# THE DEVELOPMENT OF EYE COLORS IN DROSOPHILA MELANOGASTER. FURTHER STUDIES ON THE MUTANT CLARET[1]

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## INTRODUCTION

THESE studies were undertaken in order to repeat and supplement the experiments of BEADLE and EPHRUSSI (1936, 1937) and EPHRUSSI and BEADLE (1936b) which provided the evidence upon which these authors postulated the existence of $ca^+$ substance as one of three specific diffusible substances assumed to be necessary for the differentiation of wild type eye color in *Drosophila melanogaster*.

The first portion of this paper is primarily concerned with a re-examination of this evidence and the presentation of new data bearing on the question of whether or not $ca^+$ substance exists as an entity distinct from $v^+$ and $cn^+$ substances. [For information concerning the chemical identification of $v^+$ hormone see BUTENANDT, WEIDEL, and BECKER (1940), TATUM and BEADLE (1940), and TATUM and HAAGEN-SMIT (1941).] This problem has led to the consideration of other aspects of the differentiation of eye color involving the mutant claret. One of these aspects is the effect of the mutation $ca^+ \rightarrow ca$ on the quantity of the pigment components making up the eye color of *D. melanogaster*. A second portion of this paper presents in connection with data bearing on the latter problem an outline of a general method for the extraction and measurement of the eye-color pigments of Drosophila.

Doubt concerning the existence of $ca^+$ substance has been expressed by GOTTSCHEWSKI and TAN (1938) and by BEADLE, ANDERSON, and MAXWELL (1938). Recently, LUERS and STUBBE (1940) have sought to determine the content of free $ca^+$ substance in the blood of *D. melanogaster* and two other species of Drosophila. Their results will be discussed later.

Evidence has now accumulated which invalidates the original argument upon which the existence of $ca^+$ substance was based. This negating evidence consists, in part, in the correction of certain observational errors made in the original experiments. The recognition of these errors permits a different interpretation to be placed upon the results of certain optic-disc transplantations involving, especially, wild type and the mutants claret and vermilion.

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When $ca^+$ substance was postulated, the following transplantation results were considered valid[3] (See BEADLE and EPHRUSSI 1936, 1937): (1) + in *ca*→*ca*, (2) *v* in *ca*→*v*, (3) *cn* in *ca*→*cn*, (4) + in *v*→+, (5) + in all eye color mutants except claret→+.

Based on this interpretation of the color of the implants, the argument for the existence of $ca^+$ substance is as follows: A wild type optic disc evidently requires a substance from some other part or parts of the fly's body in order to develop wild type pigmentation. This substance is lacking in a claret host. Since a vermilion fly lacks both $v^+$ and $cn^+$ substances, but, as a host, allows a wild type optic disc to develop wild type pigmentation, vermilion must contain the substance lacking in the claret host. Because transplants of vermilion and cinnabar optic discs were not modified toward wild type when transplanted to claret hosts, $ca^+$ substance was assumed to be a precursor to the formation of $v^+$ substance in the reaction chain, ($ca^+$ substance)→$v^+$ substance→$cn^+$ substance.

It was soon necessary to adapt this conception to a new observation—namely, that a wild type optic disc grown in a claret host was not always phenotypically claret but occasionally approached wild type in color. Specific experiments designed to clarify the meaning of this observation were carried out by EPHRUSSI and BEADLE (1936b) as a result of which they interpreted the color of wild type discs grown in claret hosts to be phenotypically like claret only when the transplantations were made within 80 hours after egg-laying (at 25°C). If the same transplantations were made shortly before puparium formation (about 106 hours after egg-laying), the resulting implant was phenotypically close to wild type. They interpreted these results as indicating the presence of a critical period in the larval life of a wild type fly, between 80 and 106 hours after egg-laying, during which $ca^+$ substance moved from the body to the eye. They inferred that after this critical period the transplantation of a wild type optic disc to a host unable to supply this substance would not necessarily modify the normal course of pigment development in the disc.

Along with these results EPHRUSSI and BEADLE (1936b) also reported that additional and more sensitive tests showed that the claret host does

[3] Standard genetic symbols for the mutants of *Drosophila melanogaster* are used; for their significance consult MORGAN, BRIDGES, and STURTEVANT (1925). A list of the races mentioned along with their symbols follows: +, wild type (an inbred stock of +Oregon-R was used in all experiments); *bw*, brown; *ca*, claret; *car*, carnation; *cd*, cardinal; *cm*, carmine; *cn*, cinnabar; $g^2$, garnet-2; *p*, pink; *rb*, ruby; *st*, scarlet; *se*, sepia; *v*, vermilion; *w*, white; $w^e$, eosin.

In addition, a shorthand notation employed by BEADLE and EPHRUSSI (1937) for designating transplantations of optic discs is also used. For example, *v* in +→+ means that an operation has been performed in which a genetically vermilion optic disc has been transplanted to a wild type host, has differentiated, and upon comparison with implants from the control operations, (*v* in *v*) and (+ in +), shows no essential difference in its pigmentation from that found in the implant from the (+ in +) control operation. For details concerning the technique of transplantation in Drosophila see BEADLE and EPHRUSSI (1936) and EPHRUSSI and BEADLE (1936a).

not completely lack $v^+$ and $cn^+$ substances as originally supposed. The bearing of these new observations on the general scheme relating the three postulated diffusible substances was referred to in their paper, but a detailed discussion was not given, possibly because data available at that time were considered inadequate to merit such a discussion.

Shortly afterward, EPHRUSSI and BEADLE (cited in EPHRUSSI and CHEVAIS 1938) found that wild type optic discs grown in vermilion hosts were not quite autonomous in their pigment development. EPHRUSSI and CHEVAIS (1938), who repeated this transplantation, pointed out that the difference in pigmentation between wild type implants grown in vermilion hosts and the control implants was slight. They accounted for this "limited self-differentiation" of the wild type optic disc as being due to the disc's inability to produce enough $v^+$ hormone to completely satisfy its pigment requirement.

Pertinent information on hand when the present studies were begun may be summarized by re-stating the transplantations listed above in the following manner:

(1) + in *ca* → *ca* (before critical period)
(1) + in *ca* → Close to + (after critical period)
(2) *v* in *ca* → Intermediate between *v* and +
(3) *cn* in *ca* → Intermediate between *cn* and +
(4) + in *v* → "limited self-differentiation" of +

Data bearing on the fifth member of the series of transplantations listed earlier are presented in table 1.

BEADLE, ANDERSON, and MAXWELL (1938) took note of the inconsistencies in the evidence for $ca^+$ substance as indicated in the following footnote quoted from their paper: "Experiments as yet unpublished suggest that the so-called '$ca^+$—substance' of Drosophila does not exist as a distinct substance but what may be called the 'claret effect' is one aspect of the action of either $v^+$ or $cn^+$ substance."

The unpublished data mentioned in this footnote were turned over to the writer by PROFESSOR G. W. BEADLE in the summer of 1939 and are presented in the experimental portion of this paper (see table 1).

## MATERIAL AND METHODS

The wild type stock and the mutants used are listed in footnote 3. All flies were cultured at 25°C.

