A RECONSIDERATION OF THE MODE OF DEVELOPMENT OF
THE BAR EYE OF DROSOPHILA MELANOGASTER

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INTRODUCTION

The sex-linked semi-dominant mutation, Bar, of Drosophila melanogaster (Tice 1914) when homozygous reduces the facet number of the compound eye from approximately 770 (the facet number of the wild type) to about 75. When heterozygous, it reduces the facet number to about 350. Its discovery afforded geneticists an effective tool for the quantitative study of gene action. Only one year after the announcement of its discovery, Zeleney and Mattoon (1915) showed it to be sensitive to selection—that is, to changes in the genotypic environment. Seyster (1919) showed that in Bar facet number varied inversely with temperature and that temperature was effective only during the larval period. To explain his data, Seyster postulated “the existence of a chemical factor or determiner which acts as an inhibitor of facet formation and that, at a higher temperature, the speed of reaction is much greater than at a lower.” This pioneer work of Seyster, which unfortunately was interrupted by the first World War, opened the floodgates for a number of experiments designed to measure the effect of temperature on the development of the Bar eye, with the intention of finding an explanation of the mode of action of the Bar mutant.

Kraftka, in a series of three papers, published in 1920, confirmed and extended Seyster's work to show that temperature was effective during only a limited portion of the larval period. He suggested “... the decrease in facet number to be due to an inhibitor, the temperature coefficient of which differs from that of the normal facet-producing reaction ...” and that “... the Bar eye factor comes into play after about three-fourths of the larval period is finished.” Subsequent work showed that the temperature effective period (T.E.P.) varies in duration (percentage of larval life) and position from temperature to temperature (Driver 1926 and others). For example, at 20°C the T.E.P. occupies approximately 25 percent of larval life, while at 27°C it includes only about 15 percent (Driver 1931).

More recently, Margolis (1934, 1935a, 1935b, 1936) very carefully repeated the temperature work on Bar. His conclusions in the main confirm those of the earlier workers. Margolis (1935a) presented in schematic

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1 Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.
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form a hypothetical system of reactions to explain the action of the Bar mutant. This scheme was based largely on the conclusions of KRAFKA as well as on MARGOLIS's own data. It postulates a substance or substances "... whose quantity at a particular time determines the number of facets," the so-called "facet precursor," and that Bar initiates (MARGOLIS 1935a) or accelerates (MARGOLIS and ROBERTSON 1937) a chain of reactions resulting in a product which combines with the "facet precursor," thus reducing its quantity and consequently the facet number. The interaction of the two "products," the "facet precursor" and the "Bar substance" occurs during the T.E.P. and is limited by the extent of that period. The limits of the T.E.P. are determined by two factors: (a) its onset is determined by the formation of the products resulting from the chain of reactions initiated or accelerated by Bar (CHEN 1929) observed that at the early stages of larval development the eye discs of Bar and wild type larvae are not different, while at later stages they are markedly so); (b) its end depends upon the "great many... processes of general development which determine when differentiation of the optic discs into ommatidia may take place" (MARGOLIS 1935a). KRAFKA (1924) placed this time at three to four days after oviposition.

HUXLEY (1935) offered an alternative hypothesis to that of KRAFKA and MARGOLIS. He claimed that the data on Bar indicate "... that the main difference between Bar and full eye is brought about by differential growth during late larval life." HUXLEY's hypothesis led him to draw the same conclusions concerning the growth curve of Bar eye discs as did GOLD SCHMIDT (see below) with the one difference that HUXLEY recognized the possibility that Bar might reduce the size of the eye disc before as well as during the T.E.P. The main reduction, through the alteration of the growth rate, would occur, however, during the T.E.P.

MEDVEDEV (1935) compared the growth curves of glass2, eyeless2, Lobec, and wild type. He found that the eye discs of the mutants were smaller than those of wild type at 36 hours of larval life. (He started his measurements at 24 hours of larval life, but due to the small size of the cephalic complex at this time, it seems to the present author that these data are unreliable.) He found further that all four types had the same growth rate for the remainder of their development.

The observations on the T.E.P. coupled with those of KRAFKA and CHEN on the morphology of the eye disc lead GOldSchmidt (1938) to believe that a comparison of the growth curves of Bar and wild type eye discs would yield quite different results from those derived by MEDVEDEV. GOldSchmidt, in discussing the Bar case (1938, page 31), says "... certain experimental facts point to the possibility that in this case a phenomenon may occur similar to that observed in the vestigial case, viz.,
a secondary though rather early destruction of already formed eye material." The expectation in the case of Bar, then, would be that with the onset of the T.E.P. the Bar eye discs would become relatively smaller than the wild type, although both would continue to increase in size. If the conclusions discussed above concerning the growth curve of Bar eye discs are true, we would have rather forceful evidence that the definitive effect of Bar on facet number occurs during the T.E.P.—that is, during a limited and well defined period of larval development. It should then be possible to proceed from this point to study by other means—for example, chemical and histological—the exact nature of the Bar reaction.

Clearly then, the answer to the question "When does the definitive effect of Bar on facet number occur?" would be extremely useful in seeking an answer to the larger question "How does Bar affect facet number?" Each of these questions actually includes a series of smaller questions as follows:

(1) When does the effect of Bar on facet number occur? Is it before, during or after the T.E.P., etc.?

(2) How does Bar limit facet number? Is it by an effect on growth rate as postulated by Huxley and Goldschmidt; or by growth limitation as Sinnott and his students have found for fruit size in various Curcurbits (see Sinnott and Dunn (1935) for review); or by resorption of already formed material as in Brachyury and Taillessness in the house mouse (Chesley 1935, Gluecksohn-Schoenheimer 1938); or by destruction (cytolysis) of already formed material as Goldschmidt postulates for the vestigial case in Drosophila melanogaster (Goldschmidt 1937); or does Bar affect facet number simply by reducing the initial capital of cells, etc.?

The method employed to seek answers to these questions consisted primarily in a comparison of the growth curves of the eye discs of Bar and wild type. This method involves the assumption that there is a direct and consistent relation between the size of the eye disc in the mature larvae and the size of the eye of the imago as measured by facet number. That such an assumption is valid is adequately shown by the observations of such correlation for the mutants glass², eyeless², Lobe⁶ (Medvedev 1935), Bar (Chen 1929), and the Bar "alleles" (Steinberg and Abramowitz 1938) as well as for the wild type.

