr. Belg. **71**: 54–74.

1937b Développement des couleurs des yeux chez la Drosophile: influence des implants sur la couleur des yeux de l'hôte. Bull. Biol. Fr. Belg. **71**: 75–90.

HARNLY, M. H., AND EPHRUSSI, B., 1937 Development of eye colors in Drosophila: time of action of body fluid on cinnabar. Genetics **22**: 393–401.

KHOUVINE, Y., EPHRUSSI, B., AND HARNLY, M. H., 1936 Extraction et solubilité des substances intervenant dans la pigmentation des yeux de *Drosophila melanogaster*. C. R. Acad. Sci. Paris. **203**: 1542.

MORGAN, T. H., 1930 The apparent inheritance of an acquired character and its explanation. Amer. Nat. **64**: 97–114.

SCHULTZ, J., 1935 Aspects of the relation between genes and development in Drosophila. Amer. Nat. **69**: 30–54.

STURTEVANT, A. H., 1932 The use of mosaics in the study of the developmental effect of genes. Proc. Sixth Intern. Congress Genetics **1**: 304–307.

THIMANN, K. V. AND BEADLE, G. W., 1937 Development of eye colors in Drosophila: extraction of the diffusible substances concerned. Proc. Nat. Acad. Sci. **23**: 143–146.

WRIGHT, S., 1932 Complementary factors for eye color in Drosophila. Amer. Nat. **66**: 282–283.