Transplantation operations were performed by the method of EPHRUSSI and BEADLE (1936a), and in all cases where semi-quantitative estimations of $v^+$ and $cn^+$ hormones were made, vermilion-brown and cinnabar-brown test larvae were used as described by TATUM and BEADLE (1938).

In testing whole pupae for content of $v^+$ hormone, a method for securing

TABLE 1

*Differentiation of wild type optic discs implanted into various eye-color mutant hosts. Under the heading "number of individuals" in this and certain other tables to follow, the four sex combinations and total are given in the order: female in female, female in male, male in female, male in male, and total.**

| EXP. NO. | HOST | HOURS AFTER EGG-LAYING | TIME TO PUPARIUM FORMATION (HRS.) | NUMBER OF INDIVIDUALS | PHENOTYPE OF IMPLANTS |
|---|---|---|---|---|---|
| 71 | *ca* | 70–74 | 38–39 | 6-12-0-0:18 | Lighter than +; not *ca* |
| 72† | *ca* | 67–71 | 41–42 | 4-1-0-0: 5 | 2 ♀♀ darker than 71; 2 ♀♀ and 1 ♂ about same as 71 |
| 73 | *ca* | 66–70 | 44–45 | 6-4-0-0:10 | Lighter than + |
| 74 | *ca* | 90–93 | 20–21 | 4-5-0-0: 9 | Same as above (73) |
| 75 | *ca* | 108–111 | 2–3 | 7-3-0-0:10 | Some darker than above, but not + |
| 76‡ | *ca* | 66–70 | — | 2-3-0-0: 5 | Very close to 73 |
| 77§ | *ca* | 66–70 | — | 1-1-0-0: 2 | 1 disc same as lightest +; but darker than 73. 1 disc lighter than +; but darker than average of 73 |
| 78 | *v* | 68–72 | 41–43 | 10-2-4-6:22 | Slightly lighter than + |
| 79 | *v* | 102–105 | 8–9 | 5-0-4-1:10 | Same as 78 |
| 80 | *v* | 68–71 | 45–46 | 1-4-3-3:11 | Slightly lighter than + |
| 81 | *ca* | 69–72 | 44–45 | 1-3-1-3: 8 | Same as 80 |
| 82 | *car* | 71–74 | — | 9-3-6-3:21 | Lighter than + |
| 83 | *cm* | 71–73 | 45–46 | 2-6-3-3:14 | Not *cm*; *ca*-like but not the same |
| 84 | $g^2$ | 67–70 | 45–46 | 2-3-0-0: 5 | Lighter than +; not $g^2$ |
| 85 | $g^2$ | 71–73 | 42–43 | 0-0-3-5: 8 | Same as 84 |
| 86 | *p* | 70–74 | — | 6-8-3-5:22 | Lighter than +; not *p* |
| 87 | *rb* | 69–72 | 42–43 | 2-4-4-3:13 | *ca*-like; close to *rb* |
| 88 | *rb* | 94–96 | 18–19 | 2-4-3-7:16 | Same as 87 |
| 89 | *cd* | 64–68 | 45–46 | 1-3-1-0: 5 | Same as + control |
| 90 | *cn bw* | 70–75 | 42–43 | 10-2-4-7:23 | Same as + control |
| 91 | *ma* | 64–67 | 50–51 | 7-2-0-0: 9 | Same as + control |
| 92 | *se* | 68–71 | — | 5-3-0-0: 8 | Same as + control |
| 93 | *se* | 75–78 | — | 0-0-4-4: 8 | Same as + control |
| 94 | *w* | 70–73 | — | 6-0-0-3: 9 | Same as + control (possibly lighter??) |
| 95 | *w* | 92–98 | 18–27 | 5-6-0-0:11 | Same as + control |
| 96 | *st* | 68–73 | 44–46 | 6-1-4-4:15 | Slightly lighter than +, but close |

* Data kindly furnished by PROFESSOR G. W. BEADLE, STANFORD UNIVERSITY.

† Two + malpighian tubes inplanted 24–26 hours after optic disc transplantation.

‡ One + fat body implanted 42–44 hours after optic disc implantation.

§ Four + malpighian tubes implanted 43–44 hours after optic disc implantation.

bacteriologically sterile larvae (TATUM and BEADLE 1939) was adapted to the feeding technique of BEADLE and LAW 1938). The procedure was as follows: Eggs of wild type and claret were collected over a 24-hour period and allowed to develop on an excess of food. Thirty-six to 60 hours after

puparium formation the pupae were washed, counted, and killed by immersion in boiling water for 45 seconds. The pupae were then transferred aseptically to sterilized 8-dram shell vials and thoroughly mashed. Two drops of a thick suspension of dried brewer's yeast in 2 percent sucrose solution were added to this pulp in order to ensure an excess of food, to inhibit any starvation effect, and to prevent dessication. Bacteriologically sterile vermilion-brown test larvae, 72–76 hours old, were transferred to these vials and on emergence classified as to eye-color modification by comparing them with the genetic eye-color standards of TATUM and BEADLE (1938).

A method for the extraction and measurement of the eye-color pigments is described later in this paper.

## CONCERNING CA$^+$ SUBSTANCE

### *Analysis of data received from G. W. Beadle, Stanford University*

BEADLE re-examined the pigmentation of wild type optic discs recovered from transplantations to claret and various other eye-color mutant hosts. His results are summarized in table 1, where the experiments are arranged in three groups.

Transplants of wild type optic discs to claret are listed in the upper section. In all cases the implant was "lighter than wild type" in color and not-claret. The ages tested range from 45 to 2 hours before puparium formation. These results are at variance with those obtained by EPHRUSSI and BEADLE (1936b) in that the phenotype of a wild type implant grown in a claret host could not be construed as claret. The evidence at hand shows that the earlier interpretation of the color of (+ in *ca*) implants was at fault in that the "lighter than wild type" appearance of the implant in some cases was judged to be phenotypically claret.

In addition to the ordinary optic-disc transplantations, three experiments were performed in which either wild type Malpighian tubes or wild type fat-bodies were implanted along with the optic disc (see legend and footnotes in table 1) in order to determine whether the optic disc would approach wild type pigmentation when these organs, known to be sources of $v^+$ hormone, were present. In the case of an added fat-body, two of the optic discs were darker than those from the (+ in *ca*) controls. With one pair of Malpighian tubes the result was doubtful. Four added Malpighian tubes clearly affected the color of the implant but did not make it so dark as most of the (+ in +) controls. These results indicate that if the amount of $v^+$ hormone in the claret host is increased, the wild type implant is able to use it.

In the middle section data are given for transplants of wild type discs, first to vermilion alone, and then to vermilion and claret for direct comparison of the implants. No differences in pigmentation could be detected

between implants from either of these hosts, and the phenotype was "lighter than wild type."