Preliminary reports of various aspects of this work were given in Drosophila Information Service 11, at the Seventh International Congress of Genetics and at the Genetics Society meetings in Woods Hole, August 1940.

MATERIALS AND METHODS

The growth curves of the eye discs were determined by the technique originally described by Medvedev (1935). The method consists of making
camera lucida drawings of the living eye discs immediately after they are
dissected in a drop of Ringer's solution from larvae of known ages. Planim-
eter measurements of the areas of these drawings are then made. Because
the discs are slightly curved it is necessary to flatten them by gentle
tapping with a needle. Variation in the flattening, of course, is a source of
error in the measurements. Other sources of error are the inaccuracies
introduced in the camera lucida drawings and in the measurements of
these drawings. These errors, however, are random and tend to cancel out.
This is shown by the fact that the measurements can be reproduced.

The ages of the larvae were determined as follows: eggs were collected
over a 24 hour period at 25 ± 1°C by the method described by SCHWEITZER
(1935). Newly hatched larvae were collected over a two hour period. Con-
sequently all ages recorded in the paper are from hatching and are accurate
to within ± one hour. The larvae were raised at 27 ± 1°C (unless otherwise
indicated) in three inch Petri dishes containing 15 to 20 cc of the following
food: 2 percent agar, plus 12.5 percent of molasses in H2O, seeded 24 hours
before use with two drops of a heavy yeast suspension. Thirty larvae were
placed in a dish.

Measurements of the eye discs were made at 12 hour intervals from 36
hours after hatching until 84 hours after hatching at which time approxi-
mately one-half of the larvae had pupated.

Eye discs (cephalic complex) of 24 hour old larvae were not measured,
because the magnitude of the error of measurement introduced by the
minute size of the discs at this time makes such measurements meaning-
less. In addition, even at a magnification of 100X it is not possible to be
certain that it is the cephalic complex and not some other tissue that is
being measured.

The following stocks of Drosophila melanogaster were employed: (1)
A Florida wild type strain, which had been inbred for more than 100 gen-
erations; (2) an inbred Bar strain which had been rendered isogenic with
the wild type by repeated back-crossing and which, by the beginning of
this experiment, had been inbred for approximately 100 generations.

No measurements of the cephalic ganglia of the larvae were made, be-
cause preliminary examination indicated that Bar probably did not affect
the size of the cephalic ganglia. This agrees with MEDVEDEV’S observations
that the mutants eyeless², Lobe⁵, and glass² do not affect the size of the
cephalic ganglion even though they have a marked effect on facet number
and optic disc size. The observations that glass² (JOHANSEN 1924), eyeless²
(RICHARDS and FURROW 1925, DERRICK 1928), and Bar (KRAFKA 1924)
reduce the optic tract in the adult brain indicate that an investigation of
the nature of the dependence of the adult brain on the eye would be
fruitful.
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Other materials and techniques used will be described below.

DATA

In view of the fact that both CHEN (1929) and MEDVEDEV (1935) offer extensive descriptions of the cephalic complex and of the eye discs of *Drosophila melanogaster* and that these discs closely resemble the discs of Melaphagus described by WEISMANN (1864), such description will be omitted here. CHEN offers excellent drawings of both Bar and wild type eye discs. The present data entirely confirm MEDVEDEV's description of the course of development of the wild type eye disc with respect to its gross morphology. As in MEDVEDEV's work, it was found that the cephalic complex does not separate into eye and antennal discs until some time between 36 and 48 hours after hatching. MEDVEDEV, in discussing the mutant eye discs, states that the "Morphological resemblance of the imaginal disc and the adult eye consists in the very early appearance of the asymmetry of the former, beginning from the separation of discs from the cephalic complex." It will be recalled that MEDVEDEV worked with *ey*² and *Lc*, both of which are very asymmetrical phenotypically. Bar, on the other hand, is quite symmetrical; in fact, it is identical in shape, but not in size, with the wild type eye disc (CHEN 1929 and the present work). A discussion of the histological structure of the eye disc will be reserved for a later section of the paper.

Tables 1 and 2 present the data obtained from the measurements of the various eye discs. The data show clearly that growth is rapid at first and then gradually falls off. This is further demonstrated in figure 1 and table 3. Figure 1 is based on the data of table 2, while table 3 is calculated from the data in table 2. This change in growth rate had been observed previously by MEDVEDEV and also by ENZMANN and HASKINS (1938) for the eye discs and the dorsal ganglia and is, of course, what has been observed repeatedly in all measurements of this type.

Comparison of the growth curves of Bar and wild type eye discs

The Bar eye disc, or rather the Bar cephalic complex, is clearly and significantly smaller than that of the wild type at 36 hours of larval life. The mean area of the camera lucida drawings of the Bar eye discs equals 151.4 ± 6.3 sq. mm, while that of the wild type equals 177.8 ± 4.8 sq. mm (table 2 and fig. 1). The difference in size between the Bar and wild type eye discs is equally clear at 48 hours (Bar = 345.3 ± 6.7, wild type = 543.6 ± 9.1). At this age the optic discs may be directly compared with each other, since at this time the cephalic complex has separated into the eye and antennal discs. Reference to tables 1 and 2 will show that the differences in size are equally marked at 60, 72, and 84 hours of larval life.
Areas in square millimeters of the camera lucida drawings of +, B and B; m(B) eye discs taken from larvae at the ages indicated in the table. Magnification = 140 X. Temperature = 27 ± 1°C.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>36 hours</th>
<th>48 hours</th>
<th>60 hours</th>
<th>72 hours</th>
<th>84 hours*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ φ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>182.8 ± 5.8</td>
<td>22.0</td>
<td>47</td>
<td>171.1 ± 8.0</td>
<td>27.6</td>
</tr>
<tr>
<td>B</td>
<td>154.5 ± 6.3</td>
<td>22.4</td>
<td>40</td>
<td>153.6 ± 4.9</td>
<td>19.6</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>133.3 ± 3.8</td>
<td>20.9</td>
<td>54</td>
<td>143.0 ± 5.9</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>572.9 ± 11.7</td>
<td>15.3</td>
<td>57</td>
<td>489.7 ± 14.4</td>
<td>16.5</td>
</tr>
<tr>
<td>B</td>
<td>362.0 ± 6.8</td>
<td>10.7</td>
<td>33</td>
<td>332.7 ± 7.5</td>
<td>14.9</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>336.6 ± 14.2</td>
<td>24.2</td>
<td>33</td>
<td>340.9 ± 6.0</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>946.5 ± 19.8</td>
<td>14.8</td>
<td>52</td>
<td>938.5 ± 15.5</td>
<td>9.4</td>
</tr>
<tr>
<td>B</td>
<td>773.7 ± 9.8</td>
<td>9.5</td>
<td>56</td>
<td>720.9 ± 16.5</td>
<td>11.0</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>629.9 ± 10.6</td>
<td>11.0</td>
<td>42</td>
<td>642.4 ± 12.2</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1138.9 ± 25.9</td>
<td>14.3</td>
<td>40</td>
<td>1127.8 ± 40.9</td>
<td>20.7</td>
</tr>
<tr>
<td>B</td>
<td>914.9 ± 11.6</td>
<td>8.6</td>
<td>46</td>
<td>803.5 ± 15.1</td>
<td>10.9</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>854.0 ± 12.5</td>
<td>9.4</td>
<td>41</td>
<td>836.9 ± 13.8</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1347.0 ± 42.4</td>
<td>19.5</td>
<td>38</td>
<td>1345.6 ± 32.5</td>
<td>15.0</td>
</tr>
<tr>
<td>B</td>
<td>1005.8 ± 20.7</td>
<td>11.3</td>
<td>30</td>
<td>895.0 ± 27.3</td>
<td>17.7</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>1036.4 ± 13.1</td>
<td>8.4</td>
<td>45</td>
<td>1023.2 ± 20.0</td>
<td>11.1</td>
</tr>
</tbody>
</table>

* At this time approximately one-half the larvae had pupated. Only those larvae which had not pupated were used; consequently these values may be lower than what would have been obtained from a random sample.
† This value appears to be too low as evidenced by comparison with the females and also by comparison with the values in table 5. See the text for further discussion.

Relation to the T.E.P.

Driver (1926) has shown that at 27°C the T.E.P. begins at about the 60th hour of egg-larval life and ends 15 hours later—that is, after 75 hours of egg-larval life are completed. According to Powsner (1935) the length of the egg stage at 27°C in the wild type line with which he worked is 13 hours. If it is assumed that this is true, or approximately true for the Bar stock used in these experiments, the T.E.P. at 27°C begins at sometime between 45 and 50 hours of larval life and ends after approximately 60 to 65 hours of larval life are completed. It is clear, therefore, that the measurements which were made at 36 hours after hatching were made well before the T.E.P. began, while those made at 48 hours of larval life were made at approximately the time of onset of the T.E.P. It will be recalled that
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Table 2

Same as table 1 except that the values given here are derived from the combined male and female data.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>MEAN ± σM</th>
<th>CV</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>177.8 ± 4.8</td>
<td>24.3</td>
<td>82</td>
</tr>
<tr>
<td>B</td>
<td>154.1 ± 4.0</td>
<td>22.8</td>
<td>77</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>136.5 ± 3.2</td>
<td>21.1</td>
<td>81</td>
</tr>
<tr>
<td>48 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>543.6 ± 9.1</td>
<td>15.7</td>
<td>88</td>
</tr>
<tr>
<td>B</td>
<td>345.3 ± 6.7</td>
<td>17.1</td>
<td>77</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>337.9 ± 6.8</td>
<td>18.1</td>
<td>81</td>
</tr>
<tr>
<td>60 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>943.4 ± 13.3</td>
<td>13.0</td>
<td>85</td>
</tr>
<tr>
<td>B</td>
<td>758.3 ± 7.3</td>
<td>8.6</td>
<td>79</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>655.5 ± 8.0</td>
<td>11.0</td>
<td>76</td>
</tr>
<tr>
<td>72 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1134.0 ± 23.1</td>
<td>17.3</td>
<td>72</td>
</tr>
<tr>
<td>B</td>
<td>867.6 ± 9.2</td>
<td>9.4</td>
<td>80</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>845.7 ± 9.3</td>
<td>9.8</td>
<td>80</td>
</tr>
<tr>
<td>84 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1346.3 ± 26.5</td>
<td>17.3</td>
<td>77</td>
</tr>
<tr>
<td>B</td>
<td>946.9 ± 17.9</td>
<td>15.1</td>
<td>64</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>1038.8 ± 11.3</td>
<td>9.6</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 3

Growth increments of +, B and B; m(B) eye discs. (Calculated from the data of table 2.)

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>48/36</th>
<th>60/48</th>
<th>72/60</th>
<th>84/72</th>
<th>AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>3.06 ± 0.09</td>
<td>1.74 ± 0.04</td>
<td>1.20 ± 0.02</td>
<td>1.19 ± 0.03</td>
<td>1.80 ± 0.26</td>
</tr>
<tr>
<td>B</td>
<td>2.24 ± 0.07</td>
<td>2.19 ± 0.07</td>
<td>1.14 ± 0.02</td>
<td>1.09 ± 0.02</td>
<td>1.67 ± 0.20</td>
</tr>
<tr>
<td>B; m(B)</td>
<td>28.4 ± 0.08</td>
<td>1.88 ± 0.04</td>
<td>1.33 ± 0.02</td>
<td>1.23 ± 0.02</td>
<td>1.73 ± 0.23</td>
</tr>
</tbody>
</table>

according to the hypothesis as expressed by Goldschmidt (1938)—that is, "a secondary . . . destruction of already formed eye material"—the eye discs of Bar and wild type may be expected to be the same size before the T.E.P. and to become different only after the T.E.P. has begun. This change in relationship is accredited to the presence of different growth rates in the two forms during the T.E.P. The data show conclusively that the Bar eye discs are smaller than the wild type at 36 hours of larval life—that is, well before the T.E.P.