In the lower section the mutants used as hosts to wild type optic discs are grouped into two classes—(1) those in which the implant attained full wide type pigmentation and (2) those in which the implant failed to do so. Since the operations were done at different times, all possible direct comparisons could not be made. Control implants of host constitution were available, however, and in all instances were distinguishable from the wild type implants. Presumably, the implants designated as "lighter than wild type" were similar to each other and to those from claret and vermilion hosts. Thus, it was found that certain eye-color mutants, carnation, carmine, garnet-2, pink, and ruby gave results essentially similar to claret —that is, the "claret effect" was not limited to claret.

The other group of eye-color mutants, cardinal, maroon, sepia, white, and the double recessive, cinnabar-brown, were not distinguishable from wild type as hosts to a wild type implant. The result with scarlet was doubtful.

All of the eye-color mutant hosts here giving the "claret effect" were known to contain less $v^+$ hormone than wild type (BEADLE and EPHRUSSI 1937). In view of this correlation the original argument for the existence of $ca^+$ substance is no longer tenable.

## TRANSPLANTATION OF (+ in *ca*) AND (+ in +) INVOLVING HOSTS AND DONORS OF DIFFERENT AGES

There still remained some question about (+ in *ca*) implants attaining a pigmentation phenotypically close to wild type as reported by EPHRUSSI and BEADLE (1936b). A series of transplantations of (+ in *ca*) and (+ in +) in which host and donor were of the same age was made both to check on previous observations and to compare with those from the same combinations in which host and donor were of different age. The data are contained in table 2. In all the equal-age combinations the wild type implant recovered from the claret host was lighter red in color than the control implant of comparable age. These implants were simultaneously compared with the age-difference combinations. The latter, along with those equal-age combinations with which they were directly compared, are distinguished in table 2 by a code indication of the age relation between host and donor. Those transplantations which have no code indication are equal-age combinations not used in these comparisons but made to complete the series at close age-intervals up to puparium formation. The specific comparisons made in each case are stated in the last column of the table. In addition, figure 1 illustrates graphically the transplantation combinations and cross-comparisons made in specific experiments.

Within the limits of the age-differences tested, a wild type optic disc

TABLE 2

*Transplants of wild type optic discs to claret and wild type hosts. In column four under the heading "code," Y = young, and O = old. For further explanation see text. The numbers in parentheses give the mean age of the larvae at the time of transplantation.**

| EXP. NO. | CONSTITUTION AND AGE IN HOURS OF DONOR | HOST | CODE | NUMBER OF INDIVIDUALS | PHENOTYPE OF IMPLANTS (COMPARISONS) |
|---|---|---|---|---|---|
| 513 | +(93) | +(93) | Y in Y | 2-0-0-0:2 | Same as 508, 510, and 512, but darker than 514 |
| 503 | +(103) | +(103) | O in O | 2-0-0-0:2 | Darker than 500 and 501 |
| 508 | +(113) | +(113) | O in O | 2-2-1-2:7 | Same as 513, 510, and 512 |
| 525 | +(121) | +(121) | — | 5-0-0-0:5 | Darker than 524; different from *ca* |
| 510 | +(91) | +(114) | Y in O | 2-0-0-0:2 | Same as 512 and 508 |
| 512 | +(115) | +(92) | O in Y | 0-2 0 0:2 | Same as 510 and 508 |
| 514 | +(94) | *ca* (94) | Y in Y | 2-0-0-0:2 | Lighter than 510, 512, and 513; same as 515 |
| 500 | +(96) | *ca* (96) | — | 0-0-1-0:1 | Lighter than 503; |
| 501 | +(97.5) | *ca* (97.5) | — | 1-0-0-0:1 | Same as 500 |
| 517 | +(115) | *ca* (115) | O in O | 1-0-0-0:1 | Lighter than +; same as 516 |
| 535 | +(119) | *ca* (119) | — | 6-1-0-0:7 | Lighter than + |
| 524 | +(120) | *ca* (120) | — | 3-0-0-0:3 | Lighter than 525; different from *ca* |
| 530 | +(122) | *ca* (122) | — | 1-3-0-0:4 | Lighter than + |
| 547 | +(124) | *ca* (124) | — | 3-1-0-0:4 | Lighter than + |
| 516 | +(90) | *ca* (114) | Y in O | 4-0-0-0:4 | Same as 517 |
| 515 | +(117) | *ca* (94) | O in Y | 2-0-0-0:2 | Same as 516 |

* The mean age was calculated from the mean time of the egg-laying period to the mean time of the transplantation period. In this and the following tables in which the ages of larvae are given, the maximum deviation from the mean is $\pm 2.5$ hours.

grown in a wild type host is readily distinguishable from one grown in a claret host regardless of the age combination of the latter with respect to donor and host. Since the age of the youngest larvae involved in these transplantations exceeded by 10 to 15 hours that point which EPHRUSSI and BEADLE (1936b) assumed to be the beginning of the critical period for the movement of $ca^+$ substance from body to eye, the constant difference between (+ in +) and (+ in *ca*) implants cannot be explained by their hypothesis.

## COMPARATIVE MEASUREMENTS OF $v^+$ HORMONE IN WILD TYPE AND CLARET

As mentioned above, claret along with a number of other eye-color mutants is characterized by having a reduced amount of $v^+$ hormone as compared to wild type. Since this fact is here used to account for the so-called "claret effect," some idea of the magnitude of the difference between claret and wild type in this respect seemed desirable. Measurements of the amount of $v^+$ hormone released by transplants of Malpighian tubes and fat-bodies were therefore made. In addition, crushed whole pupae of wild

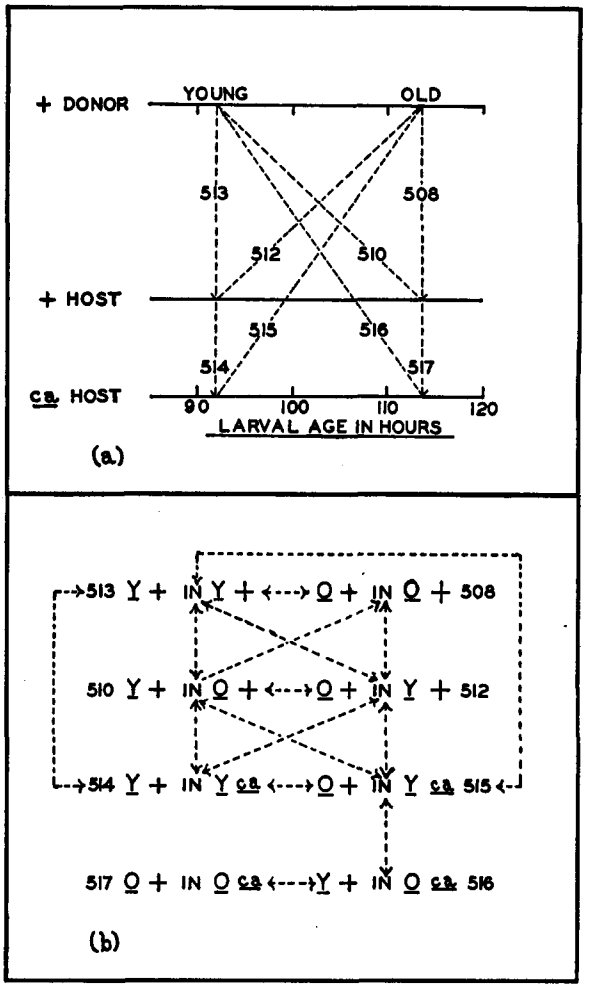


FIGURE 1.—(a) Diagram indicating age relations between host and donor for the transplantations recorded in table 2.—(b) Diagram showing the comparisons made between implants in the experiments recorded in table 2; see table 2 for significance of abbreviations.

type and claret were tested as to their content of $v^+$ hormone by feeding them to vermilion-brown test larvae.

### (a) *Transplantation of Malpighian tubes and fat-bodies*

BEADLE (1937a, 1937b) tested both the Malpighian tubes and fat-bodies of wild type and most of the eye-color mutants for $v^+$ and $cn^+$ hormones. His tests were made before a semi-quantitative method for the evaluation of hormone activity was available. Results of the present tests are recorded in table 3.

In terms of unit hormone activity (TATUM and BEADLE 1938), wild type Malpighian tubes are some 19 times as active as those of claret.

TABLE 3

*Tests for the release of $v^+$ hormone. The units of hormone per implant are calculated on the basis of the maximum color modification attained by the vermilion-brown test animal.*

| IMPLANT | NUMBER OF INDIVIDUALS | MODIFICATION OF THE HOST'S EYES | | | |
|---|---|---|---|---|---|
| | | COLOR VALUES | | UNITS PER IMPLANT | RATIO + TO *ca* |
| | | MEAN | RANGE | | |
| Malpighian Tubes: | | | | | |
| *ca* | 5-2-0-0: 7 | 0.34 | (0.3–0.5) | 0.65 | 19.2 |
| + | 6-2-1-2:11 | 3.72 | (3.5–4.0) | 12.50 | |
| *Fat Bodies: | | | | | |
| *ca* | 7-3-3-2:15 | 2.4 | (1.8–2.5) | 3.5 | 2.3 |
| + | 3-2-0-1: 6 | 3.1 | (2.5–3.5) | 8.2 | |

* The fat-body transplantations were made at the UNIVERSITY OF OREGON, and the work was assisted by a grant-in-aid from the General Research Council, Oregon State System of Higher Education.

When fat-bodies are considered on the same basis, the difference between claret and wild type, although in the same direction, is not so striking. Wild type fat-bodies release slightly more than twice as much $v^+$ hormone as do those of claret. From this it is evident that the Malpighian tubes and fat-bodies are affected differentially in their relation to $v^+$ hormone by the mutation $ca^+ \rightarrow ca$.

### (b) *Feeding experiments with pupae of + and ca*

The details of the procedure employed in this test are given above, under the section Materials and Methods, and the results are recorded in table 4.

If either mean color-values or maximum units of hormone are used as a basis of comparison, the ratio of wild type to claret is 2.8. It may be inferred from this rather crude test that between 36 and 60 hours after puparium formation a wild type pupa contains about three times as much $v^+$ hormone as a claret pupa of the same age.

### *Transplants of* (+ in *ca*) *and* (+ in *w*; *ca*)

It is known that a wild type optic disc can produce some $v^+$ hormone itself and that this amount is not enough for its total pigment requirement (E. B. CLANCY 1940). BEADLE's data (see table 1) show that its pigment is still only partially differentiated when it is grown in hosts (*ca*, *cm*, et al.) containing reduced amounts of $v^+$ hormone. Moreover, BEADLE's experiments (see footnotes table 1) in which wild type Malpighian tubes or fat-bodies served as additional sources of $v^+$ hormone indicate that complete pigment differentiation is possible in an otherwise claret milieu. A possible objection to the latter experiments is that the sources of extra $v^+$ hormone

TABLE 4

*Data showing the effect on the eye color of vermilion-brown test anima s of feeding crushed pupae of wild type and claret.*

| EXPER. NO. | VIAL NO. AND STOCK | NUMBER OF INDIVIDUALS ♀♀ | ♂♂ | TOTAL | COLOR VALUES MEAN | RANGE | MAXIMUM UNITS HORMONE |
|---|---|---|---|---|---|---|---|
| 721a | (1) *ca* | 5 | 4 | 9 | 0.62 | (0.5–0.8) | 0.85 |
| | (2) *ca* | 2 | 7 | 9 | 0.52 | (0.5–0.6) | 0.70 |
| | (3) *ca* | 7 | 6 | 13 | 0.51 | (0.3–0.7) | 0.78 |
| | (4) *ca* | 2 | 6 | 8 | 0.40 | (0.3–0.6) | 0.70 |
| 722a | (1) *ca* | 5 | 2 | 7 | 0.80 | (0.5–1.0) | 1.00 |
| | (2) *ca* | 2 | 2 | 4 | 0.60 | (0.4–0.7) | 0.78 |
| | (3) *ca* | 3 | 3 | 6 | 0.58 | (0.5–1.c) | 1.00 |
| | | 26 | 30 = | 56 | m=0.58 | | m=0.83 |
| 721b | (1) + | 4 | 4 | 8 | 1.80 | (1.5–2.1) | 2.50 |
| | (2) + | 2 | 6 | 8 | 1.90 | (1.5–2.0) | 2.30 |
| | (3) + | 3 | 3 | 6 | 1.20 | (0.8–1.8) | 2.00 |
| | (4) + | 4 | 3 | 7 | 2.00 | (———) | 2.30 |
| 722b | (1) + | 7 | 3 | 10 | 1.50 | (0.8–2.1) | 2.50 |
| | (2) + | 4 | 3 | 7 | 1.70 | (1.0–2.1) | 2.50 |
| | | 24 | 22 = | 46 | m=1.68 | | m=2.35 |
| Control | *v; bw* | 4 | 3 = | 7 | 0.0 | | |

TABLE 5

*Transplants of wild type optic disc to claret and white-claret hosts. All transplantations were made shortly before puparium formation.*

| EXPER. NO. | HOST | NUMBER OF INDIVIDUALS | PHENOTYPE OF IMPLANTS |
|---|---|---|---|
| 528 | *w* | 4-2-0-0:6 | Same as 531; darker than 530 |
| 529 | *w* | 0-0-4-2:6 | Same as 531; darker than 530 |
| 530 | *ca* | 1-3-c-c:4 | |
| 531 | + | 2-1-0-0:3 | |
| 534 | *w; ca* | 3-2-0-0:5 | Not so dark as 536; darker than 530 |
| 535 | *ca* | 6-1-0-0:7 | Lighter than 534 |
| 536 | + | 2-3-0-0:5 | |
| 546 | *w; ca* | 4-3-0-0:7 | 4 lighter than 548; 3 same as 548* |
| 547 | *ca* | 3-1-0-0:4 | Lighter than either 546 or 548 |
| 548 | + | 2-2-0-0:4 | |

* See text for further notes on this experiment (546 and 548).

may also supply $ca^+$ substance. To obviate such criticism, transplants of wild type discs to white-claret and claret were compared on the assumption that by introducing the white gene the effective concentration of $v^+$ hormone would be increased by whatever amount is ordinarily utilized in claret pigmentation. This assumption is an adaptation of principles worked out by EPHRUSSI and CHEVAIS (1937, 1938) in their studies on the relations between production, utilization, and release of diffusible substances in eye-color development.