Huxley (1935), as stated above, did recognize the possibility that Bar might reduce the size of the cephalic complex (eye disc) before the T.E.P.,
although he, like Goldschmidt, postulated that the main effect of Bar would be detected as a reduction of the growth rate during the T.E.P. A comparison of the Bar and wild type eye discs at the beginning and end of the T.E.P. should afford a simple and direct test of this assumption. If the Bar eye discs have a slower rate of growth during the T.E.P. than do those of the wild type, the ratio of the area of the Bar discs at the end of the T.E.P. to that at the beginning should be less than the same ratio for the wild type. It has been shown above that probably all of the T.E.P. at 27°C falls between the 45th and 65th hours of larval life. Consequently a comparison of the ratios of Bar at 60 to Bar at 48 hours with that of wild type at 60 to wild type at 48 hours is almost exactly what is required. The column headed 60/48 of table 3 contains the necessary data. The ratio for Bar is $2.19 \pm 0.05$; that for wild type is $1.74 \pm 0.04$. The growth rate of Bar, contrary to what would have been predicted from the hypothesis, is higher rather than lower than that of wild type. (It will be shown below that these values are probably the same.)

The growth rates of Bar and wild type between 60 and 72 hours and
between 72 and 84 hours are statistically the same, the corresponding values being 1.14 and 1.09 for Bar and 1.20 and 1.19 for wild type (table 3). It is clear, therefore, that there is no reduction in the growth rate of the Bar eye discs relative to that of the wild type eye discs either during or after the T.E.P. It can be shown by elementary algebra that the apparent greater growth rate of wild type as compared to that of Bar during the 36 to 48 hour interval is due in large part and perhaps entirely to the fact that at 36 hours the cephalic complex has not separated into optic and antennal discs while at 48 hours it has (the antennal discs of Bar and wild type are the same). Since the actual size of the antennal disc at 36 hours cannot be determined, no direct comparison of the growth rates of Bar and wild type optic discs can be made for the 36 to 48 hour interval.

The above data indicate that the growth curve of the Bar eye discs bears the same relationship to that of the wild type as do those of glass², eyeless², and Lobe³. Consequently, neither of the hypotheses mentioned above explains the data and both must therefore be discarded. A more extensive discussion of these data will be presented below.

Comparison of the growth curves of Bar and modified Bar eye discs

The mutant m(B) is a semi-dominant modifier of Bar. It was found originally in two B;px sp males with exceptionally large eyes and has been maintained in a stock having this genotype. Subsequent work has shown the modifier to be located in the second chromosome approximately six units to the left of px. It has also been shown that m(B) does not have an effect on facet number in the absence of Bar or one of its 'alleles' (Steinberg in press). For the purposes of this paper px (plexus) and sp (speck) may be ignored (Steinberg in press). This stock was inbred but had not been backcrossed to either the Bar or wild type stocks.

At 25°C modified Bar males have 220 facets; the females have 141 facets. In the Bar stock used in these experiments the males have 74.2 facets and the females 75.2 facets at 25°C. At 29°C the facet number of the modified Bar males is 169.7 and that of the females 114.5; those of Bar are 38.0 and 35.7 in the males and females respectively (Steinberg in press). It is apparent that an increase of temperature has less effect on facet number in the case of modified Bar than in the case of Bar. No counts of facet number were made at 27°C. Nevertheless, it may be concluded that the facet numbers of Bar and modified Bar at this temperature are intermediate between those at 25° and 29°C. Equally, the difference in facet number between Bar and its modified form will be intermediate to that at 25° and 29°C. Therefore, it was expected that the eye discs of modified
Bar would be larger than those of Bar, since previous work had shown a correlation between facet number and disc size. Tables 1, 2, 3, and 4, and figure 1 present the pertinent data.

Considering the sexes at each age separately (table 1), the females show the following relationships: at 36 hours there is a barely significant difference (Diff. = 21.2 ± 7.3) which is in the opposite direction to that expected (B = 154.5 ± 6.3, and B;m(B) = 133.3 ± 3.8); at 48 hours the discs are the same size statistically (Diff. = 28.4 ± 15.7); at 60 and 72 hours the discs are significantly different in size, but contrary to expectations, the Bar discs are larger than the modified Bar (B = 773.7 ± 9.8 and 914.9 ± 11.6, and B;m(B) = 629.9 ± 10.6 and 854.0 ± 12.5); at 84 hours the discs are again statistically the same (Diff. = 30.6 ± 24.5); the males on the other hand show significant differences in size at 60 and 84 hours of age only. At 60 hours the Bar eye discs are larger (B = 720.9 ± 16.5, and B;m(B) = 642.4 ± 12.2), while at 84 hours the modified Bar eye discs are larger (B = 895.0 ± 27.3, and B;m(B) = 1023.2 ± 20.0). Because of subsequent measurements made on Bar eye discs from mature larvae, it is believed that there was some undetected experimental error involved in the measurement of the male Bar eye discs at 84 hours. These measurements were made to test a hypothesis advanced in a later section of this paper. They will be discussed in that light below. For the present, it will suffice merely to indicate that measurements were made of the eye discs of mature Bar larvae which had completed their development at either 20°, 25° or 28°C, after spending the first 24 hours of their larval development at 25°C. All the larvae were therefore of the same physiological age. The data are presented in table 5. The magnification here was 146 diameters as compared with 140 diameters in the earlier measurements. The correction factor for translating the values of table 1 into those of table 5 is therefore (146)^2/(140)^2, or 1.09. This gives a corrected value of 1096.3 for the 84 hour old Bar females and 975.6 for the corresponding males. The corrected value for the females agrees very well with the values of table 5, while in the case of the males it is clearly much lower. Since all three of the mean areas shown in table 5 are statistically the same, and since all three are larger than the corresponding value for the Bar males given in table 1, it seems probable that the true value for the disc area is closer to those of table 5 than that of table 1.