Transplants were first made to white, claret, and wild type. Implants (see table 5) from (+ in *w*) and (+ in +) were indistinguishable. Implants from (+ in *w*; *ca*) were darker than those from (+ in *ca*), but not so dark as those from white or wild type hosts. An exception, in which the implants were as dark as those from wild type hosts, was found in experiment 546 (see table 5). In this experiment all donor discs were derived from wild type females. The protocol is as follows:

(+ ♀ discs in *w; ca*)

| | *Hosts* | *Implants* | |
|---|---|---|---|
| (a) | 3 ♀ ♀ | medium-sized discs, dark in color | |
| | 1 ♂ | small-sized disc, dark | |
| (b) | 1 ♀ | large-sized disc | All lighter than above. |
| | 2 ♂ ♂ | large-sized discs | |

The discs from (a) were indistinguishable from those of experiment 548 (+ in +), while those from (b) were slightly lighter in color. All of the latter were darker, however, than implants from experiment 547 (+ in *ca*). It appears obvious that the size of the implant to some extent determines the intensity of color attained in a given hormone environment, but this does not detract significance from the fact that some wild type implants attained full wild type pigmentation in white claret hosts.

To explain these results it is assumed that, because it completely blocks pigment formation, the presence of the white gene in the double recessive white-claret raises the concentration of $v^+$ hormone to a level where its utilization by the wild type implant in forming additional brown pigment may be detected by this method of observation. The amount of $v^+$ hormone made available to the implant by this means is not sufficient for the attainment of full wild type pigmentation unless the implant is smaller than usual for these transplants.

### PIGMENT MEASUREMENTS

After the above experiments were under way an opportunity to extend them arose when equipment became available for measuring eye-color pigment. The studies reported in this section represent part of an attempt to

characterize the action of the claret gene in relation to the general scheme of eye-color development outlined by BEADLE and EPHRUSSI (1936), EPHRUSSI and CHEVAIS (1938), and BEADLE and TATUM (1941).

Attention is called to the pioneering work of SCHULTZ (1932, 1935), MAINX (1937, 1938), and BECKER (1939) with Drosophila eye pigments which showed that the + pigment consists of two types of pigments: the brown and the red components and thus formed the working basis upon which the methods employed here were devised.

The action of the mutant gene claret on the pigment components of the eye was studied (1) by comparing the amount of red and brown pigment contained in the eyes of wild type and claret and (2) by noting the kind and quantity of pigment present in the eyes of double recessive stocks made up of claret plus some other eye-color gene known to block the formation of either the red or brown pigment component. The latter technique is essentially the same as that used by MAINX (1938) in his more general analysis of gene action.

*Method for extraction and measurement of pigment*

Eggs of the desired stocks were collected over 24-hour egg-laying periods on food contained in small metal trays. The larvae when 30 to 48 hours old were transferred (100 to 125 larvae per bottle) to culture bottles containing the standard medium enriched with dried brewer's yeast. It was hoped that this procedure would provide optimal growth conditions (at 25°C and a relative humidity of 70–80 percent) and that a characteristic maximal size would be attained which might be considered fairly constant for flies of a given genetic constitution. In the early experiments no other attempt to control the size factor was made. In some of the later experiments an indirect check was made by weighing male sibs of the flies used for extracts. Males were used instead of females because of the variable weight of developing eggs in the latter. All extracts were made from the heads of females, and care was taken to secure a random sample of the flies hatching from any given series of bottles. Decapitation was performed 24 to 48 hours after emergence, and an effort was made to use only uninjured heads. Each extract was ordinarily prepared from 100 heads. In a few instances where the pigment content was known to be low, 200 to 500 heads were used. In all cases, however, the values recorded in tables 6 to 10, inclusive, are based either directly on the pigment extracted from 100 heads contained in 10.0 cc of solvent, or have been calculated to that base.

The measurements of dissolved pigment were made with an Evelyn Photo-electric Macro-colorimeter manufactured by the Rubicon Company, Philadelphia. They are recorded in terms of "Photometric Density," a value directly proportional to the relative concentration of pigment, as

defined in the manual accompanying this instrument. Absorption curves for solutions of the eye pigments were obtained from DR. E. L. TATUM (unpublished) and a filter selected which allowed passage of wave lengths of light corresponding to the region of maximum absorption. The particular filter used with the above colorimeter bears the code number, 440, indicating in millimicrons the wave length of maximum transmission. Dilution curves for solutions of the pigments in the solvents described below were supplied by PROFESSOR G. W. BEADLE (unpublished). When necessary, extracts were diluted to a concentration range where the relation between "Photometric Density" and concentration is strictly linear.

In preparing pigment extracts from the heads of wild type and claret the aim was to remove the water-soluble red pigment first and then to remove by means of a different solvent the relatively insoluble brown pigment. This was known to be soluble at room temperature in 2N hydrochloric acid (BECKER 1939) and in an anhydrous solution of 1.0 percent H Cl in methyl alcohol (E. L. TATUM, unpublished). All extractions of the brown pigment reported here were made at room temperature with the latter solvent. For convenience it will be referred to as "Solvent B."

Various attempts were made to quantitatively extract the red pigment from intact whole heads by treating them with water. None of these trials was successful, and the details need not be related here. The solvent finally used for removing the red pigment was a solution of 30 percent ethyl alcohol acidified with H Cl to pH 2.0. When this solvent was tested on the heads of vermilion, scarlet, and cinnabar flies, it removed the pigment readily and completely. It will be referred to as "Solvent A."

When Solvent A was originally tested on the heads of the mutant brown, it was concluded (unjustifiably, it now appears) that the brown pigment characterizing the eye color of this mutant was quite insoluble in this solvent. However, after soaking the heads for almost two months in Solvent A, a value was obtained which corresponds to about 15 percent of the total amount of brown pigment extractable with Solvent B. For this reason the method of double-extraction described below for the removal of pigment from wild type and claret is definitely open to criticism. Corrections in the pigment values obtained for wild type and claret could be made, utilizing the maximum value obtained for the control. This has not been done, because critical evidence necessary to justify such a procedure is lacking.

The procedure in the case of wild type and claret was to place the intact heads immediately after decapitation into a small homoeopathic vial containing 2 to 3 cc of Solvent A. Several days later (12 to 38 days is the range for the experiments reported) the extract containing most of the pigment was quantitatively removed and a fresh supply of solvent added. This was

removed after a few days, combined with the first extract, filtered under reduced pressure through ground glass, and brought up to 10.0 cc volume with the pure solvent. The relative amount of pigment contained in this volume of extract was then measured either directly or after dilution to an appropriate concentration as explained above. After this treatment for the removal of the red pigment with Solvent A, the heads were rinsed for a few seconds in absolute methyl alcohol, and 2 to 3 cc of Solvent B were added for removal of the residual brown pigment. Shortly before taking measurements, the brown pigment was reduced by the addition of a few crystals of sodium hydrosulfite.

The extraction of brown pigment was usually accomplished in two to three days, although in some experiments the heads were exposed to the solvent for a longer time. Comparable experiments indicated that the brown pigment solutions were quite stable at room temperature, permitting considerable latitude in time for handling the extracts. This is not true for solutions of the red pigment at room temperature. A collateral experiment carried out during the time that most of the pigment measurements were made showed that the percentage concentration of pigment decreased with time. Comparable solutions of red pigment were kept at room temperature, at 25°C, and in the refrigerator (6° to 8°C) and measured from time to time during the course of 68 days. The data are contained in table 11, and self-explanatory curves are given in figure 2. Here again, corrections in the original measurements have not been made because of the lack of pertinent data. In all cases involving the red pigment the lapse of time (in days) between decapitation and measurement is indicated in the tables.

After the heads of vermilion, scarlet, and cinnabar are extracted with Solvent A, they appear white when examined under a low power binocular microscope, indicating that practically complete removal of the pigment has been effected. The same is true after extraction of the brown pigment with Solvent B from the heads of brown and brown-claret. In contrast, a residual pinkish color may be noted in the heads of wild type and claret after they have been given the double extraction described above. If the heads are extracted with Solvent B alone, they are white. It therefore appears that the treatment with Solvent A in some way interferes with the subsequent extraction by Solvent B. The pinkish color of the chitin and soft parts of the head may be evidence of retained red or brown eye pigment, but this is not certainly known, and in any case the amount left is extremely small.

## RESULTS OF PIGMENT MEASUREMENTS

Pigment measurement data are contained in tables 6 to 10, inclusive. Table 10 summarizes in terms of mean values and percentage comparisons the data of tables 6 to 9 and will be useful for reference.

TABLE 6

*The relative amount of red and brown pigment contained in extracts of intact whole heads of various stocks of Drosophila melanogaster. In this and the following three tables (table 6–9, inclusive) the numbers followed by an asterisk are the mean values for the pigment extracts used for comparative purposes in table 10.*

| LOT NO. | EXPER. NO. | STOCK | PHOTOMETRIC DENSITY | |
|---|---|---|---|---|
| | | | RED PIGMENT (SOLVENT A) | BROWN PIGMENT (SOLVENT B) |
| B-1 (37) | 595 | *bw* | 0.020 | |
| B-2 (56) | 670–1 | " | 0.014 | |
| | | | m=0.017* | |
| D/B-1 | 672–1 | *v; bw* | 0.0055 | |
| (38) | 672–2 | " | 0.0055 | |
| | | | m=0.0055* | |
| | (BEADLE) | *v; bw* | | 0.0088 |
| | " | " | | 0.0066 |
| | | | | m=0.0077* |
| | (BEADLE) | *cn bw* | | 0.0044 |
| | " | " | | c.0044 |
| | | | | m=0.0044* |
| A-2 | 61[illegible] | +Ore-R | 0.848 | 0.1308 |
| (29) | [illegible]18 | " | 0.822 | 0.1382 |
| | 619 | " | 0.875 | 0.1352 |
| | 620 | " | 0.862 | 0.1163 |
| | 621 | " | 0.848 | 0.1249 |
| | | | m=0.851 | m=0.1291 |
| A-3 | 663–1 | " | 0.862 | 0.1065 |
| (50) | 663–2 | " | 0.888 | 0.0969 |
| | 663–3 | " | 0.862 | 0.1024 |
| | 663–4 | " | 0.848 | 0.0996 |
| | | | m=0.865 | m=0.1013 |
| | | | m=0.858* | m=0.1152* |
| C-1 | 606 | *ca* | 0.1264 | 0.0315 |
| (12) | 609 | " | 0.1235 | 0.0339 |
| | 610 | " | 0.1278 | 0.0339 |
| (21) | 614 | " | 0.1235 | 0.0339 |
| | | | m=0.1253 | m=0.0333* |
| C-2 | 622 | " | 0.1163 | |
| (29) | 624 | " | 0.1264 | |
| | 625 | " | 0.1337 | |
| | | | m=0.1255 | |
| | | | m=0.1254* | |

TABLE 7

*The relative amount of red pigment contained in extracts of the heads of vermilion, cinnabar, and their double recessive combination with claret.*

| LOT NO. | EXPER. NO. | STOCK | PHOTOMETRIC DENSITY (L) RED PIGMENT (SOLVENT A) |
|---|---|---|---|
| D-1 | 612 | *v* | 0.656 =0.656 |
| (12) | | | |
| D-2 | 615 | " | 0.615 |
| (29) | 616 | " | 0.527 |
| | | | m=0.571 |
| D-3 | 664 | " | 0.706 =0.706 |
| (16) | | | |
| | | | m=0.645* |
| D/C-1 | 631 | *v; ca* | 0.1110 |
| (27) | 632 | " | 0.1135 |
| | | | m=0.1123 |
| D/C-2 | 637 | *v; ca* | 0.1086 |
| | 638 | " | 0.1036 |
| | | | m=0.1061 |
| | | | m=0.1092* |
| E-2a | 643 | *cn* | 0.783 |
| (27) | 644 | " | 0.809 |
| | 645 | " | 0.809 |
| | 646 | " | 0.809 |
| | | | m=0.802 |
| E-2b | 650–1 | *cn* | 0.888 |
| (28) | 650–2 | " | 0.862 |
| | | | m=0.875 |
| E-3 | 665–1 | *cn* | 0.942 |
| (19) | 665–2 | " | 0.942 |
| | 665–3 | " | 0.942 |
| | | | m=0.942 |
| | | | m=0.873* |
| E/C-1 | 633 | *cn; ca* | 0.1575 |
| (27) | 634 | " | 0.1566 |
| | | | m=0.1570 |
| E/C-2a | 676–1 | *cn; ca* | 0.1397 |
| (38) | 676–2 | " | 0.1397 |
| | | | m=0.1397 |
| | | | m=0.1484* |

TABLE 8

*The relative amount of brown pigment contained in extracts of the heads of the mutant brown and the double recessive, brown-claret.*

| LOT NO. | EXPER. NO. | STOCK | PHOTOMETRIC DENSITY (L) BROWN PIGMENT (SOLVENT B) | WEIGHT ♂♂ MG/100 |
|---|---|---|---|---|
| B, B/C | 724-1 | *bw* | 0.1135 | 23.4 |
| (3) | 724-2 | *bw* | 0.1121 | |
| | | | m=0.1128* | |
| | 724-3 | *bw; ca* | 0.0327 | 23.0 |
| | 724-4 | *bw; ca* | 0.0327 | |
| | | | m=0.0327* | |
| A, B-1 | 723-5 | *bw* | 0.1135 | 23.0 |
| (3) | 723-6 | *bw* | 0.1135 | |
| | | | m=0.1135* | |
| B/C-2 | 690-1 | *bw; ca* | 0.0327 | 23.5 |
| (25) | | *bw; ca* | 0.0327 | |
| | | | m=0.0327* | |