It will be noticed that the only age at which there is a significant statistical difference in disc size in both sexes between Bar and modified Bar is at 60 hours of larval life (table 1) and that contrary to expectation, Bar is larger than modified Bar. Table 3 shows that during the interval of 48 to 60 hours of larval life Bar has a faster growth rate than either wild type or modified Bar; while during the interval of 60 to 72 hours, Bar has a
slower growth rate than either wild type or modified Bar. Table 4 shows
that the ratio of wild type to Bar at 60 hours is significantly smaller than
that at 48 or 72 hours. Furthermore, only at 60 hours is there a significant
difference between the ratios of wild type to Bar and wild type to modified

Table 4

<table>
<thead>
<tr>
<th>AGE</th>
<th>RATIO</th>
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<tbody>
<tr>
<td>36</td>
<td>1.15±0.05</td>
</tr>
<tr>
<td>48</td>
<td>1.57±0.05</td>
</tr>
<tr>
<td>60</td>
<td>1.24±0.01</td>
</tr>
<tr>
<td>72</td>
<td>1.31±0.03</td>
</tr>
<tr>
<td>84</td>
<td>1.42±0.04</td>
</tr>
</tbody>
</table>

Bar. In addition, the growth curve of the Bar eye discs as plotted in figure
1 shows only one point very much out of line with that of the wild type—
namely, that at 60 hours. All the other points fit fairly closely to a line
drawn parallel to that of the wild type growth curve. For these several
reasons it appears likely that the measurements of the Bar eye discs at 60
hours are too large—that is, that the larvae were actually physiologically
older than 60 hours. This could easily be due to an undetected rise in
temperature. Since there is no consistent size difference between the eye
discs of Bar and modified Bar, the conclusion that they are identical in
size and exhibit the same growth pattern seems warranted.

Table 5

Areas in square millimeters of the camera lucida drawings of Bar eye discs from mature larvae
which had spent the first 24 hours of their development at 25°C and the remainder at the indicated tem-
peratures. (Magnification = 146×.)

<table>
<thead>
<tr>
<th>TEMPERATURE</th>
<th>Φ Φ</th>
<th>Φ Φ</th>
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<tbody>
<tr>
<td>20°C</td>
<td>1112.5±18.5</td>
<td>10.7</td>
</tr>
<tr>
<td>25°C</td>
<td>1104.0±19.0</td>
<td>11.0</td>
</tr>
<tr>
<td>28°C</td>
<td>1098.7±21.5</td>
<td>11.7</td>
</tr>
</tbody>
</table>

The possibility remains, however, that the difference in facet number
between Bar and modified Bar is not great enough to be detected as a size
difference in the eye discs under the conditions of this experiment. The
data of table 6 showing the measurements of eye discs taken from mature
double Bar (BB) and infra Bar (B1) larvae raised at 25°C clearly demon-
strate that this is not so. These larvae were raised under exactly the same
culture conditions as were the wild type, Bar and modified Bar larvae
Areas of BB and B\textsuperscript{i} eye discs from mature larvae raised at 25\textdegree C. (Magnification=146\times.)

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>( ? ? )</th>
<th>( ? ? )</th>
<th>( \sigma \sigma )</th>
<th>( \sigma \sigma )</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MEAN ( \pm \sigma_m )</td>
<td>( C_v )</td>
<td>( N )</td>
<td>MEAN ( \pm \sigma_m )</td>
</tr>
<tr>
<td>( BB )</td>
<td>931.5( \pm ) 18.5</td>
<td>12.9</td>
<td>42</td>
<td>948.4( \pm ) 18.7</td>
</tr>
<tr>
<td>( B^i )</td>
<td>1311.5( \pm ) 29.0</td>
<td>12.8</td>
<td>31</td>
<td>1168.9( \pm ) 21.5</td>
</tr>
</tbody>
</table>

except for temperature, which in this case was 25\textdegree C instead of 27\textdegree C. Measurements were made when approximately one-half of the larvae in the dish had pupated. When raised at 25\textdegree C, the \( BB \) stock used has 28 facets (STEINBERG in press). The \( B \) stock when raised at 27\textdegree C probably has between 50 and 60 facets as indicated by work on other \( B \) stocks with similar facet numbers to that of the stock used here (cf. Margolis 1935a). It is clear then that a decrease of approximately only 35 facets (or 50 percent) may result in a detectable decrease in disc size. The \( B^i \) eye discs are clearly larger than either the \( B \) or \( B;\text{m}(B) \) eye discs (table I, 6). The \( B^i \) stock used here has 345 facets at 25\textdegree C (STEINBERG in press). Therefore, an increase of approximately 300 facets (400 percent) if Bar is taken as the base, or of approximately 125 facets (50 percent) if the modified form is taken as the base, may be easily detected as an increase in disc size. There remains, then, the fact that although Bar and its modified form differ by about 150 facets (200 percent), a number which we have seen may lead to a detectable difference in disc size, no difference was found.

**Histological**

ENZMANN and HASKINS (1938) studied the histological development of the eye discs of wild type larvae. They reported that at 26 hours (presumably from the time of egg laying, although this is not made clear in the paper; nor is the temperature stated) “... the anlage appears to consist of rod-like elements each of which contains one to three nuclei.” These elements increase in number, they report, as development proceeds until in older larvae “... their number approaches that of the number of ommatidia in adult flies.” It is perhaps worth noting that although the authors report the time of origin of these ‘elements’ to be 26 hours, in their table 2 they give the number of ‘elements’ present at 18 and 21 hours.

KRAFKA (1924) reported that histological examination of the eye discs of larvae 24 hours before they pupate showed that “... the rudiments of the ommatidia are fixed...” He found that “... four terminal cells and six basal cells arranged around a deeply staining axis, form a cylindrical unit that is repeated over the entire inner surface of the imaginal disc.” He concluded that it was this differentiation of the rudiments of the omma-
tidia which determined the end of the T.E.P. In view of the findings to be reported here, it is worth noting that although Enzmann and Haskins (1938) studied the same and later stages than Krafska, they did not report the finding of structures such as he reported.

Larvae were raised in Petri dishes at 25° and 27°C as recorded above. Groups of larvae were fixed at various ages after hatching from the egg. The fixatives used were Carnoy II (glacial acetic one part, chloroform three parts, and absolute alcohol six parts) at 50°C and hot aqueous Bouin (90° to 100°C). It was soon found that Carnoy and particularly a modification of Carnoy consisting of glacial acetic two parts, chloroform three parts, and absolute alcohol five parts gave much better fixation than did Bouin, and hence most of the work was done with Carnoy or the modification reported above. The larvae were sectioned at 5, 7, or 10 micra, the last being used most often. The slides were stained in either Gentian violet or Delafield’s haemotoxylin with eosin as a counter stain.