TABLE 9

*See text for discussion of data listed here.*

| LOT NO. | EXPER. NO. | STOCK | PHOTOMETRIC DENSITY RED PIGMENT (SOLVENT A) | PHOTOMETRIC DENSITY BROWN PIGMENT (SOLVENT B) | WEIGHT ♂♂ MG/100 |
|---|---|---|---|---|---|
| A, C-1 | 691-1 | +Ore-R | 0.996 | 0.1308 | 23.7 |
| (17) | 691-3 | " | 0.996 | 0.1337 | |
| | | | m=0.996* | m=0.1322* | |
| | 691-2 | *ca* | 0.1457 | 0.0223 | 21.2 |
| | 691-4 | " | 0.1472 | 0.0188 | |
| | | | m=0.1464 | m=0.0205 | |
| Corrected for weight difference between ♂♂ | | | m=0.1636* | m=0.0229* | |
| Pigment Ratios: | | | | | |
| $\frac{ca}{+\text{Ore-R}}$ | | | $\frac{0.1636}{0.996}\times 100 = 16.4\%$ | $\frac{0.0229}{0.1322}\times 100 = 17.3\%$ | |

Control experiments are recorded in the upper section of table 6. Experiments Nos. 595 and 670-1 were independent determinations of the action of Solvent A on the heads of brown flies. In one experiment (595) the heads were extracted for 37 days, and in the other (670-1) for 56 days. The mean value of the two lots is 0.017, which corresponds to approximately 15.0

TABLE 10

*Comparative summary of the data contained in tables 6–9.*

| STOCK | RED PIGMENT MEAN (L) | RED PIGMENT PERCENTAGE | BROWN PIGMENT MEAN (L) | BROWN PIGMENT PERCENTAGE |
|---|---|---|---|---|
| +Ore-R | 0.858 | 100.0 | 0.115 | 100.0 |
| *ca* | 0.125 | 14.6 | 0.033 | 28.7 |
| *+Ore-R | 0.996 | 100.0 | 0.132 | 100.0 |
| *ca* | 0.164 | 16.5 | 0.023 | 17.4 |
| *v* | 0.645 | 100.0 | — | — |
| *v; ca* | 0.109 | 16.9 | — | — |
| *cn* | 0.873 | 100.0 | — | — |
| *cn; ca* | 0.148 | 17.0 | — | — |
| *bw* | — | — | 0.113 | 100.0 |
| *bw; ca* | — | — | 0.033 | 29.2 |
| *ca* | — | — | 0.033 | 29.2 |
| +Ore-R | — | — | 0.115 | 100.0 |
| *bw* | — | — | 0.113 | 98.2 |
| *ca* | — | — | 0.033 | 28.7 |
| *bw; ca* | — | — | 0.033 | 28.7 |
| +Ore-R | 0.858 | 100.0 | — | — |
| *v* | 0.645 | 75.2 | — | — |
| *cn* | 0.873 | 101.8 | — | — |

* Data shown in table 9, and discussed in text.

TABLE 11

*Data showing the effect of temperature and age on extracts of the red pigment made with Solvent A. See text and figure 2.*

| DAYS | REFRIGERATOR 6°–8°C PHOTOMETRIC DENSITY (L) | REFRIGERATOR 6°–8°C (L) IN % | ROOM 20°–23°C PHOTOMETRIC DENSITY (L) | ROOM 20°–23°C (L) IN % | INCUBATOR 25°±0.5°C PHOTOMETRIC DENSITY (L) | INCUBATOR 25°±0.5°C (L) IN % |
|---|---|---|---|---|---|---|
| 0 | 0.710 | 100.0 | 0.710 | 100.0 | 0.710 | 100.0 |
| 4 | 0.710 | 100.0 | 0.699 | 98.5 | 0.699 | 98.5 |
| 14 | 0.710 | 100.0 | 0.668 | 94.1 | 0.658 | 92.7 |
| 30 | 0.688 | 96.9 | 0.629 | 88.6 | 0.620 | 87.3 |
| 68 | 0.673 | 94.8 | 0.573 | 80.7 | 0.542 | 76.4 |

percent of the total value obtained when heads of the same mutant are extracted with Solvent B. On the basis of other evidence (see BEADLE and TATUM 1941) the particular allele of *bw* used here should block rather completely the formation of any water-soluble red pigment. Assuming this to

be true, the result of extracting brown heads with Solvent A may mean (1) that the water-insoluble brown pigment is to a certain extent (slowly) soluble in 30 percent acid ethyl alcohol, (2) that the eye pigment differentiated by the *bw* allele used here consists of two components one of which is soluble in Solvent A and the other not, (3) that under the conditions of the experiment, chemical changes occurred in the medium which rendered a portion of the otherwise insoluble brown pigment soluble in Solvent A.

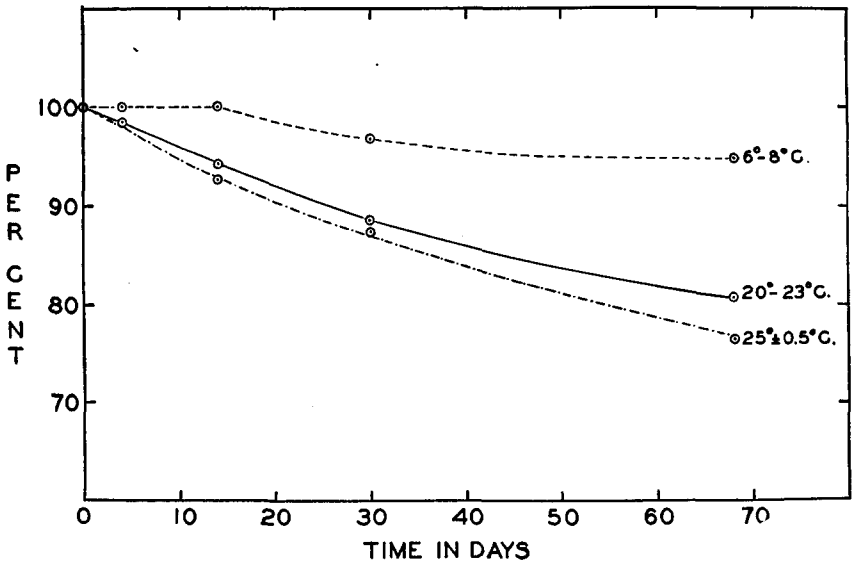


FIGURE 2.—Graphical representation of data given in table 11.

There probably are other alternatives, but the data on hand permit of no choice in explanations.

The other control experiments show the relative amount of colored material that can be extracted from the heads of stocks so constituted genetically as to block the formation of both the brown and the red pigment components. In the case of vermilion-brown extracted with Solvent A (672-1), the value obtained is about 0.5 percent of the maximum amount of colored material extractable from wild type or cinnabar with this solvent. On the other hand, vermilion-brown extracted with Solvent B gives a value which is approximately 6.7 percent of that obtained for wild type or brown with the same solvent. Cinnabar-brown extracted with Solvent B gives a lower value, corresponding to 3.8 percent of that from brown. This agrees with the observation that the eyes of the double recessive, cinnabar-brown, appear whiter than those of vermilion-brown. It is not known whether or not these basic values of colored material represent dissolved pigment derived from granules contained in the ommatidial cells, basement membrane, or from pigments unrelated to the eye.

The lower sections of table 6 give the data on wild type (+Oregon-R) and claret. The pigment relations between these two stocks given in terms of percent are shown in table 10. According to these data the claret mutant contains only 14 to 15 percent as much pigment extractable with Solvent B as does wild type.

Table 7 records measurements taken on stocks in which no brown pigment component is present, hence all extractions were made with Solvent A. The purpose here was to determine the action of the *ca* gene on the red pigment component alone. Comparisons in terms of percentage are given in table 10. The combination of claret with vermilion or cinnabar reduces the content of pigment to approximately 17 percent of that present in either alone.

Table 8 gives the data and table 10 the comparisons of the reciprocal arrangement wherein the effect of claret on the brown pigment component is demonstrated. The combination brown-claret contains about 29 percent as much pigment extractable with Solvent B as does brown alone. This value is almost identical with that obtained for the differential between wild type and claret with respect to the same pigment component and tends to encourage confidence in the validity of the double extraction method involving the use of Solvent A. However, in a final experiment (691, table 9) in which wild type and claret were raised in the same bottle, the sib males weighed, and the mean color values corrected for the weight difference of the males of the two stocks, there was no such agreement. In this experiment the relation between claret and wild type with respect to the red pigment is probably not significantly different from that previously noted. With respect to the brown component, however, the ratio is definitely different, claret in this experiment having only 17.3 percent as much pigment as compared to the 29 percent previously noted. This new value, 17.3 percent, is very similar to the values obtained for the differential action of the mutant gene claret on the red pigment component.

## DISCUSSION

EPHRUSSI and CHEVAIS (1938) used the term "*l'autodifférenciation limitée*" to characterize the type of development a wild type optic disc undergoes when transplanted to a vermilion host. It should be clear that the so-called "claret effect" refers to this limited self-differentiation of the wild type optic disc. As shown by EPHRUSSI and CHEVAIS (1938) and by experiments reported in the first section of this paper, the wild type optic disc is dependent on sources other than its own tissues for enough $v^+$ hormone to develop its characteristic pigmentation. So far as is now known, $v^+$ hormone is concerned only in reactions leading to the formation of the

brown pigment component of Drosophila eye color; consequently, one might assume that the "lighter than wild type" appearance of wild type optic discs grown in claret and vermilion hosts is due to a deficiency for the normal complement of brown pigment. Although wild type optic discs can produce $v^+$ hormone (E. B. CLANCY 1940) when grown in vermilion hosts, the question arises as to whether or not such discs contain any brown pigment at all. To answer this, (+ in *v*) implants were extracted with Solvent A to remove the red pigment and compared under a binocular microscope with (+ in +) implants treated in the same way. Wild type discs from vermilion hosts show a definite residuum of brown pigment, but the amount present is less than that in (+ in +) implants. It was noted above that wild type implants recovered from vermilion (lacking $v^+$ and $cn^+$ hormones) and from claret (some $v^+$ and $cn^+$ hormones) hosts were indistinguishable by the ordinary method of observing such implants. There are at least two alternative explanations for this fact—either the quantity of brown pigment formed by virtue of the additional hormone supplied by the claret host is so small that a difference cannot be detected by the eye, or a definite concentration level of hormone in the host is necessary for the formation of additional brown pigment.

No account has thus far been taken of the possibility that the red pigment component of a wild type disc may be qualitatively or quantitatively affected by its development in hosts giving the "claret effect." Since the red pigment acts as a pH indicator and can be reversibly reduced to a colorless compound (see BEADLE and TATUM 1941), it is certainly possible that the particular reddish hue ("lighter than wild type") observed is conditioned by the pH and/or the oxidation-reduction potential of the medium in which the disc develops, or in which it is observed. Preliminary measurements (unpublished) of the red pigment from (+ in +) and (+ in *ca*) implants, however, indicate that there is no difference in the amount of red pigment they contain, but the data are inadequate for a proof of this point.

The claret gene is evidently concerned in reactions involved in the production (or destruction) of $v^+$ hormone. It is also concerned in the utilization of hormone in the production of pigment, since claret optic discs produce more $v^+$ hormone than they use in developing claret pigmentation (EPHRUSSI and BEADLE 1937). The latter fact makes it clear that the amount of $v^+$ hormone produced by claret eye tissue is not the limiting factor in determining the quantity of the brown pigment component in claret eye color. Since both pigment components are affected by the mutation $ca^+ \rightarrow ca$, the claret gene is evidently related in its action to the common step controlled by the white gene in the scheme of eye-color develop-

ment outlined by BEADLE and TATUM (1941). Any attempt to assign a single primary action to the claret gene in this scheme must relate reactions involved in the production of $v^+$ hormone to those controlled by the white gene.

LUERS and STUBBE (1940) have reported the results of tests for free $ca^+$ substance in the body fluid of the wild types of several species of Drosophila, including *D. melanogaster*. As test implants they used the optic discs of the double recessive, $w^e$; *ca*, of *melanogaster*. They found that the test implant developed a pigmentation only slightly darker than the control implant (one unit as measured on a Ridgway Color Chart). Eosin implants are three Ridgway units darker than eosin-claret. LUERS and STUBBE conclude that at the ages at which larvae are normally employed for these transplantations the wild type of all three species contains an insufficient amount of $ca^+$ substance [to permit development of the eosin phenotype?].

In view of the fact that the mutant gene claret limits the utilization of $v^+$ hormone to less than can be produced by its own tissues, a completely negative result in the case of these tests would have been more easily explained. The fact that some modification of the $w^e$; *ca* implant was apparently observed is not easily accounted for without further investigation.

## SUMMARY

Two main problems are considered (1) the validity of evidence for the existence of $ca^+$ substance, and (2) the quantitative effect of the mutant gene claret on the eye-color pigments of *D. melanogaster*.

Results concerned with the first of these questions show that (1) the type of incomplete pigment differentiation exhibited by a wild type optic disc when grown in a claret host is nonspecific and occurs in other hosts known either to lack $v^+$ hormone or to contain it in reduced amount as compared to wild type; (2) differences in developmental age between the wild type implant and the claret host are not responsible for the partial pigment differentiation of the implant; (3) in comparative tests for $v^+$ hormone (release by Malpighian tubes and fat-bodies, and feeding tests of crushed whole pupae) claret gives lower values than wild type; (4) wild type optic discs grown in white-claret hosts (assumed to contain more $v^+$ hormone than claret) approach wild type pigmentation very closely.

In view of these results, the original argument for assuming the existence of $ca^+$ substance is no longer valid.

In connection with the second problem, a method for the extraction and measurement of Drosophila eye pigments is described along with the presentation of pigment measurements.

The data obtained in a series of measurements of eye-color pigment on wild type, claret, and double recessives of claret with vermilion, cinnabar,

and brown show that the mutant gene claret acts in such a way as to reduce the quantity of the red pigment to about 17 percent, and the brown pigment to about 28 percent of that present in wild type.

The relation of the claret gene to the scheme of eye-color development in Drosophila outlined by BEADLE and TATUM (1941) is discussed briefly.

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