**Wild type eye discs**

Figures 2 through 10 are photographs of representative sections from larvae of various ages. At 24 hours after hatching (27°C) the eye disc is leaf shaped (Medvedev 1935, and fig. 2, 3, 4). Contrary to Chen (1929), who reported that the optic stalk first begins to develop at 40 hours, the present study shows that at 24 hours a well developed optic stalk is present and that it is already attached to the brain (fig. 2, 3, 4; also cf. Weismann 1864, fig. 19b). Kaliss (1939) has shown that the frontal sac is already present at 12 hours of embryonic life—that is, after one-half of the embryonic life is completed. This observation is supported by Patterson’s (1929) X-ray experiments in which mosaics of the entire eye or of one-half of the eye were obtained only when very early egg stages were treated. Consequently, it is not surprising that the optic stalk should be developed as early or earlier than 24 hours rather than as late as Chen indicated.

The cephalic complex at this time (24 hours) is several cell layers thick. The cells are large, long, and fusiform. They overlay one another somewhat as do shingles on a roof. Beyond this there is no visible organization into a pattern. At 48 hours the antennal and optic discs are clearly formed as individual structures (fig. 6). They are connected by a thin layer of cells. There has been a tremendous increase in cell number, but no special organization of cells into larger units has occurred. In fact the cell arrangement in the eye and limb discs is remarkably similar (fig. 5, 6, 7).

By 72 hours at 27°C the discs have grown considerably. The antennal disc has undergone much folding as have also the wing and limb discs (see Auerbach 1936 for a description of development of the wing and limb discs). The eye disc has increased greatly (table 1, 2). At this time
the organization of the cells into larger units may be seen for the first time. The units appear to consist of clusters of four cells. The clusters are regularly arranged in a pattern quite similar to that of the ommatidia. It is probably because of this that Kraflka, who first saw these structures, believed them to be the forerunners of the ommatidia themselves. This may be true, but in the author's opinion, the question had best be left open until further study actually affords direct evidence in support of this assumption, particularly since Bodenstein (1938) claims that the ommatidia do not differentiate until after pupation. At 72 hours the clusters are relatively few in number, closely approximated to each other, and not very distinct. In 96 hour old larvae (temperature = 25°C; these larvae are physiologically the same age as 84 hour old larvae raised at 27°C) these units have greatly increased in number. They are now for the most part widely separated from each other although still arranged in very regular rows (fig. 8). That they are superficial structures with a thickness of about ten micra may be seen by a comparison of figures 8, 9, and 10 (these figures are photographs of three adjacent serial sections of the eye disc, figure 8 is the most superficial). That each unit is a cluster of four cells may be seen more readily in the older larvae (fig. 11). Thus far it has not been possible to confirm Kraflka's (1924) statement that 24 hours before pupation "... four terminal cells and six basal cells arranged around a deeply staining axis, form a cylindrical unit that is repeated over the entire inner surface of the imaginal disc." The present study has not revealed any more than four cells in any cluster, nor has it shown a deeply staining axis. At no stage were any structures resembling those reported by Enzmann and Haskins observed.

Bar eye discs

Through the first 48 hours (temperature = 25°C) of larval life, the Bar eye disc is histologically the same as the wild type. The first difference was noted at 72 hours (temperature = 25°C) after hatching. Bar unlike the wild type showed no evidence whatsoever of the organization of the cells into clusters. Histologically the disc appears not to differ from one which is 48 hours old. What is even more startling is the fact that at 96 hours (temperature = 25°C) there is still no sign of any organization of the cells into the clusters described for the wild type (fig. 12, 13). Twenty-two larvae were examined at 72 hours and 23 at 96 hours. As yet no study of later stages (prepupal and pupal) has been made, consequently the possibility remains that the organization of the cells into clusters occurs later.

It was pointed out above that Kraflka concluded that it was the formation of the clusters of cells ("rudiments of the ommatidia"—Kraflka) that determined the end of the T.E.P. This appears entirely unlikely in view of the present observations. In Bar no differentiation was observed
FIGURE 2.—Longitudinal section through brain and cephalic complex of a 24 hour old wild type larva showing the optic stalk extending to brain. Sectioned at 20μ. 300X.

FIGURE 3.—Same as figure 2. 1000X.

FIGURE 4.—Longitudinal section through another 24 hour old wild type larva showing cephalic complex and optic stalk. Sectioned at 10μ. 300X.

FIGURE 5.—Photograph of a portion of figure 6. 420X.

FIGURE 6.—Frontal section of a 48 hour old wild type larva.
Compare eye and wing discs. 260X.

FIGURE 7.—Longitudinal section of a 48 hour old wild type larva.
Compare eye and limb discs. 260X.

List of abbreviations used in figures 2 through 7: A antennal disc, B brain, E cephalic complex in 24 hour old larvae; eye disc in older larvae, L limb disc, P pharynx, S optic stalk, T trachea, W wing disc.
FIGURES 8, 9 and 10.—Successive sections through the eye disc of a 96 hour old wild type larva. 260X.

FIGURE 11.—Photograph of the central portion of the eye disc of figure 8 showing details of the structure of the cell clusters. 1000X.

FIGURE 12.—Photograph of a portion of figure 13. E eye disc. 420X.

FIGURE 13.—Longitudinal section of the brain and cephalic complex of a 96 hour old Bar larva. A antennal disc, B brain, T trachea. 200X.
in any larval stage, yet the T.E.P. ends about 24 hours before puparium formation (at $25^\circ$C). In wild type on the other hand differentiation into what Kraffa has called “rudiments of the ommatidia” occurs at least as early as 72 hours after hatching, and probably earlier. Margolis and Robertson (1937) found that the T.E.P. in wild type extended over the entire larval period, up to prepupa formation. Regardless of what role these structures play in the development of the eye, it is certain that their formation does not determine the end of the T.E.P.

A more complete discussion of the role of these “cell clusters” in the differentiation of the eye especially with regard to the differences between Bar and wild type will have to be deferred until histological observations on prepupal and pupal stages have been made.

**DISCUSSION**

The study of the growth curves of Bar, modified Bar, and wild type eye discs showed that the discs of both of the former are already smaller than wild type at 36 hours after hatching, the earliest stage examined, and that from this point on they grew at the same rate as wild type. Since this age is prior to the T.E.P. at $27^\circ$C, it is obvious that Bar changes the size of the eye discs before the T.E.P. Furthermore, since the growth rate of the Bar eye discs from 36 hours until puparium formation is the same as that of the wild type, it must be concluded that Bar does not affect growth during this period. The data, however, do not offer any direct evidence on whether or not Bar affects the growth rate prior to this period.

Kaliss (1939) has shown that the frontal sac is present when only approximately one-half of the embryonic period is completed. Therefore, at $27^\circ$C the frontal sac—cephalic complex—has been present and growing for about 42 hours by the time 36 hours of larval life are completed. Consequently, it is possible that the growth rate of Bar is lower than that of wild type prior to 36 hours of larval life and the same as that of wild type after this point. If this is true for Bar, it would also be the likely explanation for the mutants studied by Medvedev (1935)—that is, eyeless, glass, and Lobec. But at 36 hours of larval life the eye discs of these mutants are all different in size from each other. There are several possible explanations to account for the origin of these differences in disc size. It may be assumed that each mutant exhibits a different growth rate from all the others during this period; or that they all have the same growth rate during this period, slower than that of the wild type, and that each maintains this reduced rate for varying periods of time, and henceforth grows at the same rate as the wild type. Such shifts in growth rates as are assumed in these two alternatives are, to the author's knowledge, unprecedented and seem to be quite unlikely.
The difference in disc size might have arisen through the later origin of the cephalic complex of the mutants as compared to wild type. No histological study of the early embryology of the mutant forms has as yet been made, consequently no direct evidence can be brought to bear on this question. The observation that although differentiation has occurred in the eye discs of wild type by 72 hours and is extensive at 96 hours (fig. 8, 9, 10) none has occurred in Bar (fig. 12, 13) would seem to argue in favor of this assumption. However, if the cell clusters are really the precursors of the ommatidia as Krafa assumed, one would expect many fewer in Bar than in wild type; hence it might be possible that, although present, they were missed. Only further study can answer this question. But if this explanation is invoked to explain the size difference of the four mutants (B, ey², gl², and L⁶), we are faced with the necessity of assuming that the developmental pattern of Drosophila melanogaster is an extremely labile one so that the relative time of origin of a structure may be shifted very easily. In view of the known mosaic nature of the Dipteran egg (see Richards and Miller 1937 for review) and in view of the many experiments on amphibia showing the non-labile nature of the time relationships in the developing organism, this does not seem likely.

A third possible explanation is that the capital of cells involved in the initial formation of the cephalic complex is different in the various mutants and that the growth rate is the same in all the mutants throughout development. This explanation does not involve any of the difficulties inherent in the first two. The necessity for an extremely large number of different growth rates (proportional to the number of mutants affecting eye size) maintained for varying lengths of time is obviated, as is the necessity for assuming that the time of origin of the cephalic complex relative to the general growth pattern is so labile as to be almost haphazard. In addition, there is abundant evidence to show that the initial cell number of an organ does vary. Finally, the growth curve data on Bar, modified Bar, eyeless², glass², and Lobe⁶ indicate that the growth rates of the eye discs of all these mutants, at least during that part of the egg-larval period when they can be measured, are identical with each other and with wild type. For these reasons, the assumption that Bar reduces the size of the eye discs and ultimately the facet number by reducing the initial capital of cells forming the cephalic complex seems the most likely.

The work on the T.E.P. shows that the final facet number is not completely determined at the time of origin of the eye anlage but that it may be varied considerably by changes in temperature during a specific period of larval development. The above data and discussion indicate that the T.E.P. is not the period during which Bar acts to reduce facet number (Krafa, and others). It is probable that the T.E.P. is simply the period during which the facet number may be influenced by temperature.
Before discussing the data further, it will be necessary to review some facts of the development of the imago.

Weismann (1864), in his remarkable study of the imaginal discs of Musca vomitoria, showed that the eye disc gives rise to a good deal of the head in addition to the eye. Beadle and Ephrussi (1936) and Chevais (1937) have shown this to be true for Drosophila melanogaster as well. Experiments of the author in which the antennal and optic discs of mature larvae were separated before transplantation into larvae of the same age show that in addition to the eye, the eye disc forms all of the head with the exception of the antennae and the mouth parts. Sturtevant (1927) has pointed out that the region which is not faceted in the Bar eye but is so in the wild type is chitinous and appears exactly like the rest of the chitinous head.

The experiments of Howland and Child (1935) in which eye and head defects in Drosophila imagos were obtained as a result of injuries to a localized region in four hour old eggs, and those of Patterson cited above, support the assumption that the cephalic complex is determined very early in embryonic development. Kaliss's (1939) observation that the frontal sac is present when only one-half of the embryonic development is completed lends further support to this assumption.

Direct evidence that the cephalic complex is determined after 24 hours of larval life was afforded by experiments in which the cephalic complex was transplanted from larvae of this age to 48 hour old hosts. Four sets of transplants were made as follows: wild type into wild type, wild type into Bar, Bar into wild type, and Bar into Bar. In every case the implant was autonomous (table 7). The wild type cephalic complex gave rise to a wild type eye regardless of the host and likewise the Bar cephalic complex gave rise to a Bar eye regardless of the host. The other structures formed by the cephalic complex (see above) were also shown by these experiments to have been determined at the time of transplantation. These experiments indicate not only that the eye is determined by 24 hours of larval development, but also that the Bar eye disc is already determined to form eye, but more specifically Bar eye. This conclusion is supported by the data

<table>
<thead>
<tr>
<th>DONOR</th>
<th>HOST</th>
<th>IMPLANT</th>
<th>N</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>B</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>B</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 7
Transplants of 24 hour old eye discs into 48 hour old hosts. Temperature = 27 ± 1°C.
on the growth curve which indicate an early effect of Bar on the eye disc. What remains undetermined is just what size of Bar eye will be formed.

As pointed out in the introduction to this paper, it has been shown that an increase in temperature leads to a decrease in facet number in Bar flies and that temperature is effective during only a limited period of the larval stage. It was also pointed out that these facts lead to a hypothesis which assumed the existence of some mechanism inhibiting facet formation and which is effective during the T.E.P. This mechanism, it was supposed, had a higher $Q_{10}$ than that of the general growth processes of the organism, and hence at higher temperatures fewer facets were formed.

Sturtevant (1925) reported the occurrence of an allele of Bar, infra-Bar ($B^i$) which had a facet number intermediate to that of Bar and wild type. Luce (1926, 1931, 1935) studied the effects of temperature on this mutant. He found that contrary to what was found for Bar, an increase in temperature yielded an increase in facet number. Here again as in Bar, temperature was effective during only a limited period of the larval stage. To explain these facts, Luce assumed that in $B^i$ as in $B$ there is a process inhibiting facet formation, but that unlike the process in Bar, this process has a lower $Q_{10}$ than that of the general growth processes of the larva.

For Bar, at any rate, it seems highly unlikely that the postulated "facet inhibiting reaction," if it exists at all, is responsible for the major reduction in facet number, unless its time of action be assumed to be at a stage much earlier than that of the T.E.P. But this is unjustified, since the "facet inhibiting reaction" was invoked to explain the T.E.P. It is possible of course to retain the "facet inhibiting reaction" as an explanation of the T.E.P., but to assign a secondary role to it—that is, to have it function in the determination of the final size of the Bar eye, but not in deciding whether the eye will be the wild type or Bar.

The development of the Bar eye may be pictured as follows: by 24 hours of larval development, and in view of the mosaic pattern of the Dipteran egg and of the early origin of the frontal sac, very likely much earlier than this, the phenotype of the Bar eye is already determined; this determination, however, is not complete but labile so that the facet number may be shifted, within limits, to either a greater or smaller number. Several factors are already known which do this. Temperature is of course the most commonly known and used; others are pure oxygen atmosphere (Margolis 1939) and a nitrogenous extract of Calliphora larvae when fed to Bar larvae (Ephrussi, Khouvne, and Chevais 1938; Chevais and Steinberg 1938). It is noteworthy that both these agents are effective at times other than that at which temperature is effective. Facet number may be affected by increasing or decreasing the length of the growth period of the eye disc relative to that of the larva in which it arose by transplanting the
disc into a younger or older host (Beadle and Ephrussi 1936; Boden- 
stein 1939). The present discussion, however, is concerned only with 
those cases in which the disc remains in its normal position.

As pointed out above, this shifting of the facet number is possible be- 
cause determination is not complete until late in development. The labilely 
determined tissue may form either head chitin or eye (facets, ommatidia). 
If this be correct, an increase or decrease in facet number in the Bar eye 
caused by a change in temperature should not involve a change in the 
size of the eye disc. The following experiment was performed to test this 
assumption. Eggs from genotypically Bar females were collected over a 
24 hour period at 25 ± 0.25°C. Newly hatched larvae were collected over 
two hour intervals. They were permitted to continue the first 24 hours of 
their development at 25°C. At the end of this time the larvae were divided 
into three groups: one group was transferred to 28 ± 1°C, a second to 
20 ± 1°C, the third was left in the 25°C incubator. The larvae were per- 
mitted to continue their development at these temperatures until approxi- 
mately one-half the larvae in a particular Petri dish had pupated. In this 
way a uniform physiological age was obtained. The eye discs of the remain- 
ing larvae were then measured as described above. Because the entire 
T.E.P. was spent at the temperature at which the larvae had completed 
their development, the facet numbers of each of the groups should cor- 
respond to that characteristic for the temperature at which development 
was completed. Facet number should vary, therefore, from approximately 
50 (corresponding to 28°C) to approximately 125 (corresponding to 20°C), 
a range sufficiently large to yield disc size differences as was shown above. 
Table 5 shows the data derived from the measurements of the areas of 
these eye discs. There can be no doubt that the data show no significant 
difference between the areas of the eye discs of either sex. The eye discs 
of the females vary in size from 1098.7 ± 21.5 to 1112.5 ± 18.5; those of the 
males vary from 1007.5 ± 10.0 to 1057.5 ± 28.3. The difference between 
these two latter values is 49.9 ± 30.0 and is not significant. Furthermore, 
it should be noted that in the males the area at 25°C is smaller than that 
at 28°C, although not statistically so (table 5). The data, therefore, are 
in agreement with what was predicted by the hypothesis and to that 
extent confirm the hypothesis.

The modified Bar eye discs were shown to be identical in size with the 
Bar eye discs throughout the period of observation despite the fact that 
the facet number of the former is considerably higher than that of the 
latter. It will be recalled that m(B) is a modifier of Bar which has no 
phenotypic expression in the absence of Bar or one of its alleles. It has 
been shown for at least one extrinsic modifier of Bar—namely, tempera- 
ture—that a change of facet number brought about through its action
does not affect the disc size. There is no \textit{a priori} reason for assuming that intrinsic modifiers of Bar may not act in the same way—that is, shift labilely determined tissue in one direction or another so as to increase or decrease the facet number. It is proposed that $m(B)$ is an intrinsic modifier of facet number in Bar which acts by causing more of the labilely determined tissue to form facets than would have done so in the absence of $m(B)$. The explanation of how the modifier causes this result must await further experimentation.

It is indeed a pleasure to acknowledge my indebtedness to Dr. L. C. Dunn for his many helpful suggestions and for his sustained interest during the progress of these experiments.

**SUMMARY**

The growth curves of Bar, of Bar in association with a second chromosome modifier of Bar ($m(B)$), and of wild type eye discs were determined from 36 hours after hatching until, but not including, puparium formation.

All three eye discs showed the same growth rate throughout the period of observation.

Bar and modified Bar were the same size throughout the experiments. Both were smaller than wild type at 36 hours, the earliest stage measured. This is before the onset of the T.E.P.

Histological examination of the eye discs showed the presence in the wild type eye discs of ‘cell clusters’ consisting of four cells each at 72 hours of larval life (temperature $= 25^\circ C$) and indicated that these increased in frequency between 72 and 96 hours. No such ‘cell clusters’ were found in Bar even at 96 hours.

The time of origin of the optic stalk was placed at no later than 24 hours after hatching (temperature $= 27 \pm 1^\circ C$).

Some possible explanations of the mode of development of the Bar eye were discussed. The following seemed the most likely: the Bar eye was determined very early in development. This determination was labile so that facet number could be changed, within limits, by extrinsic and intrinsic modifiers. Changes in facet number brought about in this way would not affect disc size. This was tested for temperature and shown to be true.

It was assumed that the $m(B)$ is an intrinsic modifier of Bar which affects the distribution of the labilely determined tissue in such a way that more of it goes to form facets than in the absence of $m(B)$.

**LITERATURE CITED**

DEVELOPMENT OF BAR EYE


* Original not seen by author.


